The 1st Joint CSHP and SH Workshop 2019

Gran Meliá Hotel, Xi'an, China

Agenda

Time	Topic	Presenter	Chairs
Friday, October 18, 2019	**Registration** The Gran Meliá Hotel, Lobby		
Saturday, October 19, 2019 The Gran Meliá Zhen Guan Ballroom			
8:00-8:10 am	**Welcome and Program Overview**	Zhe Wang, MD PhD Magdalena Czader, MD PhD	
SESSION ONE: Chronic EBV Infections and Borderline Lymphoproliferations			
8:10 - 8:45 am	EBV positive T/NK cell lymphoproliferative diseases in China	**Dr. Xiaoge Zhou**	Dr. Weiping Liu Dr.Yaso Natkunam
8:45 - 9:35 am	Case Presentations (Type 1: 4 cases)		
8:45 - 8:57 am	Type1 Case No. 1443295	Fang Liu	
8:58 - 9:10 am	Type1 Case No. 1447527	Sophie Song	
9:11 - 9:23 am	Type1 Case No. 1455032	LiMin Gao	
9:24 - 9:35 am	Type1 Case No. 1469082	Atif Saleem	
9:35 - 9:50 am	Session 1 case summary	Dr. Weiping Liu and Dr. Yaso Natkunam	
9:50 - 10:10 am	**Coffee/Tea Break**		
10:10 - 10:45 am	Pathogenesis of Chronic active infection of EBV and novel therapy on horizon	**Dr. Jianfeng Zhou**	Dr. Weiping Liu Dr.Yaso Natkunam
SESSION TWO:	**EBV-Related Lymphomas**		
10:45 - 12:00 am	Case Presentations (Type 2: 6 cases)		Dr. Eric Hsi Dr. Xiaoqiu Li
10:45 - 10:57 am	Type2 Case No. 1452890	Yuta Tsuyuki	
10:58 - 11:10 am	Type2 Case No. 1440633	WenQing Yao	
11:11 - 11:23 am	Type2 Case No. 1455401	Eric Hsi	
11:24 - 11:35 am	Type2 Case No. 1360218	Yu Li	
11:36 - 11:48 am	Type2 Case No. 1367619	Qingling Zhang	
11:48 - 12:00 am	Type2 Case No. 1456909	Yukiko Kitagawa	
12:00 am – 1:20 pm	**Lunch** The Duo restaurant at the Gran Meliá		
1:20 - 1:55 pm	EBV-related lymphomas, non-PTLD-type	Dr. John Goodlad	Dr. Eric Hsi Dr. Xiaoqiu Li
1:55 - 2:50 pm	Case Presentations (Type 2: 4 cases)		

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1:55 - 2:07 pm	Type2 Case No. 1455146	Kennosuke Karube	
2:08 - 2:20 pm	Type2 Case No. 1395845	Fang Yu	
2:21 - 2:33 pm	Type2 Case No. 1454386	Jagmohan Sidhu	
2:34 - 2:50 pm	Type2 Case No. 1454893	Xiang-Nan Jiang	
2:50 - 3:05 pm	Session 2 case summary	Dr. Eric Hsi and Dr. Xiaoqiu Li	
SESSION THREE:	**HIV and HHV8-Related Lymphomas**		
3:05 - 3:40 pm	HIV and HHV8-related lymphomas	**Dr. Amy Chadburn**	Dr. Amy Chadburn Dr.Girish Venkataraman
3:40 - 3:55 pm	**Coffee/Tea Break**		
3:55 - 5:00 pm	Case Presentations (Type 3: 5 cases)		Dr. Amy Chadburn Dr.Girish Venkataraman
3:55 - 4:07 pm	Type3 Case No. 1441462	Xiaohui Zhang	
4:08 - 4:20 pm	Type3 Case No. 1447539	Alexander Nobori	
4:21 - 4:33 pm	Type3 Case No. 1453907	Wang Xueju	
4:34 - 4:46 pm	Type3 Case No. 1455006	Christos Masaoutis	
4:47 - 5:00 pm	Type3 Case No. 1455126	Kennosuke Karube	
5:00 - 5:15 pm	Session 3 case summary	Dr. Amy Chadburn and Dr.Girish Venkataraman	
5:15 - 6:00 pm	Treatment of HIV-related lymphomas	**Dr.Paul Rubinstein**	
6:30 - 8:30 pm	**Gala Dinner** The Gran Meliá; other possible venue pending		
Sunday, October 20, 2019 The Gran Meliá Zhen Guan Ballroom			
SESSION FOUR:	**Other Infectious Agent-Associated Lymphomas**		
8:00 - 8:50 am	Keynote Lecture: Viral lymphomagenesis, host susceptibility, and updates on vaccines against lymphomagenic viruses (e.g., EBV and HIV vaccines).	**Dr.Richard Longnecker**	Dr. Sophie Song Dr. Zhe Wang
8:50 - 9:30 am	Case Presentations (Type 4: 3 cases)		
8:50 - 9:02 am	Type4 Case No. 1455111	Kennosuke Karube	
9:03 - 9:15 am	Type4 Case No. NA	Girish Venkataraman	
9:16 - 9:30 am	Type4 Case No. NA	Magdalena Czader	
9:30 - 9:45 am	Session 4 case summary	Dr. Sophie Song and Dr. Zhe Wang	
9:45 - 10:05 am	**Coffee/Tea Break**		
SESSION FIVE:	**Multidisciplinary Case Presentations and Discussions**		

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10:05 - 11:20 am	Case Presentations (MDT: 5 cases)		Dr.Magdalena Czader Dr. Paul Rubinstein Dr. Jianfeng Zhou
10:05 - 10:23 am	MDT Case No. 1456578	Sarah Ondrejka	
10:24 - 10:42 am	MDT Case No. 1450517	ZiHang Chen	
10:43 - 11:00 am	MDT Case No. 1395848	Fang Yu	
11:00 - 11:18 am	MDT Case No. NA	Minh Yen T. Mays	
11:19 - 11:37 am	MDT Case No. 1450723	Lisa Rimsza	
11:37 - 11:50 am	Session 5 case summary	Dr. Magdalena Czader	
11:50 - 12:00 am	Close remark	Dr. Magdalena Czader and Dr. Zhe Wang	
12:00 - 1:20 pm	**Lunch** The Duo at the Gran Meliá		

Contents

EBV positive T/NK cell lymphoprolifertive diseases in China

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October 19, 2019 Xi'an

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Part 1

Review of EBV positive T/NK cell lymphoprolifertive diseases

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Backgroud

- EBV is ubiquitous virus
- Infects ＞90% population in the world
- no symptoms
- Infectious mononucleosis
- Related malignancies
- and others

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EBV associated LPD in WHO lassification

2008	2017
1. EBV+DLBCL in elder	1. EBV+DLBCL, NOS
2. DLBCL with chronic imflammation	2. EBV+mucocutaneous ulcer
3. lymphomatoid granulomatosis	3. DLBCL with chronic imflammation
4. plasmablastic lymphoma	4. lymphomatoid granulomatosis
5. primary effusion lymphoma	5. plasmablastic lymphoma
6. HHV8 associated LPD	6. primary effusion lymphoma
7. Burkitt lymphoma	7. HHV8 associated LPD
8. classic Hodgkin's lymphoma	8. Burkitt lymphoma
9. immunodeficiency-associated LPDs (PID, HIV, PTLD, others)	9. classic Hodgkin lymphoma
10. aggressive NK cell leukemia	10. immunodeficiency-associated LPDs (PID, HIV, PTLD, others)
11. systemic EBV+T-cell LPD of childhood	11. aggressive NK cell leukemia
12. hydroa vacciniforme-like lymphoma	12. systemic EBV+T cell lymphoma of childhood
13. extranodal NK /T cell lymphoma, nasal type	13. CAEBV of T/NK cell type, systemic form
	14. hydroa vacciniforme-like LPD
	15. severe mosquito bite allergy
	16. extranodal NK /T cell lymphoma, nasal type

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Other EBV associated lymphoid diseases

B cell	T cell
1. infectious mononucleosis	1. infectious mononucleosis@ #
2. acute EBV+lymphadenopathy	2. acute EBV+lymphadenopathy
3. acute EBV+ naso/pharyngitis	3. acute EBV+ naso/pharyngitis
4. EBV+B-LPD^:	4. EBV+ HLH
（1）EBV+ lymphoid hyperplasia	5. severe infectious mononucleousis
（2）EBV+ polymorphic extranodal LPD	6. EBV+ nodal NK/T cell lymphomas
（3）EBV+ polymorphic nodal LPD	7. EBV+ nodal CTL lymphoma*

^Blood. 2011;117(18):4726-4735

@Arai A. et al. Int J hematol 2014;99: 671
#Xie JL. et al Virchows Arch 2019;474:219
*Kato S. et al Histopathol 2012;61:186
Attygale AP, et al Histopathol 2014;64:171

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Possible states of EBV associated lymphoid diseases

acute infection	chronic active infection （lymphoproliferative）	lymphomas
Latent infection	EBV+B-LPD:	Burkitt
IM	- CAEBV-B type	EBV+DLBCL
EBV lymphadenopathy	EBV+T-LPD:	HL
	- CAEBV-T/NK type	NK/T lymphomas
	- HV-like LPD/lymphoma	etc.
	- mosquito bite allergy	

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An international meeting concerning EBV lymphoproliferative disease in non-immunocompromised hosts, in Washington, USA, 8–9 Sept. 2008.

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EBV+ LPD in Non-Immunocompromised Hosts Washington,Sept. 8-9, 2008

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EBV-associated lymphoproliferative diseases in non-immunocompromised hosts: Clinicopathological and biological features

Disease	Lineage Clonality	Primary Age Group	Epidemiological Features	Clinical Features	Related Entities & Comments
CAEBV B-cell type	B-cells Polyclonal/ Monoclonal	Children/ Young Adults	More common in western countries, very rare	Fever, systemic symptoms with organ involvement e.g. pneumonitis, uveitis, hepatitis; splenomegaly, adenopathy, hypergammaglobulinemia	Chronic/ persistent infectious mononucleosis with organ involvement
EBV+ Large B-cell lymphoma of the elderly (senile EBV LPD)	B-cells Monoclonal	Adults, > 60 yrs	No ethnic or geographic predilection	Usually extranodal Skin, GI tract, lung Aggressive clinical course	EBV-positive lymphoid hyperplasias in the elderly
Lymphomatoid Granulomatosis	B-cells Oligoclonal/ Monoclonal	Adults Median ~ 40 yrs	More common in Western countries	Extranodal Predominantly lung, also kidney, liver, CNS, skin	May also occur with immunodeficiency disorders
CAEBV T-cell/ NK-cell types (an umbrella term that encompasses specific diseases below)	T-cells NK-cells Monoclonal> Oligoclonal/polyclonal	Children/ Less often young adults	Asians: mainly Japan, Taiwan, Korea Native Americans in Mexico, Central, South America	Fever, hepatosplenomegaly, thrombocytopenia, lymphadenopathy; also hydroa vacciniforme, severe mosquito bite allergy, systemic EBV-positive T-cell LPD of childhood	T cell disease has poorer long term prognosis than NK cell disease
Hydroa vacciniforme Hydroa vacciniforme/HV-like lymphoma	T-cells Oligoclonal/ Polyclonal Monoclonal	Children/ Less often young adults	As above for T/NK cell CAEBV	Papulovesicular rash with ulceration Sun exposed or unexposed areas of skin May regress in adulthood or progress to systemic disease with hepatic failure and lymphoma	Some cases of severe hydroa vacciniforme may be monoclonal and overlap with HV-like lymphoma
Severe mosquito bite allergy	NK-cells Clonality Undetermined	Children/ Less often young adults	As above for T/NK cell CAEBV	Hypersensitivity to mosquito bites with ulcers and necrosis, High IgE	More indolent than Hydroa vacciniforme
Systemic EBV+ T-cell LPD of childhood	T-cells Monoclonal	Children/ Less often young adults	As above for T/NK cell CAEBV	Fever, lymphadenopathy, hepatosplenomegaly, HPS, DIC, hepatic failure Aggressive course	Severe CAEBV - 75% of cases of systemic CAEBV are clonal and overlap with systemic EBV+ T-LPD of childhood

Abbreviations: CAEBV, chronic active EBV-infection; HPS, hemophagocytic syndrome; DIC, disseminated intravascular coagulation; LPD, lymphoproliferative disease

Ann Oncol. 2009;20(9):1472-82.

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Nomenclature for Epstein–Barr virus+ T-cell and NK-cell lymphoproliferative diseases

Classification of EBV+ T/NK LPD (2012 EAHP Workshop)	Classification of mature T-cell / NK-cell neoplasms (2008 WHO)	Classification for EBV+ T/NK LPD of childhood type (2012 Asian hemocytopathology Workshop)
CAEBV Systemic (T, NK)	Systemic T-cell LPD of childhood*	CAEBV disease-type T/NK-cell LPD
CAEBV Hydroa vacciniforme (T)	Hydro vacciniforme-like lymphoma	Polymorphic/polyclonal
CAEBV MBH (NK)	Mosquito-bite hypersensitivity	Polymorphic/monoclonal
Systemic, malignant EBV+ LPD	Aggressive NK-cell leukemia	Monomorphic/monoclonal
Aggressive NK-cell leukemia/ lymphoma (NK)	Systemic T-cell LPD of childhood	HV -like T-cell LPD
Systemic EBV type + T-cell LPD	Extranodal NK/T-cell lymphoma	Hydroa vacciniforme
Extranodal NK/T-cell lymphoma, nasal type		Classic type
Nodal T/NK-cell lymphoma		Severe type
		HV-like T-cell lymphoma
		Mosquito-bite hypersensitivity
		Aggressive NK-cell leukemia
		Systemic EBV+ T/NK-cell LPD of childhood type
		Extranodal NK/T-cell lymphoma

*Monoclonal LPD among chronic active EBV infection. EBV, Epstein–Barr virus; LPD, lymphoproliferative diseases; NK, natural killer.

Journal of Dermatology 2014; 41: 29–39

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Table 14.01 Classification of EBV-positive T-cell and NK-cell proliferations WHO 2017

Diagnosis	Usual patient age group(s)
EBV-positive haemophagocytic lymphohistiocytosis (benign, may be self-limited)	Paediatric, adolescent
Systemic CAEBV	Paediatric, adolescent
Cutaneous CAEBV, hydroa vacciniforme-like lymphoproliferative disorder	Paediatric, adolescent
Cutaneous CAEBV, severe mosquito bite allergy	Paediatric, adolescent
Systemic EBV-positive T-cell lymphoma	Paediatric, adolescent
Aggressive NK-cell leukaemia	Adult
Extranodal NK/T-cell lymphoma, nasal type	Adult
Nodal peripheral T-cell lymphoma, EBV-positive[a]	Adult

CAEBV, chronic active EBV infection.

[a] Included within the category of peripheral T-cell lymphoma, NOS.

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EBV+ T/NK cell lymphoprolifertive diseases of childhood

- Acute/fulminant: Systemic EBV+T cell lymphoma of childhood
- Chronic, active: Chronic active EBV infection of T/NK cell type
  - systemic CAEBV: Chronic active EBV infection of T/NK cell type
  - cutaneous CAEBV: Hydroa vacciniforme-like LPD disorder
  - cutaneous CAEBV: Severe mosquito bite allergy

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1. Systemic EBV+ T cell lymphoma of childhood（CSEBV+TL）

- It occurs prevalently in children/young adults in Asia, Central and South America
- symptoms: high fever, hepatosplenomegaly, liver dysfunction, pancytopenia, etc.
- clinically fulminant, systemic, severe（complications: HLH, DIC, mutiorgan failure, etc.）
- following: 1、mostly primary EBV infection and 2、rarely CAEBV
- involve：blood, BM, liver, spleen, lymph node, skin, and so on.

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1. Systemic EBV+ T cell lymphoma of childhood（CSEBV+TL）

- morphology: scattered or focal mild atypical small to medium-sized lymphocyte infiltration.
- phenotype: mostly CD8+cytotoxic T cells, or some CD4+ T cell.
- EBV detection: EBV-DNA copies elevated, VCA-IgM+/- in serum, EBER+ in tissues,
- with monoclonal TCR gene rearrangement in cells
- EBV： monoclonal（4=2-CAEBV、1-T-NHL、1-HLH）
- Prognosis：rapidly progress to death (in days-weeks)
- ICD-O：9724/3
- Synonym：CSEBV+T-LPD, Fatal IM, fulminant EBV+T-LPD of childhood, Severe CAEBV, fulminant/fatal HLH

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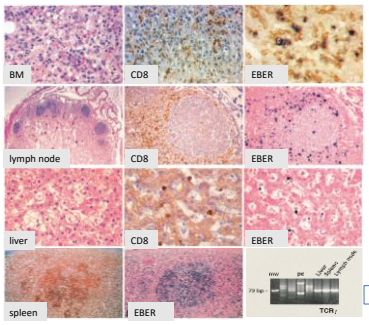

from: WHO 2008

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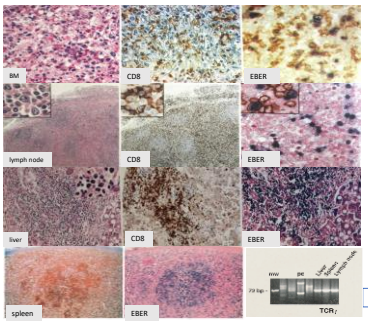

from: WHO 2017

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2. Chronic active EBV infection of T/NK cell type, systemic form (systemic CAEBV-T/NK)

Diagnostic criteria:

•clinical presentation：IM-like symptoms：

- fever
- hepatosplenomegaly
- lymphadenopathy

- clinical course > 3 months
- Histological evidence of orgain disease
- EBV+：DNA >$10^{2.5}$copies/mg in blood, or EBER+ in tissues
- Exclusion：immunodeficiency, malignacy or autoimmune disorders

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Morphology of CAEBV-T/NK

The cells show variable morphology, and mostly with mild atypia. small cells > medium >large

- lymph node: paracortex expanding, follicular hyperplsia or narrow or disappear, focal necrosis, debris
- extranode: variable number of infiltrating cells in scatter or focal. focal necrosis, debris
  - liver: lymphocytes infiltrating in sinus of liver.
  - spleen: lymphocytes infiltrating in red pulp
  - BM: normal or lymphocytes increased
  - Skin：infiltrates surrounding vessels and appendages of dermis

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Immunophenotype and genotype

- T cell 59%
- NK cells 41%
- Both T and NK 4%
- B cell: rare
- CD4+ >> CD8+ CTC

- monoclonal > polyclonal and oligoclonal
- EBER+

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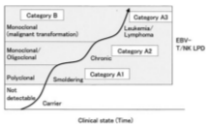
Grading of CAEBV

Categories	Cytology	Clonality	Nature
A1	polymorphic	polyclonal	proliferation
A2	polymorphic	monoclonal	boardline
A3	monomorphic	monoclonal	lymphoma
B	monomorphic	monoclonal	lymphoma



Ohshima, et al. Pathol Int, 2008;58:209

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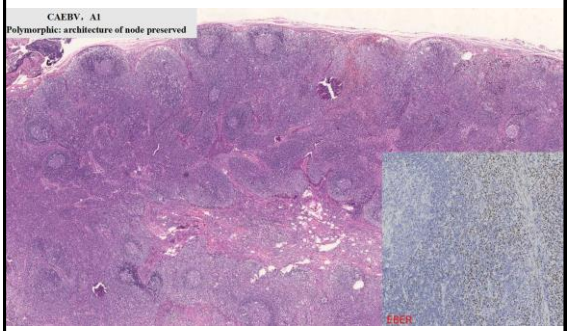

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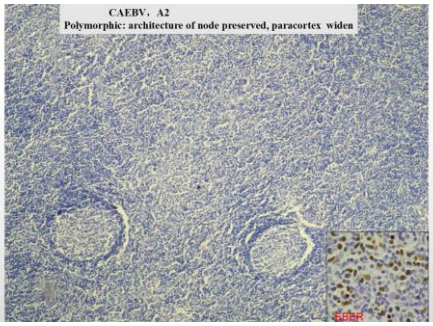

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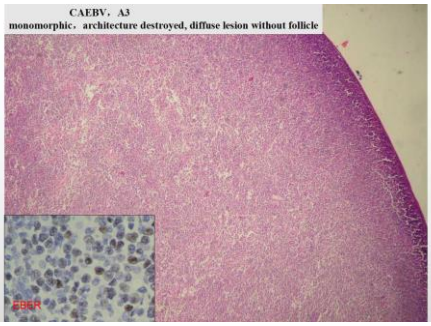

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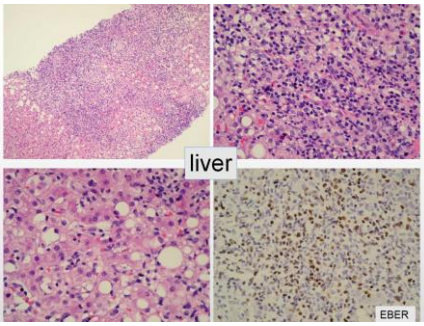

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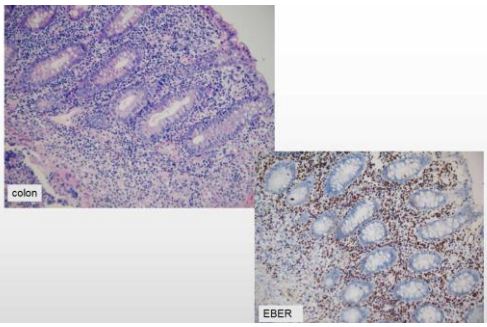

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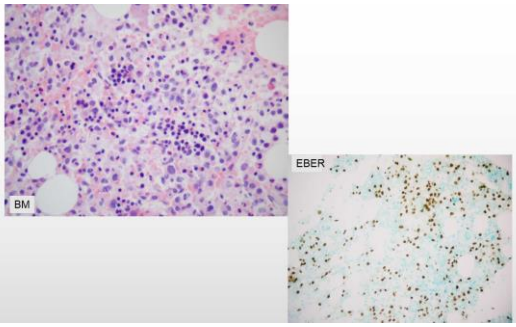

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Cutaneous CAEBV:
3、Hydroa vacciniforme-like LPD (HV-LPD)
4、Severe mosquito bite allergy（SMBA）

- Children, long clinical course,with high fever
- skin lesions: papulovesicular eruption，necrosis, ulceration, crust, scarring
- epidermal reticular degeneration, spongiotic vesiculation,
- atypia of infiltrating cells in dermis varies, most are small to medium-sized
- most CD4+ or CD8+ T cells and rare NK cells
- TCR: polyclonal or oligoclonal or often monoclonal
- spontaneous regression in early phases,and may progress to CAEBV or lymphoma
- type of HV-LPD: classic HV; severe HV/HV-like T cell lymphoma

- SMBA：larger ulceration, CD56+, IgE elevated, may progress to NK/T lymphoma/ANKL

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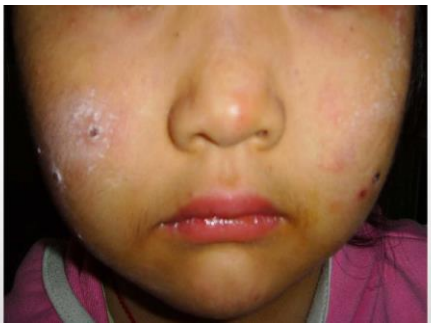

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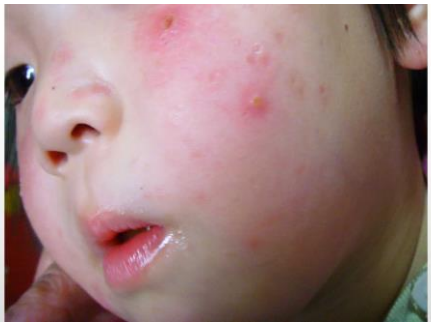

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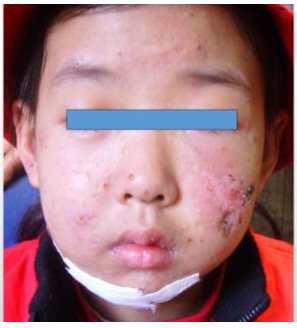

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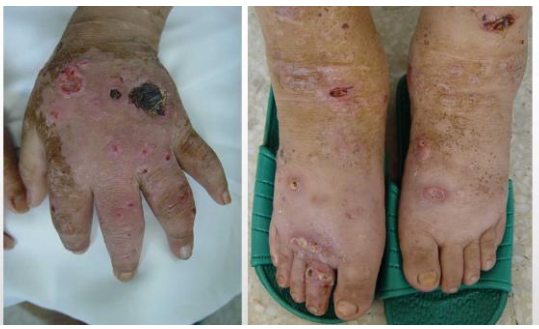

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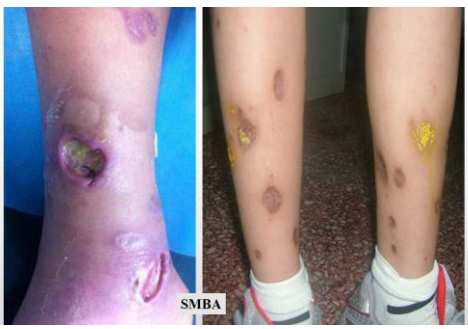

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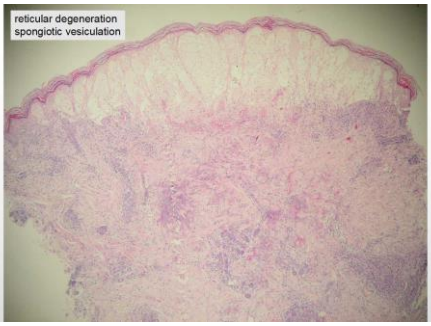

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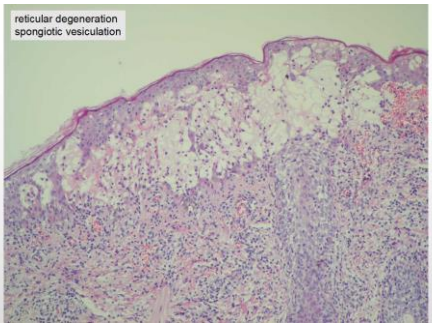

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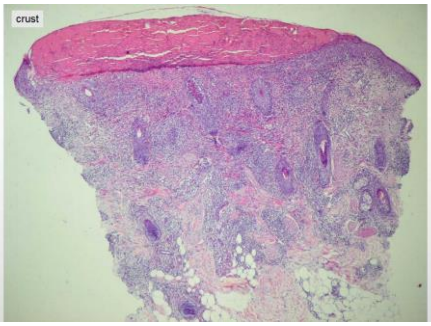

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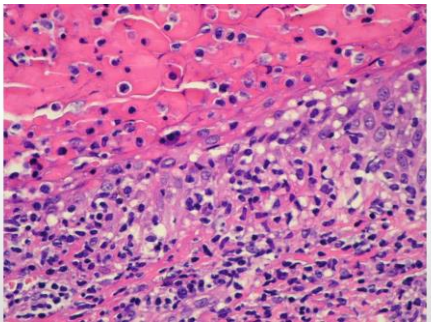

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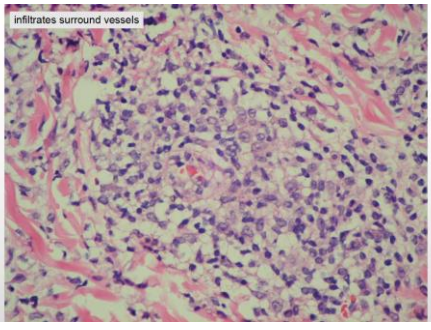

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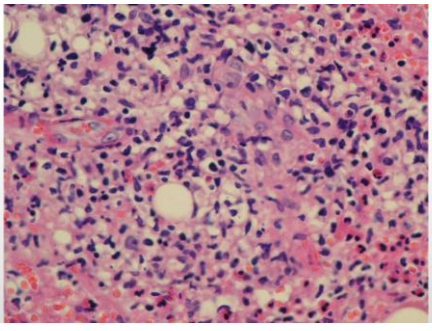

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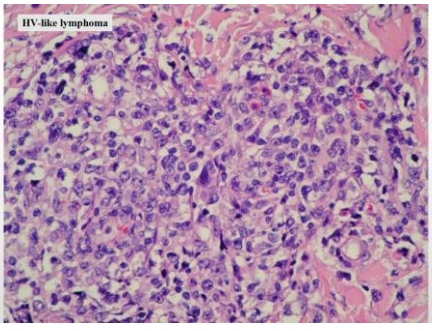

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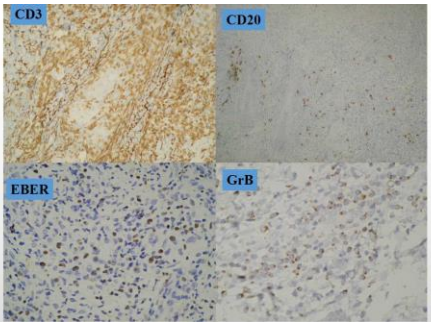

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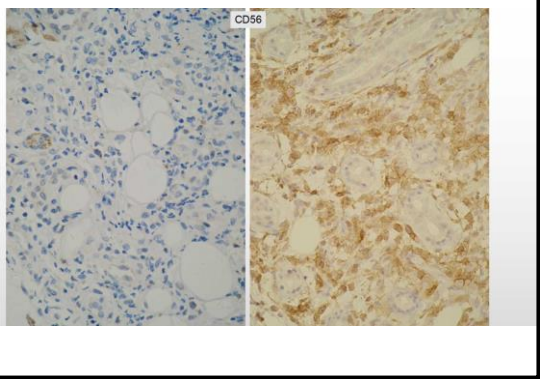

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Part 2

EBV positive T/NK cell LPD in China

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156 cases of Chinese CAEBV-T/NK type

zhang et al. Chin J Pathol. 2018, 47(6):407

Table 1 clinical data, morphological grading of 156 Chinese cases of CAEBV-T/NK

items	gender (No. of case)		age (year)		course of disease(month)		clinical manifestation (No. of cases)			grading of morphology			time of fellow-up (month)		outcome (No. of case)	
	Male	female	mean	median	mean	median	fever	lymphadenopathy	hepatosplenomegaly	G1	G2	G3	mean	median	dead	alive
systemic	82	39	26.8	20	10.3	6	100	97	64	19	55	47	16.5	9	34	87
HV	12	6	11.5	7	56.1	24	14	6	8	9	7	2	70.5	70	3	15
SMBA	6	3	11.8	9	26.7	24	8	5	3	4	3	2	20.6	10	4	5
local*	6	2	11.8	14	32.5	17	0	0	0	1	3	4	15.0	17	3	5
total	106	50	23.4	14	17.7	20	122	108	75	33	68	55	22.9	24	44	112

*include: 1 case of giant ulcer of skin without association with SMBA, 1 case of stomach, 4 cases of intestine, 2 cases of gum.

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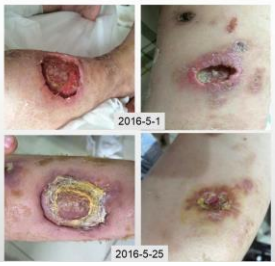
Giant ulcer of skin without history of mosquito bites

- 6 year old boy,
- reccurent giant ucler in skin of lower extremities.
- Dexamethasone is effective.



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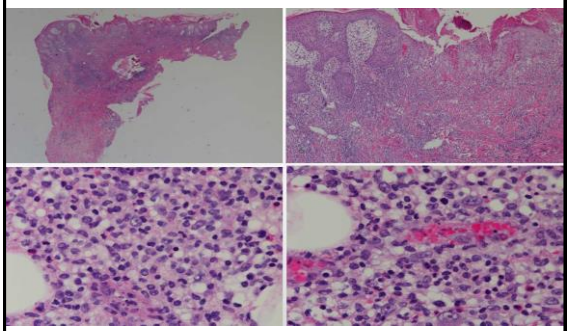

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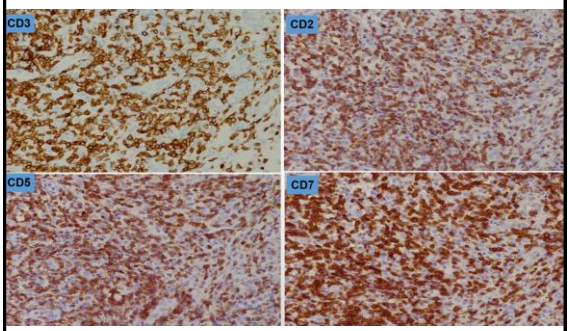

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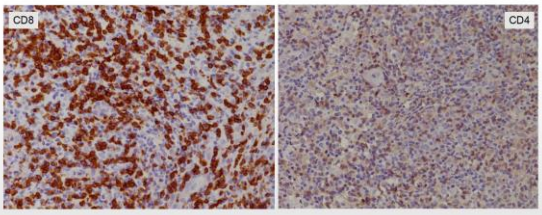

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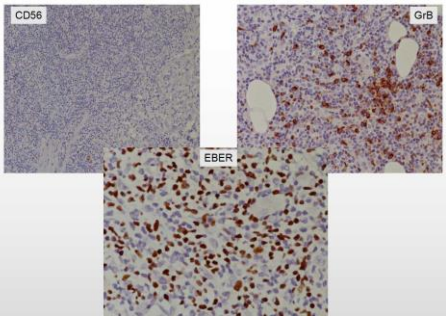

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VOLUME 31 | SUPPLEMENT 2 | MARCH 2018

MODERN PATHOLOGY

1601 Acute Epstein-Barr virus infections mimicking T/NK cell lymphoma in lymph node: are there infectious mononucleosis, T/NK cell type

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Background: Infectious mononucleosis (IM) is a benign self-limited disease with Epstein-Barr virus (EBV) primary infection that results in a polyclonal B cell proliferation and T and natural killer (T/NK) cell cellular immune response. It is not clear whether there is IM with EBV infection in predominantly T and NK cells. The aim of our study is to display IM-like cases of acute EBV infection of T/NK cells, and to analyze their clinicopathological features.

Design: 7 cases of children and young people from China with acute EBV infections of T/NK cells was retrospectively analyzed using HE stain for morphology, immunohistochemistry for phenotype, EBV encoded small RNA (EBER) in situ hybridization for EBV status and TCR,coupled with clinical data study and follow-up.

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Results: (1)Five patients were male and two were female. The age of the patients ranged from 10 months to 19 years with a median age of 5.9 years. They were healthy as ordinary people before sick. Sudden onset was seen in all the patients with highfever as first symptoms followed by lymphadenopathy and hepatosplenomegaly. The course of the diseases before diagnosis ranged from 22 days to 1.5 months. All patients received conservative treatment but not chemotherapy. Only one child died of serious complications after diagnosis in 3 months. Other patients achieved long-term clinical observation and follow-up did not relapse.(2)All the samples were lymph nodes which architecture were destructed. There were numerous infiltrating lymphocytes with medium and large in size in the lesion, with focal to extensive coagulative necrosis obviously. Mitotic figures were easily found, most of cells showed highly atypia.(3)The majority of lymphocytes in the lesion expressed CD3 and Granzyme B or TIA-1, but not CD5 in all cases. CD56 was expressed in numerous cells in 5 cases. EBER was detected in medium to large-sized cells (50 to over 100 cells per high power field) in all cases. TCR gene rearrangement was performed in 6 cases, in which monoclonal rearrangement was found in 4 cases.

FOR TABLE DATA, SEE PAGE S75, FIG. 1601

Conclusions: Acute EBV infection of T/NK cell could express malignant features mimicking T/NK cell lymphoma pathologically as well as benign features mimicking IM clinically. The findings indicated that there may be infectious mononucleosis, T/NK cell type in China. Special clinical information (such as children and young people, sudden onset, short disease course, systemic symptoms, acute EBV infection, etc.) are the main clues for diagnosis and evaluation of prognosis.

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The clinical data of the 7 cases of acute EBV infection

Case no	sex / Age	course of disease	Clinical symptoms	blood test	EBV detected in blood	Follow-up (month)
1	F/10y	1m	fever, hepatosplenomegaly, lymphadenopathy	WBC 5.9×10⁹/L, HB 90g/L, PLT 51×10⁹/L, CRP 97.2mg/L	EBV-CA IgM+ EBV-CA IgG+ EB-DNA2.0×10⁴copy/mL EBV-PCR+	5,Alleviation,but the fourth month dead for MOF suddenly
2	M/2y	1m	fever, hepatosplenomegaly, lymphadenopathy	WBC9.7×10⁹/L,HB71g/L,PLT11×10⁹/L, CRP135mg/L,LDH892U/L, FERR 1044ng/L, ALT 163U/L, AST 235U/L	EBV-CA IgM+ EBV-CA IgG+	20,Alleviation
3	M/10y	1.5m	fever, hepatosplenomegaly, lymphadenopathy pleural effusion, ascites,pneumonia	WBC 2.7 ×10⁹/L, HG 112 g/L, PLT118 ×10⁹/L	EBV- CA IgM- EBV- CA IgG+	18,Alleviation
4	M/5y	1m	fever, hepatosplenomegaly, lymphadenopathy Pleural effusion,pneumonia	WBC2x10⁹/L, HG 94 g/L, PLT 140x10⁹/L CRP 111mg/L,FERR1178ng/mL, LDH893U/L, AST 51.8U/L	EB-DNA1.0×10⁴copy/ml	12,Alleviation
5	M/10m	1m	fever, hepatosplenomegaly, lymphadenopathy	WBC3.2×10⁹/L,HB-106g/L,PLT258.4x10⁹/L CRP 3mg/L, LDH 754U/L,AST61.8 U/L	NA	36, Alleviation, grow fatter and higher
6	F/1y	20d	fever, hepatosplenomegaly, lymphadenopathy	WBC1.36x10⁹/L,HB102g/L,PLT60x10⁹/L, ALT 29IU/L, AST 39IU/L, LDH 1670 U/L, CRP1.43mg/L	EBV- CA IgM- EBV- CA IgG+ EB-DNA1.08×10⁴copy/ml	19, Alleviation, grow fatter and higher
7	M/5y	1.5m	fever, hepatosplenomegaly, lymphadenopathy	WBC3.3x10⁹/L,HB90g/L,PLT19x10⁹/L, ALT378U/L, AST 238IU/L, FERR 1495.5ng/L, CRP 31mg/L	EBV- CA IgG+ EBV-PCR+	6, Alleviation

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The data of the 7 cases of morphology and phenotypies and genetics

Case no	structure destruction	components of cells	atypia	Coagulative necrosis	CD3	CD5	CD56	TIA-1	Granzyme B	CD30	CD4	CD8	EBNA2	EBER(HPF)
1	Yes	L	H	Yes	+++	+	+	+	+	+	+++	-/+	+	50□100
2	Yes	M	M	No	+++	-	-	+	NA	-	-/+	-/+	+	>100
3	Yes	L	H	Yes	+++	+	+	NA	+	-/+	-/+	++	-	50□100
4	Yes	L	H	Yes	++	-	+	+	NA	-	-	++	+	>100
5	Yes	L	H	Yes	+++	+	-	NA	+	-	-/+	+++	NA	>100
6	Yes	L	H	Yes	+++	-	+	+	+	-	-	+++	NA	>100
7	Yes	L	H	No	+++	+	+	+	+	+	-	-/+	NA	>100

TCR gene rearrangement: monoclonal in 4/6 cases

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Int J Clin Exp Pathol 2017;10(9):9802-9807
www.ijcep.com /ISSN:1936-2625/IJCEP0057465

Case Report

Infectious mononucleosis caused by EBV infection with clonal cytotoxic T cells in an infant patient

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Received May 15, 2017; Accepted July 13, 2017; Epub September 1, 2017; Published September 15, 2017

Abstract: Infectious mononucleosis (IM) is a self-limiting lymphoproliferative disease usually caused by Epstein-Barr virus (EBV) infection, which predominantly infects B lymphocytes in adults. IM that occurs in T cells infected with EBV is rare and only a few cases have been reported worldwide. We describe a rare case of IM in an infant with an EBV infection predominantly in clonal cytotoxic T cells. A cervical node taken from a 10-month-old male infant was retrospectively analyzed. The architecture of the lymph node was effaced mostly with focal to extensive coagulative necrosis. In some areas, numerous infiltrating lymphocytes were detected, expressing CD2, CD3, and granzyme B, but lacked CD5 and CD56. EBV-encoded small RNAs were detected in the majority of lymphocytes. Furthermore, the T cell presented with T-cell receptor rearrangements. The patient did not do any radiation and chemotherapy. Thirty-two months follow up showed that the patient was well. We speculate IM can occur in infants through EBV infection of clonal cytotoxic T cells.

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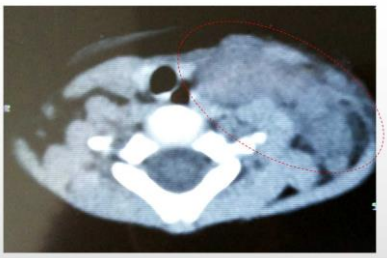

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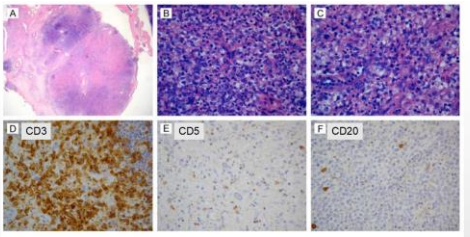

Figure 1. A. Architecture of lymph nodes showing that they were mostly effaced. The capsule of the node was thick and the lesion involved the peripheral tissue of the node (hematoxylin and eosin [HE]; original magnification, × 40). B. Infiltrating lymphocytes were of medium size, round with mildly irregular nuclei, inconspicuous or small nucleoli and dispersed chromatin (HE; original magnification, × 400). C. Some lymphocytes were larger in size with small nucleoli. There were some macrophages with abundant pale cytoplasm containing nuclear debris (HE; original magnification, × 400). D-F. Lymphocytes in the lesion expressed CD3, with only a few scattered lymphocytes that expressed CD5 and CD20 (IHC; original magnification, × 400).

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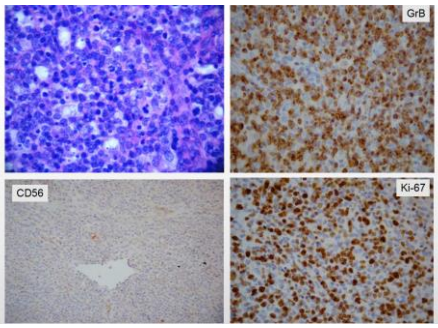

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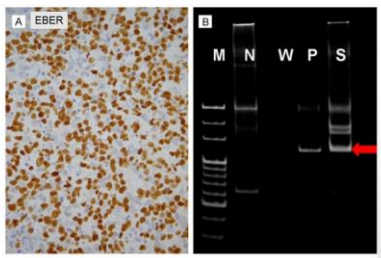

Figure 2. A. EBERs were detected in the majority of lymphocytes, approximately 70-80% of all cells. Original magnification, × 400. B. T-cell receptor gene rearrangement for Vβ + Jβ2. The arrow indicates the monoclonal band. (M: marker, N: negative, W: water, P: positive, S: sample.)

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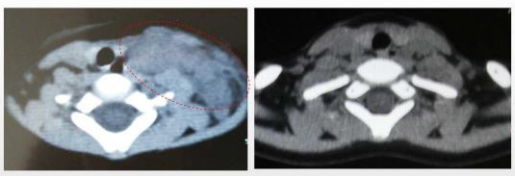

2013-10-29 2015-8-16

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Similar cases were also reported from Japan and Taiwan

Int J Hematol (2014) 99:671–675
DOI 10.1007/s12185-014-1568-4

CASE REPORT

Infectious mononucleosis accompanied by clonal proliferation of EBV-infected cells and infection of CD8-positive cells

Ayako Arai · Takeshi Yamaguchi · Honami Komatsu · Ken-ichi Imadome · Morito Kurata · Kaoru Nagata · Osamu Miura

Int J Clin Exp Pathol 2013;6(1):105-109
www.ijcep.com /ISSN:1936-2625/IJCEP1208012

Case Report

Infectious mononucleosis mimicking malignant T-cell lymphoma in the nasopharynx: a case report and review of the literature

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Virchows Archiv (2019) 474:219–226
https://doi.org/10.1007/s00428-018-2458-7

ORIGINAL ARTICLE

Acute Epstein–Barr virus-positive cytotoxic T cell lymphoid hyperplasia in the upper aerodigestive tract, mimicking extranodal natural killer/T cell lymphoma, nasal type

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Received: 28 October 2017 / Revised: 29 October 2018 / Accepted: 21 November 2018 / Published online: 28 November 2018
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Abstract

To describe the clinicopathological features of nine patients with acute Epstein–Barr virus (EBV)-positive cytotoxic T cell lymphoid hyperplasia (EBV+TLH) in the upper aerodigestive tract, in which initial findings led to a preliminary misdiagnosis of extranodal NK/T cell lymphoma, nasal type (ENKTL). A series of nine cases of EBV+TLH in one Chinese institution over a 9-year interval was retrospectively analyzed. Median age was 16 years (range 5–29 years) with a M:F ratio of 5:4. All patients were previously healthy with an acute onset period of < 1 month. Six patients (66%) presented with masses or polypoid protrusions in the upper aerodigestive tract. Nasopharyngeal symptoms, cervical lymphadenopathy, and fever were found in 89%, 78%, and 56% of patients, respectively. In seven cases, morphology mainly showed small-sized irregular cells and in two cases medium-to-large cells. In all cases, the cells diffusely expressed cytoplasmic CD3 and at least one marker for cytotoxic granules, but were negative for CD56. CD5 expression was detected in eight cases (89, 89%). In all cases, double staining for CD3 and EBER indicated that most T cells were infected with EBV. T cell receptor gene rearrangement was performed in five cases and all showed polyclonal results. All patients achieved complete remission within 1 month after diagnosis without any chemoradiotherapy and were followed up 19–124 months without recurrent disease. EBV+TLH in the upper aerodigestive tract is occasionally observed in China. The histopathologic features of EBV+TLH can mimic ENKTL. EBV+TLH should be taken into consideration as a potential diagnosis when the disease duration is short, spontaneous remission is achieved without intervention, and when histology shows infiltration with EBV-infected T lymphocytes.

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Table 1 Clinical characteristics and outcomes in 9 cases of EBV-positive T cell lymphoid hyperplasia in upper aerodigestive tract

Case no.	Age (years)/Sex	Biopsy site	Onset to biopsy interval (days)	Clinical presentation				
				Stuffy nose	Pharyngeal discomfort	Rhinorrhea	Headache	Fever
1	16/F	Nasal mucosa	30	Y	N	N	N	N
2	16/M	Nasal mucosa	30	Y	N	N	Y	N
3	22/F	Pharyngeal mucosa	20	N	Y	N	N	Y
4	17/M	Nasal mucosa	20	Y	N	Y	N	N
5	15/M	Nasal mucosa	30	Y	N	N	N	N
6	29/F	Nasal mucosa	10	Y	Y	Y	N	Y
7	9/M	Nasal mucosa	20	Y	N	N	Y	Y
8	16/M	Nasal mucosa	30	N	N	N	N	Y
9	5/F	Nasal mucosa	10	Y	N	N	N	Y

Case no.	Clinical presentation: Lymph node enlargement	Clinical presentation: Hepatosplenomegaly	Observation of nasopharyngoscopy	Computed tomogram manifestation	Treatment	Outcome	Length of follow-up (months)
1	Y (bilateral cervical)	N	0.8 × 0.3 cm mucosa polypoid protrude in left nasal cavity	ND	Anti-inflammatory	CR	124
2	N	N	0.7 × 0.6 cm mucosa mass in bilateral nasal cavity	ND	Anti-inflammatory	CR	121
3	Y (bilateral cervical)	N	Pharyngeal mucosal swelling with surface erosion	Soft tissue of nasopharyngeal posterior wall thickening, no bone destruction	Anti-inflammatory	CR	110
4	N	N	0.8 × 0.6 cm mucosa mass in bilateral nasal cavity	ND	Antiviral	CR	108
5	Y (bilateral cervical)	N	0.6 × 0.6 cm nasal mucosa polypoid protrude with pale yellow exudate on the surface	ND	Anti-inflammatory	CR	62
6	Y (bilateral cervical)	N	Nasal mucosa congestion and swelling	ND	Anti-inflammatory	CR	46
7	Y (bilateral cervical)	N	1.4 × 1.2 cm mucosa mass in bilateral nasal cavity	ND	Anti-inflammatory	CR	42
8	Y (left cervical and right retro-auricular)	Y	Nasopharyngeal posterior wall thickening, swelling, surface is not smooth	Nasopharyngeal swelling and pharyngeal crypt disappearance, no bone destruction	Antiviral	CR	20
9	Y (left cervical)	N	1.3 × 1.0 cm mucosa mass in left middle meatus	Soft tissue of nasopharyngeal posterior wall and lateral wall protrude, no bone destruction	Antiviral	CR	19

CR, complete remission; *F*, female; *M*, male; *Y*, yes; *N*, no; *ND*, not done

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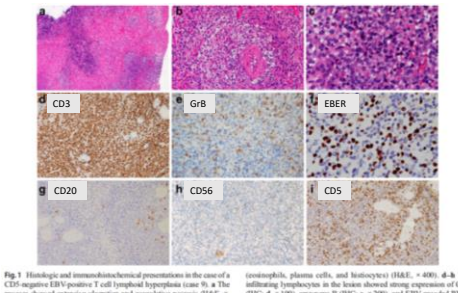

Fig. 1 Histologic and immunohistochemical presentations in the case of a CD5-negative EBV-positive T cell lymphoid hyperplasia (case 9). **a** The mucosa showed extensive ulceration and coagulative necrosis (H&E, × 40). **b** Lymphocytes surrounding the vessel (H&E, × 200). **c** Lesion with an infiltration of small lymphocytes admixed with inflammatory cells (eosinophils, plasma cells, and histiocytes) (H&E, × 400). **d–h** The infiltrating lymphocytes in the lesion showed strong expression of CD3 (IHC; **d**, × 100), granzyme B (IHC, **e**, × 200), and EBV-encoded RNAs (ISH; **f**, × 400) but were negative for CD20 (IHC; **g**, × 100), CD56 (IHC; **h**, × 200), and CD5 (IHC; **i**, × 100) expression

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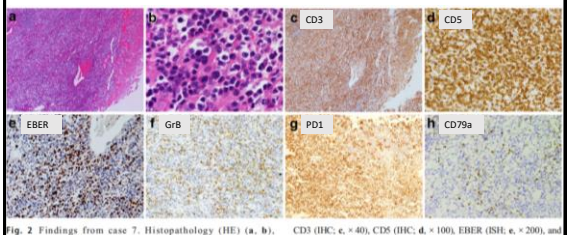

Fig. 2 Findings from case 7. Histopathology (HE) (**a**, **b**), immunohistochemical staining (**e**–**d**, **f**–**h**), and EBERs (EBV-encoded RNAs) (**e**) of case 7. **a** Mucosa with diffusely infiltrated lymphoid cells and erosion of the surface epithelium (× 40). **b** Lymphoid cells of various sizes, ranging from small to large (× 630). **e**–**f** Many cells positive for CD3 (IHC; **c**, × 40), CD5 (IHC; **d**, × 100), EBER (ISH; **e**, × 200), and granzyme B (IHC; **f**, × 100). **g** and **h** Numerous T cells show positive staining for PD-1 (IHC; **g**, × 100) and negative staining for CD79a (IHC; **h**, × 200)

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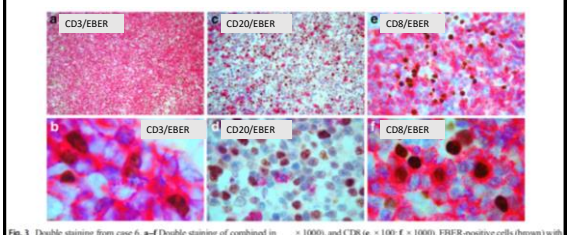

Fig. 3 Double staining from case 6. **a**–**f** Double staining of combined in situ hybridization for EBV-encoded RNAs (EBERs) with immunostaining against CD3 (**a**, × 100; **b**, × 1000), CD20 (**c**, × 100; **d**, × 1000), and CD8 (**e**, × 100; **f**, × 1000). EBER-positive cells (brown) with expression of CD3 (red) and CD8 (red); EBER-positive cells (brown) without the expression of CD20 (red) in the membrane and cytoplasm

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Classification of EBV+T/NK cell lymphoproliferative diseases in China (Proposal)

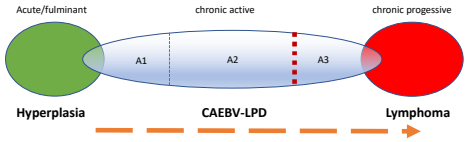
	Systemic		Local		
Acute	Acute EBV infection (IM)-T/NK Acute severe EBV infection: - EBV+HLH - fulminant IM - CSEBV-TL* ANKL*		Lymphadenitis nasopharyngitis Tonsillitis Hepatitis? pneumonia? encephalitis?		
Chronic active	Systemic CAEBV-T/NK		Cutaneous/Mucosal CAEBV-T/NK		
	children	adults	HV-T/NK	SMBA-T/NK	others
	G1 G2 G3*	G1 G2 G3*	G1 G2 G3*	G1 G2 G3*	G1 G2 G3*
Chronic progressive	Extranodal EBV+CTL* Extralnodal EBV+NK/TL* Nodal EBV+CTL* Nodal EBV+NK/TL*				

*: tumour, IM=Infectious mononucleosis, HLH=hemophagocytic lymphohistiocytosis, CSEBV-TL= Systemic EBV+T cell lymphoproliferative disease in childhood, ANKL=Aggressive NK cell leukemia, CAEBV=Chronic active EBV infection, LPD=Lymphoproliferative disease, HV=Hydroa vacciniform, SMBA=Severe mosquito bite allergy, G=Grade, TL=T-cell lymphoma, CTL=Cytotoxic T cell lymphoma,

67

Possible relationship between EBV+T/NK-LPD and lymphoid hyperplasia and lymphoma

- EBV+LPD is different from both typical benign reactive hyperplasia and typical EBV associated lymphoma, but it overlaps with both. These two overlapped areas are the current difficulties in pathological diagnosis, and it is not easy to distinguish them. In diagnosis, more clinical information is needed for comprehensive analysis.



68

Differential diagnosis

69

CAEBV Grade: A2 or A3

- A1: polymorphic+polyclonal=hyperplasia
- A2: polymorphoic+monoclonal=borderline
- A3: monomorphic+monoclonal=lymphoma

Suggestion: add parameters of immunophenotypes and clinical date, for instance, whether is there a T cell antigen loss, and more serious clinical manifastation

70

EBV+T/NK-LPD or NK/TL

- A1:Polymorphic+polyclonal=hyperplasia
- A2:Polymorphoic+monoclonal=borderline
- A3:monomorphic+monoclonal=lymphoma

onset：systemic
chronic active, reccurent

Atypia+(clonal-/+)=lymphoma

onset：local
chronic progressive

71

The EBV+T-LPD case with course duration 1 to 3 months: IM or CAEBV?

IM	CAEBV

• chirldren/young aduts
• acute onset
• short course, mostly < 1 month
• primary EBV infection:
  • VCA-IgM+
  • low affiniy VCA-IgG+
• reactive paracortical expansion with IB cells
• CD8+ >> CD4+
• EBER+ and EBNA2+
• self-limited outcome
	•clinical presentation：IM-like symptoms：
	- fever
	- hepatosplenomegaly
	- lymphadenopathy
	• clinical course > 3 months
	• Histological evidence of orgain disease
	• EBV+：DNA >$10^{2.5}$copies/mg in blood, or EBER+ in tissues
	• Exclusion：immunodeficiency, malignacy or autoimmune disorders

When the two diseases cannot be distinguished, the first is to favor IM.

72

Comparison of CSEBV+TCL and Primary EBV+ hemophagocytic lymphohistocytosis(HLH)

	CSEBV+TL	Primary EBV+HLH
children/young adults	+	+
Acute onset	+	+
Fulminant	+	+
Systemic	+	+
Primary EBV infection	+/-	+
CTL	+	+
CD8+	+/-	+
monoclonal for TCR*	19/21 (90%)cases	41/55 (75%)cases
Abnormal karyotype*	2 cases	12 cases
HLH	+	+
Prognosis	dead	Survival（90%）

*Int J Clin Exp Pathol 2014;7(9):5738-5749。

73

Comparison of cases of CSEBV+TCL between China and Peru

	cases of CSEBV-LPD from China	cases of CSEBV-LPD from Peru
children/young adults	+	+
Acute onset	+	+
Fulminant	+	+
Systemic and lymphadenopathy	+	+
Primary EBV infection	+/-	+
CTL	+	+
CD8+	+/-	-
monoclonal for TCR*	4/6 (67%)cases	3/6 (50%)cases
Abnormal karyotype*	N	N
HLH	+	+
Prognosis	Survival（86%）	All dead

74

Summary

- EBV infections are widespread in China.
- All the EBV-associated lymphoproliferative diseases in WHO classification can be seen in China.
- EBV+LPD in China is more diverse and complex, especially EBV+ T/NK-LPD.
- There are huge challenges in diagnosis and treatment of EBV+ T/NK-LPD.

75

Thank you

76

Chinese Society for Hematopathology | Society for Hematopathology

Gran Melia Hotel
Xi'an, P.R. China
Oct. 19-20, 2019

The 1st Joint CSHP/SH Workshop and CSHP2019

INFECTIOUS AGENT-ASSOCIATED LYMPHOID PROLIFERATIONS

Foshan hospital ,Sun yat-sen university
The first people's hospital of Foshan

Fang Liu

20191019 XI' AN

1

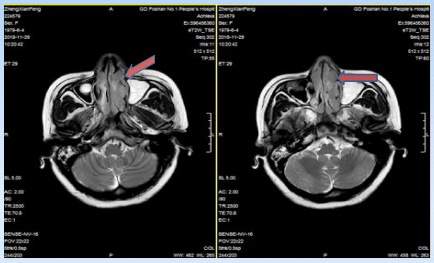
Clinical case

39 yrs old Female

- ◆Persistent nasal obstruction without obvious cause 1 months ago.
- ◆No snot blood, but the sense of smell significantly decreased.

Four biopsies with half a year

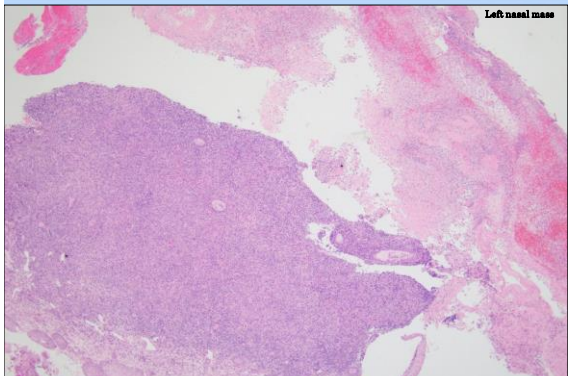
2



There were multiple soft tissue nodular opacities in bilateral nasal meatus, nasal vestibule and right wall of nasopharynx, involving in nasal septum, bilateral inferior turbinate, left maxillary sinus orifice, right pharyngium tube round pillow, right levator of palate.

3

2018.11.12 first nasal biopsy



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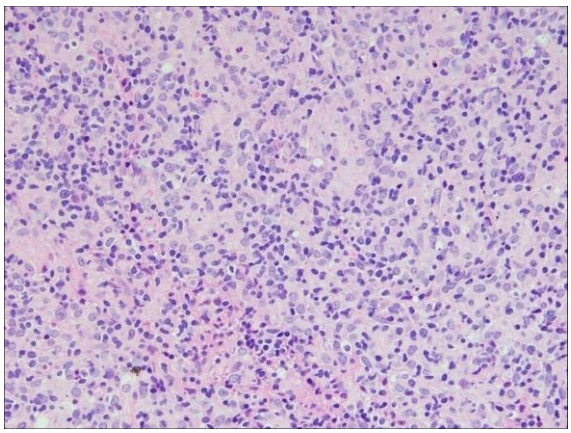

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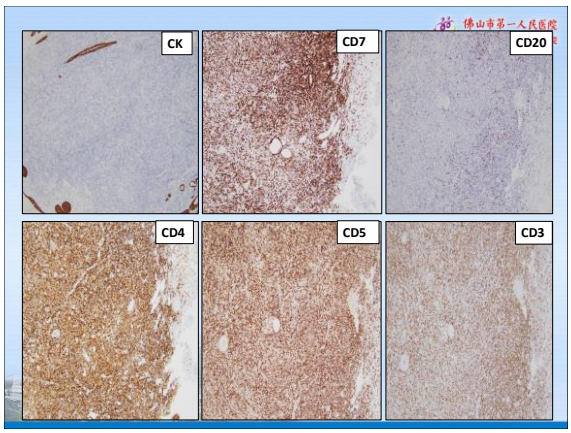

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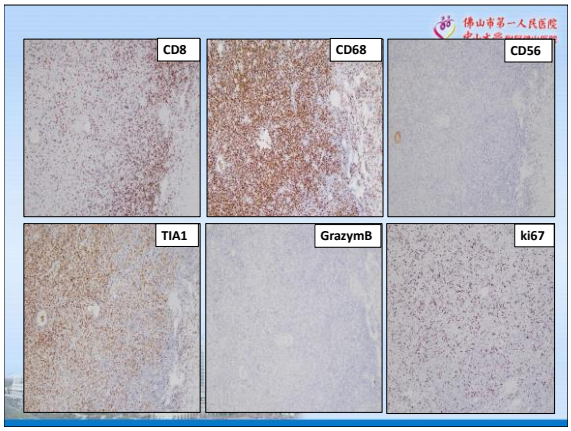
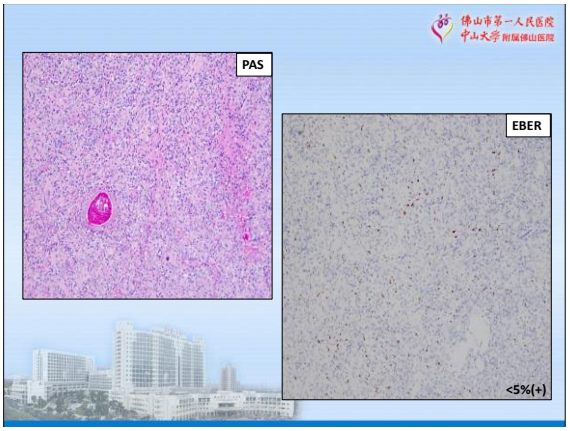
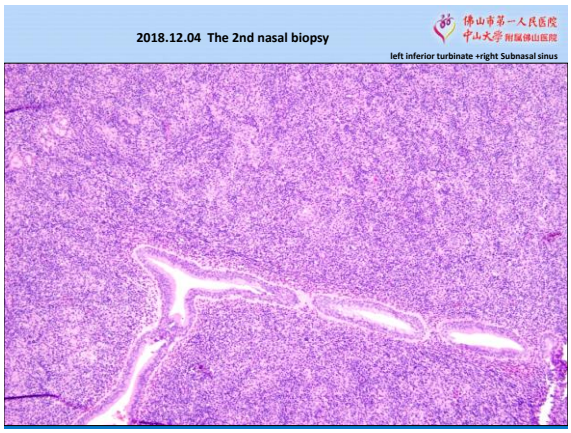

CD8
CD68
CD56
TIA1
GrazymB
ki67

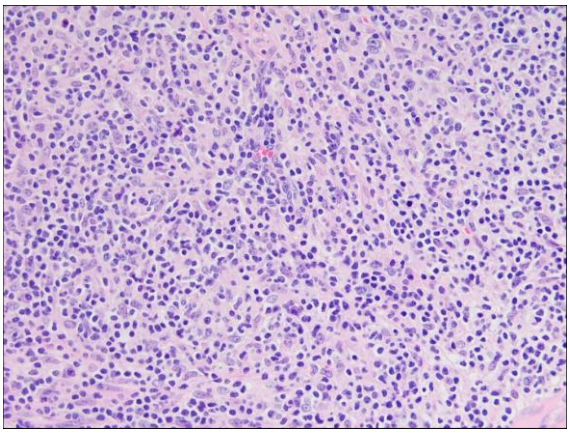
7


PAS
EBER
<5%(+)

8


2018.12.04 The 2nd nasal biopsy
left inferior turbinate +right Subnasal sinus

9



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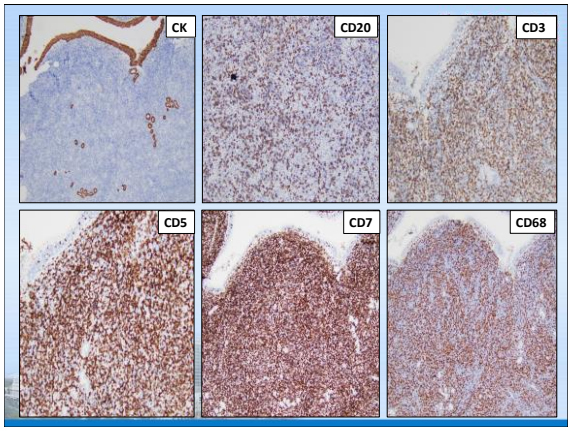
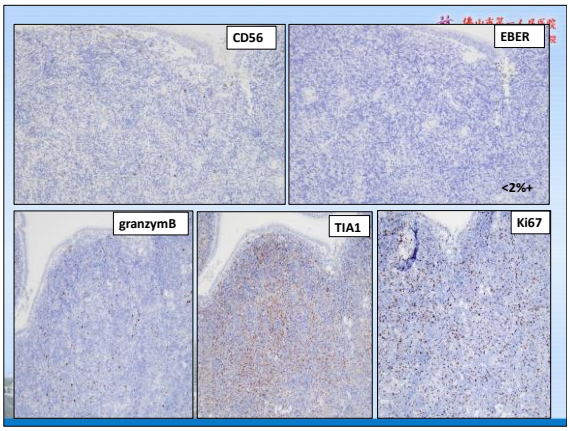
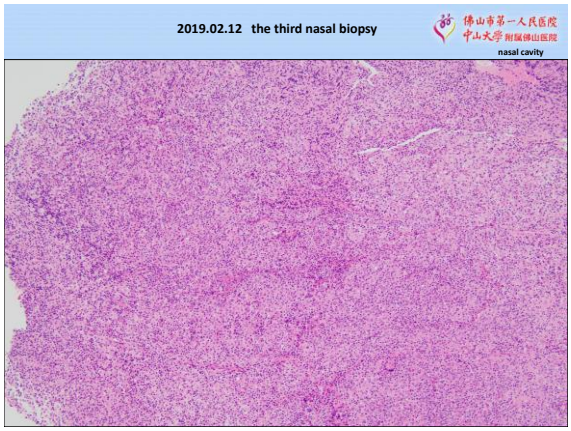

CK
CD20
CD3
CD5
CD7
CD68

11


CD56
EBER
<2%+
granzymB
TIA1
Ki67

12



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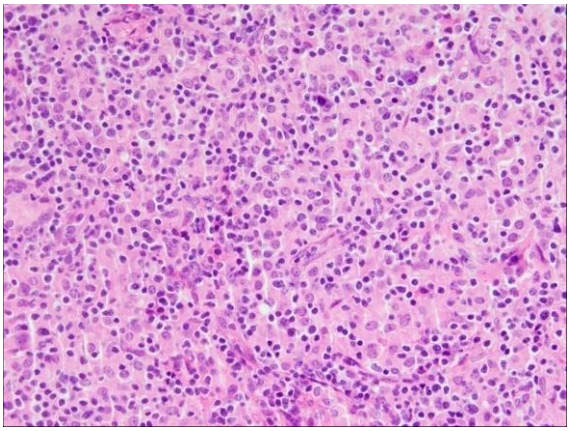

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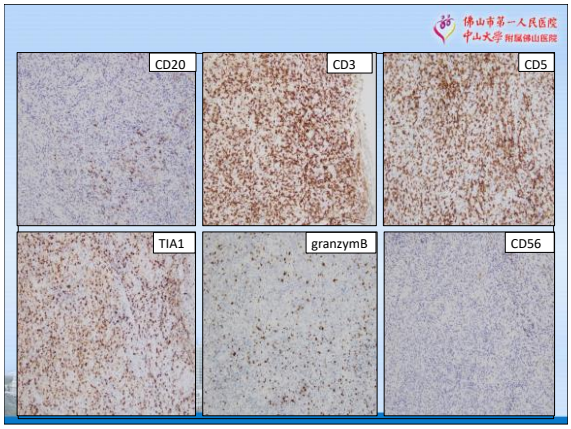

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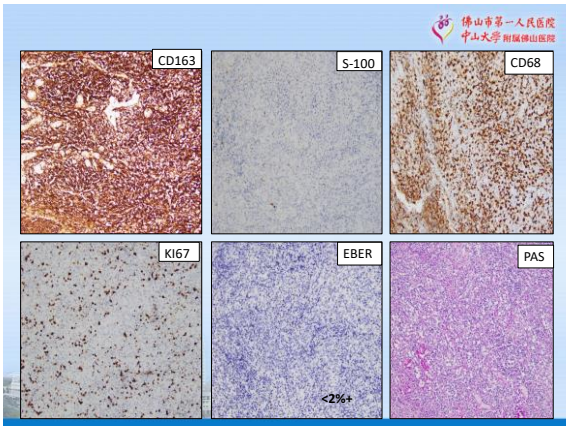

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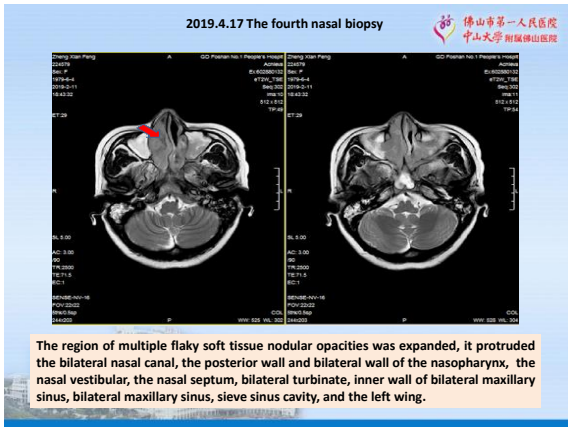

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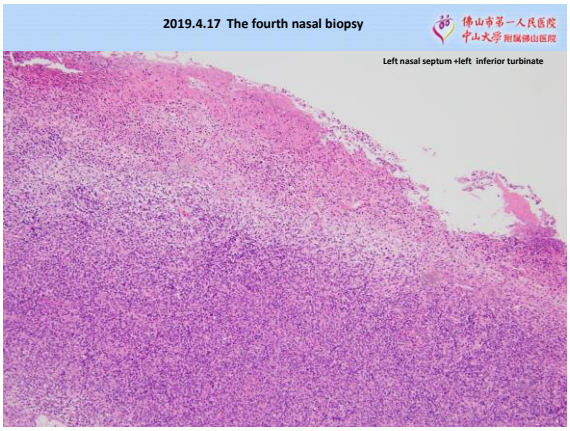

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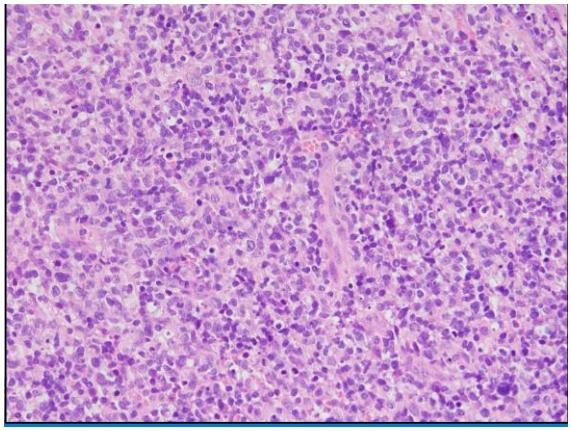

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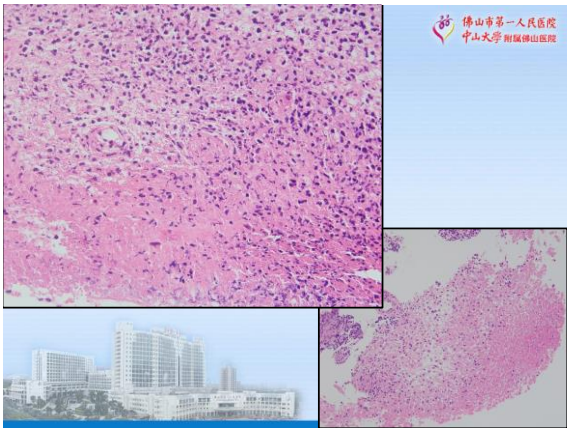

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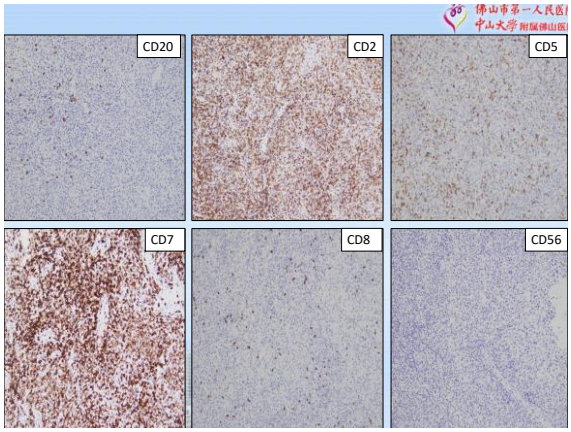

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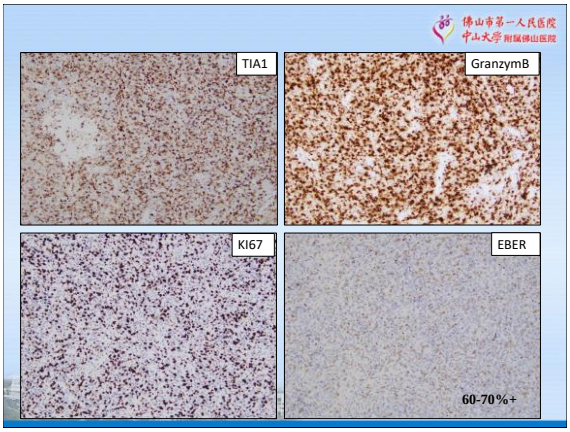


22

Final diagnosis

Chronic active EBV infection with progression to more aggressive disease overlapping extranodal NK/T cell lymphoma, Nasal Type.

The patient presented hepatosplenaomegaly at this time.
The level of EBV-DNA was higher than normal.

23

Pathological states of EBV positive T/NK cell lymphoprelipheration disorders (LPD)

Category A1	Polymorphic LPD composed of T-cells (CD8 > CD4) or NK-cells and rare B-cells without clonal proliferation of EBV-infected cells (infectious mononucleosis-like pattern).
Category A2	Polymorphic LPD with clonal proliferation of EBV-infected cells.
Category A3	Monomorphic LPD (either peripheral T-cell lymphoma or NK-cell lymphoma/leukemia) with clonal proliferation of EBV-infected cells.
Category B	Fulminant form; monomorphic LPD (peripheral T-cell lymphoma) with clonal proliferation of EBV-infected cells.

Categories A1–A3 are associated with severe chronic active EBV infection. Category B is a fulminant form immediately after the primary EBV infection, or fulminant one with immediate transformation in the course of CAEBV, which could not detected clinically.

Ohshima K,et al. Pathol Int, 2008

24

- Categories A：

A1（polymorphic/ polyclonal）

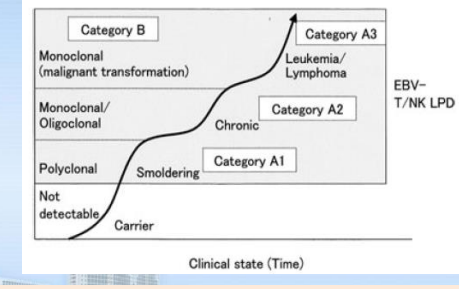
A2（polymorphic/monoclonal ）

A3（ monomorphic/monoclonal ）

*possibly constitute a continuous spectrum and all these kinds of states are equivalent to CAEBV

Ohshima k ,et al. Pathol Int, 2008

25



Clinical states and clonality of EBV-associated T/natural killer-cell lymphoproliferative disorder (EBV-T/NK LPD).

Ohshima k ,et al. Pathol Int, 2008

26

T/NK-CAEBV diagnositic criteria

◆ >3 months , recurrent disease with severe symptom
-fever
-hepatosplenomegaly

◆Antibody titer of serum EBV increased
-Anti-VCA-IgG≥5120, Anti-EA-IgG≥640 or Anti-EBNA<2
-and/or EBV copy number increase（≥102.5mg）

◆The number of T/NK cells with EBER positivity increased .

◆Histological evidence of major organ involvement
-interstitial pneumonia
-reduced bone marrow hematopoietic cells
-hymenatitis
-lymphadenitis
-persistent hepatitis or splenomegaly.

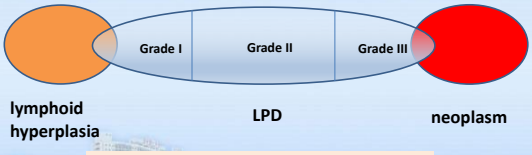
◆Other disease, such as lymphoma, immunodeficiency, and other infections were excluded.

27

EBV (+) T/NK cell LPD：

-May constitute a continuous spectrum, which including reactive lymphoid hyperplasia、borderline、neoplasm。

* clinical information is needed.



The relation ship of EBV+LPD and neoplasm

Xiaoge Zhou, et al. 中华病理学杂志,2016

28

EBV+T/NK-LPD

NK/T

Onset：Systematic

Onset：local

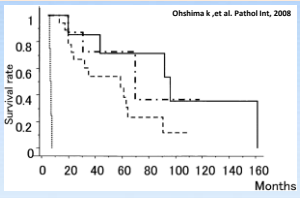
- A1: polymorphic + polyclonal = hyperplasia
- A2: polymorphic + monoclonal =boderline
- A3: monomorphic+ monoclonal =neoplasm

atypia+(clonal +/-)= neoplasm

Xiaoge Zhou, et al. 中华病理学杂志, 2016

29

Overall survival of patients with EBV-associated T/NK cell



high mortality and high morbidity

Treatment of this case (2019.09.18)

◆P+Gemox (Peimendase, gemcitabine, oxaliplatin) regimens for 6 courses
◆the mass of nasal cavity became smaller
◆CT : Partial response (PR)

30

Acknowledgement

Beijing friendship hospital, Capital medical university
Xiaoge Zhou

Foshan hospital ,Sun yat-sen university
Ni xiao, Weiwei Hu, Jiongyuan Chen, Haiyan Shi, Qianwen Yan

31

Joint CSHP and SH Workshop 2019

Session One

Sophie Song, MD PhD

Dept. of Pathology and Laboratory Medicine
UCLA Medical Center
Los Angeles, CA, USA

1

Clinical Presentation

- 70 year-old male with systemic lymphadenopathy for two years
- No other symptoms
- No significant laboratory abnormalities
- Previous LN biopsies performed at outside institutions have been non-diagnostic
- The most recent LN excision is sent to UCLA for consultation

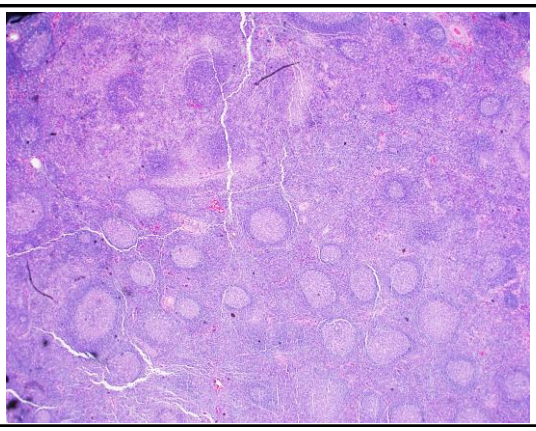
2

Specimen

- Excisional biopsy of left axillary lymph nodes

(performed at the OSH and sent to us for consultation)

3



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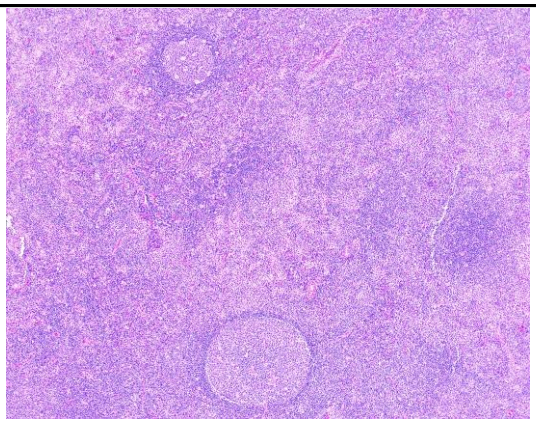

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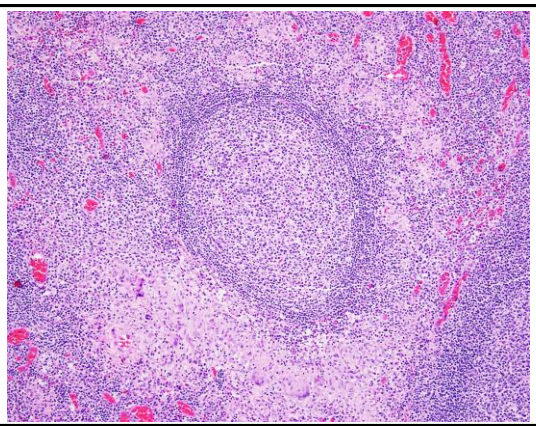

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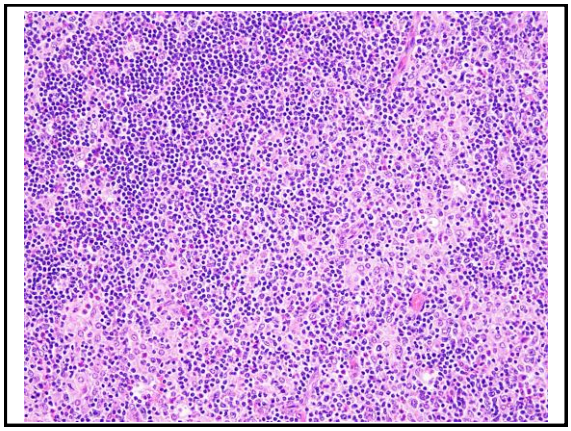

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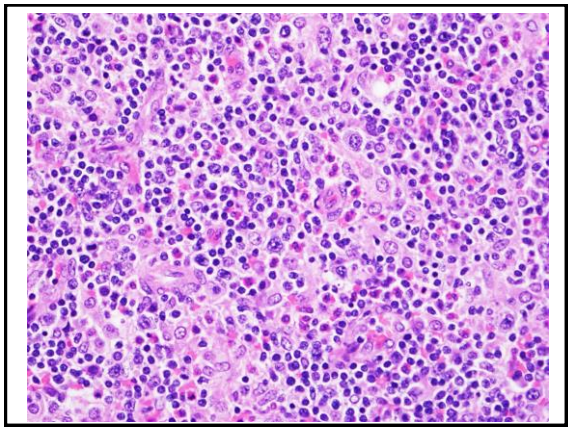

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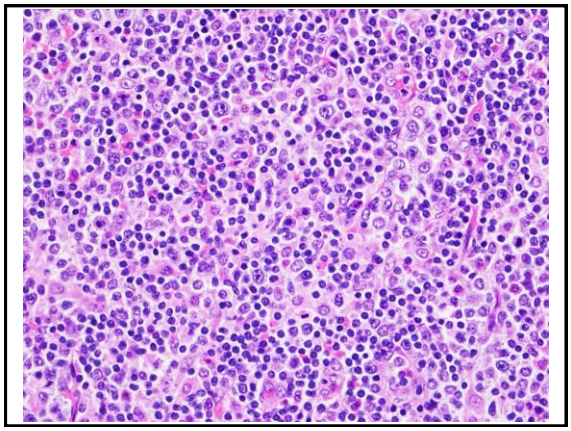

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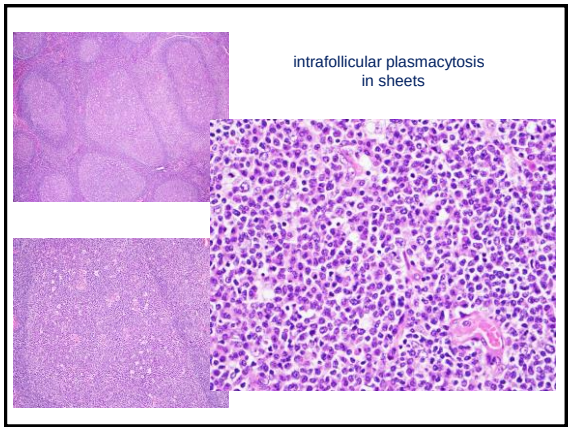
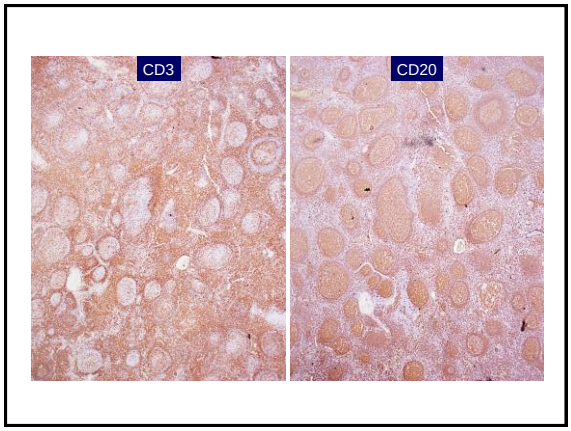

intrafollicular plasmacytosis
in sheets

10


CD3
CD20

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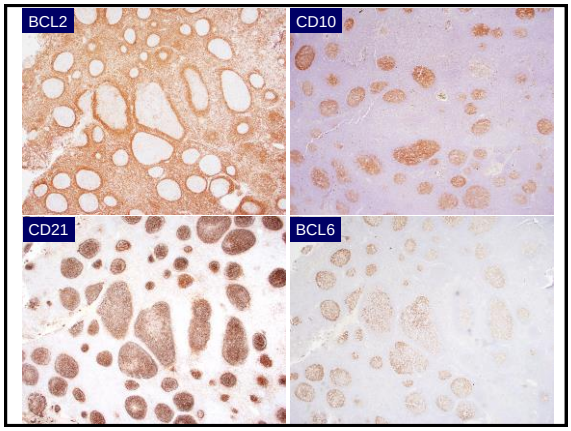
BCL2
CD10
CD21
BCL6

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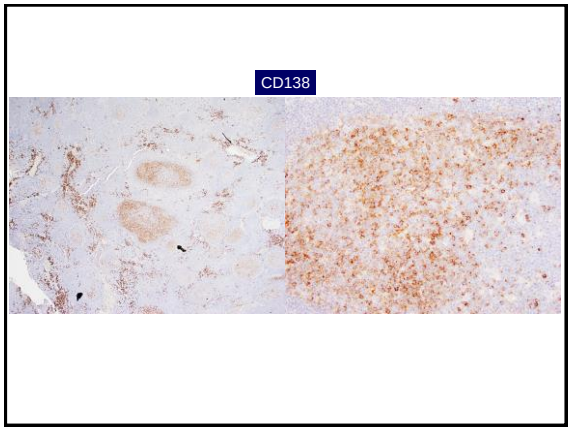

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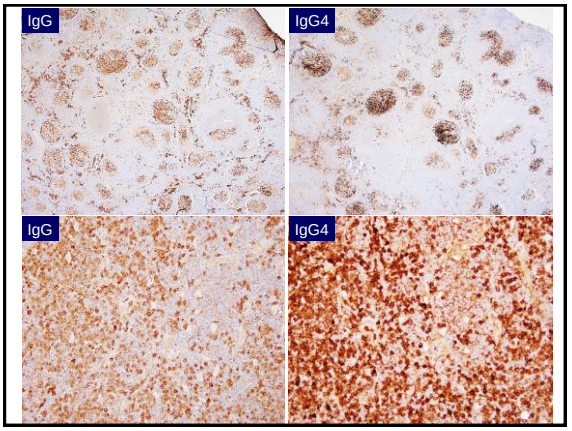

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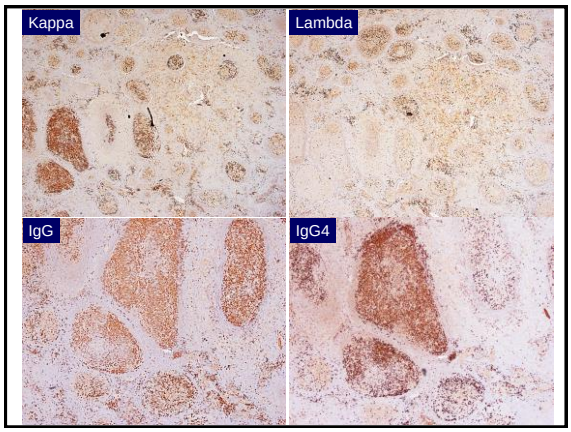

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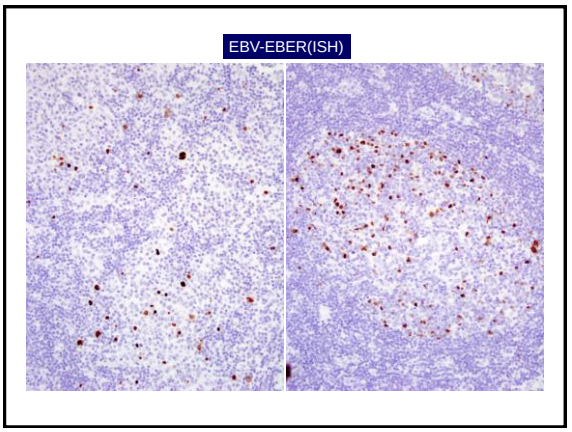

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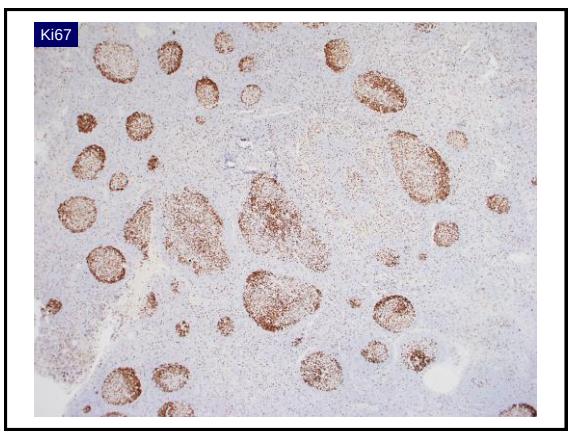


17

Follow-up

- Result of serum IgG4 became available in June 2019, and it is 185 mg/dL (normal 135 mg/dL)

18

Summary of LN Findings

- Preserved architecture with significant expansion
- Marked follicular hyperplasia
- Variable interfollicular expansion with mixed cell types
- Perifollicular and interfollicular granulomata
- Sheets of IgG 4 plasma cells inter- and intrafollicular; appear kappa-restricted in very focal intrafollicular areas
- Variable EBV-EBER positivity; appears more in areas of granulomata

19

Proposed Diagnosis

IgG4-related lymphadenopathy, EBV-positive

Panel Diagnosis

IgG4-related lymphadenopathy, EBV-positive

20

IgG4-Related Lymphadenopathy

- 80% of extranodal IgG4-RD
- Localized or systemic LAD without constitutional symptoms
- Appear before, concurrent with, or after dx of extranodal IgG4-RD
- IgG4 plasma cells ≥ 100 per HPF, and IgG4/IgG ratio ≥ 40%
- Lack of storiform fibrosis in most types; absent obliterative phlebitis in all

21

IgG4-Related Lymphadenopathy

	Histological type	Distribution of IgG4+ plasma cell	LAD
I	Multicentric Castleman disease-like	Interfollicular	Systemic
II	Reactive follicular hyperplasia-like	Interfollicular	Localized
III	Interfollicular expansion & immunoblastosis	Interfollicular	Systemic
IV	PTGC-type	Intragerminal center	Localized/Systemic
V	Inflammatory pseudotumor (IPT-) like	Interfollicular	Localized

(Adopted from Sato Y, Yoshino T, Int J Rheumatol 2012)

22

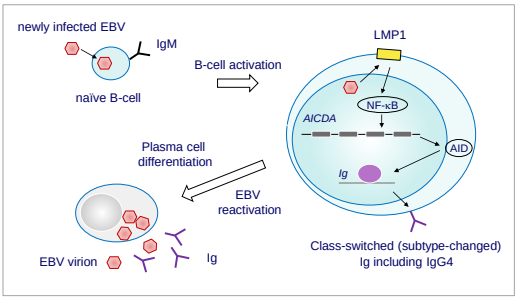
EBV and IgG4-Related Lymphadenopathy

Diagnosis	No. of Cases	EBV-EBER(ish)+
IgG4-related LAD	31	18 (58%)
extranodal IgG4-RD	24	5 (21%) (P = 0.006)
other reactive LNs	22	4 (18.1%) (similar age; P = 0.002)
[AITL	10	(significantly fewer IgG4; P < 0.001)]

(Takeuchi M et al., Am J Surg Pathol 2014)

23

Pathway for EBV Reactivation-Induced Ig Production



(Adopted with modification from Nagata K et al., Viral Immunol 2018)

24

Diagnostic Pitfalls and Overlaps with EBV+ B-Cell and other LPDs

Histology of IgG4-related LAD	Mimics
Multicentric Castleman disease-like	MCD, other immune-mediated conditions, and hyper IL-6 syndrome
Reactive follicular hyperplasia-like	FL
Interfollicular expansion & immunoblastosis	EBV-positive DLBCL, NOS; AITL
PTGC-type and perifollicular granuloma	NLPHL, FL
Sheets of plasma cells	Plasma cell neoplasm, and B-cell lymphoma with plasmacytic differentiation

25

Acknowledgement

- Dr. Robert Veve, referring pathologist
  Medical Laboratory Services Medical Group, INC.
  Murrieta, CA 92562, USA
- Dr. Ramesh Kumar, hematologist/oncologist
  The Oncology Institute of Hope and Innovation
  Murrieta, CA 92562, USA

26

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27

Chronic Active EBV infection with hemophagocytic lymphohistiocytosis and progression to Aggressive NK Cell Leukemia

Li-min Gao[1], Sha Zhao[1], Zi-Hang Chen[1], Qiang Li[2], Wei-Ping Liu[1]

1. West china hospital of SiChuan university
2. West china second university hospital

2019-10

1

Clinical History:

- A 7-year-old boy had recurrent fever and mosquito bite allergy for about 3 months 1 year ago.
- Recurrent fever occurred for 2 month before he admitted to hospital without obvious inducement, the highest temperature was 39℃.
- The left cervical lymph node enlargement and was 1.2 cm in diameter. The liver extends below the costal margin 6cm and the spleen extends below the costal margin 4cm.
- Excision biopsy of the left cervical lymph node was performed in 2017-08-08.
- 4 monthes later, the patient had fever again and peripherial blood separated lymphcytes smear was performed in 2017-12-28.

2

Laboratory examination:

↓ WBC: 1.2×10^9/L, HGB: 68g/L, PLT: 15×10^9/L. ANC: 0.03×10^9/L.

↓ NK cell activity: 15.82% (Normal: 28%-36%).

↑ Soluble CD25 > 44000pg/ml (Normal < 6400 pg/ml).

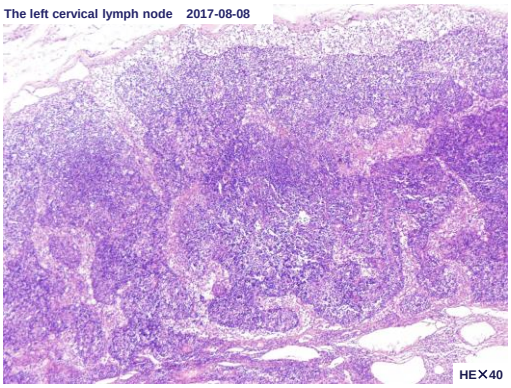
↑ CD163: 2249ng/ml (Normal: 88-902pg/ml).

↑ Ferritin: 1471.3ng/ml (Normal: 7~140ng/ml).

↑ EBV-DNA loading: 2.34×10^4. (Normal < 400 copies/ml).

3

The left cervical lymph node 2017-08-08



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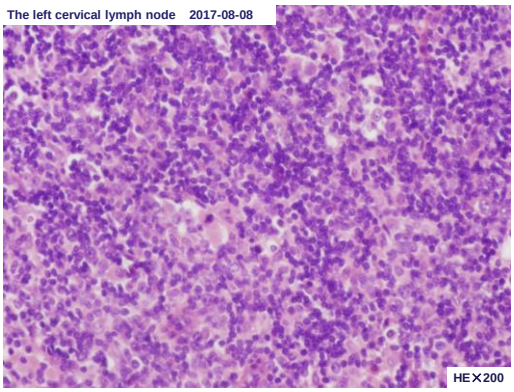
The left cervical lymph node 2017-08-08



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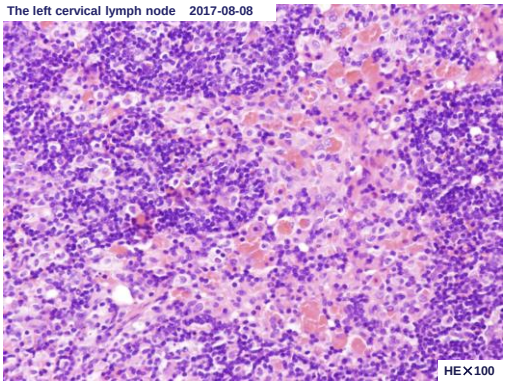
The left cervical lymph node 2017-08-08



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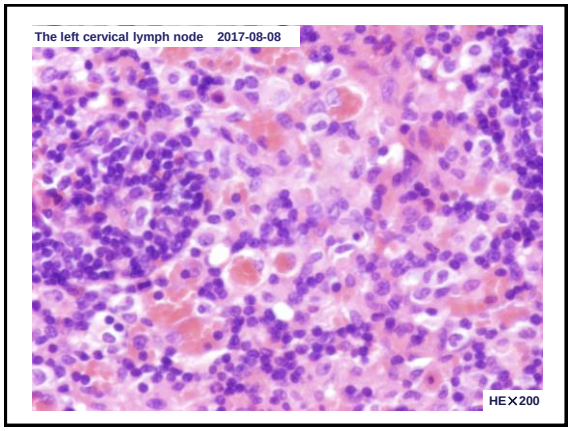
The left cervical lymph node 2017-08-08
HE×200

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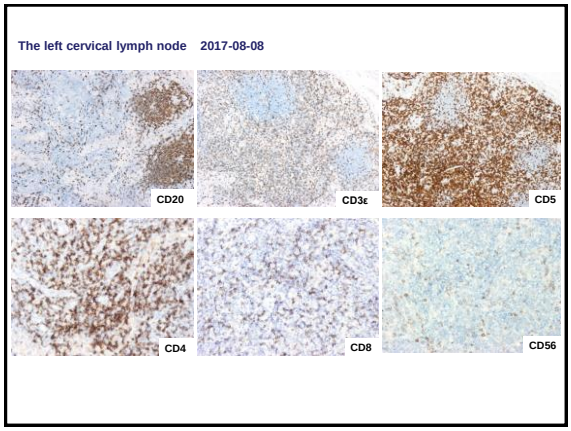
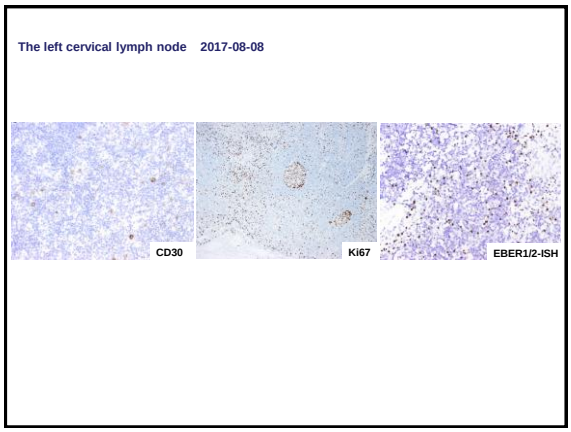
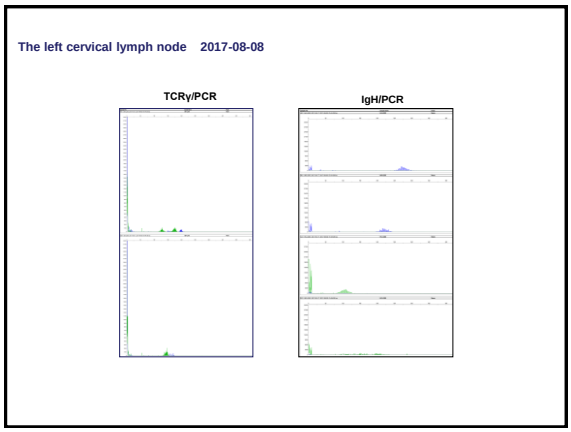

The left cervical lymph node 2017-08-08
CD20
CD3ε
CD5
CD4
CD8
CD56

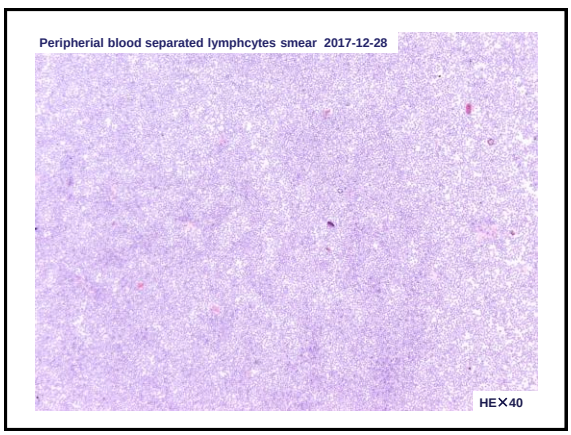
8


The left cervical lymph node 2017-08-08
CD30
Ki67
EBER1/2-ISH

9


The left cervical lymph node 2017-08-08
TCRγ/PCR
IgH/PCR

10


Peripherial blood separated lymphcytes smear 2017-12-28
HE×40

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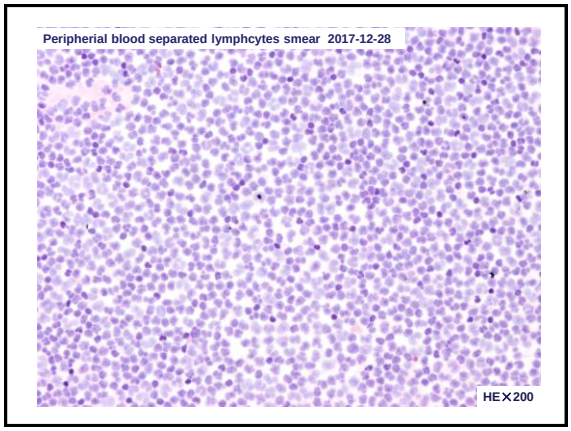
Peripherial blood separated lymphcytes smear 2017-12-28
HE×200

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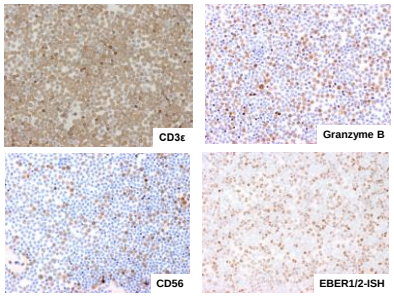
Peripherial blood separated lymphcytes smear 2017-12-28



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Cytogenetics:

UNC13D gene heterozygous mutation was detected in the patient and patient' father.

- UNC13D is a gene associated with familial hemophagocytic lymphohistiocytosis (FHLH).

14

Proposed Diagnosis:

The left cervical lymph node:

Chronic active EBV infection of T-and NK cell type with hemophagocytic lymphohistiocytosis. (2017-08-08)

Peripherial blood separated lymphcytes:

Aggressive natural killer cell leukemia. (2017-12-28)

15

Follow up:

The patient received allogeneic hematopoietic stem cell transplantation in December 2017 and is in good condition.

16

Time axis of disease development and outcome

6 May 2016	8 July. 2017	25 Sep. 2017	27 Dec.2017	10 June 2019
Rash Mosquito bite allergy Recurrent fever	Recurrent fever LN enlargement Hepatosplenomegaly	Normal	Fever	Normal
Chemotherapy			allo - HSCT	
CD4=20.8%↓ CD8=10.8%↓ CD4:CD8=1.9 NK cell=60.1%↑	EBV-DNA: 2.34×10^4 EBV-VCA-IgM(-)	EBV-DNA:312 EBV-VCA-IgM(-) CD4=38.8% CD8=20.8% CD4:CD8=1.9 NK cell=24.5%	EBV-DNA: 1.21×10^5 EBV-EA (-) EBV-VCA-IgM(-) EBV-VCA-IgG(+) EBNA(+) CD4=5.3% CD8=4.8% CD4:CD8=1.1↓ NK cell=89.4%↑	EBV-DNA＜400 CD4=26.8% CD8=30.1% CD4:CD8=0.9 NK cell=6.9%↑
CAEBV&HLH			ANKL	

17

Interesting Feature(s) of Submitted Case:

1. **This is a rare case of aggressive NK cell leukemia in a child.**
2. **This case had showed the process of Chronic active EBV infection of T-and NK cell type with hemophagocytic lymphohistiocytosis progressing to aggressive NK cell leukemia.**
3. **This is a rare case of aggressive NK cell leukemia cured by allogeneic stem cell transplantation.**

18

1. Chronic active EBV infection (CAEBV) of T-cell or NK-cell type (CAEBV-T/NK):

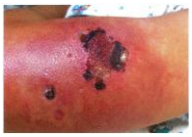
- CAEBV-T/NK is a systemic EBV-positive polyclonal, oligoclonal, or monoclonal lymphoproliferative disorder.
- CAEBV-T/NK shows varying degrees of clinical severity depending on the host immune response and the EBV viral load.
- Hemophagocytic Syndrome occur in **24%** of CAEBV-T/NK cases.
- CAEBV-T/NK progress to ENKTL or ANKL occurs in **16%** of cases.

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues（2017）

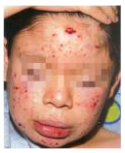
19

2. Different Features of localized versus systemic chronic active EBV infection.

Localized CAEBV:



Severe mosquito bite allergy.



Hydroa vacciniforme-like lymphoproliferative disorder.

Systemic CAEBV:

Fever, hepatosplenomegaly, and lymphadenopathy, with or without cutaneous manifestations diseases.

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues（2017）

20

3. Hemaophagocytic Lymphohistiocytosis (HLH)

Diagnostic Criteria of HLH
Fever (peak temperature of > 38.5° C for > 7 days)
Splenomegaly (spleen palpable > 3 cm below costal margin)
Cytopenia involving > 2 cell lines (Hb < 9 g/dL, absolute neutrophil count < 100/μL, platelets < 100,000/μL)
Hypertriglyceridemia (fasting triglycerides > 2.0 mmol/L or > 3 standard deviations more than normal value for age) or hypofibrinogenemia (fibrinogen < 1.5 g/L or > 3 SD less than normal value for age)
Hemophagocytosis (in biopsy samples of bone marrow, spleen, or lymph nodes)
Low or absent natural killer cell activity
Serum ferritin > 500 μg/L
Elevated soluble interleukin-2 (CD25) levels (>2400 U/mL or very high for age)

- Hemophagocytic lymphohistiocytosis can be diagnosed if there is a mutation in a known causative gene or if at least 5 of 8 diagnostic criteria are met.
- The orange label is the diagnostic criteria that the case meets.

21

3. Familial Hemaophagocytic Lymphohistiocytosis (FHL)

- FHL caused by an underlying genetic defect in infants

Familial HLH subtypes and associated gene defects

HLH Subtype	Genetic defect	Protein function
FHL1	9q21.3-locus 6	unknown
FHL2	*PFR1*	pore forming cytolytic protein
FHL3	*UNC13D*	secretion of cytolytic granules
FHL4	*STX11*	intracellular vesicle transport
FHL5	*(UNC18B)*	membrane fusion

- **All of these genes encode proteins involved in intracellular vesicle trafficking, and their mutations lead to defective cytotoxicity**

Zuzana T et al. *Intensive Care Med.* 2016. 30(7): 401–412.

22

4. Aggressive Natural killer cell leukemia（ANKL）:

- The median onset age of ANKL is 40 years and is rare in children.

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues（2017）

- This patient recovered after allogeneic hematopoietic stem cell transplantation.

ORIGINAL ARTICLE (*Am J Surg Pathol* 2016;40:836–846)

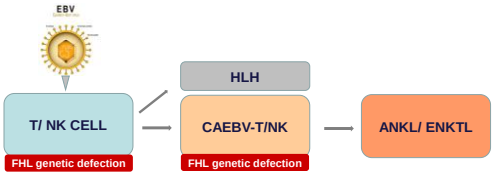
Clinicopathologic Characterization of Aggressive Natural Killer Cell Leukemia Involving Different Tissue Sites

Li-Min Gao, PhD, Shu Zhao, PhD,* Wei-Ping Liu, MD,* Wen-Yan Zhang, PhD,* Gan-Di Li, PhD,* Can Kaçlk, PhD,†‡§ Xiao-Zhou Hu, PhD,†‡ Wing C. Chan, PhD,† Yuan Tang, PhD,* Wen-Shuang Ding, PhD,* Jia-Qi Yan, PhD,* Wen-Qing Yao, PhD,* and Jian Chao Wang, MD**

- **35 cases of ANKL were reported by the Department of Pathology, West China Hospital.**
- **The median age of onset was 35 years. The median survival time was 43 days.**

23

Conclusion



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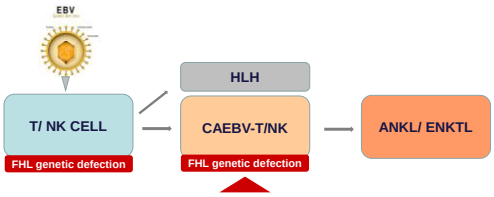
Conclusion



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Thanks For Your Attention

26

Hydroa Vacciniforme-Like Lymphoproliferative Disorder

Submitters: Yasodha Natkunam and Atif Saleem

Authors: Gillian Heinecke, MD, Sebastian Fernandez-Pol MD, PhD, P. Mayan, MD, K. Martinez, MD, Mauricio Siliezar, MD, Sandra Delgado Perez, MD, Kerri E Rieger, MD, PhD

1

History

8 year old Guatemalan boy of Mayan descent presented with progressive ulcerated papulonodules

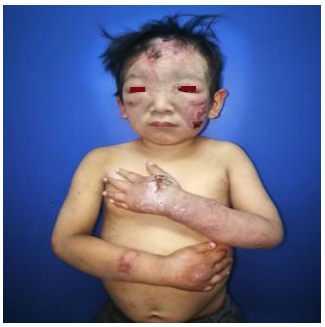
- Involved his face, upper and lower extremities, chest, upper back and occasionally his abdomen
- Initially started on his scalp 4 years earlier and had gradually worsened with time
- He also complained of facial swelling and fevers
- No other systemic symptoms
- His parents are farmers, patient often helped out on the farm

2

Physical Exam Findings

- Erythematous plaques with central ulceration and necrotic crust as well as peripheral atrophic scarring on predominantly sun exposed skin
- Few papules and plaques on his trunk
- Marked facial edema
- Scattered pitted scars on face

3



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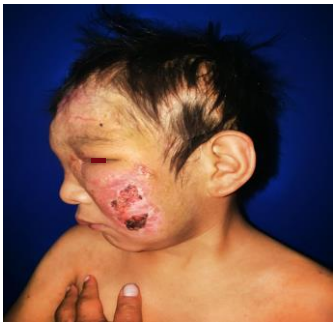

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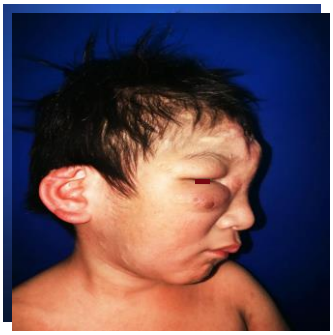

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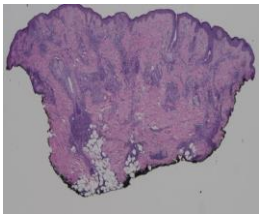
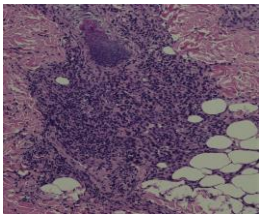

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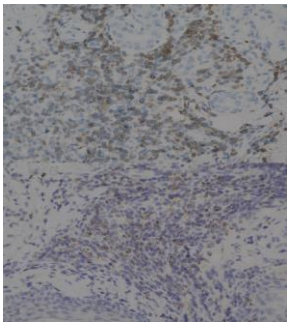
CD3

TIA1



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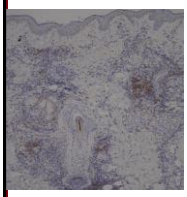
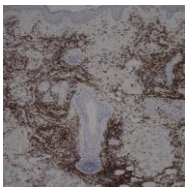
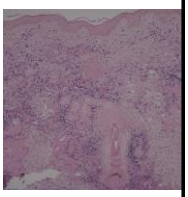

CD4 CD8 EBV

9

Diagnosis

Hydroa Vacciniforme-Like Lymphoproliferative Disorder

10

Histopathology Differential

TABLE 3. Comparative Analysis of HV-LPD, Systemic EBV+TCL of Childhood, SPTCL, GDTCL, and Extranodal NK/TCL, Nasal Type (NK/TCL)

	HV-LPD	Systemic EBV+TCL of Childhood	SPTCL	GDTCL	NK/TCL
Age of patients	Pediatric	Pediatric; some adults	Adult; some pediatric	Adult	Adult
Ethnicity	Asians, parts of Central and South America	Asians, parts of Central and South America	No ethnic or regional preference	No ethnic or regional preference	Asians, parts of Central and South America
Sun-related	Yes	Sometimes	No	No	No
EBV positivity	Yes	Yes	No	No	Yes
CD56 expression	Yes, often	Yes, often	No	No	Yes

EBV indicates Epstein-Barr virus; GDTCL, gamma delta T-cell lymphoma; HV-LPD, hydroa vacciniforme-like lymphoproliferative disorder; NK, natural killer; SPTCL, subcutaneous panniculitis-like T-cell lymphoma; TCL, T-cell lymphoma.

Sundram U. Adv Anat Pathol. 2019 Mar;26(2):93-113.

11

Hydroa Vacciniforme-Like Lymphoproliferative Disorder (HV-LPD)

- Previously categorized as hydroa vacciniforme and hydroa vaccinforme-like lymphoma
  - 2016 revised 4th edition of WHO both groups now classified as HV-LPD
  - A chronic active EBV infection
- Most cases occur in children and adolescents from Asia and Native Americans from Mexico, Central and South America
- Mean age of diagnosis is 8 years old, male predominance (2.3:1)
- Eruption usually starts minutes to hours after sun exposure
- Can have eye and oral involvement
- Facial edema tends to occur in more severe cases
- Systemic symptoms such as hepatosplenomegaly, lymphadenopathy, fever and weight loss can occur
  - Frank extracutaneous lymphoma classified as Systemic EBV+ T cell lymphoma of childhood

12

Chronic Active EBV Infection of T- and NK-Cell Type, Systemic Form

- Approximately 50% present with infectious mononucleosis-like illness, with accompanying symptoms:
  - Skin rash (26%)
  - Severe mosquito bite allergy (33%)
    - Erythema, bullae, ulcers, necrosis, scarring
  - Hydroa vacciniforme-like eruptions (10%)
    - Papulovesicular eruption in sun-exposed areas→ulceration/scarring

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Hydroa Vacciniforme-Like Lymphoproliferative Disorder (HV-LPD)

- Prognosis:
  - Can have an aggressive course with fatal outcome, especially in Latin American patients
- Management:
  - Sun avoidance and sun protection
  - Chemotherapy effects can be transient
  - Immunomodulating agents such as prednisone, cyclosporine, hydroquinone, and thalidomide have also shown temporary improvement

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Presentation and Clinical Course in Central American vs. East Asian Populations

- Periorbital and perioral edema prominent in Latin American populations compared to East Asia
- Latin American cases demonstrate increased cellular pleomorphism and Ki-67 proliferation index microscopically
- Majority of Latin American cases did not survive compared to a majority of East Asian cases which did survive

Liu Y et al. JAAD. 2019 Jan 14.

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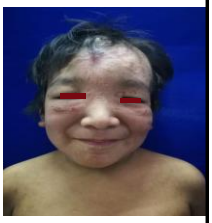
Clinical Course



- Workup found hepatic steatosis – no other systemic involvement
- Started initially on prednisone, acyclovir and dicloxacillin
- Vincristine added given persistent symptoms
- Now with marked improvement in ulcerations, facial swelling and fevers

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Conclusion

- In 2016 revised 4th edition of WHO – Hydroa vacciniforme and hydroa vacciniforme like lymphoma are now HV-LPD
- Patients from Asia and Native Americans from Mexico, Central and South America more likely to have severe disease
- Immunomodulating agents as well as chemotherapy can be used for treatment

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References

1. Gru AA, Jaffe ES. Cutaneous EBV-related lymphoproliferative disorders. Semin Diagn Pathol. 2017 Jan;34(1):60-75.
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4. Levoska MA, Cohen JI, Manoli I, et al. Recurrent scarring papulovesicular lesions on sun-exposed skin in a 22-year-old man. J Am Acad Dermatol. 2018 Mar;78(3):637-642.
5. Quintanilla-Martinez L, Ridaura C, Nagl F, et al. Hydroa vacciniforme-like lymphoma: a chronic EBV+ lymphoproliferative disorder with risk to develop a systemic lymphoma. Blood. 2013 Oct 31;122(18):3101-10.
6. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J (Eds): WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th edition). IARC: Lyon 2017.
7. Liu Y, Ma C, Wang G, Wang L. Hydroa vacciniforme-like lymphoproliferative disorder: Clinicopathologic study of 41 cases. Journal of the American Academy of Dermatology. 2019 Jan 14.

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18

A case of MTX-associated EBV+ B-cell LPD with rapidly lethal clinical course, challenging differential diagnosis of EBV+ mucocutaneous ulcer

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Akira Satou, Shigeo Nakamura

Department of pathology and Laboratory Medicine,
Nagoya University Hospital, Nagoya, Japan

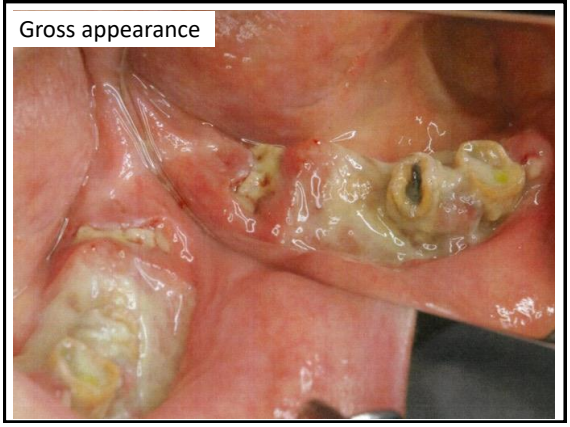
1

Initial presentation

- A 75-year-old woman who had a past medical history of rheumatoid arthritis.
- She was treated with an administration of methotrexate for six years.
- She presented with gingival swelling, the biopsy of which was done for the diagnosis.

2

Gross appearance



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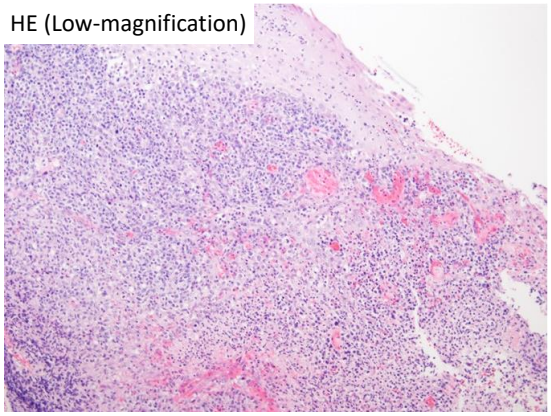
HE (Low-magnification)



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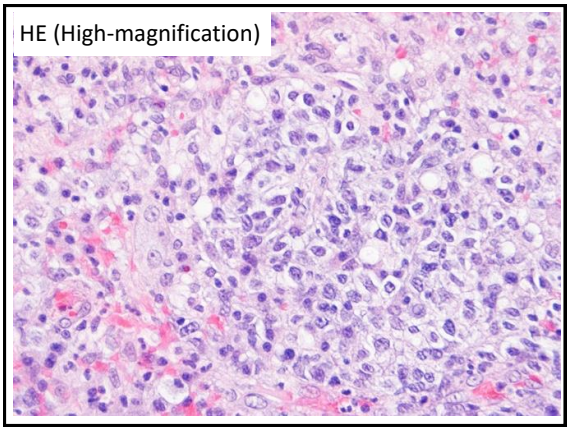
HE (High-magnification)



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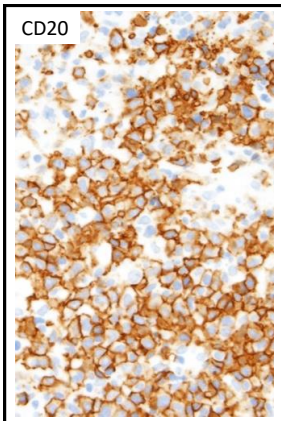
CD20



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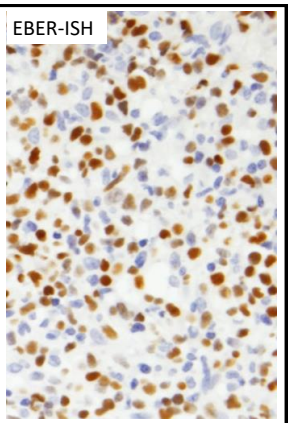


6

Diagnosis (biopsy)

EBV+ diffuse large B-cell lymphoma (DLBCL)

(preferred diagnosis of EBV+ mucocutaneous ulcer with large cell predominant areas)

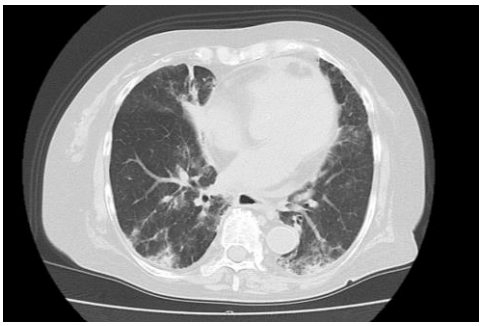
7

Disease course

- 1 month after biopsy, the patient was admitted to our hospital because of leukopenia and renal dysfunction.
- Respiratory function and general condition rapidly deteriorated in the clinical course.
- She died 6 days after admission.

8

CT scan (at the time of admission)



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Blood test (at the time of admission)

WBC	800 /mm³	BUN	84.9 mg/dl
RBC	3.54x10⁶ /mm³	Creatinine	2.28 g/dl
Hemoglobin	10.2 g/dl	AST	96 U/l
Hemoglobin	31.8 %	ALT	56 U/l
Platelet	122x10³ /mm³	LDH	363 U/l
Total protein	5.5 g/dl	ALP	42.7 U/l
Albumin	2.8 g/dl	γ-GTP	140 U/l
Glucose	132 mg/dl	Amylase	98 U/l

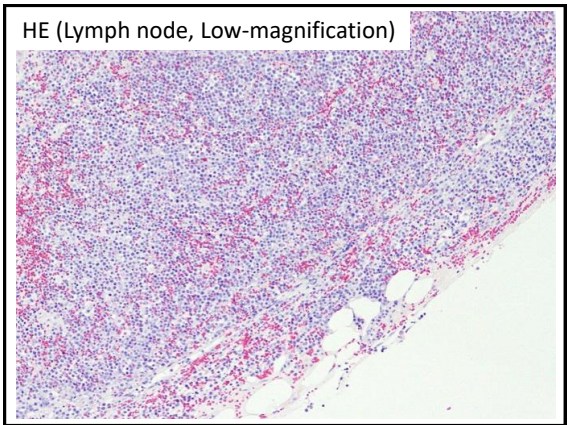
10

Autopsy

- Ulcerative lesion in mandibular gingiva to buccal mucosa
- Lymphadenopathy
  (cervical, parabronchial, mesenteric lymph node)

11

HE (Lymph node, Low-magnification)



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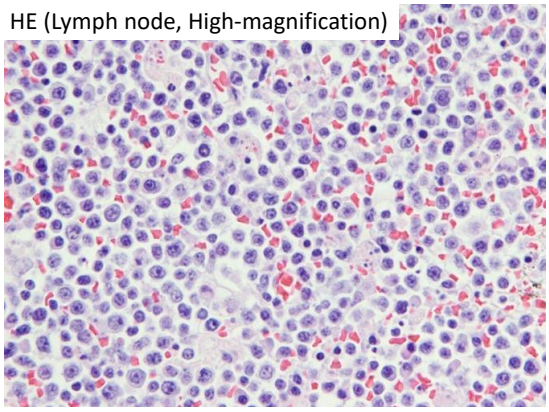
HE (Lymph node, High-magnification)



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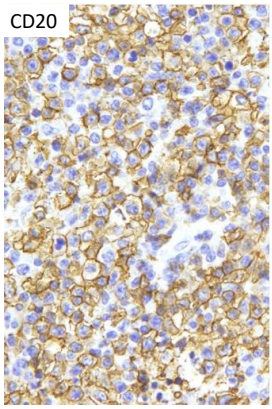

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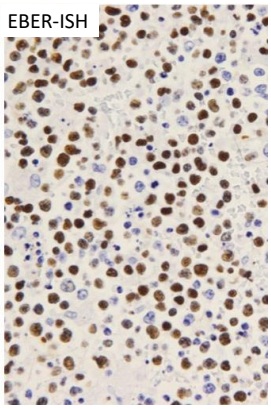

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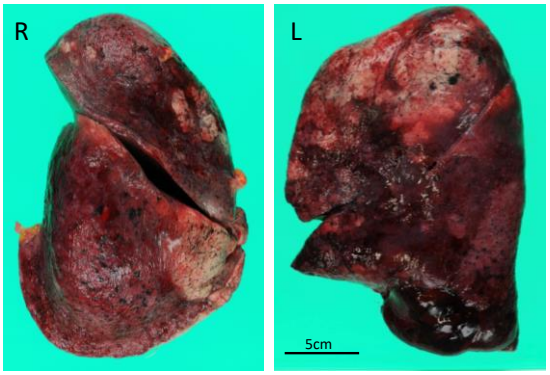
Gross appearance (Lung)



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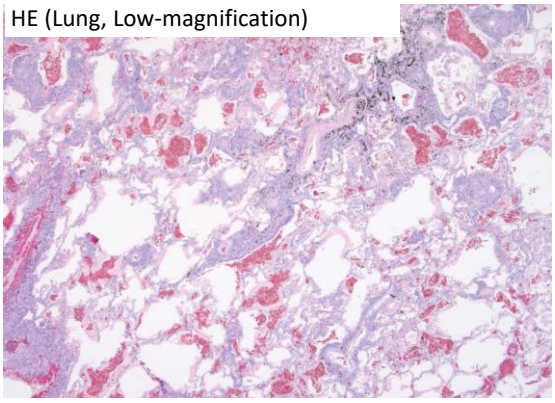

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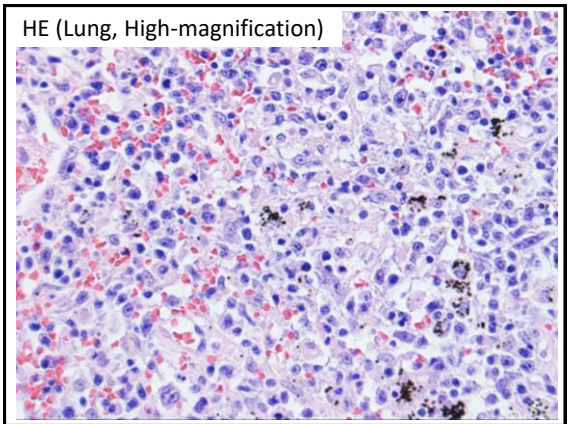


17

Diagnosis (Autopsy)

EBV+ diffuse large B-cell lymphoma

(MTX-associated EBV+ B-cell lymphoproliferative disorder,
monomorphous subtype,
masqueraded with EBV+ MCU-like lesion in the process)

18

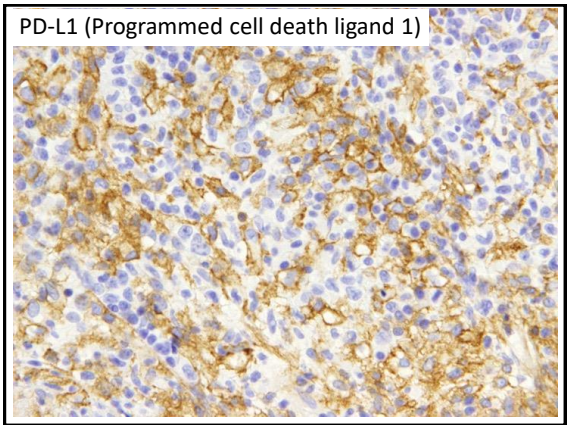
EBV-positive mucocutaneous ulcer (EBV+ MCU)

- Occurs in patients with various forms of immunosuppression.
- Responds to reduction of immunosuppressive therapy, often resulting in spontaneous regression.
- Some cases resemble diffuse large B cell lymphoma.

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. (2017)

19

PD-L1 (Programmed cell death ligand 1)



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Conclusion

- The present case rapidly progressed into a systemic disease as contrasted with ordinal EBV+ MCU patients.
- Neoplastic PD-L1 expression on tumor cells was well documented in EBV+ DLBCL of young patients, featured by a node-base disease with favorable clinical course, by Nicolae et al. (Elaine S. Jaffe's group), suggesting an immune evasion in the pathogenesis.

Nicolae et al. *Blood* 2015;126(7):863-872

21

Conclusion (continued)

- Our case lacked this neoplastic PD-L1 expression, indicating an immunodeficiency in the host.
- Much attention should be requested on the diagnostic approach for EBV+ DLBCL and related diseases (localized vs. systemic; and immune evasion vs. immunodeficiency associated)

22

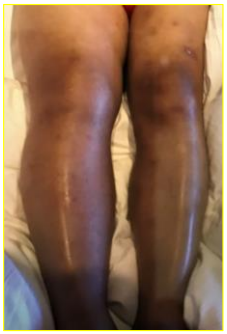
Angioimmunoblastic T cell lymphoma with severe, generalized cutaneous involvement and EBV infection

Wen-Qing Yao, Sha Zhao, Wei-Ping Liu

Department of Pathology, West China Hospital, Sichuan University

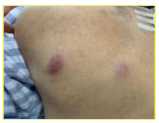
1

Clinical History



- 64 years old male
- CC: right leg swelling and erythematous subcutaneous nodules on trunk and limbs for 3 months.
- Painless nodules, no fever
- PE: painless subcutaneous nodular ranging from 1 to 2 cm in diameter.
- Circulating EBV DNA:

1.05×10^4 copies/ml



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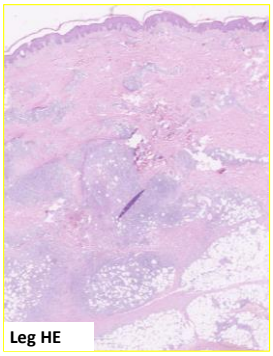
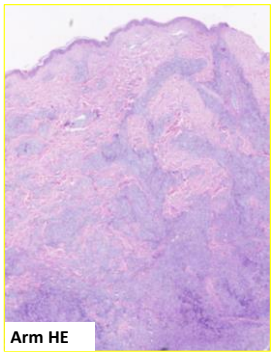
Skin biopsies



Leg HE



Arm HE

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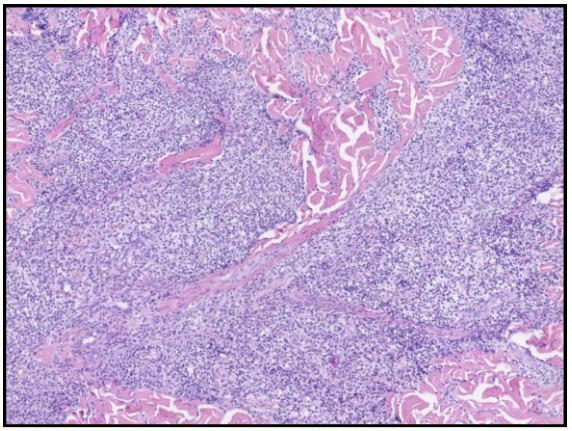

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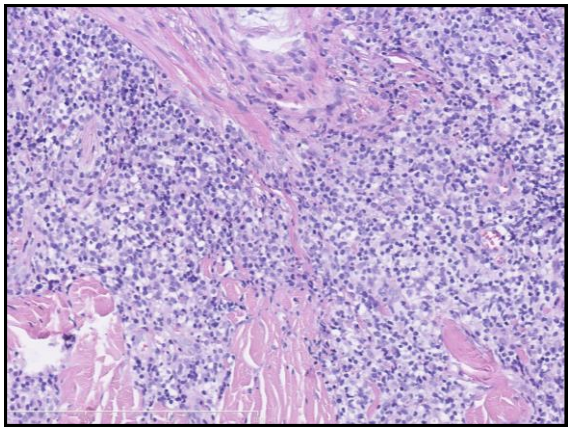

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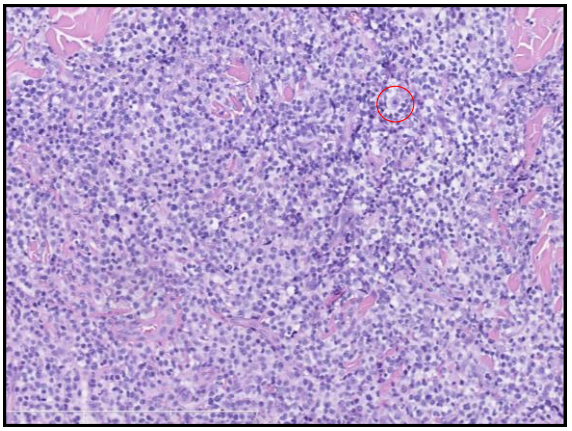

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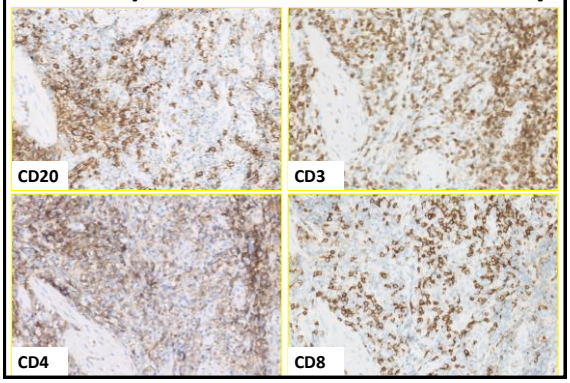
Skin biopsies: immunohistochemistry



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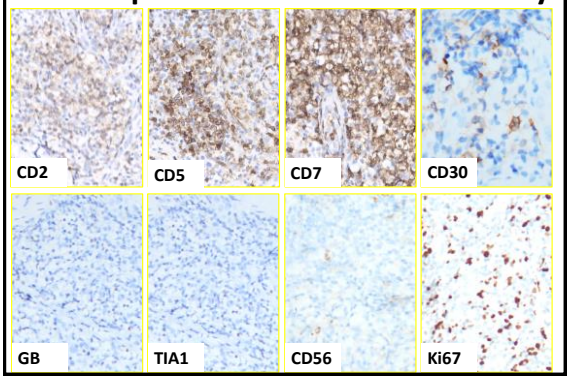
Skin biopsies: immunohistochemistry



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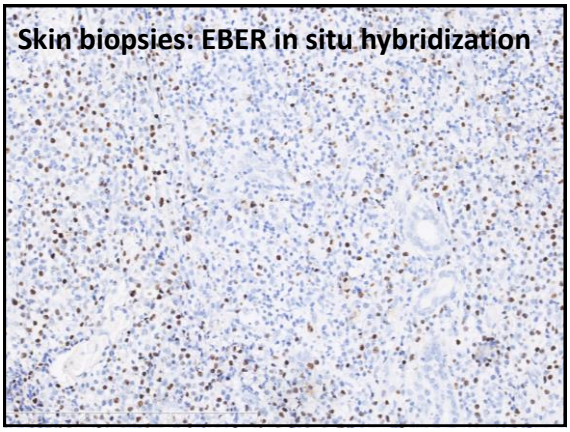
Skin biopsies: EBER in situ hybridization



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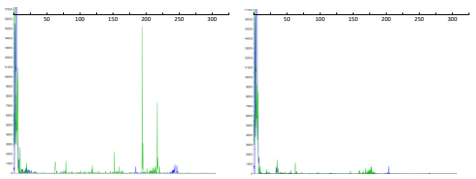
TCR clonal analysis: clonal TRG rearrangement



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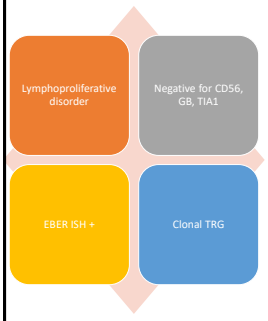
Diagnosis



- Local hospital: panniculitis-like lesion
- Pathological consultation: EBV associated lymphoproliferative disorders, atypical lymphoid hyperplasia (ALH)
- Differential diagnosis including:
  - Extranodal NK/T cell lymphoma, nasal type
  - Systemic EBV-positive T-cell lymphoma of childhood
  - Systemic chronic active EBV infection of T/NK type

LN biopsy is highly recommended!

11

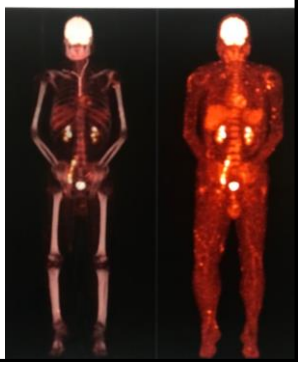
West China Hospital

Lab test:

Items	Results	Reference
LDH	168 IU/L	110-220
Circulating EBV DNA	2.23×10^3 copies/ml	Negative
B2 microglobulin	3.24 mg/L	0.70-1.80

PET/CT:

- generalized lymphadenopathy: max 3.4cm×2.6cm, max SUV 8.34
- multiple subcutaneous nodules: max 1.5cm×1.5cm, max SUV 4.86
- right leg swelling



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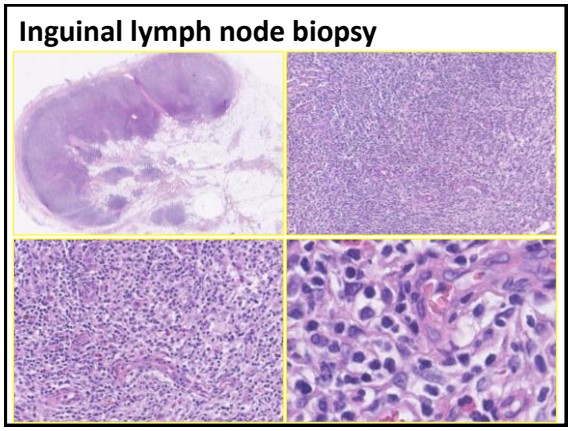

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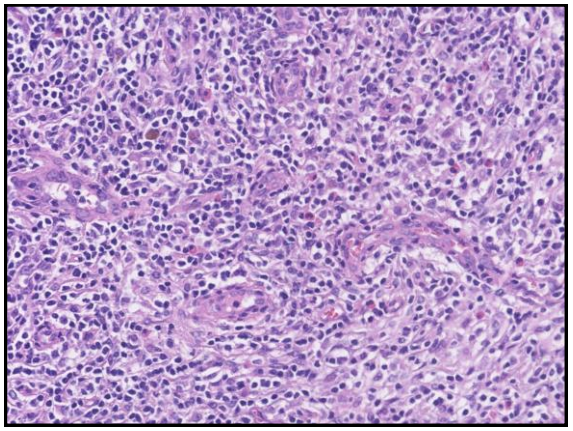

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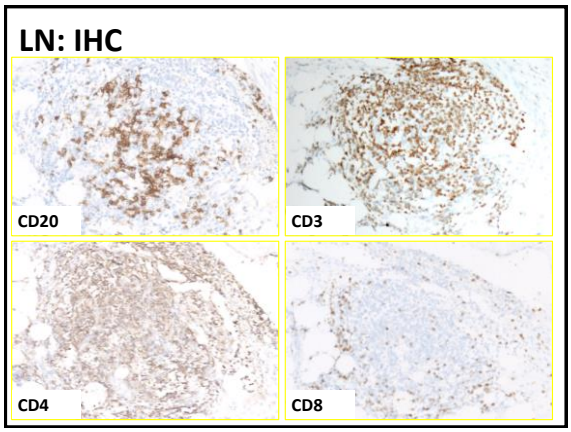

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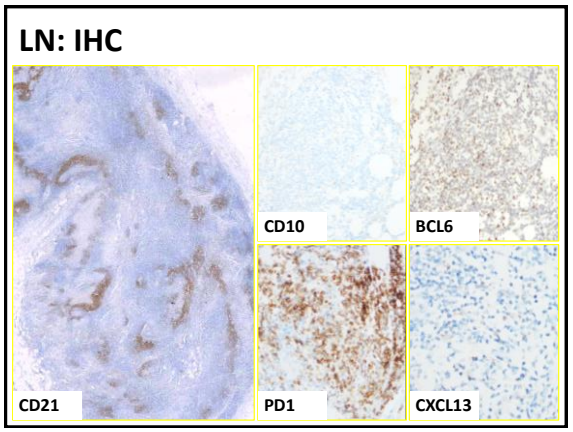

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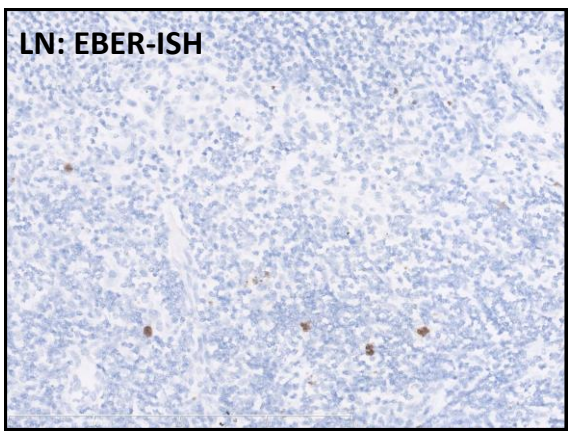

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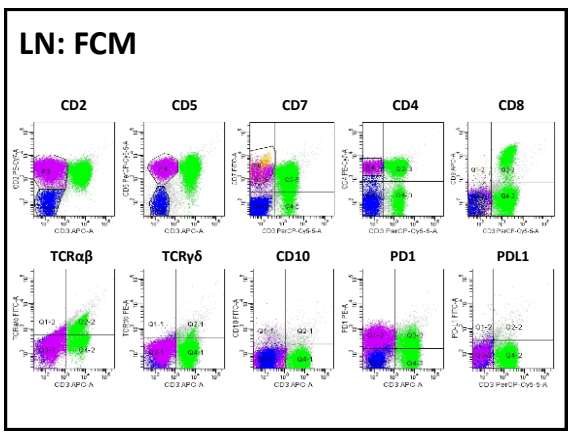
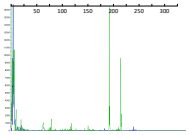


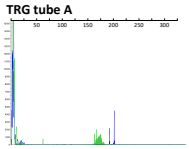
18

LN: molecular analysis

Clonal TRG rearrangement

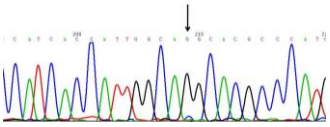


TRG tube A

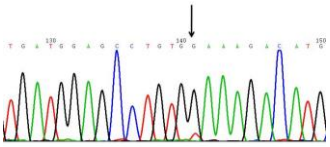


TRG tube B

IDH2: AGG>ACG, c.G515C, p.R172T



RHOA: GGA>GTA, c.G50T, p.G17V



19

LN: pathological diagnosis

Clinical Manifestation:
67 M
Generalized lymphadenopathy
Severe and generalized skin lesion

Histology:
Perifollicular hyperplasia
High endothelium vessels
Clear cells
Background inflammatory cells

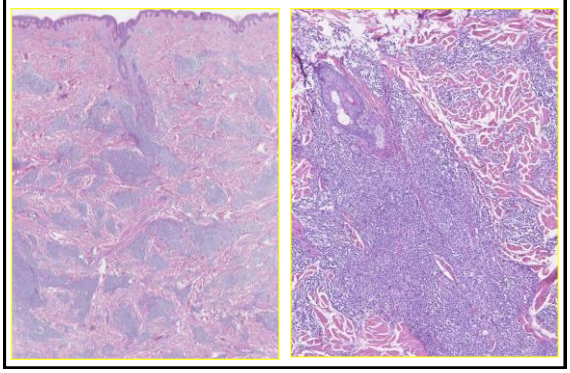
AITL

Immunophenotype:
IHC: CD20(-), CD3(+), CD4(+), CD8(-), CD10(+), BCL6(+), PD1(+), CXCL13(+)
ISH: EBER-ISH(+, 5%)
FCM: CD3(-), CD2(+), CD5(+), CD7(+), CD4(+), CD8(-), PD1(+)

Molecular:
Clonal TRG rearrangement
IDH2 (R172T)
RHOA (G17V)

20

Subcutaneous nodule biopsy



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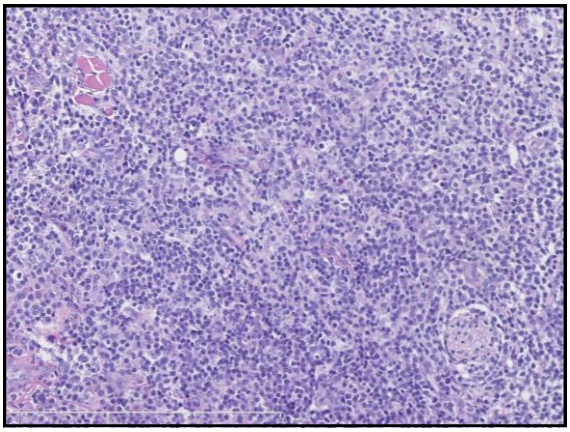

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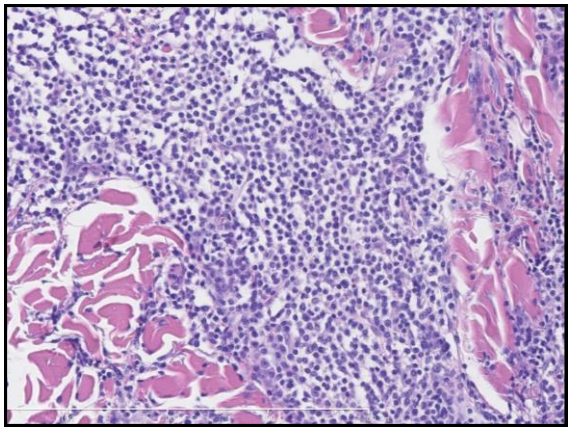
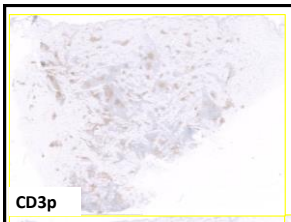


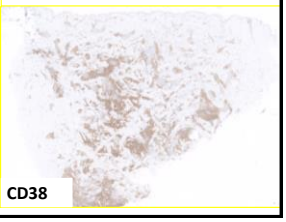
23

Subcutaneous nodule: IHC



CD3p

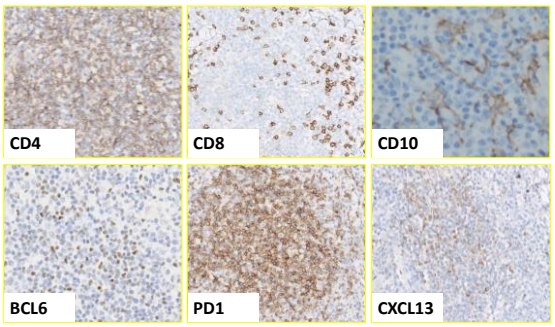
CD20



CD38

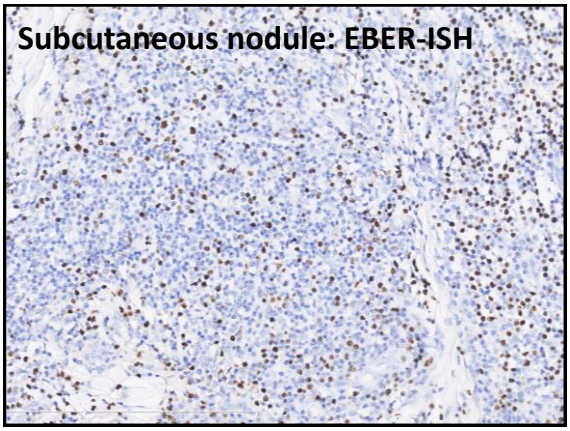
24

Subcutaneous nodule: IHC



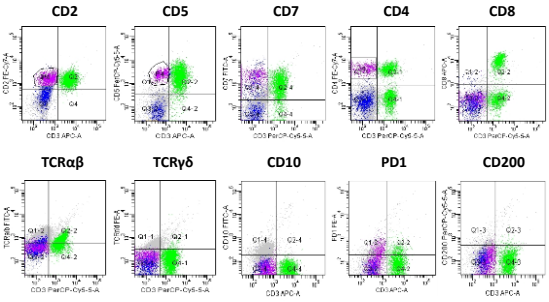
25

Subcutaneous nodule: EBER-ISH



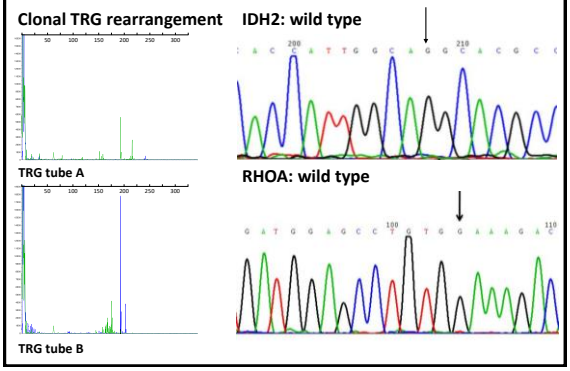
26

Subcutaneous nodule: FCM



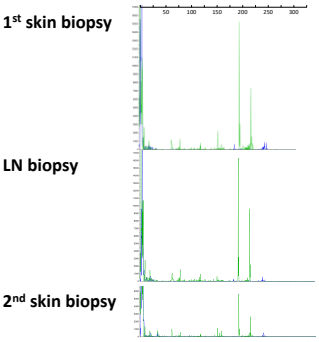
27

Subcutaneous nodule: molecular Analysis



28

TCR rearrangement analysis comparison



29

Pathological diagnosis

	Arm & leg skin	Subcutaneous nodule	Inguinal LN
Clinical manifestation	67 M, generalized lymphadenopathy & skin lesion Elevated circulating EBV-DNA and β-2 macroglobulin PET/CT: generalized lymphadenopathy, skin lesion and leg swelling		
Hypervascularity	√	√	√
Arborization vessel	√	√	√
Complex background	√	√	√
Cluster of clear cells	√	√	√
Proliferation pattern	mixed T&B cell proliferation		T predominant
IHC	TIA1-, GB-	Clear cells: CD20-, CD3+, CD4+, CD8+ CD10-, BCL6+, PD1-, CXCL13+	
FCM	NA	CD3-, CD2+, CD5+,CD7+, CD4+,CD8-, TCRαβ+, CD10-, PD1+	
EBER-ISH	multi-positive signal		Scattered positivity
TRG clonal analysis	clonal rearrangement of the same length		
IDH2/RHOA	NA	Wild type	Mutated

AITL with cutaneous involvement and EBV infection

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Conclusion

- Cutaneous involvement of AITL is seen in half of AITL patients, but severe and generalized skin lesion like leg swelling, subcutaneous nodules is rare, which may lead to misdiagnosis and/or missdiagnosis.
- Numerus EBER+ signals and plasma cells and plasmacytoid cells infiltration could be a clue for the presence of AITL.
- Skin lesion showed more EBER+ signals than lymph node.
- LN biopsy is highly recommended for patients with lymphadenopathy for a final diagnosis of AITL

31

Angioimmunoblastic T-cell Lymphoma with EBV+ Large B-cell Lymphoproliferative Disorder

Molecular Characterization of a Patient Derived Xenograft Model
Session 2: Case 7 (1455401)

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1

Disclosures

- Advisory Board/Consultant
  - Seattle Genetics, Jazz Pharmaceuticals, Celgene
- Research Support
  - Abbvie, Eli Lilly, Cellerant

2

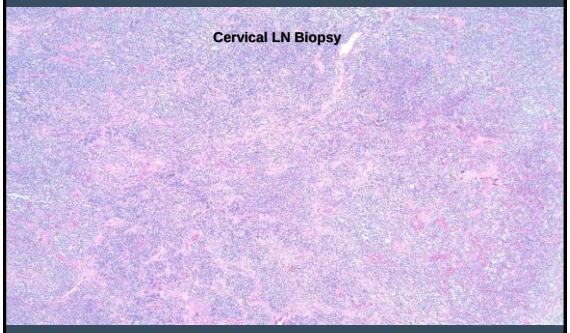
Clinical History

- A 53-year-old woman presented with fatigue, dyspnia, cervical and inguinal lymphadenopathy, and macular skin rash over face and chest.
- Computed tomography: diffuse lymphadenopathy in the neck, chest, mesentery, periaortic region, and pelvis.
- Laboratory studies showed:

  CBC: Hgb 10.1 g/dL, MCV 98.7, WBC 20.42 x 10^9/L, PLT 209 x 10^9/L .
  Differential: PMN 75, Lymph 13, Mono 7, Eo 2, Baso 1, Meta 2
  Lactate Dehydrogenase: Elevated (326 U/L)

- An axillary LN was biopsied and a diagnosis of AITL was rendered. *EBER in situ hybridization was negative*.
- Treatment: 6 cycles of CHOEP followed by ASCT 9 months after initial diagnosis.
- She developed progressive fatigue and arthralgias. PET-CT scan showed new cervical, thoracic, abdominal and pelvic lymphadenopathy.
- *A cervical LN was biopsied to confirm relapse.*

3



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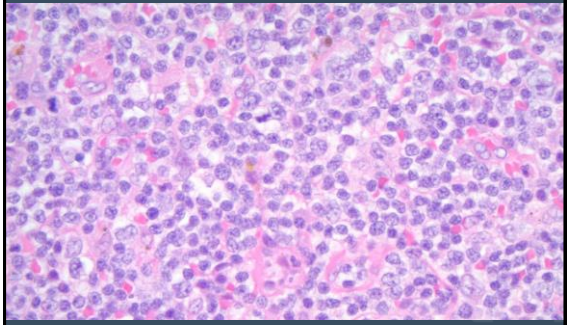

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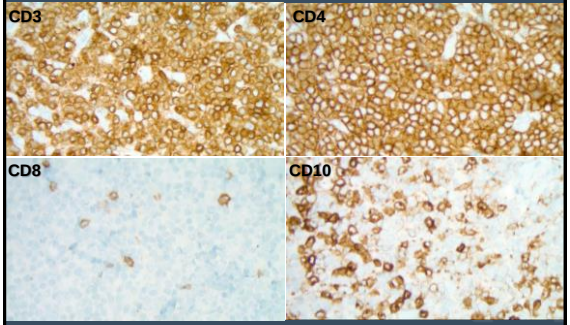

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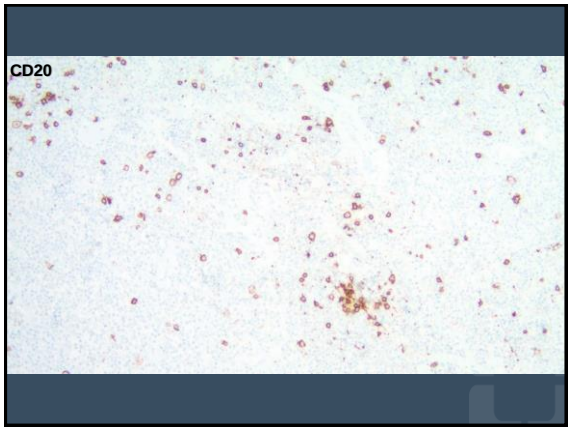
CD20

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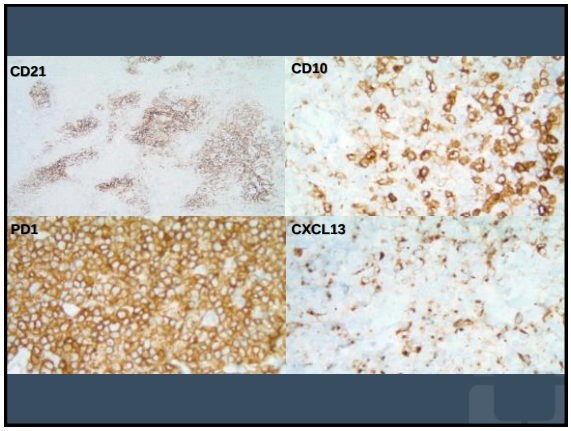
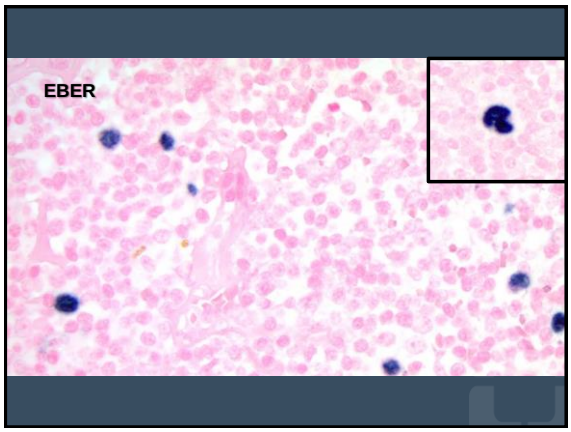

CD21
CD10
PD1
CXCL13

8


EBER

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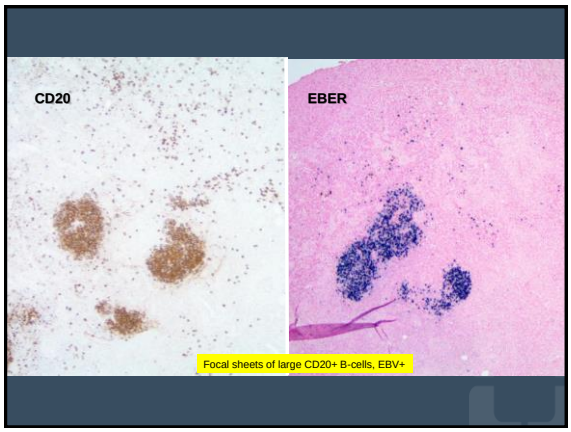
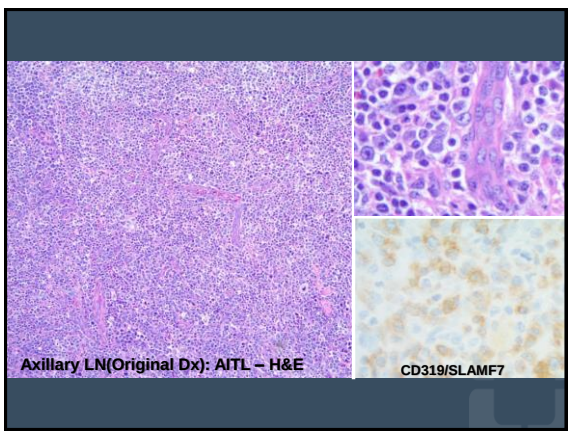
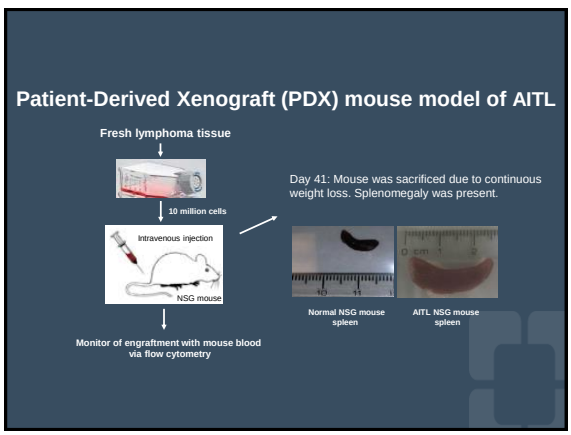

CD20
EBER
Focal sheets of large CD20+ B-cells, EBV+

10

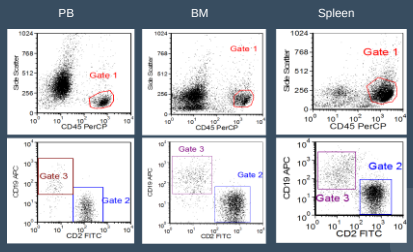

Axillary LN(Original Dx): AITL – H&E
CD319/SLAMF7

11


Patient-Derived Xenograft (PDX) mouse model of AITL
Fresh lymphoma tissue
10 million cells
Intravenous injection
NSG mouse
Monitor of engraftment with mouse blood via flow cytometry
Day 41: Mouse was sacrificed due to continuous weight loss. Splenomegaly was present.
Normal NSG mouse spleen
AITL NSG mouse spleen

12

Engraftment of human primary AITL cells in NSG mouse



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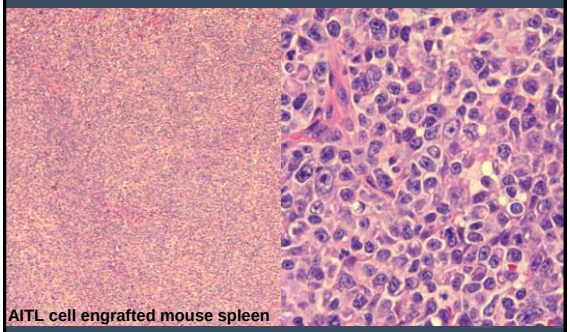

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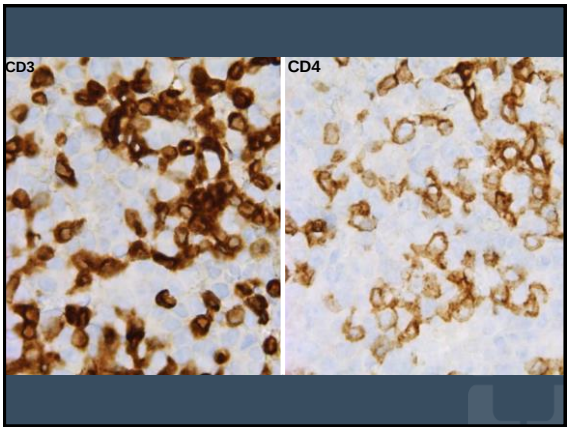

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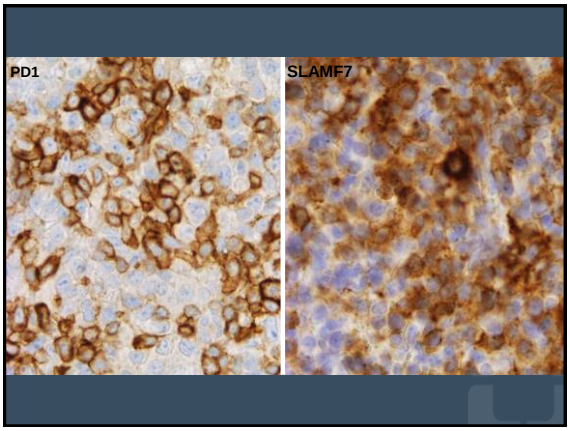

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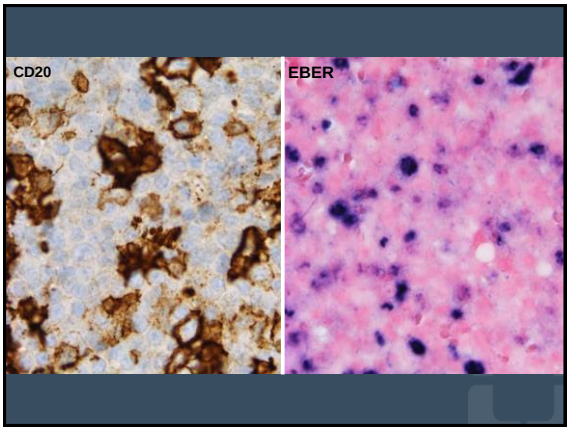
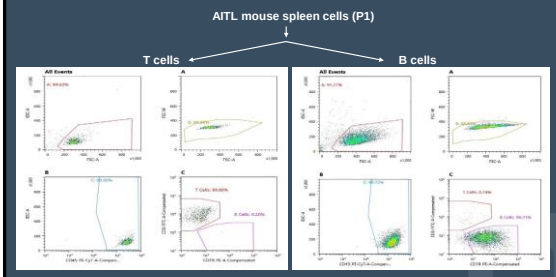


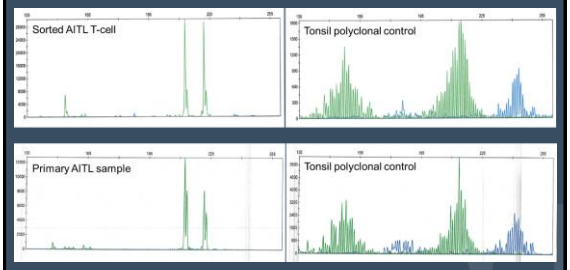
17

Sorting of primary AITL cell engrafted mouse spleen cells (P1)



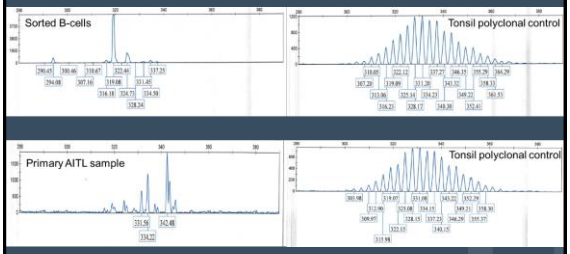
18

T-cells shown to be monoclonal by BIOMED2 PCR



19

Engrafted B-cells shown to be monoclonal by BIOMED2 PCR



20

AITL T-cell specific gene mutants (WES of Sorted T-cells)

VAF_T	Gene	Mutation	Amino acid change
38.60%	ABI3BP	nonsynonymous SNV	T506A
24.00%	CHST15	nonsynonymous SNV	P453R
29.50%	CLSTN3		exon8:c.1323+1G>A
34.10%	COL4A5	nonsynonymous SNV	R788H
18.60%	DHX40	nonsynonymous SNV	R511H
29.50%	F11	nonsynonymous SNV	G344R
22.50%	FBXO34	nonsynonymous SNV	A218S
29.10%	FNDC1**	frameshift deletion	P1138fs
27.40%	GOT1L1	nonsynonymous SNV	F211I
26.60%	JAKMIP1	nonsynonymous SNV	R121C
29.70%	MAGI2**	nonsynonymous SNV	T763I
29.80%	MAPK8IP1	nonsynonymous SNV	F567S
28.00%	MGAT4C	nonsynonymous SNV	H5N
38.20%	PAQR6	nonsynonymous SNV	H104Y
33.30%	PCDHB13	nonsynonymous SNV	L565M

VAF_T	Gene	Mutation	Amino acid change
25.80%	PLA2G6**	nonsynonymous SNV	Q538H
27.70%	PLXDC2	nonsynonymous SNV	D70G
34.00%	RHOA*	nonsynonymous SNV	G17V
32.80%	RRP9	nonsynonymous SNV	V437L
14.30%	SF1	nonsynonymous SNV	S622R
18.70%	SLC4A10	nonsynonymous SNV	R1000W
23.70%	STAT3*	nonsynonymous SNV	K658N
22.80%	STAT3*	nonsynonymous SNV	K658R
32.80%	STXBP6	stopgain	Q87X
35.40%	TET2*		exon9:c.4045-1G>C
33.20%	TET2*	stopgain	Q933X
26.70%	TTN	nonsynonymous SNV	L3554P
37.10%	TULP4	nonsynonymous SNV	G1129D
24.40%	UNC80	nonsynonymous SNV	W215L
41.90%	VAV1*	nonsynonymous SNV	I803N
28.10%	ZNF366	nonsynonymous SNV	P149S
28.40%	ZNF440	frameshift deletion	R444fs

* T-cell lymphoma related
** Mutated genes implicated in lymphoma

21

AITL EBV+ B-cell specific gene mutants (WES of Sorted B-cells)

VAF_B	Gene	Mutation	Amino acid change
34.40%	CHI3L1**	nonsynonymous SNV	I66F
41.90%	CLASP1	nonsynonymous SNV	R277W
37.60%	NLRP5	nonsynonymous SNV	L985M
36.00%	PSAPL1	nonsynonymous SNV	D480N
33.90%	RAG2**	stopgain	G221X
35.40%	REV1	nonsynonymous SNV	R852G
37.20%	SNED1	nonsynonymous SNV	G361D
37.90%	TEX15	nonsynonymous SNV	N2206K
41.20%	TRIML1	nonsynonymous SNV	P433L

** Mutated genes implicated in lymphoma

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In vivo AITL PDX model trials

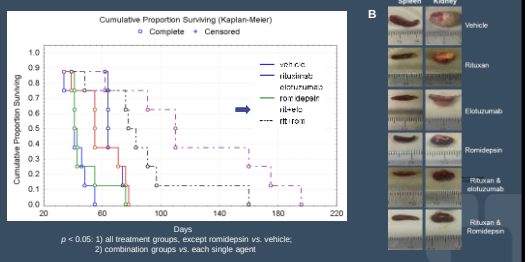
Spleen cells from second passage of the AITL engrafted mouse were injected to NSG mice. Mice were randomly assigned to six groups with 8 mice per group. After engraftment in peripheral blood was detectable, treatment was started. The groups were:

1) Vehicle control
2) Intraperitoneal injection of elotuzumab at dosing of 5 mg/kg, three times a week for three weeks
3) Intraperitoneal injection of romidepsin with dosing of 1 mg/kg, 3 times a week and continue during the study
4) One time intravenous injection of rituxan at dosing of 20 mg/kg;
5) Romidepsin plus elotuzumab
6) Rituxan plus elotuzumab

All mice were ear tagged and monitored individually throughout the studies. The Cox's *F* test in the Kaplan-Meier surviving analysis for two groups was used. *P* value of < 0.05 was considered significant.

23

Survival curves of AITL PDX mice (A) and Morphological features of mice spleens and kidneys (B)



$p < 0.05$: 1) all treatment groups, except romidepsin *vs.* vehicle; 2) combination groups *vs.* each single agent

24

Conclusions

- Angioimmunoblastic T-cell lymphoma may develop EBV+ lymphoproliferative disorders during the course of disease, even if the diagnostic biopsy is EBV-negative
- We show an incipient EBV+ large B-cell lymphoma, the clonal nature was clarified during *in vivo* expansion and whole exome sequencing.
  - The mutational profiles of the T-cell and B-cell lymphomas are distinct.
- A patient derived xenograft (PDX) NSG mouse model of AITL was established with coexisting of T and B cells proliferations.

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Conclusions

- WES identified 32 mutated genes in engrafted T cells including *RHOA G17A, TET2, VAV1* and *STAT3*.
- This is the first molecular characterization of AITL tumor cells and associated EBV+ B cells in the context of a PDX model.
- This model was used in the evaluation of agents targeting both malignant T cells and B cells and demonstrated the efficacy of elotuzumab and rituximab combination in AITL with an EBV+ B-cell lymphoma.

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Discussion

- Overview of AITL, EBV+ lymphomas in AITL, and therapy.

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Angioimmunoblastic T-cell Lymphoma (AITL)

- A lymphoma derived from Tfh cells
- Presents in middle age to elderly adults (M>F)
  - 15-30% of noncutaneous T-cell lymphomas
  - Generalized lymphadenopathy with frequent involvement of BM, Skin, Liver and Spleen
  - Systemic symptoms
- Polyclonal hypergammaglobulinemia, hemolytic anemia with cold agglutinins, rhuematoid factor and anti smooth muscle antibodies, immunodeficiency with EBV reactivation

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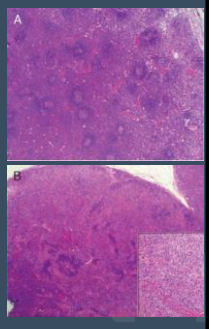
AITL – Pathology

- Small to medium sized cells with clear cytoplasm, admixed Eos, PCs, Histiocytes
  - May be located perinodal areas, form clusters near HEVs, or around reactive follicles
  - Increased vascularity
- Histological Patterns
  - Pattern 1: Limited involvement with reactive GCs and little/no expansion of FDCs
  - Pattern 2: Extensive involvement with regressed GCs, expansion of FDCs
  - Pattern 3: Diffuse involvement, no GCs, expansion of FDCs
  - Multiple patterns can coexist and change at relapse

Attygale et al *AJSP* 2007



29

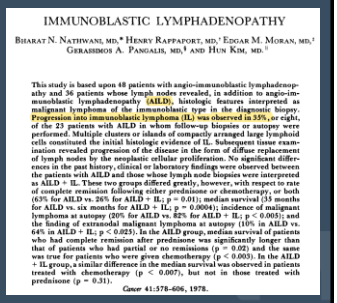
AITL

- Immunophenotype: Tfh
  - CD3, CD4, +/- pan-T-cell markers
  - CD10, BCL6, CXCL13, PD1, ICOS
  - B-immunoblasts and RS-like cells often
    - EBV+ in cases with RS-like cells
  - FDC proliferations seen with CD21 or CD23 staining
- Genetics
  - TCR rearranged: >90%; IGH rearrangements: 25-30% of cases, associated with presence of EBV+ B-cells
  - Mutations/Fusions
    - Epigenetics: *TET2* (~80%), *DNMT3A* (~ 30%), *IDH2* R172(~ 30%)
    - *RHOA* G17V (60-70%) – associated with classical pathology features
    - Signaling: *CD28, PLCG1, FYN* (5-10%)
    - *CTLA4-CD28* (not specific), *ITK-SYK* (Follicular PTCL)

30

EBV LPDs in AITL

- Long known that AIL(D)T could manifest with lymphoma of other histologies



IMMUNOBLASTIC LYMPHADENOPATHY

BHARAT N. NATHWANI, MD,* HENRY RAPPAPORT, MD,† EDGAR M. MORAN, MD,‡ GERASIMOS A. PANGALIS, MD,§ AND HUN KIM, MD.¶

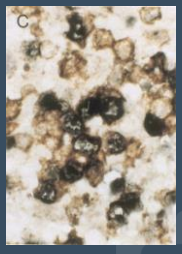
This study is based upon 48 patients with angio-immunoblastic lymphadenopathy and 36 patients whose lymph nodes revealed, in addition to angio-immunoblastic lymphadenopathy (AILD), histologic features interpreted as malignant lymphoma of the immunoblastic type in the diagnostic biopsy. Progression into immunoblastic lymphoma (IL) was observed in 33%, or eight, of the 23 patients with AILD in whom follow-up biopsies or autopsy were performed. Multiple clusters or islands of compactly arranged large lymphoid cells constituted the initial histologic evidence of IL. Subsequent tissue examination revealed progression of the disease in the form of diffuse replacement of lymph nodes by the neoplastic cellular proliferation. No significant differences in the past history, clinical or laboratory findings were observed between the patients with AILD and those whose lymph node biopsies were interpreted as AILD + IL. These two groups differed greatly, however, with respect to rate of complete remission following either prednisone or chemotherapy, or both (63% for AILD vs. 26% for AILD + IL; $p = 0.01$); median survival (35 months for AILD vs. six months for AILD + IL; $p = 0.0004$); incidence of malignant lymphoma at autopsy (20% for AILD vs. 82% for AILD + IL; $p < 0.005$); and the finding of extranodal malignant lymphoma at autopsy (10% in AILD vs. 64% in AILD + IL; $p < 0.025$). In the AILD group, median survival of patients who had complete remission after prednisone was significantly longer than that of patients who had partial or no remissions ($p = 0.02$) and the same was true for patients who were given chemotherapy ($p < 0.003$). In the AILD + IL group, a similar difference in the median survival was observed in patients treated with chemotherapy ($p < 0.007$), but not in those treated with prednisone ($p = 0.31$).

Cancer 41:578-606, 1978.

31

EBV LPD in AITL

- EBV identified in AILT in 1990s via DNA and RNA detection
- Localized to B-cells by IHC/ISH
- EBV can be found in 80-95% of cases of AITL
  - # cells by EBER varies
    - 3% no cells
    - 26% <1 cell/mpf
    - 41% 1-10 cells/mpf
    - 11% 11-100 cells/mpf
    - 19% >100 cells/mpf



Knecht H et al *Br J Haematol* 1990
Abruzzo L et al *Blood* 1993

Wess LM et al *Blood* 1992

32

EBV LPDs in AITL

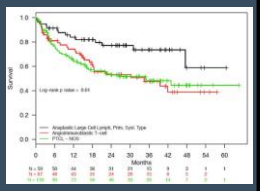
- Numerous case reports of large B-cell lymphomas or rarely HL predating, occurring with, or developing after AILT diagnosis
  - EBV present in 45% – 88% of cases
- Largest longitudinal series
  - 161 cases of AITL studied from a lymph node registry
  - 21 cases (13%) had a B-cell lymphoma (8 after [3-31 months] and 13 simultaneous)
    - 16 DLBCLs, 2 cHL, 2 LPL, 1 unclassified (CD30+)
- EBV present:
  - 6/15 DLBCLs (40%)
  - 2/2 cHL (100%)
  - 1/1 unclassified
  - 0/2 LPLs

Willenbrook K et al *Br J Haematol* 2007
Attygale A et al Am J Surg Pathol 2007
Hoffman JC et al *Hum Pathol* 2016

33

Treatment and Outcome

- Frontline therapies vary
  - Studies show benefit for anthracycline containing regimens (CHOP or CHOEP) followed by ASCT
    - 3-5 year OS for patients receiving ASCT 50-60%.
  - Unfortunately most patients are not transplant candidates
  - International T-cell Project: Only 8% of patients considered for ASCT
  - COMPLETE registry ("real world" observational registry): 40% OS
  - Front line recommendations include clinical trials



Broccoli A *Hematol Oncol Clin N Am* 2017
Carson K et al *Cancer* 2016
Federico M et al *J Clin Oncol* 2012

34

Promise of Targeted Agents

- Anti-CD30 directed therapy – brentuximab vedoitin with cyclophosphamide, doxorubicin, prednisone
  - Not histology driven (≥10% CD30 by IHC)
- Phase II data suggest activty of romidepsin in r/r AITL (subanalysis of pivotal phase II)
  - ORR for patients with AITL treated with romidepsin was 33% (9/27)
  - 6 of 9 responders achieved CR/Cru, 5 were > 1 yr
- Molecular profiling may help guide targeted therapy
  - Epigenetic mutations as biomarker for HDACi?

Broccoli A et al *Hematol Oncol Clin N Am* 2017
Horwitz S et al *Lancet* 2019
Pro B et al *Hematol Oncol* 2017

35

Acknowledgements

Jarek Maciejewski laboratory – Cleveland Clinic
Daniel Lindner – Cleveland Clinic Animal Core
Lisa Durkin – Cleveland Clinic

36

1

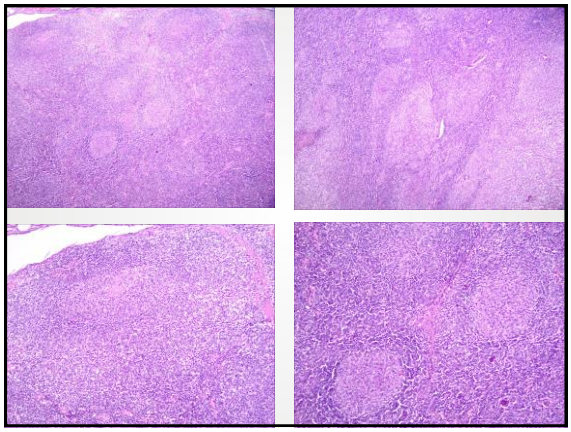
Clinical History

A 43-year-old female presented with progressive enlargement of the lymph nodes in the left lateral neck, right supraclavicular region, and the left submaxillary region for more than 20 days. The diameter of the lymph nodes was 1.0cm-2.5cm, solid, with moderate hardness and unclear boundaries, without tenderness, swelling and skin ulceration. No fever, night sweat and weight loss.

Periphal blood analysis: RBC $5.04x10^{12}$/L, HGB 145g/L, WBC 11.25 x 10^9/L, Neutrophil 79.5%, Lymphocyte 13.2%, PLT 274 x 10^{12}/L.

Macroscopic examination: Two grey-white mass, large one about 2.8×1.5×1.5 cm^3 ,small one about 2×1.3×1.3 cm^3 in size, complete surface envelope, gray-white section, soft and more delicate in texture.

2



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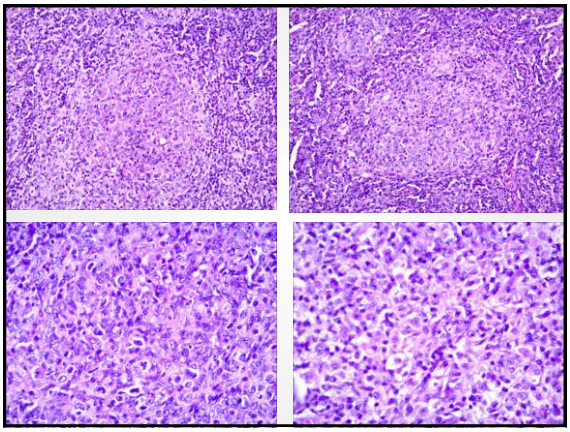

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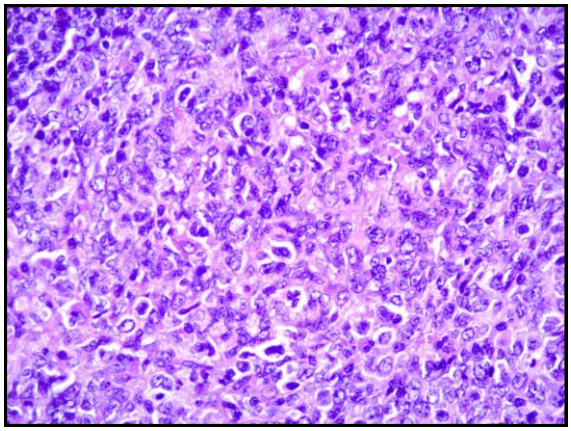

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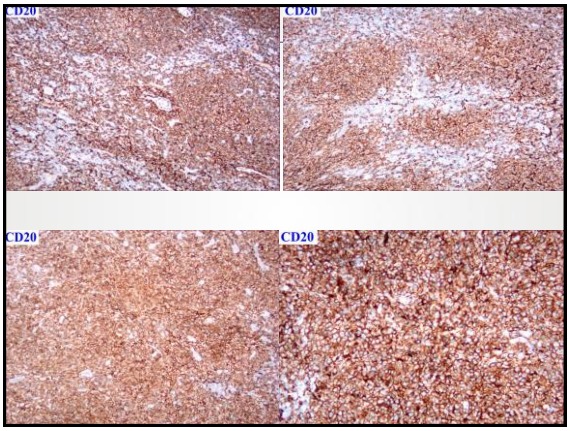

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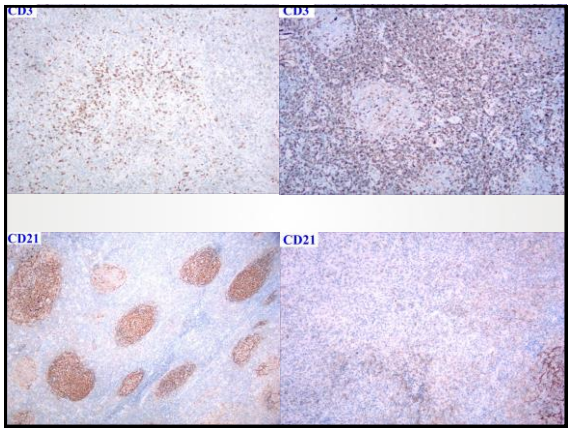
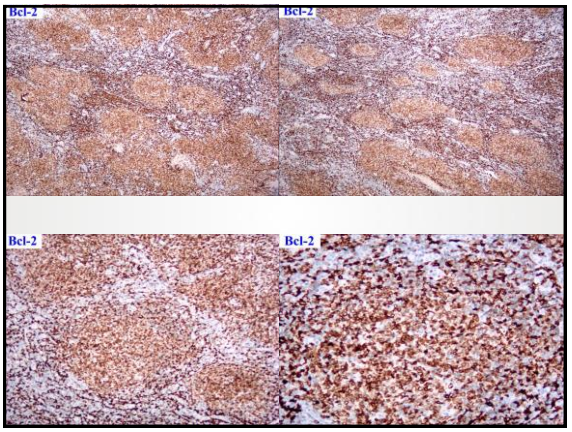
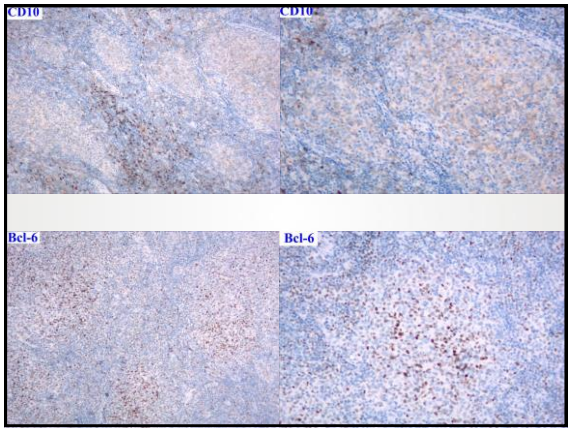

CD3
CD3
CD21
CD21

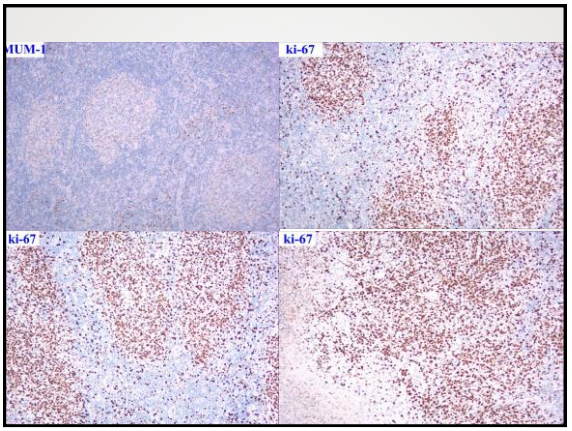
7


Bcl-2
Bcl-2
Bcl-2
Bcl-2

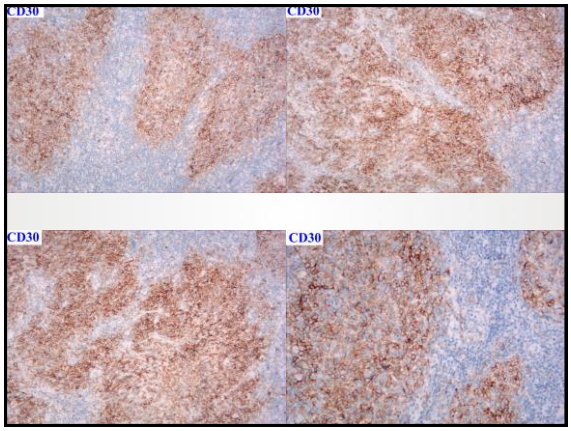
8


CD10
CD10
Bcl-6
Bcl-6

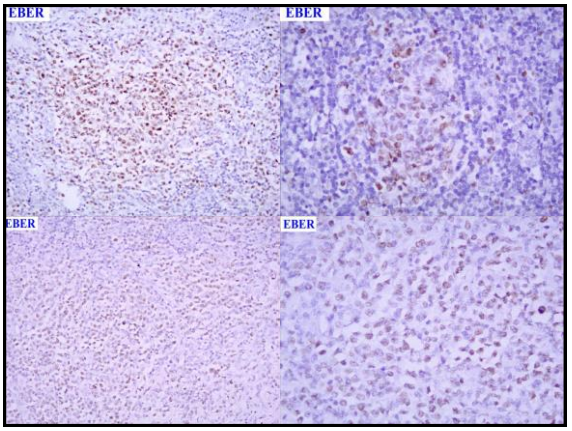
9


MUM-1
ki-67
ki-67
ki-67

10


CD30
CD30
CD30
CD30

11


EBER
EBER
EBER
EBER

12

Immunohistochemistry

Positive: CD21 & CD23: FDC(+), CD20, Bcl-6, Bcl-2, CD30, C-myc(+)10%, P53(+)10%, Ki-67 about 80%(+).

Negative: CD3, CD10, MUM-1, CD5, CyclinD1, CD68, EMA, CK, S-100, CD1a.

ISH : EBER + (both follicular areas and DLBCL areas)

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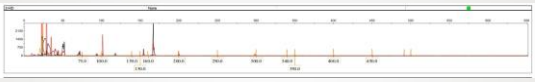
Blood EBV-DNA & EBV Ab

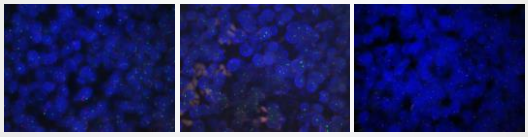
EB病毒DNA测定(EBV-DNA)	↑ 9.41E+03	拷贝/毫升	〈 5.00E+02

EBV早期抗原IgG抗体(EBV EAIgG)	0.27	AU/ml	0-2.00
EBV早期抗原IgA抗体(EBV EAIgA)	0.75	AU/ml	0-3.00
EBV衣壳抗原IgG抗体(EBV VCAIgG)	↑ >50.00	AU/ml	0-2.00
EBV衣壳抗原IgM抗体(EBV VCAIgM)	↑ 5.79	AU/ml	0-3.00
EBV衣壳抗原IgA抗体(EBV VCAIgA)	0.97	AU/ml	0-4.00
EBV核抗原IgG抗体(EBV NAIgG)	↑ >50.00	AU/ml	0-2.00

14



BCR : IgH (+) <FR2-JH, DH-JH>
Igκ (+) <Vκ-Jκ, Vk-κde+intron-κde>
IgL (-)



C-myc/IgH: (-) Bcl-2/IgH: (-) Bcl-6 break: (-)

15

Proposed diagnosis

(Left submandibular lymph nodes):Non-Hodgkin's lymphoma, conformed to EBV-positive follicular lymphoma, grade 3A, part of the region transformed to diffuse large B-cell lymphoma.

***The patients were HIV negative, and there was no clinical evidence of immunosuppression.**

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Panel Diagnosis

DLBCL NOS, FL 3A EBV+

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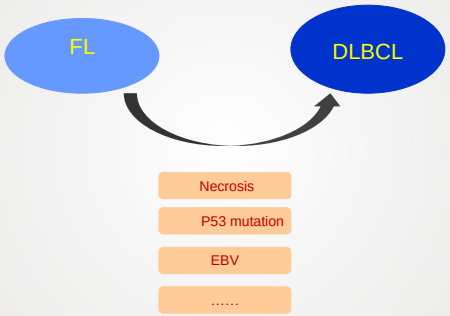

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Mod Pathol 2017 Apr;30 (4): 519-529.

Epstein-Barr virus-positive follicular lymphoma.

Mackrides N , Campuzano-Zuluaga G , Maque-Acosta Y , Moul A , Hijazi N , Ikpatt FO , Levy R , Verdun RE , Kunkalla K , Natkunam Y , Lossos IS , Vega F , Chapman J .

Abstract

Epstein-Barr virus (EBV)-associated follicular lymphoma is only rarely reported. Herein, we report the largest series analyzing prevalence and clinicopathologic characteristics of EBV-associated follicular lymphoma occurring in unselected cases. Out of 382 analyzed cases, 10 EBV-positive follicular lymphomas were identified (prevalence=2.6%, 95% confidence interval 1.3-4.0%). All EBV-positive follicular lymphomas showed EBV-encoded small RNA-positive lymphoma cells present in a follicular distribution. Of these, eight also had tissue available for testing of expression of latent membrane protein 1 (LMP1), out of which six (75%) were positive. There was a significant association with grades 3A-3B follicular lymphoma (P<0.0001) and CD30 expression (P=0.0002). EBV-positive follicular lymphomas were otherwise morphologically and immunophenotypically indistinguishable from EBV-negative cases of similar grade. Nine of the EBV-positive follicular lymphomas occurred in patients with no known history of immunosuppression, while one patient had a history of hydroxychloroquine administration for Sjögren's syndrome. The mean age in the EBV-positive and -negative follicular lymphomas was 56 (range 31-83 years) and 49 years (range 25-92 years), respectively, with no statistically significant difference. Seven of the patients with EBV-positive follicular lymphoma had additional biopsies from different time points available for review, all of which showed progression of disease in the form of progression of tumor grade. Five of these progressed to diffuse large B-cell lymphoma, only of which had tissue available for testing and was EBV-positive. Our findings suggest that EBV infection may have a role in lymphomagenesis and/or disease progression in a subset of follicular lymphomas, thereby expanding the spectrum of recognized EBV-associated B-cell lymphomas.

PMID: 27982024 [Pubmed - MEDLINE]

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Arch Pathol Lab Med 2001 Aug;125 (8): 1036-41.

CD30 expression in follicular lymphoma.

Gardner LJ , Polski JM , Evans HL , Perkins SL , Dunphy CH .

Abstract

CD30(+) anaplastic large cell lymphomas were originally described as being of T-cell, null cell, and B-cell origin. CD30, however, is not a specific marker of anaplastic large cell lymphoma and has been found to be expressed in reactive as well as neoplastic populations as a probable activation marker. In addition, CD30(+) cells have also been described in both diffuse large B-cell and follicular lymphomas (FLs), resembling the pattern seen in reactive tonsils and lymph nodes. We report an index case of FL with CD30 expression, which on initial touch preparations and flow cytometric immunophenotyping revealed a prominent population of CD30(+) cells with marked cellular pleomorphism (anaplasia) in a background of typical FL. Immunohistochemistry of the paraffin section for CD30 in our index case confirmed unequivocal CD30(+) pleomorphic cells in the malignant nodules in occasional clusters. This case prompted a study of additional cases of FL for pattern of immunoreactivity with CD30 on paraffin sections. Twenty-two additional cases of FL (grades 1-3) were retrieved for CD30 immunoperoxidase staining as in the index case. This study demonstrated 32% of the additional cases of FL had definitive CD30(+), large, pleomorphic malignant cells by paraffin immunohistochemistry. In 2 cases (9%), the pattern of immunoreactivity with CD30 showed clustering and variable staining of large cells, as our index case. This study underscores the morphologic and immunophenotypic spectrum of FL that includes CD30 staining and cellular pleomorphism.

PMID: 11473453 [Pubmed - MEDLINE]

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Table 1 Immunohistochemistry and *in situ* hybridization in cases of EBV-positive follicular lymphoma

Case	CD20	CD30 (>30%)	CD10	BCL6	BCL2	FISH t(14;18)	LMP1	EBER
1	+	+	+	ND	+	+	ND	+
2	+	+	+	+	+	−	ND	+
3	+	−	+	+	+	ND	+	+
4	+	+	+	−	+	ND	+	+
5	+	−	+	−	+	ND	+	+
6	+	−	+	+	+	ND	−	+
7	+	−	+	+	+	ND	−	+
8	+	−	+	+	+	ND	+	+
9	+	−	+	−	+	ND	+	+
10	+	+	+	ND	+	ND	+	+

+, positive; −, negative; ND, not done.

EBER + FL：10/10

CD30 + FL：4/10

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Table 2 Clinical features of patients with EBV-positive follicular lymphoma

Case	Age (years)	Clinical presentation	Immunosuppression	Initial FL grade	Stage at initial diagnosis	Initial treatment	Progression	Time from initial diagnosis to progression (years)	Treatment at disease progression	Alive/deceased at last follow-up	Follow-up time from initial diagnosis (years)
1	83	Cervical lymphadenopathy	No	3A	IVA	Expectant followed by Rituximab	Yes Grade 3A to DLBCL	3.8	R-CHOP	DOD	4.2
2	76	Abdominal lymphadenopathy	Hydroxychloroquine for Sjögren's Syndrome	1-2	IVA	None	Yes Grade 1-2 to 3A	6.1	Rituximab	DOD	14
3	60	Cervical and inguinal lymphadenopathy	No	3B	U	U	U	NA	U	Alive	9.1
4	33	Cervical lymphadenopathy	No	1-2	IVA	None	Yes Grade 1-2 to 3A	4.1	CVP	Alive	11.8
5	48	Inguinal lymphadenopathy	No	1-2	IIIA	None	Yes Grade 1-2 to DLBCL	7.2	CVP and fludarabine, idiotype vaccine, KLH, autologous BMT	Alive	17.6
6	76	Enlarged left inguinal lymph node	No	3A	U	U	U	NA	U	U	0
7	51	Mediastinal lymphadenopathy	No	3A	IVA	None	Yes Grade 3A to DLBCL	< 1	Splenectomy, CHOP, CMOPP	Alive	21.9
8	33	Enlarged left supraclavicular lymph node	No	3A	U	CVP	Yes Grade 3A to DLBCL	1.6	BACOP, XRT, BMT	DDU	10.5
9	U	U	U	3A	IIIA	U	U	NA	U	Alive	12.3
10	64	Massive splenomegaly, lymphadenopathy	No	3A	IIA	CHOP	Yes Grade 3A to DLBCL	1.1	CEPP	DOD	1.9

U, unknown; +, positive; −, negative; R-CHOP, rituximab, cyclophosphamide, hydroxydaunomycin, vincristine sulfate, prednisone; CVP, cyclophosphamide, vincristine, prednisone; KLH, keyhole limpet hemocyanin; CMOPP, cyclophosphamide, vincristine, procarbazine, prednisone; XRT, radiation therapy; BMT, bone marrow transplant; BACOP, bleomycin, epidoxorubicin, cyclophosphamide, vincristine, and prednisone; CEPP, cyclophosphamide, etoposide, procarbazine, and prednisone; DOD, dead of disease; DDU, deceased, disease status unknown at time of death; NA, not applicable.

EBV+FL, 3A→DLBCL：4/10

22

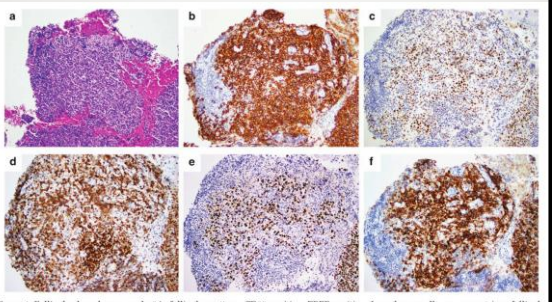

Figure 1 Follicular lymphoma, grade 3A, follicular pattern, CD30-positive, EBER-positive. Lymphoma cells are present in a follicular distribution and include increased large cells as seen on the H&E stain (**a**). Lymphoma cells are positive for CD20 (**b**), BCL6 (**c**), and BCL2 (**d**). EBER was detected in >75% of lymphoma cells in a follicular distribution (**e**). CD30 immunohistochemistry stain showing strongest expression in lymphoma cells within follicles with a distribution similar to that of EBER expression (**f**) (patient 2, Table 2). All images taken at ×200.

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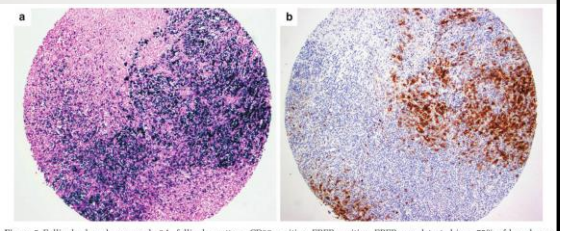

Figure 2 Follicular lymphoma, grade 3A, follicular pattern, CD30-positive, EBER-positive. EBER was detected in >75% of lymphoma cells in a follicular distribution. (**a**) CD30 immunohistochemistry stain shows strongest expression in lymphoma cells within follicles with a distribution similar to that of EBER expression (**b**). Images taken at ×200.

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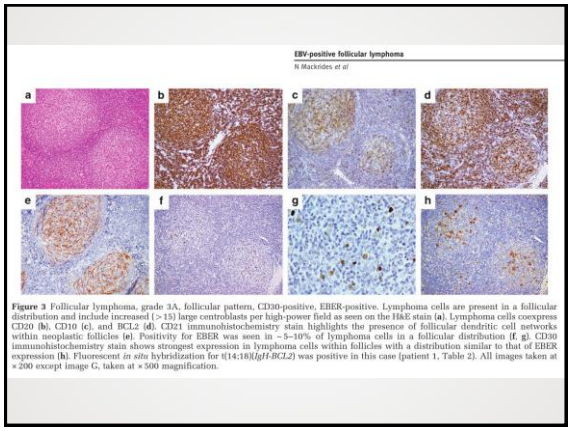

Figure 3 Follicular lymphoma, grade 3A, follicular pattern, CD30-positive, EBER-positive. Lymphoma cells are present in a follicular distribution and include increased (>15) large centroblasts per high-power field as seen on the H&E stain (**a**). Lymphoma cells coexpress CD20 (**b**), CD10 (**c**), and BCL2 (**d**). CD21 immunohistochemistry stain highlights the presence of follicular dendritic cell networks within neoplastic follicles (**e**). Positivity for EBER was seen in ~5–10% of lymphoma cells in a follicular distribution (**f**, **g**). CD30 immunohistochemistry stain shows strongest expression in lymphoma cells within follicles with a distribution similar to that of EBER expression (**h**). Fluorescent *in situ* hybridization for t(14;18)(*IgH-BCL2*) was positive in this case (patient 1, Table 2). All images taken at ×200 except image G, taken at ×500 magnification.

25

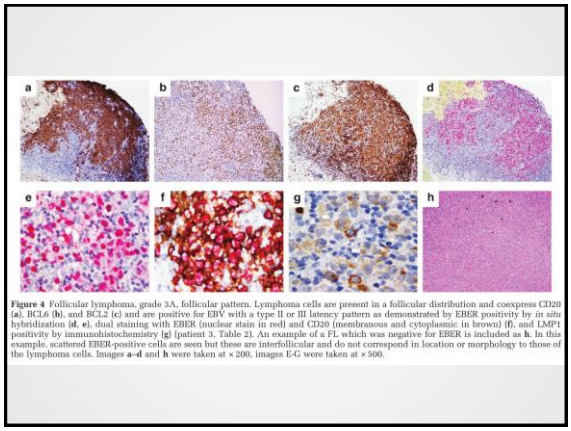

Figure 4 Follicular lymphoma, grade 3A, follicular pattern. Lymphoma cells are present in a follicular distribution and coexpress CD20 (**a**), BCL6 (**b**), and BCL2 (**c**) and are positive for EBV with a type II or III latency pattern as demonstrated by EBER positivity by *in situ* hybridization (**d**, **e**), dual staining with EBER (nuclear stain in red) and CD20 (membranous and cytoplasmic in brown) (**f**), and LMP1 positivity by immunohistochemistry (**g**) (patient 3, Table 2). An example of a FL which was negative for EBER is included as **h**. In this example, scattered EBER-positive cells are seen but these are interfollicular and do not correspond in location or morphology to those of the lymphoma cells. Images **a**–**d** and **h** were taken at × 200, images E-G were taken at × 500.

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Thanks for your attention

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THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Inflammatory polyp with EBV-positive large B-cell proliferation

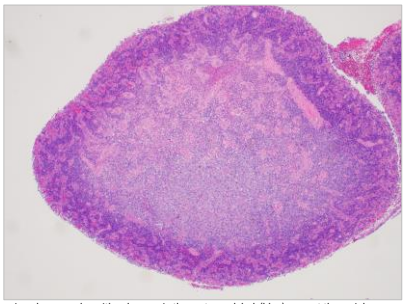
- Qingling Zhang, Zhihong Hu, L. Jeffrey Medeiros and Wei Wang
- Dept. of Hematopathology
- The University of Texas MD Anderson Cancer Center

1

Clinical History

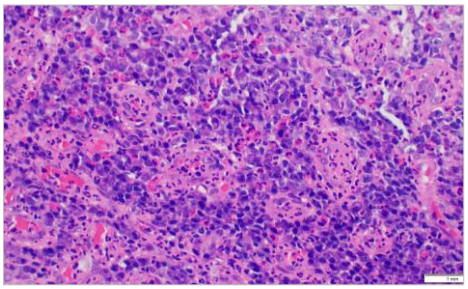
- A 61-year-old man with ulcerative colitis, treated with steroid, Lialda (Mesalamine) and Humira (Adalimumab).
- Nine months after the treatment, colonoscopy showed significant improvement, but a **2 cm pedunculated polyp** was found in the rectum.
- Polypectomy was performed.

2



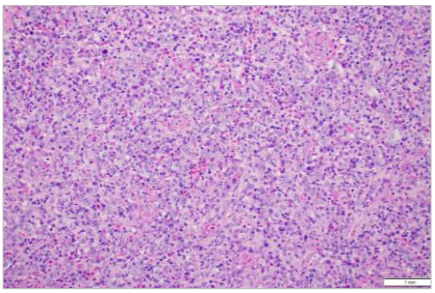
Low-power view shows a polyp with pale areas in the center and dark (blue) areas at the periphery.

3



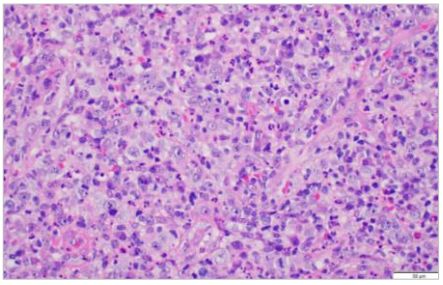
The dark (blue) areas at the periphery are composed of numerous plasma cells and scattered eosinophils and neutrophils.

4



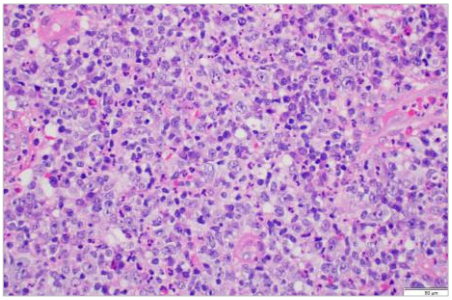
The pale areas in the center are composed of clusters to sheets of large cells with scattered inflammatory cells

5



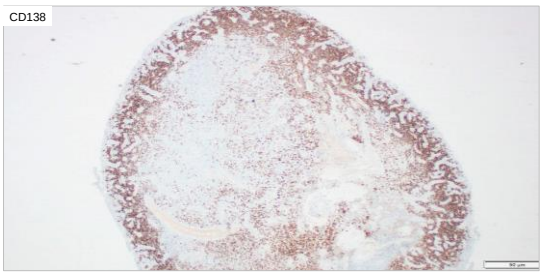
Large cells in the center areas show round to irregular nuclei, vesicular chromatin and frequent distinct nucleolus. Inflammatory cells including plasma cells and neutrophils are present in the background.

6



Another view of large cells in the center areas. Some large cells show immunoblastic morphology

7



Anti-CD138 highlights numerous plasma cells at the periphery as well as scattered plasma cells in the center areas.

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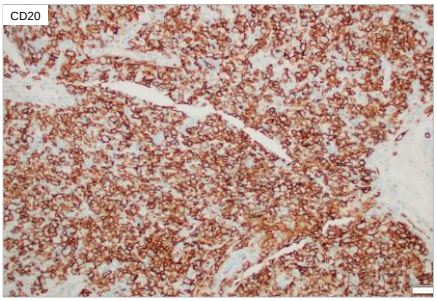
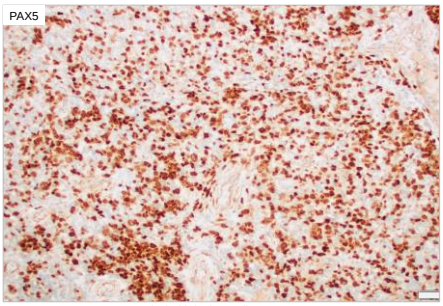


Large cells in the center areas are predominantly B cells, highlighted by CD20

9



Anti-PAX5 also highlights large cells in center areas

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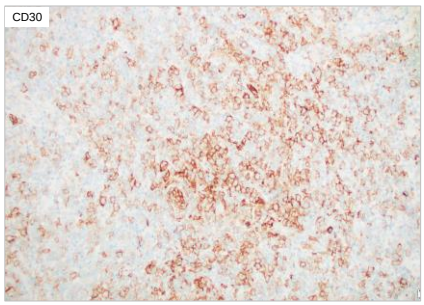

Many large cells in the center areas are positive for CD30

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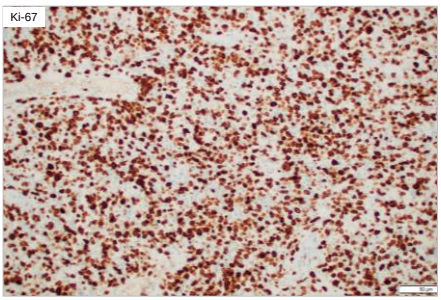
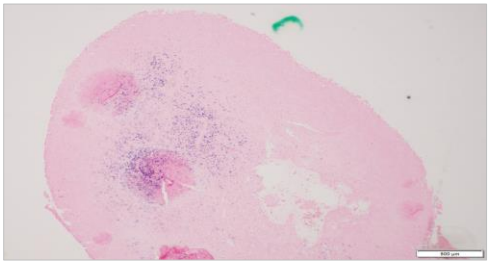


Large cells in the center areas show a high proliferation rate (80%) highlighted by anti-Ki67

12



Large cells in the central areas are positive for EBER.

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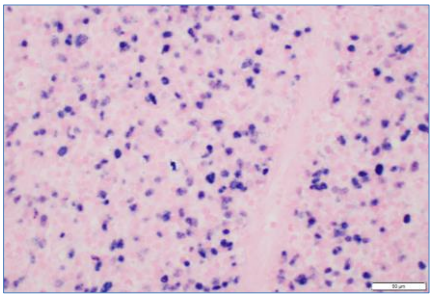

High power view highlights large cells positive for EBER

14

Summary:

Immunophenotype:

- Positive: PAX5, CD20, CD30 (subset), and Bcl-6 (subset, weak intensity)
- Negative:CD2, CD3, CD5, CD7, and Bcl-2
- Ki-67: 80-90%
- EBER(+)

Molecular:

- IGH (FR2 and FR3 primer sets) monoclonal rearrangements
- IGK monoclonal rearrangements

15

Proposed Diagnosis

Inflammatory polyp with EBV-positive large B-cell proliferation

Panel Diagnosis

EBV-positive diffuse large B-cell lymphoma in polyp

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Comment:

No matter what to call, communication with the clinical team is the key in this case:

- This large B-cell proliferation or DLBCL (either diagnosis is fine) is developed in a pedunculated polyp in the setting of ulcerative colitis.
- Potential pathogenesis: Ulcerative colitis → Inflammatory polyp → localized/compartmental immunosuppression → EBV infection → large B-cell proliferation
- Similar to fibrin-associated large B-cell proliferation/DLBCL, chemotherapy is often **not** needed.

Gruver and Hsi et al, Fibrin-associated large B-cell lymphoma: part of the spectrum of cardiac lymphomas. Am J Surg Pathol. 2012 Oct;36(10):1527-37.

17

Follow up

Case was discussed with the clinical team about the nature of this lesion:

- No drug withdrawal/tampering, no chemotherapy
- Patient continue to be **compliant with lialda daily and Humira**.
- The most recent F/U: 8 months after the polypectomy:
  - Biopsies from terminal ileum, cecum, colon and rectum:

    unremarkable mucosa with no microscopic abnormality

18

The panel asks us to discuss:

- GI lymphomas in setting of IBD treated with immune modulators.
- The differential diagnosis with EBV MCU.

19

GI lymphomas in setting of IBD treated with immune modulators:

- Difficult to determine whether due to the iatrogenic immunosuppression or underlying IBD or chance alone
- The risk of lymphoma associated with IBD itself is not increased from many studies.
- IBD therapy (anti-TNF, thiopurine) increases the risk of lymphoma occurrence.

20

GI lymphomas in setting of IBD treated with immune modulators:

- The most common lymphoma subtypes: DLBCL, FL, CHL, MZL, HSTL
- HSTL: although some studies showed increased risk of HSTL in patients treated with TNF antagonists, other studies showed no increased risk or an increased risk only when patients also received other drugs (thiopurine).

American Journal of Gastroenterology. 106(12):2146–2153

21

EBV-positive MCU

- Iatrogenic immunosuppression or age-related
- Typically indolent clinical course with spontaneous regression in some cases
- Location: skin and GI tract, no systemic lymphadenopathy, no BM involvement
- Histology: ulcer with polymorphic infiltrate beneath the ulcer (large transformed cells, Hodgkin or RS like).
- Molecular: less than half show clonal IG gene rearrangement
- **Differential diagnosis**:
  - EBV-positive DLBCL: sheets of large cells
  - Classic Hodgkin Lymphoma: Be extremely cautious to make a diagnosis of stage 1 extranodal CHL. It is extremely rare if present at all.

22

EBV-positive Follicular Lymphoma Transforming to Diffuse Large B-Cell Lymphoma

Yukiko Kitagawa, MD, PhD
Magdalena Czader, MD, PhD

Indiana University School of Medicine,
Indianapolis, IN

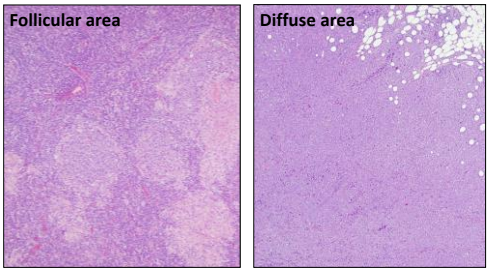
INDIANA UNIVERSITY
SCHOOL OF MEDICINE

1

Clinical History

- 58 year-old Caucasian male with a history of mixed connective tissue disease (MCTD) currently treated with hydroxychloroquine.
- Presented with right axillary and subpectoral lymphadenopathy.
- No weight loss or night sweats.
- PET/CT scan showed bulky, highly avid axillary lymphadenopathy.
- Limited core needle biopsy showed atypical EBV-positive lymphoid proliferation.
- Lymph node excision was recommended for definitive diagnosis.

2



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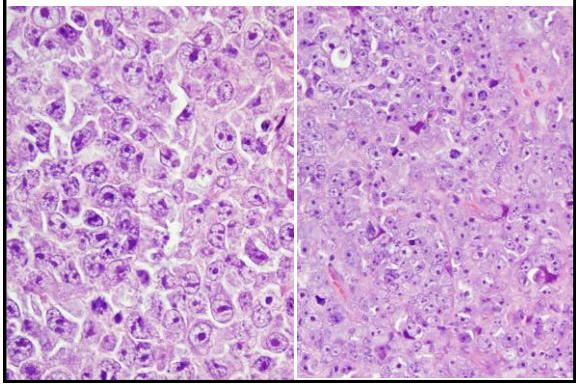

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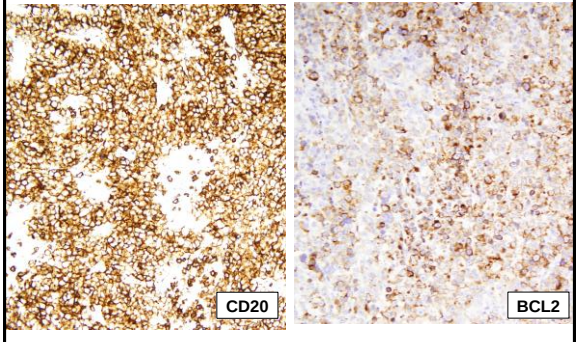

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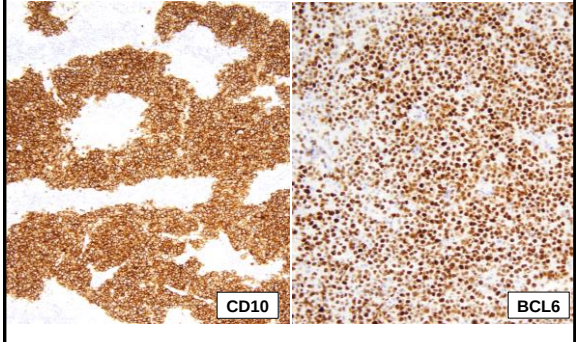

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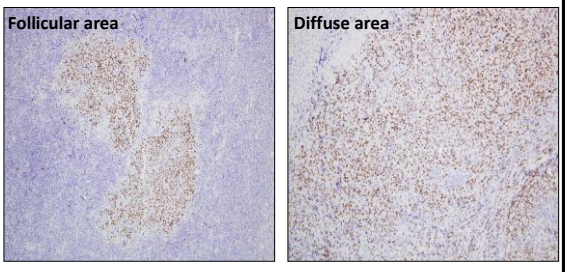
C-MYC



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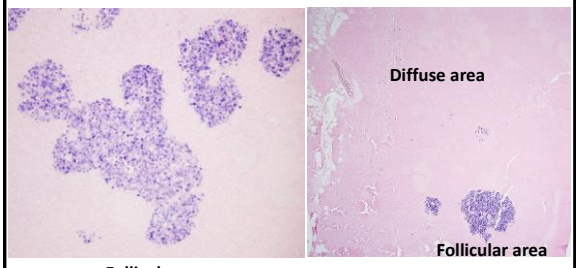
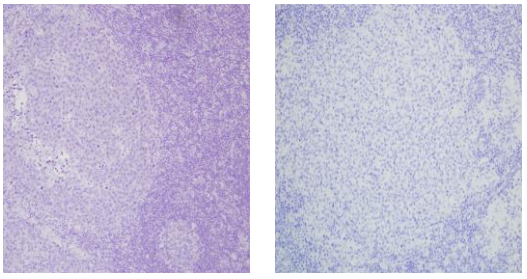
EBER *in situ* hybridization



Follicular areas

8

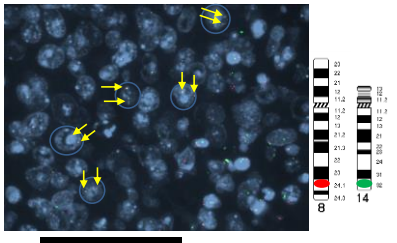
EBNA1 LMP1



Follicular areas

9

Dual Color Double Fusion (DFISH) for MYC/IGH fusion



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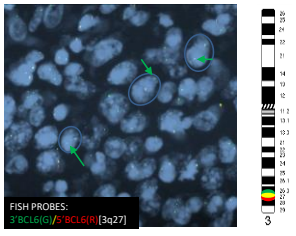
Break-Apart BCL6 = Unbalanced BCL6 Rearrangement



11

Summary of fluorescence *in situ* hybridization and EBV studies

	Follicular Areas	Diffuse Areas
BCL6	Abnormal, 58%	-
3q27; BAP		
BCL2	-	-
18q21; BAP		
MYC	Abnormal, 100%	Abnormal, 95%
IgH/MYC and BAP		
EBER (*in situ* hybridization)	+	-
LMP1	-	-
EBNA1 (immunohistochemistry)	-	-

12

Proposed Diagnosis

Follicular lymphoma, grade 3B, EBV-positive,
and diffuse large B cell lymphoma

13

Clinical History

- R-CHOP x 6 cycles with involved field irradiation.
- In remission since 2016.

14

Review of EBV-Associated B-cell Lymphomas

	Latency	EBV positivity	MYC translocation
Burkitt Lymphoma	I	Endemic 100% Sporadic 20-30% Immunodeficiency 30-40%	90%
Plasmablastic Lymphoma	0/I	50-70%	74% in EBV + cases 49% overall
EBV positive Diffuse Large B-cell Lymphoma	II /III	(100%)	4-10%
Primary Effusion Lymphoma	I/II	70%	0
EBV positive Follicular Lymphoma	I/**II**	(100%)	?

Dojcinov et al, Blood 2011, Valera et al, Am J Surg Pathol 2019, Montes-Moreno et al, Modern Pathology 2012, Liu et al, Histopathology 2012, Rezk et al, Surg Pathol,2019.

15

Prevalence of EBV-positive Follicular Lymphoma

Study	EBV-positive cases/All studied cases	Prevalence
Mackrides et al. (2017)	10/382 (EBER ISH, EBV-LMP1)	2.6%
Mackrides et al. (2018)	12/488 (EBER ISH, EBV-LMP1)	2.5%
Mundo et al. (2019)	3/50 (EBER ISH, qPCR BamHI W, EBNA1)	6%

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EBV-positive Follicular Lymphoma

- Exceedingly rare (prevalence 2.5-6%)
- Predominantly histologic grade 3, rare cases of grade 1-2.
- Latency program II, rarely I.
- Significantly associated with CD30 expression.
- Common progression to higher grade lymphoma.

Mackrides et al, Mod Pathol 2017, Mackrides et al, Am J Hematol 2018

17

Disease Progression in EBV-positive Follicular Lymphoma Cases

Reference	Age	Location	Immunosuppression	Grade at initial diagnosis	EBER	EBNA1	LMP1	Time to progression (yrs)	Diagnosis at progression
Presented case	58	Axilla subpectoral	Hydroxychloroquine	3B	+	-	-	Concurrent	Concurrent DLBCL
Granai et al.	73	Inguinal	Chemotherapy	2	+	+	-	6	DLBCL
Mackrides et al.	83	Cervical	None	3A	+	NA	NA	3.8	DLBCL
	48	Inguinal	None	1-2	+	NA	+	7.2	DLBCL
	31	Mediastinum	None	3A	+	NA	-	<1	DLBCL
	33	Supraclavicular	None	3A	+	NA	+	5.6	DLBCL
	64	Spleen/multiple	None	3A	+	NA	+	1.1	DLBCL
	76	Abdomen	Hydroxychloroquine	1-2	NA	NA	NA	6.1	FL 3A (EBER+)
	33	Cervical	None	1-2	NA	NA	NA	4.3	FL 3A (EBER+LMP1+)

Mackrides et al, Mod Pathol 2017, Granai et al, Haematologica 2019

18

Chloroquine and its Analogues vs. EBV infection

Immunomodulation

- Hydroxychloroquine inhibits dendritic cell activation by decreasing Toll-like receptor activation.
- Hydroxychloroquine reduces TNF alpha, IL-1 beta and IL-6 production.

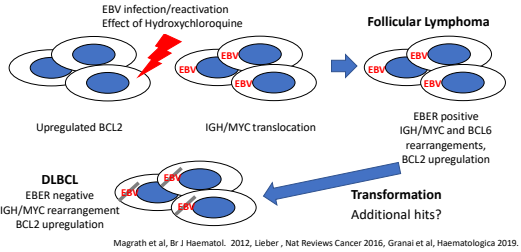
EBV reactivation

- Chloroquine causes phosphorylation of KAP1 through ATM, leading to reactivating EBV-gene replication. During lytic cycle reactivation, EBV infected cells might experience genetic instability, which may promote tumor growth.

Durcan et al, J. Autoimmun 2016, Murata, Microbiol Immunol 2014, Li et al, PLOS 2017.

19

MYC and EBV Contribute to Tumorigenesis



Magrath et al, Br J Haematol. 2012, Lieber , Nat Reviews Cancer 2016, Granai et al, Haematologica 2019.

20

Interesting Features of Presented Case

- EBV-positive follicular lymphoma is a rare entity (less than 30 cases reported)
- Latency type I or 0, exceedingly rare in EBV-positive follicular lymphomas
- Patient with a history of autoimmune disease treated with hydroxychloroquine, which has been previously linked to reactivation of EBV replication

Li et al, PLOS 2017

21

Acknowledgements

University of Wisconsin-Madison
McArdle Laboratory for Cancer Research
Dr. Shannon Kenney

Mayo Clinic
Division of Laboratory Genetics and Genomics
Department of Laboratory Medicine and Pathology
Dr. Rhett Ketterling

22

The 1st Joint CSHP and SH Workshop 2019
Gran Meliá Hotel, Xi'an, China

"EBV-related B-cell lymphomas, non-PTLD type"



John Goodlad
NHS Greater Glasgow and Clyde/University of Glasgow
QEUH & Beatson/Gartnavel Hospitals
john.goodlad@nhs.net

1

EBV-ASSOCIATED LYMPHOPRLIFERATIVE DISORDERS

Acute infection

Solely related to iatrogenic immunosuppression

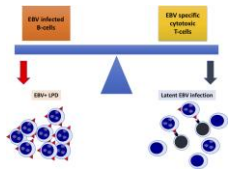
Well established lymphoma subtypes, a proportion of which are EBV+

- Plasmablastic lymphoma
- EBV-positive mucocutaneous ulcer
- EBV-positive diffuse large B-cell lymphoma, not otherwise specified
- Lymphomatoid granulomatosis
- Diffuse large B-cell lymphoma associated with chronic inflammation
  - Fibrin-associated diffuse large B-cell lymphoma

HHV8 and EBV

2

Entities to be discussed today are EBV-driven B-cell LPDs may occur in a variety of clinical settings, all resulting in reduced immune surveillance for EBV:



- Iatrogenic immune suppression
- Immune senescence
- Immune sequestered sites
- Primary/inherited immune deficiency

Important to recognize and accurately classify

- Overlapping pathologies but different biology/clinical course
- Different treatments
- May be first indication that patient is immune suppressed

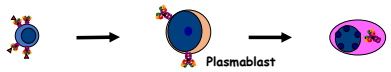
3

4

PLASMABLASTIC LYMPHOMA

5

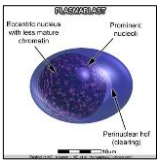
WHAT IS A PLASMABLAST?



A stage of B-cell differentiation intermediate between resting B-cell and plasma cell that follows antigen specific B-cell activation

MORPHOLOGY

- Large cells (x3-4 RBC)
- Round +/-eccentrically placed nucleus
  - Dispersed chromatin
  - Nucleoli are prominent
  - Moderate amount of basophilic cytoplasm
  - Perinuclear hof may be present



PHENOTYPE

Reflects B-cell program being switched off and plasma cell program being switched on

- **CD20 low/negative**
- **PAX5: low/negative**
- **IRF4: positive**
- **BLIMP1: positive**
- **XBP1: positive**
- **CD138: low/positive**

Santiago Montes-Moreno et al. Haematologica 2010;95:1342-1349

6

PLASMABLASTIC LYMPHOMA

First described in HIV patients as specific type of lymphoma involving oral cavity
- **Spectrum of plasmablastic lymphoma expanded in subsequent reports**

Strong association with immune compromised states
- HIV (75%)
- Iatrogenic (post organ transplant) (6%)
- HIV negative, apparaently immune competent (immune senescence?) (28%)

Many sites of involvement, predominantly extranodal
- Oral cavity
- Sinonasal cavities
- Orbit
- Skin
- GIT
- Lymph nodes relatively rarely involved

Rare neoplasm
- True incidence not known
- Approximately 2% of all HIV-related lymphoma (Carbonne A et al, Hum Pathol 2002)

Delecluse HJ et al, Blood 1997; Castillo JJ et al, Blood 2015; Carbonne A et al, Hum Pathol 2002

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CLINICAL FEATURES (i)

Most patients present between 40 and 60 years of age
- Range = 1-80 years
- HIV+ patients tend to be younger (median age = 42 years)

Percentage of patients presenting with advanced stage disease (stage III/IV) is high, particularly in HIV+ patients:
- HIV+ >75%
- Post-transplant 50%
- HIV-, 'immune competent' 25%

Presenting site varies between different clinical scenarios……

Castillo JJ et al, Blood 2015 (review and analysis of 590 published cases)

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PLASMABLASTIC LYMPHOMA: SITES OF INVOLVEMENT

Table 1. Most common sites of involvement in PBL (literature review from 1997 to 2014)

	Total Cases		HIV-positive		HIV-negative		Posttransplant		Transformed	
Sites of involvement	No.	%	No.	%	No.	%	No.	%	No.	%
Oral	260	44	176	48	65	40	6	16	11	55
Gastrointestinal	83	14	43	12	34	21	3	8	3	15
Lymph nodes	43	7	17	5	14	9	11	30	1	5
Skin	41	7	22	6	10	7	8	22	1	5
Bone	26	4	19	5	4	2	2	5	1	5
Genitourinary	23	4	12	3	6	4	2	5	3	15
Nose	18	3	11	3	6	4	1	3	—	
Central nervous system	17	3	12	3	4	2	1	3	—	
Liver	11	2	8	2	2	1	1	3	—	
Lung	11	2	9	2	1	0.6	1	3	—	
Orbit	10	2	8	2	2	1	—		—	
Spleen	5	0.8	4	1	1	0.6	—		—	
Skull	5	0.8	4	1	1	0.6	—		—	
Retroperitoneum	4	0.7	1	0.2	3	2	—		—	
Breast	3	0.5	1	0.2	1	0.6	1	3	—	
Larynx	2	0.3	2	0.5	—		—		—	
Gallbladder	2	0.3	2	0.5	—		—		—	
Choroid	1	0.1	—		1	0.6	—		—	
Heart	1	0.1	—		1	0.6	—		—	
Parotid	1	0.1	—		1	0.6	—		—	
Muscle	1	0.1	—		1	0.6	—		—	
Not specified	22	4	16	4	6	4	—		—	
Total	590		369		164		37		20	

Castillo JJ et al, Blood 2015 (review and analysis of 590 published cases)

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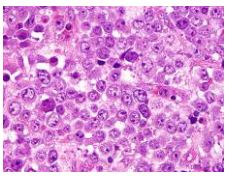
PATHOLOGY

Diffuse sheets of blast cells
- 'starry-sky' appearance common

Neoplastic cells large with moderate to abundant cytoplasm

- **Immunoblast-like cells**
  - Central oval nucleus
  - Dispersed chromatin
  - Prominent nucleolus
  - Common in oral cavity, HIV+
- **Plasmablastic/plasmacytic appearance**
  - Basophilic cyoplasm
  - Paranuclear hof
  - Eccentric nucleus
  - Prominent nucleolus
  - More common in nodal and other extranodal sites, HIV-



Castillo JJ et al, Blood 2015; Colomo L et al, Am J Surg Pathol 2004; Delecluse HJ et al, Blood 1997

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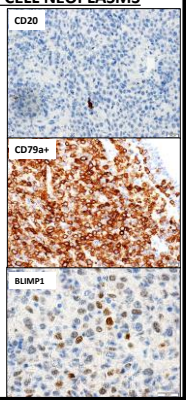
IMMUNOPHENOTYPE: SIMILAR TO PLASMA CELL NEOPLASMS

Expression of plasma cell associated antigens
- CD79A (50-85%)
- CD138
- IRF4
- BLIMP1
- XBP1
- Usually IgG+ (hypermutated)

Absent or weak expression of B-cell markers
- CD19
- CD20
- PAX5



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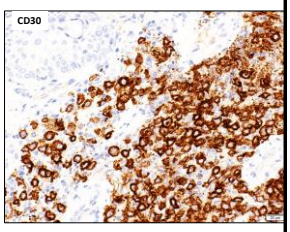
IMMUNOPHENOTYPE: SIMILAR TO PLASMA CELL NEOPLASMS

Other markers that may be positive
- EMA commonly expressed
- CD45; subset show dim expression
- CD56 (5-50%)
- MYC (50%)
- CD30 (30%)
- CD117 (rare cases reported)
- T-cell associated antigens (aberrantly expressed in rare cases; CD2, CD4)



High Ki67 (>90%)

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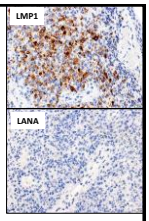
VIRUSES

EBER positive in majority of cases
- HIV+ 75%
- Post-transplant 67%
- 'Immune competent' 50%

Most cases negative for LMP1, EBNA2
- Type III latency can be seen
  - HIV+
  - Post-transplant

HHV8/KSHV8 negative

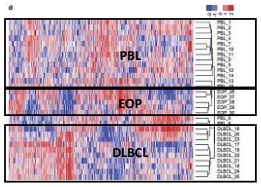


GENETICS

MYC translocation
- Approximately 2/3rds of cases

Gene expression profile
- Closer to extraosseous plasmacytoma than DLBCL

MYC overexpression during terminal B-cell differentiation
- Normally down-regulated



Valera A et al, Am J Surg Pathol 2010, Jaffe E & Pittaluga S, Hematology 2011, Castillo JJ et al, Blood 2015, Chapman J et al, Leukemia 2015

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TREATMENT AND OUTCOME

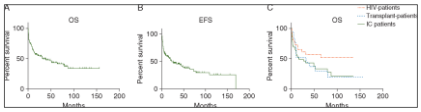
Intensive chemotherapy regimens recommended, e.g.
- EPOCH
- Hyper-CVAD
- CODOX-M/IVAC

Intrathecal methotrexate should also be considered in most patients

Recent study of 135 patients treated at major French (LYSA) centres;

Majority treated with CHOP/CHOP-like regimens (n=70/135)
- Most of rest treated with more intensive anthracyline based therapies, e.g. EPOCH, ACVBP, COPADM

Median OS = 32 months; possibly slightly better for HIV+



Tchernonag E et al, Annals Oncol 2017

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EPSTEIN-BARR VIRUS POSITIVE MUCOCUTANEOUS ULCER

EBV Positive Mucocutaneous Ulcer—A Study of 26 Cases Associated With Various Sources of Immunosuppression

Stefan D. Dojcinov, MD, FRCPath, Girish Venkataraman, MD,† Mark Raffeld, MD,† Stefania Pittaluga, MD, PhD,† and Elaine S. Jaffe, MD†*

Am J Surg Pathol 2010

(Reports of lesions that are probably EBV-MCU present in contemporaneous and historical literature, particularly in associated with methotrexate)

Localized development of slowly evolving well defined indurated ulcers at mucosal or cutaneous sites

Arises in patients with some form of immune suppression:
- Elderly
- Patients undergoing therapeutic immunosuppression for CT disease
  - Most frequently methotrexate
- Post-transplant; solid organ > BMTx

Curry JL et al, J Cutan Pathol 2011; Au W-Y et al, Am J Hematol 2006; Kalantzis A et al, Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005

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PATHOGENESIS

- Speculated that immune surveillance is reduced to a level that is only just sufficient to maintain the EBV in a dormant state systemically
  - Age-related immune senescence
  - Iatrogenic immunosuppression
- Exposure to an additional site restricted immune modulating factor tips balance towards a localised EBV driven lymphoproliferation
- Sites at which EBV-infected cells are prevalent (e.g. Waldeyer's ring) may be particularly prone to this disruption in equilibrium

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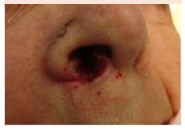
CLINCIAL FEATURES

- Well circumscribed, often painful, ulcerating lesions at mucosal or cutaneous sites
- Oropharyngeal mucosa is the most frequent site of presentation
- Cutaneous involvement often peri-oral
- Gastro-intestinal tract
  - Any part may be involved
- **No mass lesion is detectable on clinical examination or imaging**
- **No systemic lymphadenopathy and/or splenomegaly**
- **EBV-DNA is typically undetectable in peripheral blood, even in post-transplant cases, in contrast to many other types of EBV-associated lymphoproliferative disorders**

74 yr old male; Crohn's disease, Azothioprine

Drs Soumya Pandey, SH/EAHP LWS case SH2015-0344
M74, ulcerated nasal lesion



Dojcinov SD et al, Am J Surg Pathol 2010; Au W-Y et al, Am J Hematol 2006; Yamakawa N et al, J Rheumatol 2014; Hart M et al, Am J Surg Pathol 2014

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PATHOLOGICAL FEATURES

Shallow sharply circumscribed ulcers

Large transformed lymphoid cells
- Immunoblasts
- Reed-Sternberg-like cells
- **B-cells: CD30+, EBV+**

Polymorphous infiltrate in background
- Lymphocytes – small lymphocytes concentrated at ulcer base
- Plasma cells
- Eosinophils
- Histiocytes

Angioinvasion
- Present in 6/26 cases in original series
- Large lesional cells infiltrating medium sized arteries
- Surrounding necrosis

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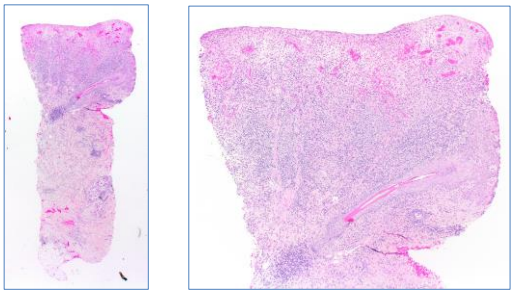

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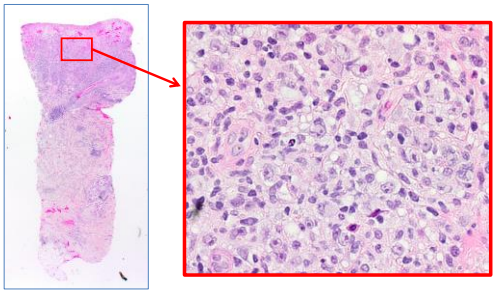

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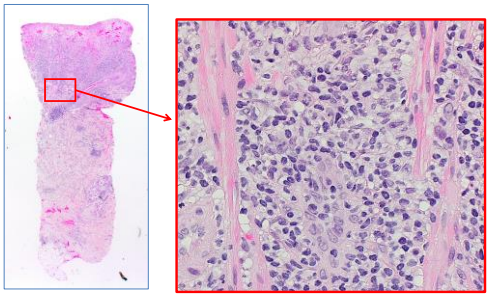

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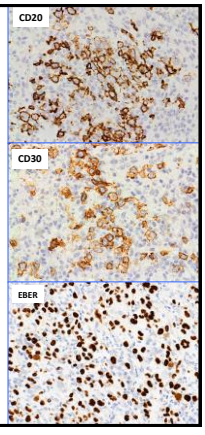
IMMUNOPHENOTYPE

Immunoblasts and RS-like cells are EBV infected B-cells
- CD20+ (can occasionally be –ve; 3/26 cases)
- CD79+, PAX5+, OCT2+, BOB1+
- CD30+
- CD45+
- MUM1/IRF4+
- EBER+
- LMP1+ (usually type II or type III latency)
- CD15+ in 10/23 cases in one series, 0/7 in another)

Many small T-cells in background and surrounding base of infiltrate
- CD4+ & CD8+
- Scattered CD8+ intermediate/large lymphocytes



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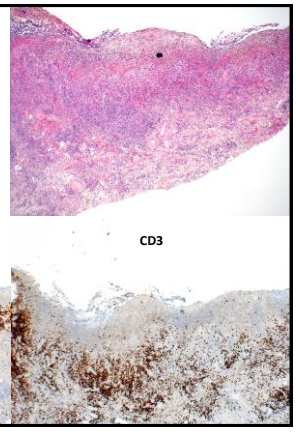
MUCOSAL SITE
- M72
- Liver transplant 14 years previously
- Recent excision of minimally invasive SCC left retromolar region
- Subsequently developed ulcer at site of operation
- Biopsied



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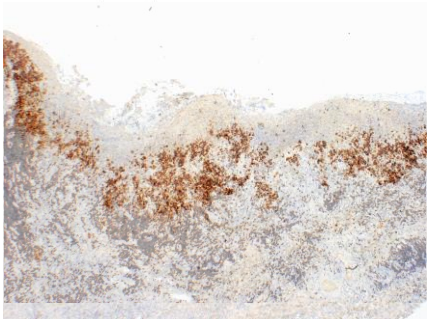
CD20

CD3



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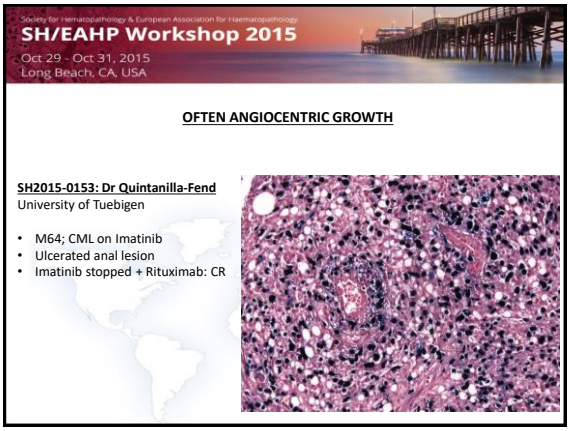

OFTEN ANGIOCENTRIC GROWTH

SH2015-0153: Dr Quintanilla-Fend
University of Tuebigen

- M64; CML on Imatinib
- Ulcerated anal lesion
- Imatinib stopped + Rituximab: CR

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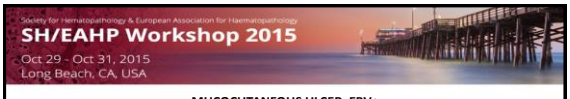


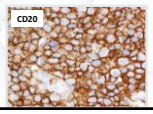
MUCOCUTANEOUS ULCER, EBV+

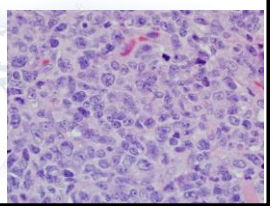
- **Spectrum of morphologies and phenotypes**

BLAST CELLS MAY BE NUMEROUS: MIMIC DLBCL

SH2015-0206: Dr Grogg
Mayo Clinic

- F49, rheumatoid arthritis on methotrexate
- Ulcerated lesion at corner of mouth
- Excised and methotrexate discontinued
- CR with no relapse





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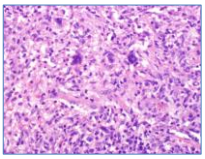
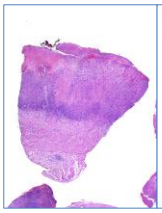
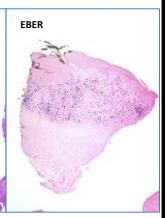
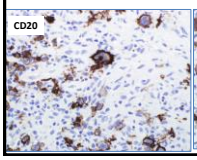
MAY RESEMBLE CLASSICAL HODGKIN LYMPHOMA

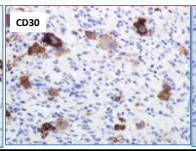
SH2015-0072: Dr Dhesi
F77, 1 year history of oral ulcer
Previous small B-cell lymphoma and ITP
Rituximab in 2004 and 2011

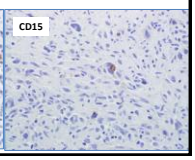












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MOLECULAR

PCR studies

- Monoclonal IG gene rearrangement in 7/18 (40%)
- Monoclonal TCR gene rearrangement in 6/16 (37.6%)
- Oligoclonal TCR gene rearrangement in 5/16 (31.2%)
  - T-cell response = restricted but reactive
  - Reflects reduced T-cell repertoire

Dojcinov SD et al, Am J Surg Pathol 2010

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TREATMENT / OUTCOME

- Indolent course although response to treatment may be variable
- Spontaneous regression in a proportion
- For some patients surgical resection sufficient
- Withdrawal of MTx / Azothoprine or reduction in immunosuppression may be required
- Single agent Rituximab probably the most 'aggressive' therapy necessary
- Patients who have persisting lesion and/or run a relapsing and remitting course do not seem to progress to more widespread disease

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LYMPHOMATOID GRANULOMATOSIS

Extremely rare EBV-associated B-cell lymphoproliferative disorder

- 1st described by Liebow and colleagues in 1972
  (Liebow AA et al, Hum Pathol 1972)
- Not recognised as a B-cell LPD until 1974
  (Guinee DG Jr et al, AJSP 1994)

Most cases seen in adults (median age = 40-50 years) but can occur in children (range = 21-74 years)

M>F, 2:1

Pittaluga S et al, WHO 2017; Katzenstein A-LA et al, AJSP 2010, Cancer 1979;
Jaffe, E.S., Wilson, W.H. Cancer Surveys 1997; 30: 233-248; Song JY et al, Am J Surg Pathol 2015

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PATHOGENESIS

Many cases have no clearly defined underlying immunodeficiency but on testing are found to have defective immune surveillance of EBV-infected B-cells, especially by CD8+ T-cells

- Abnormal T-cell subsets in peripheral blood
- Impaired T cell response to skin test antigens
  (Wilson WH et al, Blood 1996; Sordillo PP et al, Cancer 1982)

Other patients have identifiable predisposing condition

- **Hereditary**
  - Wiskott-Aldrich syndrome
  - X-linked lymphoproliferative syndrome
  - Common variable immune deficiency
- **Acquired**
  - Human immunodeficiency virus
  - Allogeneic organ transplant*
  - Other immunosuppressive drugs*

Pittaluga S et al, WHO 2017;
Katzenstein A-LA et al, AJSP 2010;
Katzenstein A-LA et al, Cancer 1979;
Song JY et al, AJSP 2015

*** Suggested by some that cases arising in these settings may be better classified as something else**

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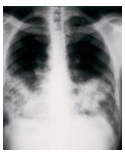
SITES OF INVOLVEMENT

LUNG

Almost always involved; for some authors lung involvement is a prerequisite for diagnosis

- Multifocal lesions
- Predilection for lower lobes



EXTRAPULOMARY SITES

- CNS 38%
- Liver 19%
- Skin 17%
- Kidney 15%

PERIPHERAL BLOOD EBV

- Almost all show evidence of prior infection (Viral capsid antigen IgG positive)
- Generally only mildly elevated EBV viral load:
  - Mean = 18 copies/ml (range = 0 – 22,727)

  (compared with PTLD: mean = 544,750 copies/ml, range = 74,000 – 3,200,000)

Song JY et al. Am J Surg Pathol 2015.39.141-56

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PATHOLOGY

Nodular lesions

- Angiocentric distribution
- Well circumscribed

Mixed mononuclear cell infiltrate

- Small lymphocytes
- Variable numbers of large lymphoid cells
  - Immunoblast-like
  - Rarely Reed-Sternberg-like
- Histiocytes
- Plasma cells

Absence of other inflammatory cells

- No neutrophils or eosinophils
- No granulomas (except in subcutaneous fat – secondary to fat necrosis)

Infiltration of blood vessel walls (often small T-cells)

Varying degrees of necrosis

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IMMUNOPHENOTYPE

Background small lymphocytes

- T-cells;
- CD4 & CD8/cytotoxic molecule+ (CD4>CD8)
  (often T-cells infiltrating blood vessel walls)

Large neoplastic blasts

- CD20+
- CD30+/-
- EBV+; EBER+, LMP1+, EBNA2+/-
  (majority of cases show type 3 latency)

CLONALITY

Clonal immunoglobulin gene rearrangement found when % of large EBV+ B-cells is high

- Clonality may not be detectable when few in number

Often oligoclonal/restricted pattern of T-cell receptor gene rearrangement

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GRADING

Grade I:
- Inconspicuous blast cells; often only seen with IHC
- <5 EBV+ cells / hpf by in situ hybridisation
- Only focal necrosis if any

Grade II:
- Occasional blasts, sometimes in small clusters
- usually 5-20 EBV+ cells / hpf; variable, may be up to 50 /hpf
- Necrosis more common

Grade III:
- Polymorphic background still present
- Numerous large atypical cells; may form small confluent sheets
- >50 EBV+ blasts / hpf
- Usually extensive necrosis

N.B. sheets of large atypical EBV+ cells without polymorphous background = DLBCL

Pittaluga S et al, WHO 2008; Wilson WH et al, Blood 1996

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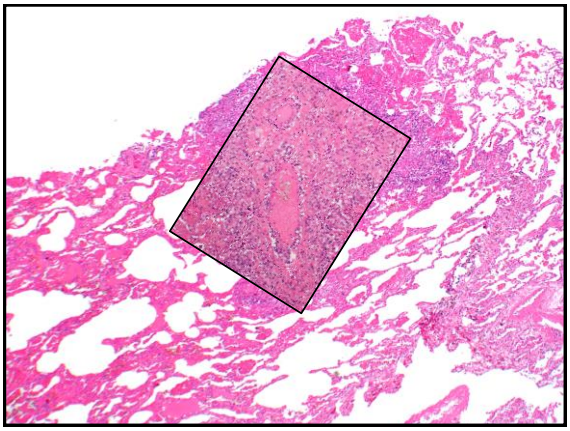

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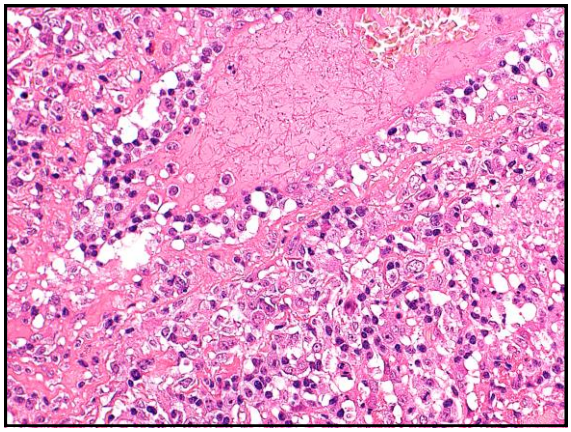

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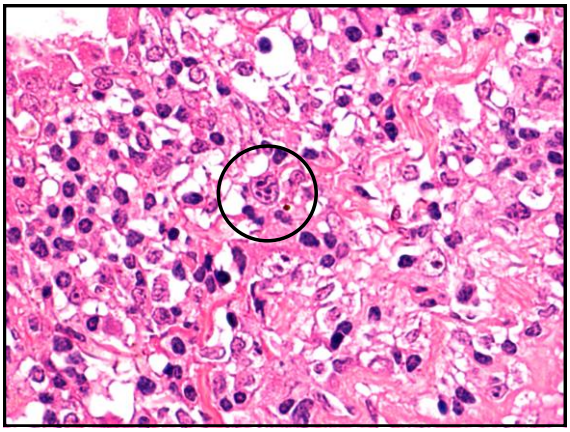

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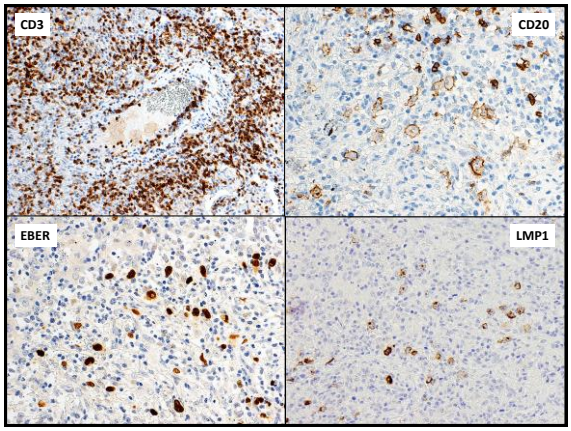

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TREATMENT AND OUTCOME

Some cases wax and wane over time and may even spontaneously regress

Most cases pursue an aggressive clinical course:
- **Median survival <2 years quoted in historical series**

Some evidence that lower grade lesions respond to less toxic therapy
Grading dictates treatment, e.g. current NCI phase 2 trial:

- **Grade 1: IFNα**
- **Grade 2: IFNα**
- **Grade3: DA-EPOCH-R**

Song JY et al, Am J Surg Pathol 2015

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EBV-POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED

Definition / Nomenclature

- Clonal EBV+ proliferation of large B-cells in apparently immunocompetent individuals
- Does not fulfill criteria for other well defined EBV+ lymphoproliferative disorders, e.g.
  - EBV+ MCU
  - Lymphomatoid granulomatosis
  - DLBCL associated with chronic inflammation
- Originally thought to be age related as a consequence of immune senescence; more recent studies report pathologically similar lesions in young individuals
- Name change to reflect these findings:
  - **EBV+ DLBCL of the elderly → EBV+ DLBCL, NOS**

Nakamura S et al, WHO 2017; Nicolae A et al, Blood 2015; Shimoyama Y et al, Cancer Sci 2008, Ok CY et al, Blood 2013

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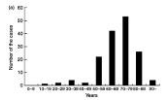
EBV-POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED

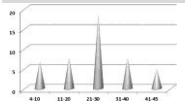
Epidemiology:

- Higher incidence reported in some East Asian populations c.f. Western populations
  - 5-15% vs <5%
- Majority present aged >50 years, peak in 8th decade
- May present at any age; Second smaller peak in third decade

Shimayoma Y et al, Cancer Sci 2008



Nicolae A et al. Blood 2015;126:863-872



Aetiology

- Immunesenescence (elderly)
- Immune modulation/escape, e.g. PDL1/PDL2 expression by tumour cells (young and old)

Beltran BE et al, Am J Hematol 2011; Chen BJ et al, Clin Cancer Res 2013; Gibson SE et al, Hum Pathol 2009; Hong JY et al, Curr Opin Oncol 2015; Nicolae A et al, Blood 2015; Castillo JJ et al, Am J Hematol 2018.93.953-962)

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EBV-POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED

Clinical:

- Nodal or extranodal sites may be involved
  - Nodal disease predominates in young (90% of cases)
- Common extranodal sites
  - Lungs
  - Gastroinestinal tract (WHO refs 1017, 3018, 3019)

Ususally high IPI at presentation

EBV usually detectable in peripheral blood

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PATHOLOGY

Large transformed cells

- Immunobasts
- Hodgkin/Reed-Sternberg-like cells

Component of reactive cells

- Lymphocytes
- Plasma cells
- Histiocytes/epithelioid cells

Broad morphological spectrum depending on proportions of neoplastic and reactive cells

- Monomorphic (DLBCL-like)
- Polymorphic
- T-cell/histiocyte rich large B-cell lymphoma-like (polymorphic/Hodgkin-like: most common in young)

FEATURES COMMON TO BOTH

- Angiocentric/angiodestructive growth
- Geographic necrosis

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PHENOTYPE

B-cell antigen positive

- CD19
- CD20
- CD79a
- PAX5

Non-germinal centre phenotype

- CD10-
- BCL6+/-
- IRF4/MUM1+

Also positive for:

- CD30 (frequently)
- CD15 (sometimes)
- Bcl2

Type II latency pattern the norm

- EBER+ (>80% of cells positive)
- LMP1+ (>90%)
- EBNA2+ (up to 36%)

GENOTYPE

No specific features

- Gains of 9p24.1 (PDL1/PDL2 loci) may be present
- IGH/MYC usually negative; if positive consider plasmablastic lymphoma
- Typically lack mutations of CD79B, CARD11, MYD88

Nicolae A et al, Blood 2015; Gebauer N et al, Leuk Lymphoma 2014; Yoon H et al, Genes Chrom Cancer 2015)

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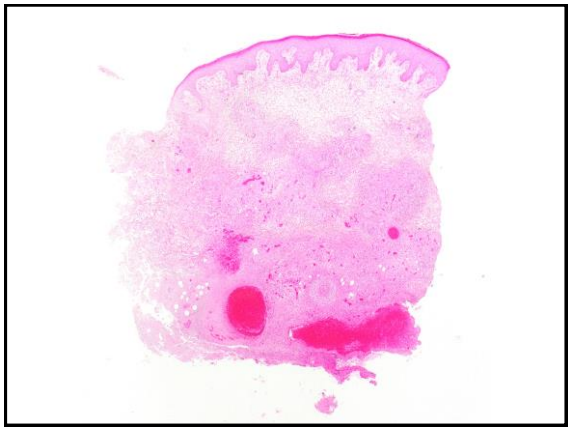

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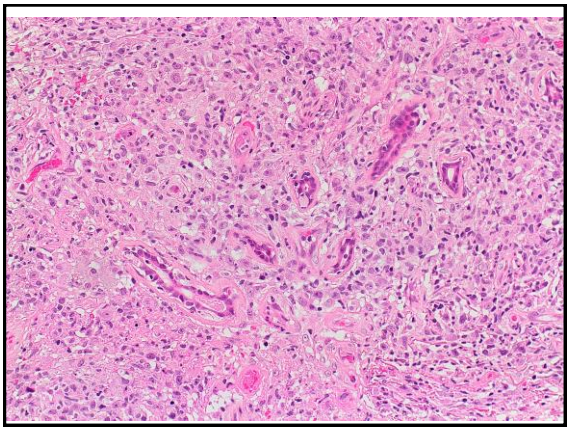

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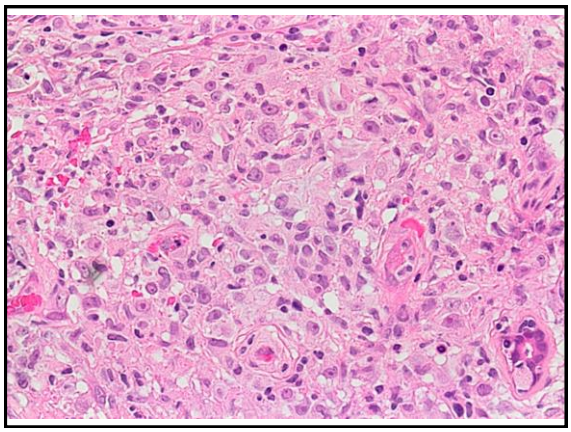

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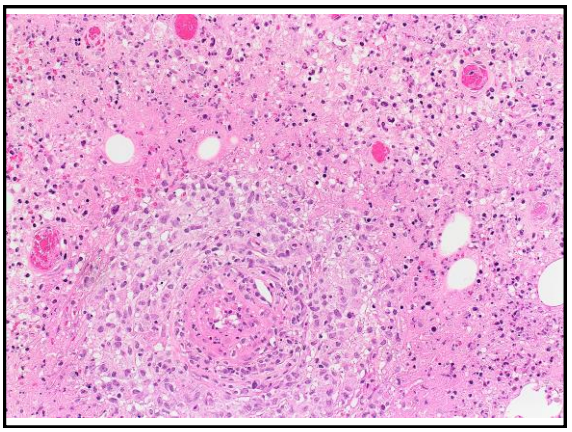

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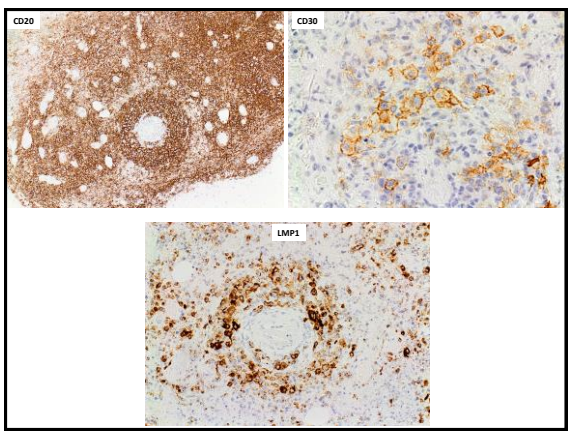

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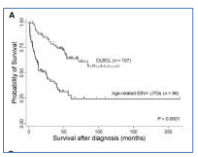
TREATMENT / OUTCOME

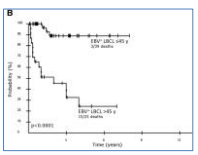
Aggressive disease in patients >50 years

- Responds poorly to conventional treatment for DLBCL (R-CHOP)
- Median survival = 2 years in elderly



Young patients have much more favourable outcome

- Long term complete remission in >80%



Oyama T et al, Clin Cancer Res 2007.13.5124
Nicolae A et al, Blood 2015.126.863-72

54

55

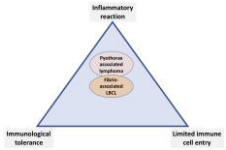
DIFFUSE LARGE B-CELL LYMPHOMA ASSOCIATED WITH CHRONIC INFLAMMATON

EBV+ lymphoma developing on background of longstanding chronic inflammation

- Usually in a confined space

Outgrowth of EBV+ clone facilitated by:

- Chronic inflammation → IL-10 → immune modulation/tolerance
- Reduced cell entry → reduced immune surveillance
- Acquired genetic abnormalities in tumour cells → Immune evasion (e.g. PDL1 overexpression)



(Kanno H et al, 1997 and 1998; Nakatsuka S et al, J Clin Oncol 2002; Petitjean B et al, Am J Surg Pathol 2002)

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DIFFUSE LARGE B-CELL LYMPHOMA ASSOCIATED WITH CHRONIC INFLAMMATION

Prototypic DLBCL-CI was pyothorax associated lymphoma

Concept of EBV-associated DLBCL arising on a background of chronic suppurative inflammation was subsequently extended to include cases arising in association with

- Chronic osteomyelitis
- Venous ulcers
- Surgical mesh implants

These lymphoproliferative disorders showed similar association with

- Chronic suppurative inflammation in a confined space
- Long latency period

Entity of diffuse large B-cell lymphoma associated with chronic inflammation

- Usually localised at presentation
- Aggressive clinical course
- Median survivals of <1 year quoted
- 5-year-survival 20% quoted

Copie-Bergman C et al, J Pathol 1997; Cheuk W et al, Am J Surg Pathol 2005; Fujimoto M et al, Pathol Int 2008; Nakatsuka S et al, J Clin Oncol 2002; Petitjean B et al, Am J Surg Pathol 2002

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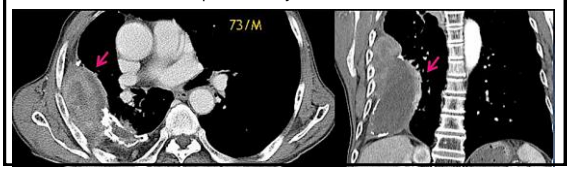
PYOTHORAX-ASSOCIATED LYMPHOMA

- First described in Japanese patients with long-standing pyothorax following artificial pneumothorax for treatment of TB
- Subsequent cases reported in Western patients
- Median time from pyothorax to lymphoma in largest published series = 37 years (range = 20-64 years)
- Typically present with:
  - Pain
  - Fever
  - Respiratory symptoms
  - Mass lesion in pleural cavity

(Nakatsuka S et al, J Clin Oncol 2002; Petitjean B et al, Am J Surg Pathol 2002)



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PATHOLOGICAL FEATURES

Present as tumour masses with invasion of surrounding structures

MORPHOLOGY

- Most cases show morphology of DLBCL
  - Rare cases have plasmablastic appearance
- May be widespread necrosis; often associated with angiocentric growth

PHENOTYPE

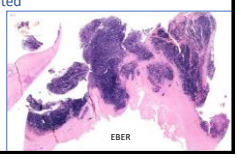
- CD20+
- CD79a+
  - (Plasmablastic cases: CD20/CD79a-, CD138+)
- CD30 (often positive)
- Aberrant T-cell antigen expression reported

(Nakatsuka S et al, J Clin Oncol 2002; Petitjean B et al, Am J Surg Pathol 2002)

Epstein Barr virus +ve: usually type III latency

- EBER+
- LMP1+
- EBNA2+

HHV8 negative



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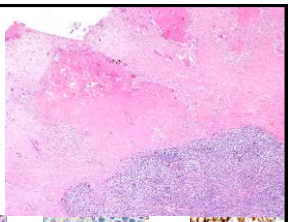
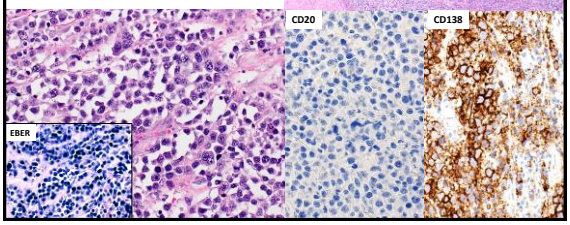
DLBCL-CI:

Plasmablastic differentiation

- M72
- Pacemaker in situ for 21 years
- Long history of intermittent fevers:
- Presented with recurrent infection and discharge from pacemaker site
- Pacemaker and 'pocket' removed





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GENETIC PROFILE

Karyotype: usually complex

Copy number alterations: *MYC* gene amplification common
TNFAIP3 (A20) sometimes deleted

Other: Down-regulation of *MHC-I* expression
TP53 mutations common (70%)
GEP different from nodal DLBCL

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FIBRIN-ASSOSIATED DIFFUSE LARGE B-CELL LYMPHOMA

Relatively recently described group of neoplasms; included in DLBCL-CI chapter in WHO 2017
- Share some features with DLBCL-CI
- Differ in some important respects:

Characteristic features:
- Stage IE EBV+ large B-cell proliferations
- Arise in confined spaces in association with fibrin
  - Thrombus
  - Cystic lesions with evidence of prior haemarrhage and organisation
- Background chronic inflammation usually present
- Suppurative inflammation **not** a feature

- Gruver AM et al, Am J Surg Pathol 2012
- Aguilar C et al, Cardiovasc Pathol 2015
- Quigley MM et al, Blood 2003
- Svec A et al, Path Res Pract 2012
- Bagwan IN et al, Cardiovasc Thorac Surg 2009

- Miller DV et al, Am J Surg Pathol 2010
- Loong F et al, Mod Pathol 2010
- Boroumand N et al, Am J Surg Pathol 2012
- Valli R et al, Int J Surg Pathol 2011
- Valli R et al, Hum Pathol 2014
- Natkunam Y, Goodlad JR et al, AJCP 2017

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FIBRIN-ASSOSIATED DIFFUSE LARGE B-CELL LYMPHOMA: CLINICAL

Initial cases described in cardiovascular system
- Aortic grafts
- Atrial thrombus
- Atrial myxoma
- Replacement heart valves

Later cases described in inner lining of cystic lesions
- Splenic pseudocyst
- Adrenal pseudocyst
- Hydrocele
- Testicular pseudocyst
- Cystic ovarian teratoma

In contrast to PAL/DLBCL-CI
- **No mass lesion** or invasive growth detected; usually an incidental finding
- Outcome appears to be excellent
  - All reported patients alive with no evidence of disease or were in CR when died of unrelated causes

Natkunam Y, Goodlad JR, et al. EBV-Positive B-Cell Proliferations of Varied Malignant Potential: 2015 SH/EAHP Workshop Report-Part 1. Am J Clin Pathol. 2017 Feb 1;147(2):129-152.

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PATHOLOGICAL FEATURES AND BEHAVIOUR

No mass lesion is found

Microscopic aggregates of large B-cells are seen lying within thrombus or in amorphous fibrinous material showing signs of previous haemorrhage, the latter lining cyst walls or lying close to the surface of atrial myxoma

Large B-cell proliferations:
- Clonal
- Post-germinal centre phenotype
- EBV-positive
- Type III latency most frequent (EBER+, LMP1+, EBNA2+; *BZLF1+)

Often chronic inflammation in cyst wall

No suppurative inflammation

***3/5 SH2015 Workshop cases BZLF1+; regulator of switch from latency to lytic EBV infection**
- **BZLF expression and type III latency implies localized alteration in host immune surveillance**

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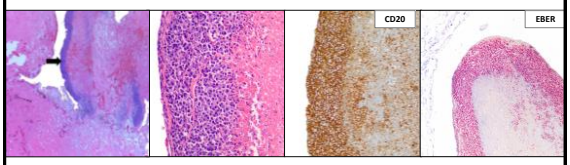
FIBRIN-ASSOSIATED DIFFUSE LARGE B-CELL LYMPHOMA

SH2015-0068
Sofia Garces, Mount Sinai Medical Center: Atrial myxoma



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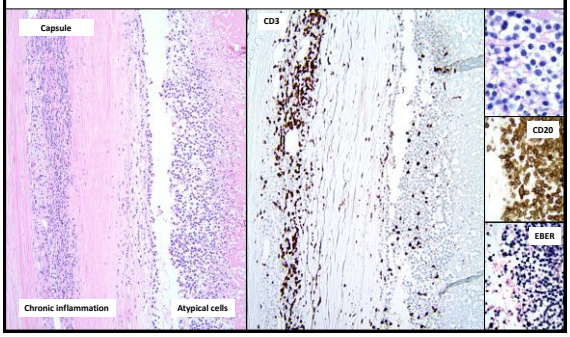
EAHP18-LYWS-461
Yan Xie, Cleveland Clinic; Adrenal pseudocyst

FIBRIN-ASSOSIATED DIFFUSE LARGE B-CELL LYMPHOMA



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Summary of Workshop cases: clinical features

Case no	Sex/AGE	Context / Site	Treatment	Outcome
SH2015-68	M50	Atrial myxoma	NA	Lost to follow-up
SH2015-147	M54	Atrial myxoma	Resection	DNED 24 m*
EAHP2018-353	F50	Atrial Myxoma	Excised	ANED 240 m
SH2015-470	M56	Cardiac thrombus	R-CHOP	ANED 46 m
SH2015-176	M56	Aortic aneurysm thrombus	R-CHOP + MTX	Current
EAHP2018-183	M74	Aortic aneurysm thrombus	R-CHOP x6	ANED 54 m
EAHP2018-129	M58	Adrenal pseudocyst	Resection	DNED 12 m
EAHP2018-461	M70	Adrenal pseudocyst	Excised	ANED 13 m
EAHP2018-551	M48	Adrenal pseudocyst	R-CHOP x6	ANED 8 m
SH2015-471	M61	Calcified renal cyst	CHOP x6	ANED 52 m
EAHP2018-280	F69	Breast implant associated	R-CHOP x3	CR, current case

* Presumed casue of death = thromboemboli; negative disease status not confirmed by autopsy

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Summary of Workshop cases: treatment and outcome

4 cases resection only
- No disease related deaths
- Median follow-up = 18.5 m
  (mean = 72 m; range = 12-240 m)

6 patients receive CHOP/R-CHOP: FU available on 4
- All alive, no evidence of disease at last follow-up
- Median follow-up = 27 months
  (mean = 40 m; range = 8-52 m)

Aggressive chemotherapy may represent overtreatment
Surgery +/- single agent Rituximab probably sufficient

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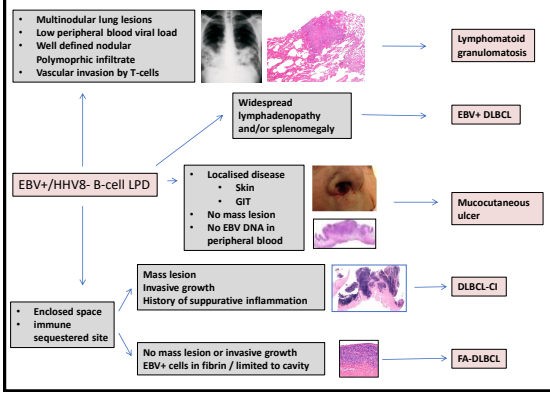
D/Dx of specific types of EBV+ LPDs



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The 1st CSHP/SH Workshop and CSHP 2019 Oct.19-20, 2019

Intravascular NK/T-cell lymphoma with multiorgan involvement

Kennosuke Karube
Dept. Pathol. and Cell Biol. University of the Ryukyus
University of the Ryukyus

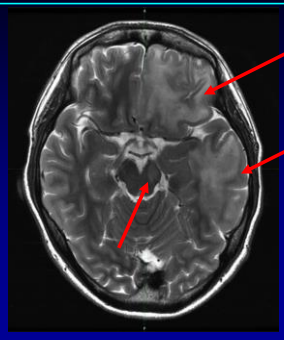
1

Case report

- 27-year old, male
- Sudden onset of syncope and generalized epilepsy
- No lymphadenopathy, no skin or bone marrow involvement
- LDH: 864 U/l, soluble IL-2 R: 2092 U/l
- HTLV-1 Ab (-), HIV Ab (-)

2

MRI



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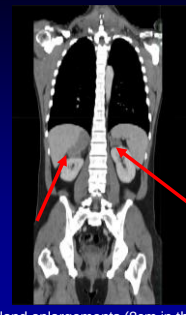
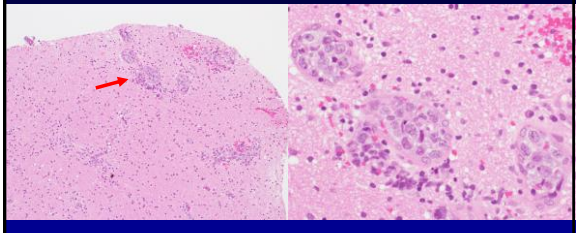
CT



Bilateral adrenal gland enlargements (8cm in the greatest diameter)

4

Brain biopsy



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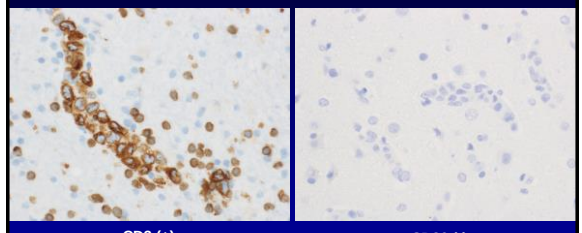
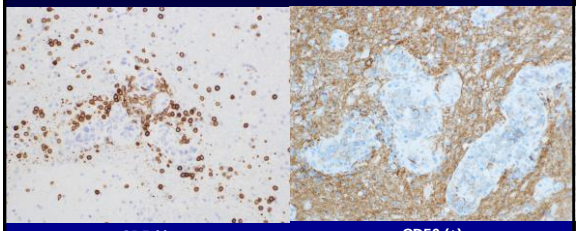
Brain biopsy



CD3 (+) CD20 (-)

6

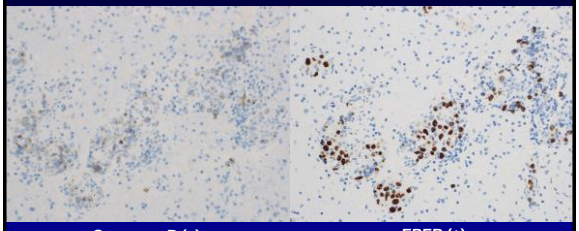
Brain biopsy



CD5 (-) CD56 (+)

7

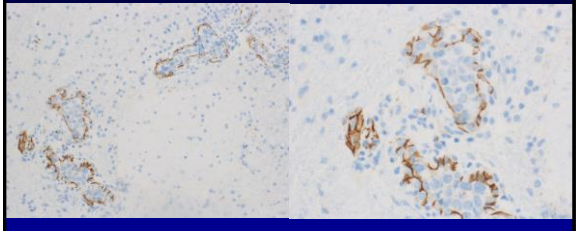
Brain biopsy



Granzyme B (+) EBER (+)

8

Brain biopsy



CD31: Lymphoma cells are confined to the blood vessels

9

Summary of immunophenotype

Positive	Negative
CD2	CD3
CD56	CD4
TIA-1	CD5
Granzyme B	CD8
EBER-ISH	CD20
CD30(partially)	ALK
	EBV-LMP1

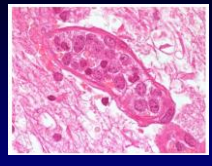
10

Proposed diagnosis
Intravascular NK/T cell lymphoma

Panel diagnosis
IVL extranodal NK/TCL, nasal type

11

Intravascular large B-cell lymphoma (IVLBCL)



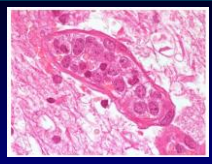
Lymphomas characterized by large B-cell lymphoid cell proliferation predominantly in lumen of the blood vessels

Three subtypes
•Classic variant
•Cutaneous variant
•Hemophagocytic syndrome-associated variant (Asian variant)

Ponzoni *Blood* 2018, Nakamura revised WHO 4th edition

12

Intravascular large B-cell lymphoma (IVLBCL)



Lymphomas characterized by large B-cell lymphoid cell proliferation predominantly in lumen of the blood vessels

Three subtypes
- Classic variant
- Cutaneous variant
- Hemophagocytic syndrome-associated variant (Asian variant)

Non-B cell phenotype is also recognized.

Ponzoni *Blood* 2018, Nakamura revised WHO 4th edition

13

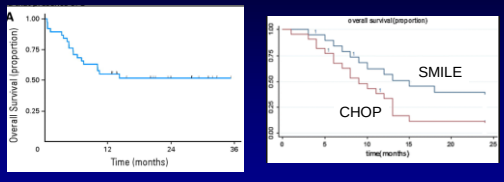
Review of intravascular lymphoma of NK/T cell type (IVNKL)

Patient	Country	Involved organ	Therapy	Follow-up (months)	Status	Reference
54/M	Italy	skin	CHOP	17	DEAD	Santucci *Cancer* 2003
41/M	USA	skin	CHOP+SCT	12	ALIVE (CR)	Wu *AJCP* 2005
47/F	USA	CNS, BM	no treatment	1	DEAD	Wu *AJCP* 2005
71/F	Taiwan	skin	no treatment	4	ALIVE (not CR)	Kuo *AJSP* 2006
40/F	Korea	skin, CNS	CODOX-M/IVAC	7	ALIVE (probably CR)	Song *JCO* 2007
63/M	Austria	skin	no information	6	DEAD	Lorenzo *AJSP* 2008
87/F	Austria	skin, liver?	no treatment	0.5	DEAD	Lorenzo *AJSP* 2008
23/F	Japan	skin, ileum, spleen	CHOP + chemo + SCT	9	DEAD	Nakamichi *Eur J Haematol* 2008
42/F	Taiwan	skin	CHOP + chemo	14	ALIVE (not CR)	Liao *Acta Derm Venereol* 2011
38/F	China	skin	CHOP	13	DEAD	Liu *AJCP* 2014
46/M	China	CNS	no treatment	2	DEAD	Xie *Leuk Lymphoma* 2015
45/M	China	skin	no information	0.5	DEAD	Wang *J Cutan Pathol* 2015
52/F	China	skin	CHOP	6	DEAD	Wang *J Cutan Pathol* 2015
32/M	China	skin	CHOP	4	DEAD	Wang *J Cutan Pathol* 2015
18/F	China	skin	CHOP	36	ALIVE (CR)	Wang *J Cutan Pathol* 2015
27/M	Japan	CNS, adrenal gland	SMILE + SCT	6	ALIVE (CR)	present case

- Reported cases are mainly from East Asia.
- Skin is the most frequent region.
- IVNKL with multi-organ involvement shows extremely poor prognosis.

14

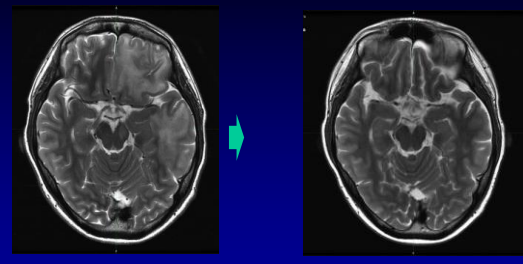
SMILE: standard chemotherapy for advanced NK/T cell lymphoma



Yamaguchi *JCO* 2011, Yang *Med Oncol* 2013

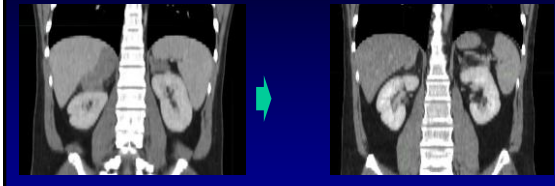
15

After chemotherapy (SMILE regimen, 2 cycles)



16

After chemotherapy (SMILE regimen, 2 cycles)



17

Intravascular NK/T cell lymphoma (IVNKL)

- Intravascular lymphoma is usually B-cell type.
- Although extremely rare, the second most frequent phenotype among IVL is NK/T cell type similar to extranodal NK/T cell lymphoma, nasal type.
- IVNKL mainly involves to the skin. IVNKL with multiorgan involvement is extremely rare, and usually shows dismal prognosis.
- The present case is the first that was treated with SMILE therapy, standard chemotherapy for advanced ENKTL. This treatment seems effective so far.

18

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

EBV-positive extramedullary plasmacytoma

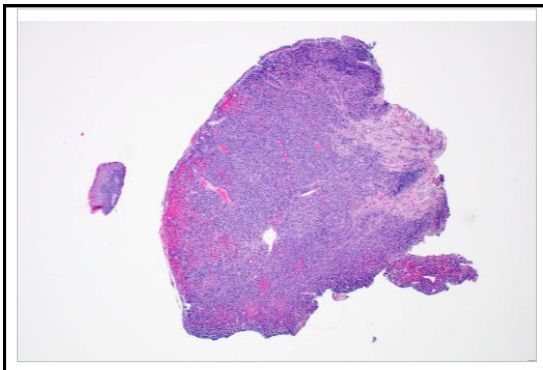
Fang Yu, Huanling Wu, L. Jeffrey Medeiros and Wei Wang
Dept. of Hematopathology
The University of Texas MD Anderson Cancer Center

1

Clinical History

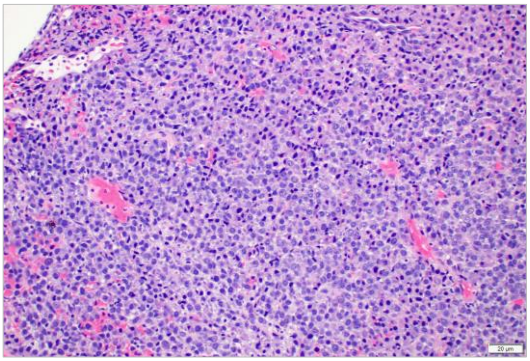
1) A 71-year-old man presented with a 1 cm mass in the nasal cavity (incidental finding during a routine annual exam).
2) He had no history of immunodeficiency, no any symptoms.
3) CBCs within normal range.
4) Bone marrow biopsy was negative for aberrant T, B or plasma cells.
5) Serum kappa and lambda light chains were in normal range. Protein electrophoresis and immunofixation were negative.
6) PET-CT scan shows no other lesions.
7) An excisional biopsy of the nasal mass was performed.

2



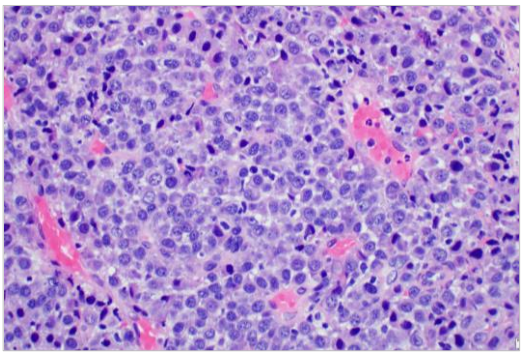
The whole lesion was excised and showed a dense and diffuse plasma cell proliferation under nasal mucosa.

3



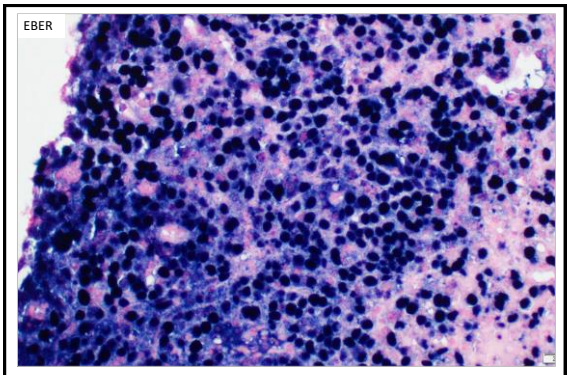
Plasma cells are small to medium sized

4



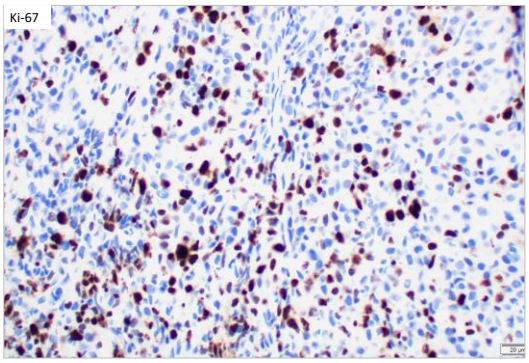
High power: Plasma cells are mature appearing without distinct central nucleolus.

5



Plasma cells are positive for EBV by EBER in situ hybridization

6



Anti-ki-67 demonstrates a maximal proliferation rate of 30%

7

Other studies not shown here:

1. **Plasma cells positive for CD138, CD79a, MUM-1 and kappa light chain by IHC.**
2. **FISH negative for MYC rearrangements.**

8

Patient's follow-up

The patient (a physician) refused any additional therapy (no local radiation therapy) and remains in remission 1 year after the biopsy.

9

Our diagnosis:

EBV-positive plasmacytoma

Panel diagnosis:

Plasmacytoma vs. PBL (EBV+)

10

The panel asks us to discuss:

1. **EBV in MM and in PBLs**
2. **Differential diagnosis between MM and PBL**

11

EBV in MM

EBV is rare in plasmacytoma and other plasma cell neoplasms

Loghavi et al. Histopathology. 2015 Aug;67(2):225-34
4 cases of EBV-positive plasmacytoma:

1. Immunocompetent
2. Localized: often head & neck areas (nasal cavity….)
3. Morphology: mature plasma cells with a prominent CD8-positive cytotoxic small T cells in the background
4. FISH: negative for MYC
5. All alive at the last F/U

Yan et al. Ann Diagn Pathol. 2017 Apr;27:1-6.
4 cases of EBV-positive plasmacytoma:

- Immunocompetent
- Local lesion without symptoms of chronic active EBV infection
- Morphology: well-differentiated morphology
- FISH: all negative for MYC rearrangement
- Prognosis: similar to EBV-negative plasmacytoma

12

EBV in PBLs

EBV is common in PBL (plasmablastic lymphoma), 60-75%

1. Often with immunodeficiency (HIV, post-transplant, iatrogenic, immunosenescence)
2. Extranodal (head & neck, GI tract)
3. Morphology: immunoblasts, plasmablasts. Mitotic figures frequent
4. MYC translocation: 50% of cases
5. Prognosis: poor

13

The differential diagnosis between EBV+ plasmacytoma and EBV+ PBL

	Plasmacytoma	PBL
Immunosuppression	no	yes
Presentation	Localized	Often high stage
Morphology	Mature plasma cells	Immunoblasts, plasmablasts
MYC rearrangement	No	Often positive
Prognosis	Good	Poor

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The differential diagnosis between EBV-negative PBL and EBV-negative plasmablastic myeloma

Can be very challenging! If you really can not separate them, call **plasmablastic neoplasm** and let clinicians struggle.

	Plasmablastic Lymphoma	Plasmablastic Myeloma
Immunosuppression	More	Less
Lytic bone lesions	No	Yes
Morphology	Immunoblasts, plasmablasts	Immunoblasts, plasmablasts
Paraprotein	Less	Often
Bone marrow involvement	Less	Often
Immunophenotype	Can not tell them apart	

15

Case # 1454386

Jagmohan S. Sidhu, MD[1]
Claude Cornwall, MD[1]
Matthew J. McGinnis, PhD FACMG[2]

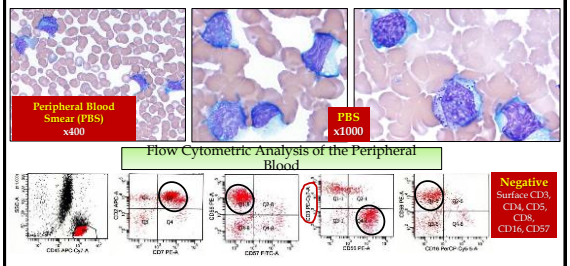
1. Department of Pathology and Laboratory Medicine, UHS Hospitals, Johnson City, NY
2. Clinical Genomics, NeoGenomics/Genoptix, Carlsbad, CA

1

Clinical Presentation

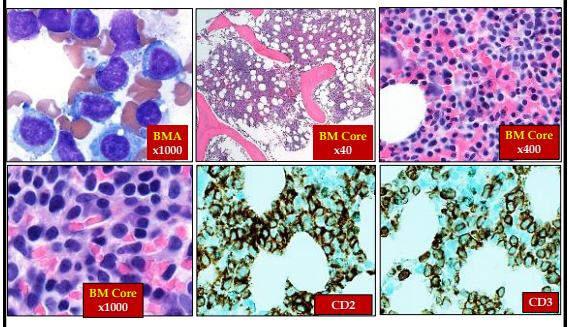
- A previously healthy 70-year-old **Caucasian** male presented to the emergency department with one-week history of progressively worsening vague abdominal pain, bloating and lack of appetite.
- He denied any fever, chills or weight loss.
- Normal physical exam
- Mild absolute lymphocytosis, moderate thrombocytopenia
- Abnormal LFTs and lactic acidosis
- Markedly elevated LDH at >4300 IU/L (ref. range: 313-618 IU/L)
- **CT Scan of Abdomen/Pelvis:** Mild splenomegaly (13 cm max. span)

2



Diagnosis: Aggressive NK cell Leukemia
Further work-up recommended

3



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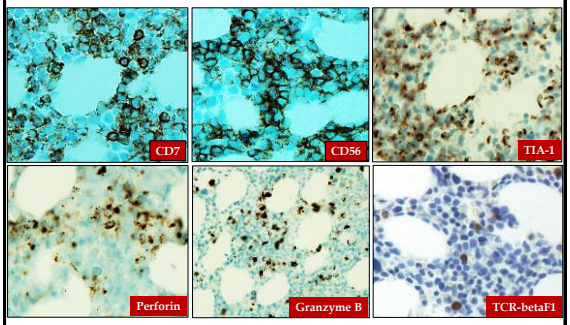

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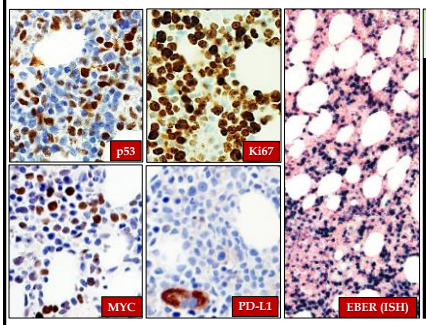
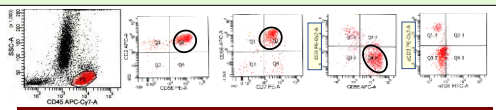


Negative IHC Stains

- CD4
- CD5
- CD8
- EBV-LMP1
- CD20
- CD30
- CD34
- β-catenin
- PD-L1

6

Flow Cytometric Analysis of Bone Marrow Aspirate



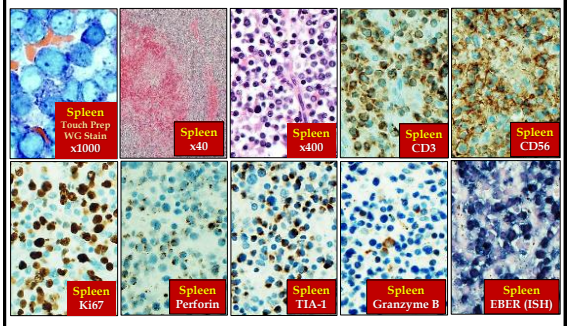
Negative

Surface CD3, cytoplasmic CD3, CD4, CD5, CD8, CD16, CD57, CD11b, TCRαβ, TCRγδ, TdT

Diagnosis and Further Work-up

- **Aggressive NK cell Leukemia**
- **EBV DNA Quantitative PCR of Serum:** >1,000,000 copies of EBV DNA/ml (Reportable range: 100 - 1,000,000 copies/ml)
- Sudden death on day 2
- **Autopsy (limited to chest, abdomen and pelvis):**
  - Exsanguination due to spontaneous splenic rupture

7



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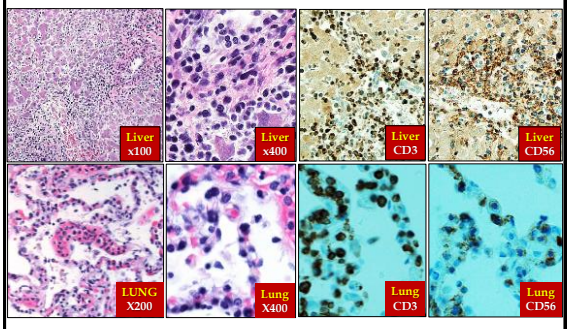
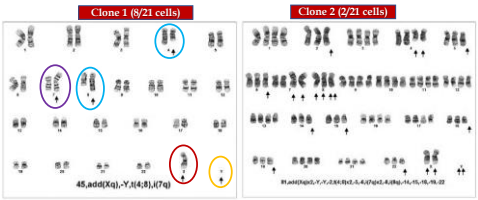


9

Cytogenetic Analysis of Bone Marrow

Chromosome Analysis

45,add(X)(q24),-Y,t(4;8)(q21;q13),i(7)(q10)[8]/81<4n>,idemx2,-2,-6,-14,-15,-18,-19,-22[2]/46,XY[11]



10

PCR for TCR Gene Rearrangement

There is no evidence of a monoclonal T-cell population (gamma) by PCR analysis, indicating a NEGATIVE result.

Results are POSITIVE for a clonal T-cell receptor Beta gene rearrangement.

Lymphoid Molecular Profile by NGS (75-Gene→173-Gene Panel)

Gene	Exons Tested	Genomic Alteration(s)	Mutation Effect	Allele Frequency	Pathogenic
KRAS	All	c.35G>C; p.G12A	MISSENSE	23%	YES
TP53	All	c.524G>A; p.R175H	MISSENSE	47%	YES
TET2	All	c.532G>T; pE178*	MISSENSE	28%	YES

Diagnosis

Aggressive NK-Cell Leukemia resulting in Spontaneous Splenic Rupture

CASE REPORT Open Access

Aggressive natural killer-cell leukemia with jaundice and spontaneous splenic rupture: a case report and review of the literature

11

Panel's Diagnosis

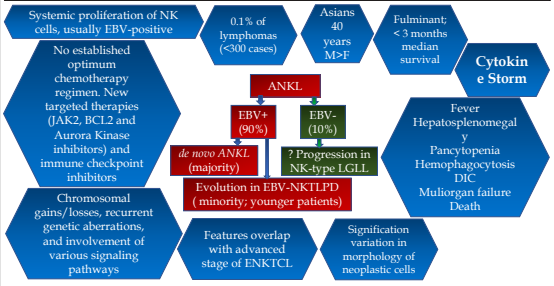
Aggressive NK-Cell Leukemia

Panel's Points of Discussion

1. Discussion of Aggressive NK-Cell Leukemia (ANKL)
2. Differential Diagnosis of ANKL
3. Mutational Landscape of ANKL

12

Discussion of Aggressive NK-Cell Leukemia (ANKL)



13

Differential Diagnosis

3 other entities are in the differential diagnosis of ANKL.

ENNKTCL, Non-nasal type	HSTCL	BPDCN
Why? Because of EBV association and significantly overlapping features in its advanced stage	**Why?** Because of hepatosplenic involvement, i(7)(q10) and a few overlapping mutations	**Why?** Because of CD2, CD7 and CD56 expression and a few overlapping mutations
How do we rule it out? Can be difficult See next two slides	**How do we rule it out?** Sinusoidal pattern of a T cell lymphoma with surface and cytoplasmic CD3 expression, and clonal TCR gene rearrangement	**How do we rule it out?** Skin nodule in an older patient, blastic morphology, expression of CD123, CD303, CD304, and absence of TCR gene rearrangement

14

Differential Diagnosis

Feature	ANKLL	ENKTCL, NON-NASAL TYPE
Median Age	40 years with 2 peaks in 3rd and 5th decade	50 years
Gender	M>F	M>F
Common Ethnic Groups	Asians	Asians; Native Americans in Mexico, Central America and South America
Common Symptoms	Fever, malaise, weight loss	Fever, malaise, weight loss
Duration of illness	Short	Short (not short for cutaneous form)
Organs Involved	Peripheral blood, bone marrow, liver, spleen	Skin, soft tissue, GI, testes (other organs less often; PB and BM in advanced stage.)
HP Syndrome	Can be present	Can be present
Cytomorphology	Variable	Variable
Necrosis/Angioinvasion	Necrosis+; Angioinvasion +/-	Necrosis+ ; Angioinvasion +
Immunophenotype	CD2+, sCD3 -, CD7+, CD16+/-, CD56+, CD57-, cCD3ε+, CGPs+, no TCR	CD2+, sCD3 -, CD7-/+, CD16-, CD56+, and cCD3ε+, CGPs+; no TCR
Chromosome Analysis	del(6)(q21-q25); 11q deletion; [i(7)(q10) is extremely rare.]	del(6)(q21-q25); i(6)(p10) [i(7)(q10) is extremely rare.]
aCGH	7p-, 17p-, 1q+	2q+, 1p-, 4q-, 5q-, 6q-, 7q-, 11q-, 15q-
TCR Gene Rearr.	None or rarely TCRγ ; TCRβ not without TCRγ	TCRγ sometimes; rarely TCRβ without TCRγ

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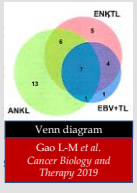
Differential Diagnosis and Mutational Landscape

Dufva O *et al.* Nat Commun. 2018 Apr 19;9(1):1568 | Huang L *et al.* Cell Res. 2018 Feb; 28(2): 172–186

Feature	Type of Mutations	ANKLL	ENKTCL, N-NT
Mutational Landscape (NGS)	**JAK-STAT Pathway**	*STAT3, JAK2*	*STAT3*
	Tyrosine	*PTPRK, PTPN4, PTPN23*	
	RAS-MAPK	*BRAF, KRAS, NRAS*	*BRAF, KRAS, NRAS*
	Epigenetic	*BCOR, KMT2D, TET2, SETD2, ARID2*	*BCOR, KMT2D, EP300, ARID1A,*
		DDX3X, FAS, TP53, MSH6, INPP5D, BRCAT, CDKN2A, SOCS1	*FAS, TP53, FOXO3, NOTCH2,*
	Tumor	*ITPKB, MEF2B* 3'-UTR truncation of *PDL1* Focal amplifications of *PDL1*	*PRDM1,* *KIT, MET, ITPKB, β*-Catenin 3'-UTR truncation of *PDL1* Focal amplifications of *PDL1*
Prognosis		**Grim**	**Usually grim**
EBV Association		**>90% patients**	**~100%**



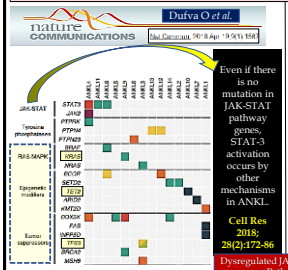
Venn diagram
Gao L-M *et al.* *Cancer Biology and Therapy 2019*

Others: Bolded Mutations: Shared | Bolded Black: More common in | Purple: Exclusively found in

16

Mutational Landscape of ANKL

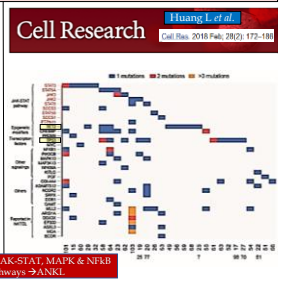
Dufva O *et al.* Nat Commun. 2018 Apr 19;9(1):1568



Even if there is no mutation in JAK-STAT pathway genes, STAT-3 activation occurs by other mechanisms in ANKL.

Cell Res 2018; 28(2):172-86

Huang L *et al.* Cell Res. 2018 Feb; 28(2): 172–186



Dysregulated JAK-STAT, MAPK & NFkB Pathways →ANKL

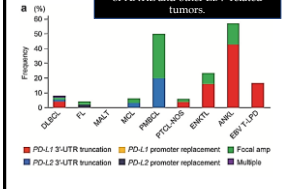
17

Mutational Landscape of ANKL

Kataoka K *et al.* Leukemia (2019) 33:1687–1699 https://doi.org/10.1038/s41375-019-0380-5

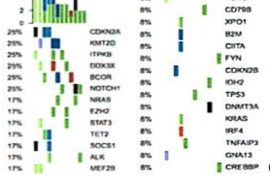
Frequent structural variations involving programmed death ligands in Epstein-Barr virus-associated lymphomas

These genetic defects in PD ligands play a pivotal role in immune-escape of ANKL and other EBV-related tumors.



Gao L-M *et al.*

Somatic mutations in *KMT2D* and *TET2* associated with worse prognosis in Epstein-Barr virus-associated T or natural killer-cell lymphoproliferative disorders



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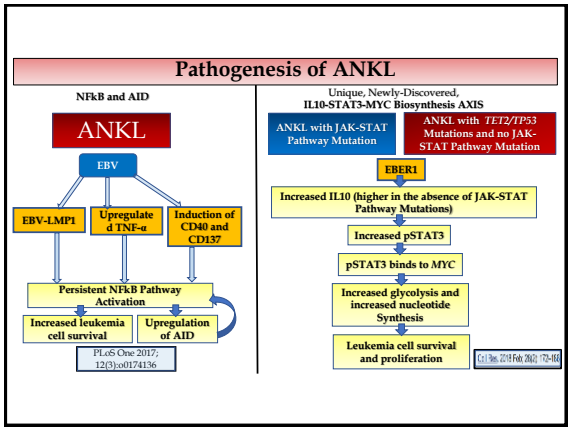
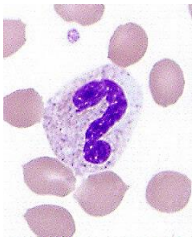

Pathogenesis of ANKL
NFkB and AID
ANKL
EBV
EBV-LMP1
Upregulated TNF-α
Induction of CD40 and CD137
Persistent NFkB Pathway Activation
Increased leukemia cell survival
Upregulation of AID
PLoS One 2017; 12(3):e0174136
Unique, Newly-Discovered, IL10-STAT3-MYC Biosynthesis AXIS
ANKL with JAK-STAT Pathway Mutation
ANKL with TET2/TP53 Mutations and no JAK-STAT Pathway Mutation
EBER1
Increased IL10 (higher in the absence of JAK-STAT Pathway Mutations)
Increased pSTAT3
pSTAT3 binds to MYC
Increased glycolysis and increased nucleotide Synthesis
Leukemia cell survival and proliferation

19



20

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Xiang-Nan Jiang M.D., Xiao-Qiu Li M.D.,PhD.

Affiliation:
Department of Pathology, Fudan University Shanghai Cancer Center, Shanghai 200032, China

E-mail:
lesleyjiang29@163.com

Case1

Clinical History:
Male,53-year-old, bloody stool for 2 mo

Biopsy Fixation Details:
Polypoid mass 3.5*3*3cm，the length of pedicle is 2.5cm

Description of Clinical Image if Any:
Colonoscopy: descending colon, a mass 40 cm away from anus

Details of Microscopic Findings:
The neoplastic spindled cells are inconspicuous or form loose whorled fascicles in the background of a prominent lymphoplasmacytic infiltrate.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
CD21, CD23, CD35, D2-40, SMA

Special Stains:
EBER in situ hybridization

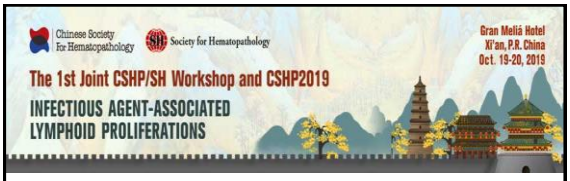
Cytogenetics:
None

Molecular Analysis:
None

Interesting Feature(s) of Submitted Case:
Rare special type of Infectious agent-association lymphoproliferation with unusual clinical, morphologic, immunophenotypic, or genetic features;

Proposed Diagnosis:
Inflammatory Pseudotumor-like Follicular/Fibroblastic Dendritic Cell Sarcoma of the digestive tract

Comments:
Inflammatory Pseudotumor-like Follicular/Fibroblastic Dendritic Cell Sarcoma typically involves the liver and spleen. Herein, we report these cases with unusual clinical features in hope to broaden the morphological spectrum and consummate the clinical data.



Case No. 1441462

Xiaohui Zhang
H. Lee Moffitt Cancer Center and Research Institute
Tampa, Florida, USA
Oct 19th, 2019

MOFFITT CANCER CENTER

1

- A-61-year-old man who presented to a local hospital with fevers, chills, fatigue, shortness of breath and left axillary mass in November 2014.
- A laboratory study revealed his WBC of 3.23 k/µL (ANC: 2.23k/uL), hemoglobin of 6.4 g/dl, MCV 86.6 fl, and platelet count of 66 k/µL.
- He also noted to have subcutaneous nodules developing over his chest, back and neck area.
- Initial CT scan of abdomen revealed an enlarged spleen and liver.
- Other imaging studies including Doppler ultrasound of lower extremities, EGD and colonoscopy, echocardiography and chest X-ray were all negative.

2

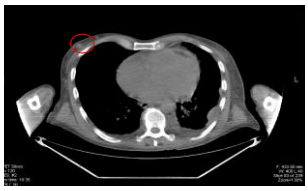
- He underwent fine needle aspiration and core biopsies (x2) performed on his left axillary and right neck mass, respectively.
- Large atypical lymphoid cells were identified.
- Per report, a flow cytometry panel was performed and showed mainly T-lymphocytes with CD4 to CD8 ratio of 1:100.
- Outside diagnosis: Aggressive peripheral T cell lymphoma

3

Visit at Moffitt

- Physical examination revealed cervical and axillary adenopathy and multiple subcutaneous masses on his trunk with the largest located in the right flank region and measuring 6 x 3 cm
- PET/CT showed hypermetabolic, non-bulky adenopathy on both sides of diaphragm. Largest measuring 2cm, with SUV ranging from 4.5-9.2.
- Additional laboratory studies showed positive serology for HIV-1 in 12/2014; The patient was started on Anti-Retroviral Therapy (ART) with Stribild.
- The patient developed dyspnea and mild pleural effusions in Jan 2015.
- A left thoracentesis was performed. The collected fluid was clear and reportedly negative for tumor cells.

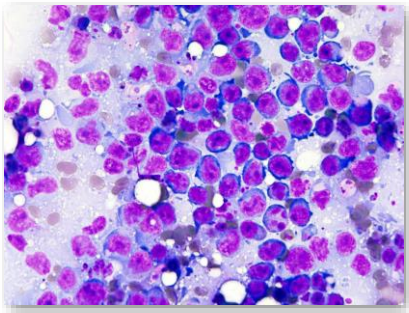
4



Contrast CT scan showed hepatosplenomegaly, mild lymphadenopathy and subcutaneous lesions in chest wall.

5

Subcutaneous nodule, left shoulder, excisional biopsy



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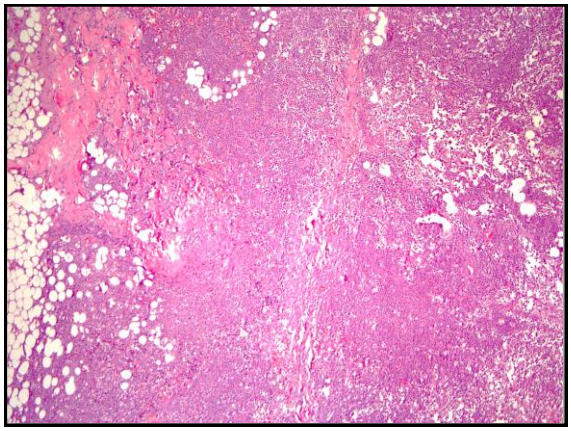

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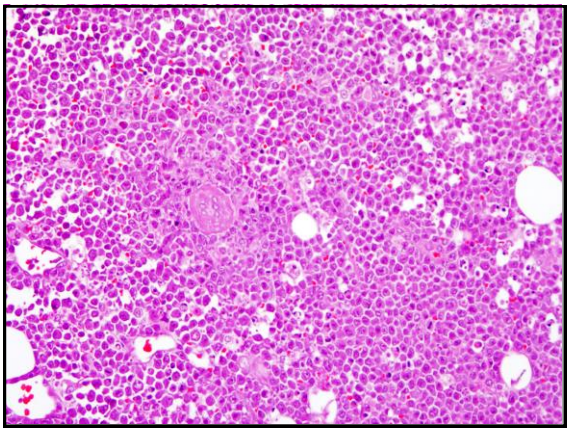

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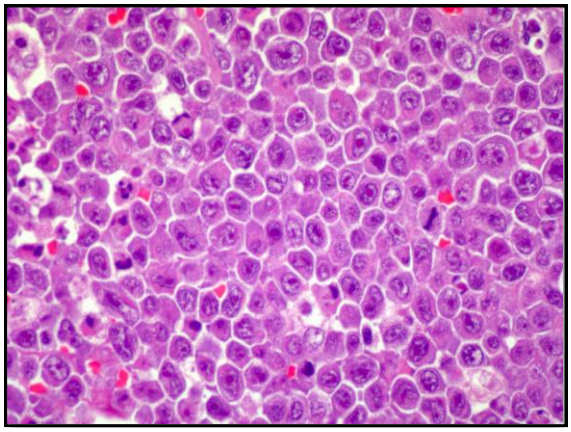


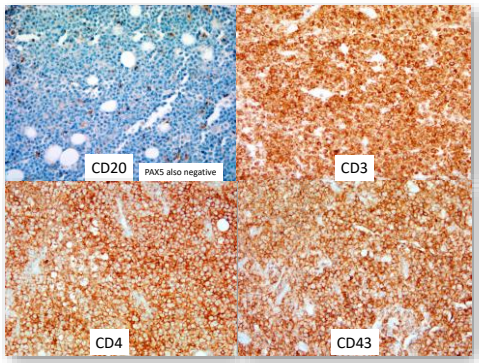
9

Flow cytometry identified a population of large cells:

- Positive for CD45, dim CD4, CD30 (subset), HLA-DR, CD38 (moderate), kappa (weak or partial)
- Negative for:
  - CD19, CD20, CD22, lambda
  - CD2, sCD3, CD5, CD7, CD8
  - CD16, CD56, CD57, TCR-αβ, TCRγδ, CD10, CD23, FMC7, CD25, CD34, PD-1, CD138

10

Immunohistochemical Study



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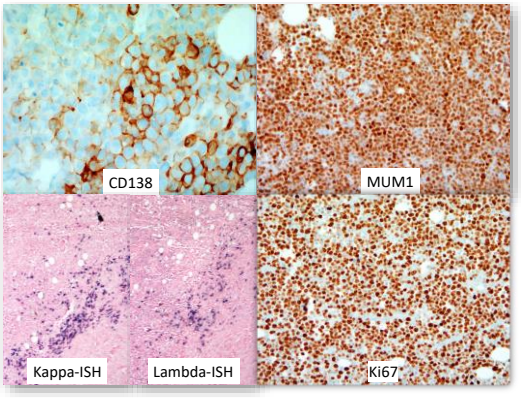

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Summary of IHC/ISH Findings

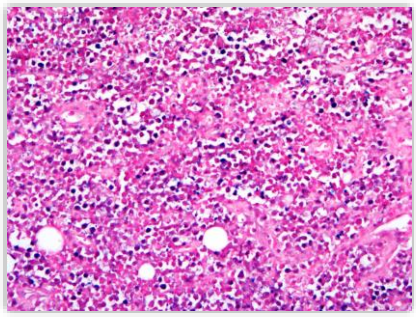
- Positive:
  - CD45
  - **CD3 (cytoplasmic)**
  - **CD4 (diffuse, strong)**
  - **CD43 (diffuse, strong)**
  - MUM-1 (diffuse strong)
  - CD138 (subset)
  - CD79a (focally, weak)
  - CD30(small subset)
  - EMA (subset)
  - Ki67 (95%)
- Negative:
  - CD20
  - PAX5
  - TCL1
  - CD2
  - CD7
  - CD8
  - TIA, Granzyme B, Perforin
  - CD56
  - ALK1
  - BCL-2
  - BCL-6

13

Differential Diagnosis:

- Peripheral T-cell lymphoma
- ~~Anaplastic large cell lymphoma~~
- ~~Extranodal T/NK cell lymphoma~~
- High grade B cell lymphoma
- Plasmablastic lymphoma
- Plasmacytoma, anaplastic variants
- Other neoplasms…

14



EBER In Situ Hybridization

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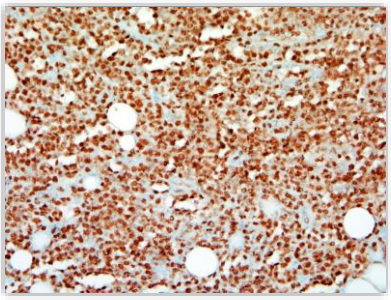
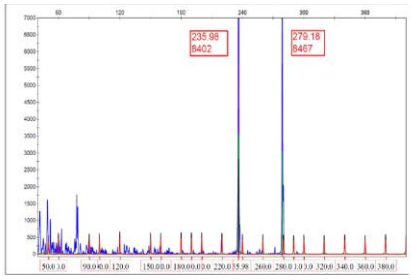


HHV8

16



PCR for immunoglobulin Kappa light chain gene rearrangement
PCR for T cell receptor β or γ gene rearrangements was negative

17

Cytogenetics

•FISH studies showed no BCL2, C-MYC, or BCL6 rearrangement

•Abnormal BCL-6 signals consistent with trisomy or tetrasomy of chromosome 3, and extra copies of chromosome 11

•The whole genome SNP microarray analysis detected three deletions including two in 13q and one in 14q, as well as duplications in chromosomes 3,4,11,13, and 19

18

Differential Diagnosis:

- ~~Peripheral T-cell lymphoma~~
- ~~Anaplastic large cell lymphoma~~
- ~~Extranodal T/NK cell lymphoma~~
- ~~High grade B cell lymphoma~~
- ~~Plasmablastic lymphoma~~
- ~~Plasmacytoma, anaplastic variants~~
- Other neoplasms…

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Diagnosis

Extracavitary Primary Effusion Lymphoma, HHV-8+, EBV+ and HIV-1-Associated

20

Follow-up

- Chemotherapy with HperCVAD was initiated in 2/2015 (x 6 cycles), achieved complete remission
- Underwent autologous hematopoietic stem cell transplant (7/8/2015)
- Last follow-up on Jun 29, 2017. No evidence of tumor recurrence.

21

DISCUSSION

- PEL typically manifests as malignant effusion in the body cavities, mostly affecting patients with immunodeficiency, most frequently HIV+ patients (70%).
- Extracavitary or solid variant of PEL occurs in lymph nodes and extranodal sites such as gastrointestinal tract, skin, lung, liver, spleen and CNS; no cavitary effusions
- Shows similar morphologic features to classic PEL.

Kalogeraki, A, et al. Diagn Cytopathol. 2015 (43): 144

22

Extracavitary PEL Immunophenotype:

- No major difference from classic PEL
  - CD45, CD30, CD38, CD138
  - Lacks Pan B cell markers and Ig light chains
- Lower expression of CD45; slightly more frequent expression of CD20, CD79a and CD138 *
- Aberrant T cell markers (CD3, CD43, and CD4) can be expressed in up to 50% of cases **
- CD3 expression can be focal, weak or strong
- May pose a diagnostic challenge

* Pan Z, et al. Am J Surg Pathol. 2012(8): 1129
**Guillet S., et al. Am J Hematol. 2016(91): 233

23

- HHV8 + in all cases.
- EBV co-infection is found in 80-90% of extracavitary cases.

- Previous report* showed extracavitary PEL has better survival when compared to classic PEL (8 vs 29 cases; 11 mo vs 3 mo).
- More recent study** suggested no significant OS difference between classic PEL and the extracavitary variant, but a better disease free survival.

* Chadburn A, et al. Am J Surg Pathol. 2004: 1401
**Guillet S., et al. Am J Hematol. 2016: 233

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Joint CSHP and SH Workshop 2019

Session Three

Sophie Song, MD PhD
Alexander Nobori, MD

Dept. of Pathology and Laboratory Medicine
UCLA Medical Center
Los Angeles, CA, USA

1

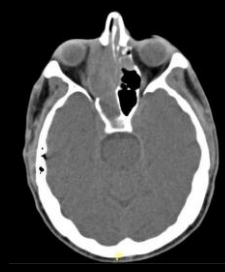
Clinical Presentation

- 52 y/o male with history of HIV and chronic kidney disease, presents with facial pain, headache, and sinus mass
- Social history
  - homeless since 2017
  - extensive travelling worldwide
  - lived in Israel from 2008 to 2014 with Bedouin nomads in the Jordan River Valley
- Physical exam: right eye proptosis and tenderness over the right sinus and right face
- Laboratory testing: pancytopenia and LDH at 254

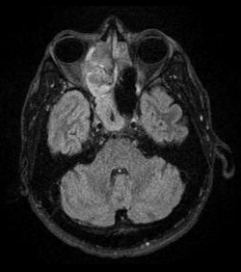
2

Imaging Studies

CT Sinus



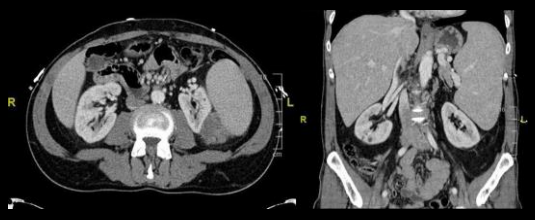
MRI Brain



3

Imaging Studies

CT Abdomen and Pelvis



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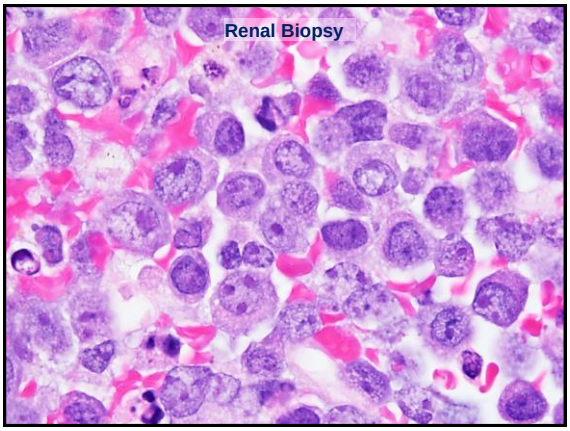
Specimens

- Renal biopsy
- Bone marrow biopsy
- Liver biopsy
- Nasal biopsy (performed at OSH, and reviewed at UCLA)

5

Renal Biopsy



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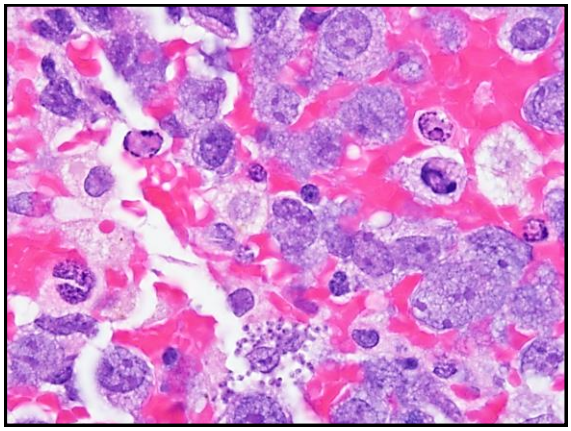

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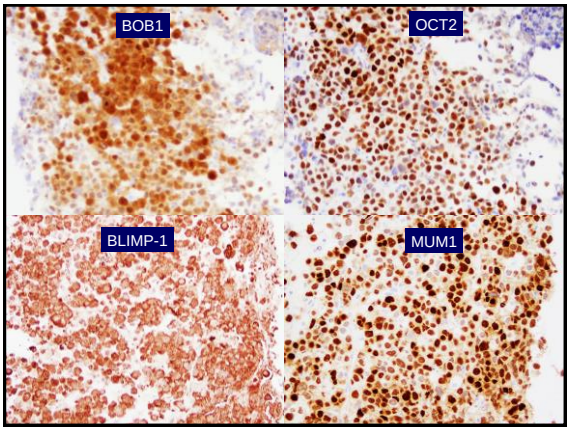
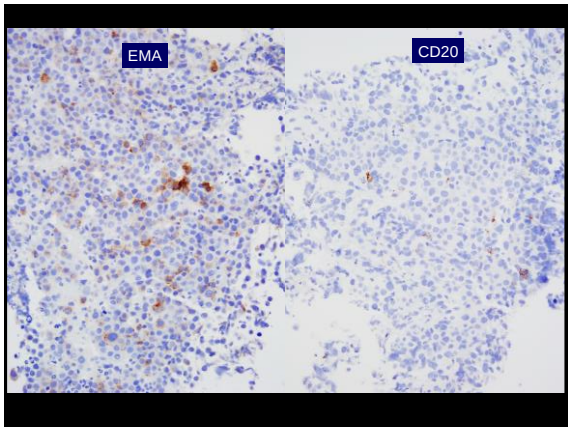

BOB1
OCT2
BLIMP-1
MUM1

8


EMA
CD20

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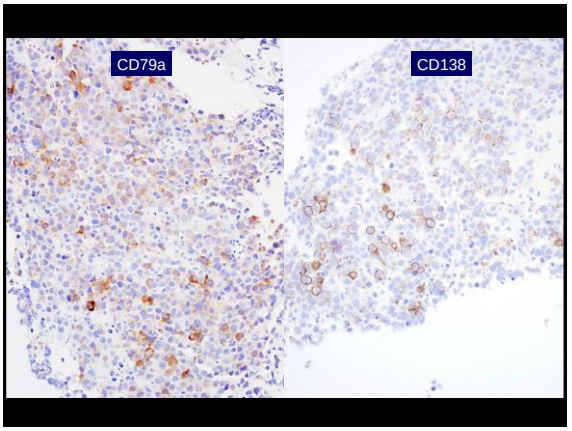
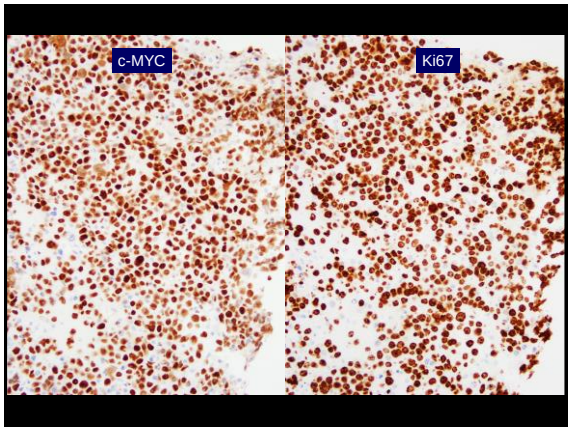
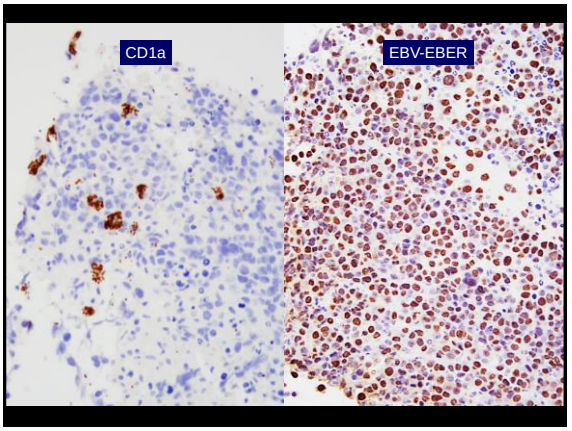

CD79a
CD138

10


c-MYC
Ki67

11


CD1a
EBV-EBER

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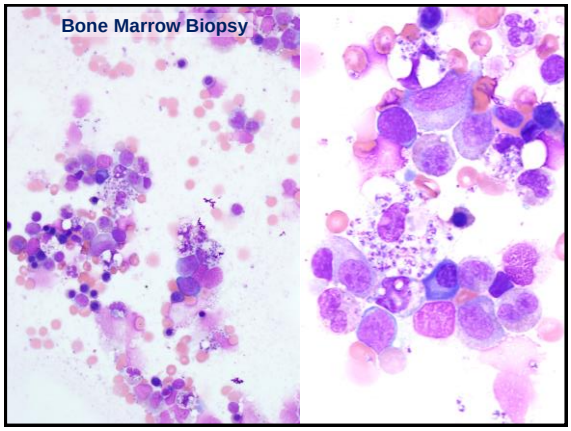

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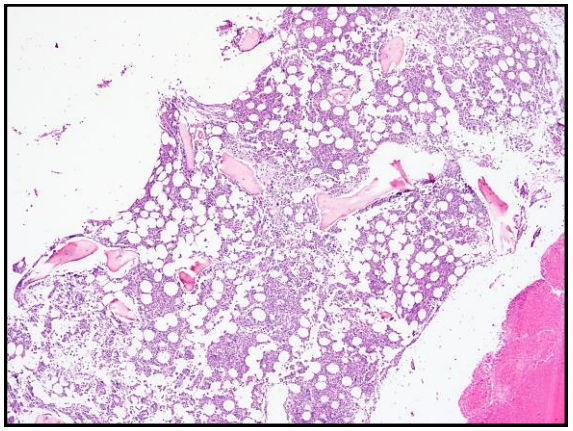

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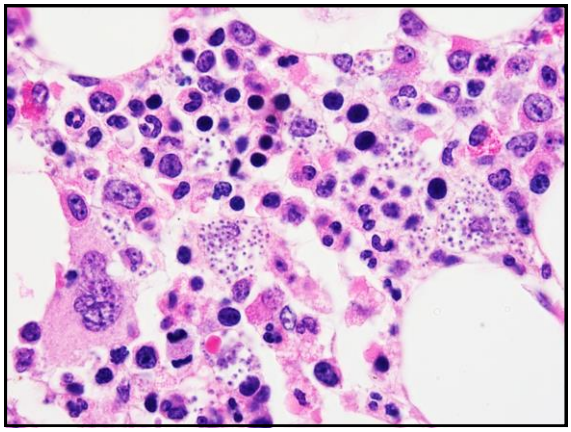

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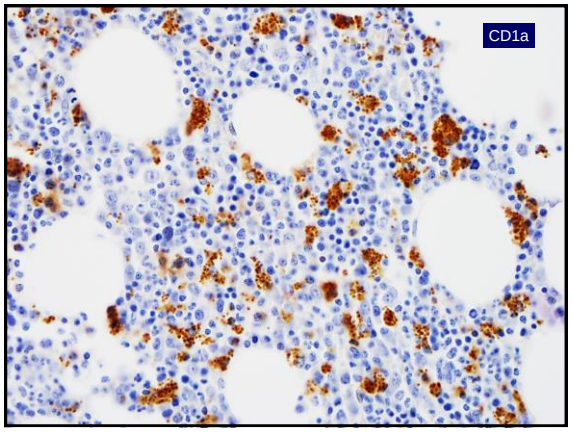

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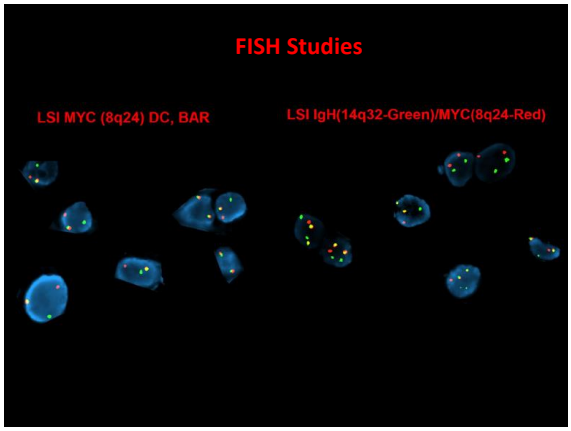

17

Case Summary

- 52-year-old man with HIV and sinus mass
- Morphology: large neoplastic cells with plasmacytic differentiation and histiocytes with Leishmaniasis amastigotes
- Immunophenotype:
  - **Positive**: CD138, BLIMP1, CD79a, CMYC, EBV-EBER(ish)
  - **Negative**: CD20, PAX5, ALK, CD45, HHV8
- FISH: t(8;14) MYC-IGH translocation

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Comparison of several large cell lymphomas

	Plasmablastic lymphoma	EBV positive DLBCL	ALK-positive large B-cell lymphoma
Clinical associations	**HIV (70%), immune suppression**	None	None
Site	Oral cavity, nasal sinuses	Nodal or extranodal (lungs, GI tract)	Nodal, mediastinal mass
Morphology	Monomorphic plasmablasts or plasmacytoid	Variable; immunoblasts and Hodgkin/ RS-like cells	Monomorphic large immunoblast-like B cells
Immunophenotype	CD20: negative PAX5: negative **CD38: positive** **CD138: positive**	**CD20: positive** **PAX5: positive** CD38: negative CD138: negative	CD20: negative PAX5: negative **CD38: positive** **CD138: positive**
EBV	Positive (60-75%)	**Positive (100%)**	**Negative (100%)**
MYC rearrangement	**Positive (50%)**	Negative	Negative

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EBV in Plasmablastic lymphoma

- 60-75% of cases positive for EBV by in situ hybridization
- EBV more frequently positive in HIV and post-transplant patients
- EBV-positive PBL has **better event-free survival** than EBV-negative PBL **(2015 Loghavi)**
- EBV-positive PBL more frequently demonstrates **MYC rearrangement (2019 Miao)**

20

References

- Yamada T, Hara T, Goto N, Iwata H, Tsurumi H. Follicular lymphoma suggested to transform into EBV-negative plasmablastic lymphoma. Int J Hematol. 2019 Jun;109(6):723-730.
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- Rodrigues-Fernandes CI, de Souza LL, Santos-Costa SFD, Silva AMB, Pontes HAR, Lopes MA, de Almeida OP, Brennan PA, Fonseca FP. Clinicopathological analysis of oral plasmablastic lymphoma: A systematic review. J Oral Pathol Med. 2018 Nov;47(10):915-922.
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21

A Case of HIV-related pulmonary lymphoproliferative disorder ——Called DLBCL?

Wang XJ, Yang SJ, Wang XM, Chen GQ, Wang LP*

Pathology Department of
China-Japan Union Hospital of Jilin University

2019.10.19 XI'AN

1

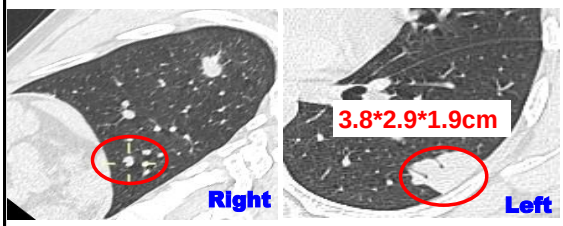
Case information

- 28 years old male, had intermittent mild fever for three months since January, 2019, and showed limited antibiotic response.
- No dry cough, night sweats and weight loss were reported.
- The patient had the history of the diarrhea, tarry stool and herpes zoster infection for two years. HIV testing is positive.

2

CT scan images

CT scan presented bilateral high intensity pulmonary nodules



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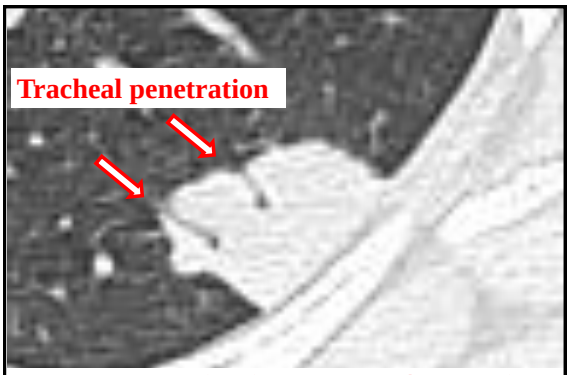


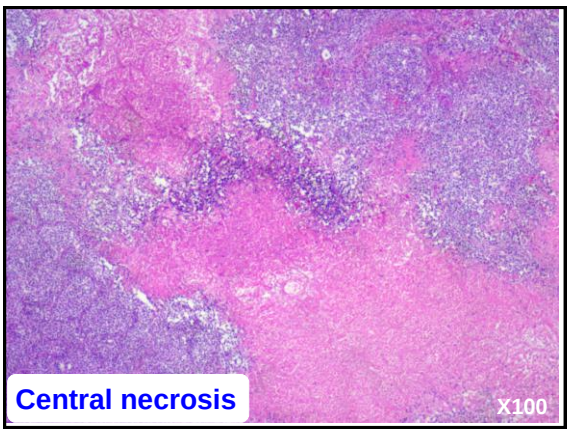
Provide a distinctive image reference to Pulmonary LPD

4

Pathology Changes

- Wedge resection of the biggest nodule in the lower lobe of left lung was submitted for pathology examination.
- Grossly, the lesion, displayed gray-yellowish cut surface in the central and whitish in the peripheral nodule.

5



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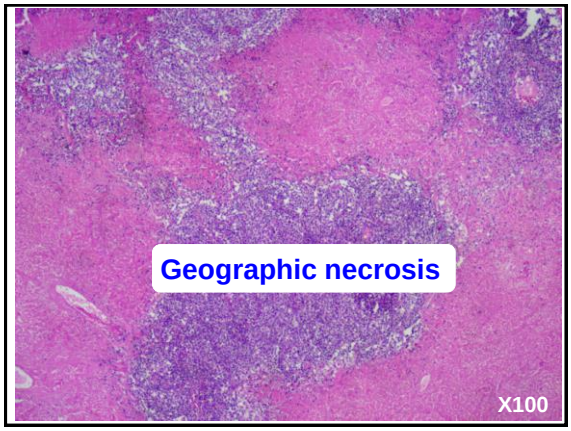
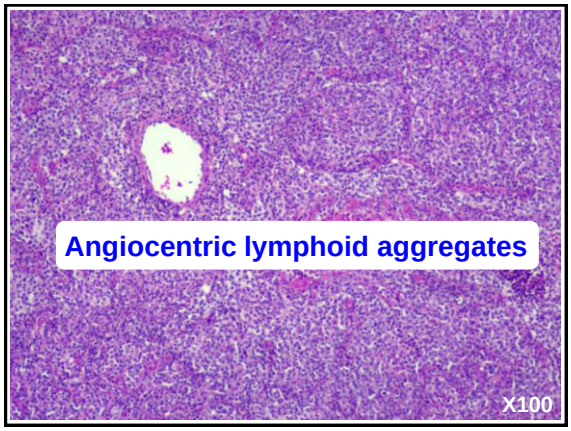
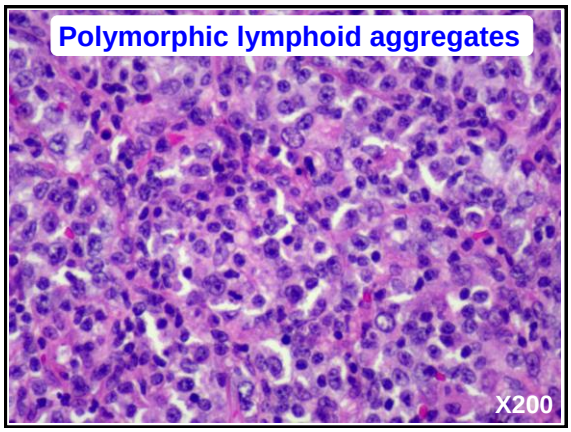

Geographic necrosis
X100

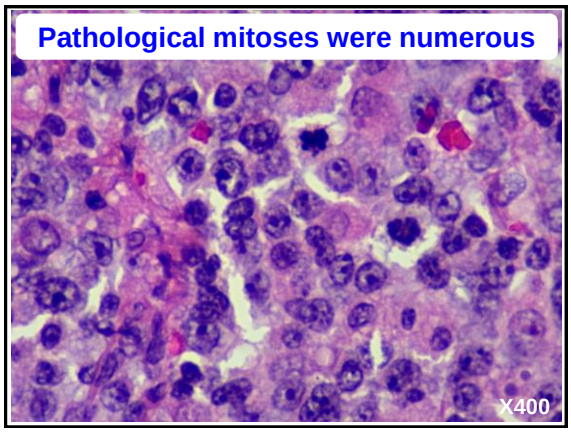
7


Angiocentric lymphoid aggregates
X100

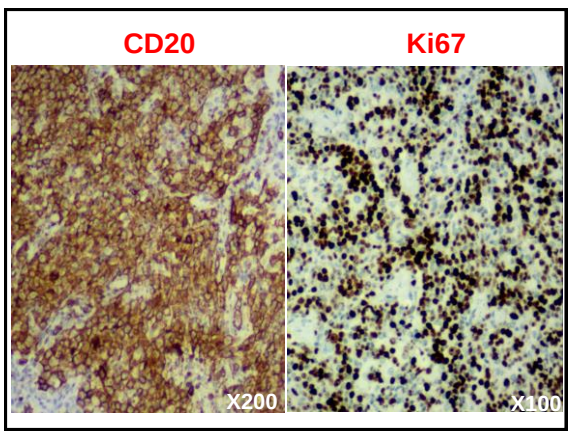
8


Polymorphic lymphoid aggregates
X200

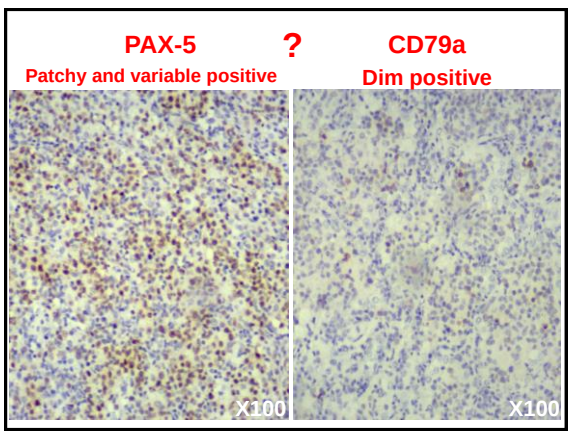
9


Pathological mitoses were numerous
X400

10

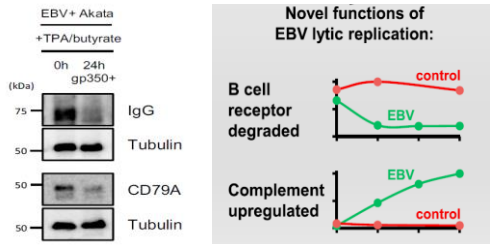

CD20
Ki67
X200
X100

11


PAX-5
?
CD79a
Patchy and variable positive
Dim positive
X100
X100

12

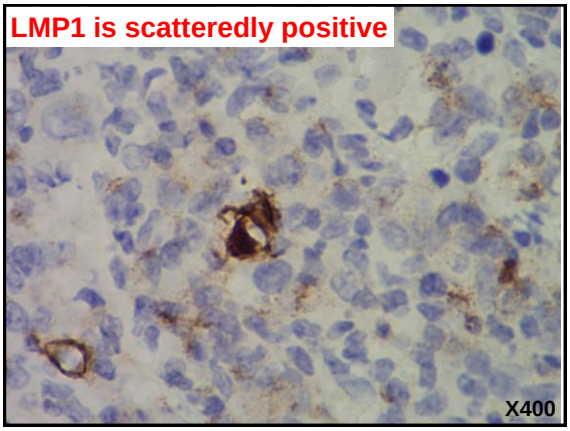
B cell receptor is degraded by EBV infection



Ersing I, Nobre L, Wang LW et al. Cell Rep. 2017 May 16;19(7):1479-1493.
A Temporal Proteomic Map of Epstein-Barr Virus Lytic Replication in B Cells.

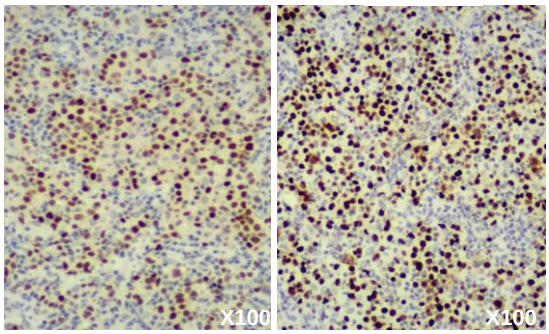
13

LMP1 is scatteredly positive



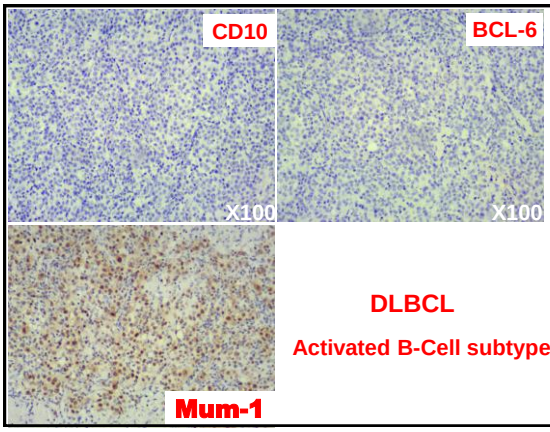
14

EBNA2 EBER



Courtesy. of Dr. Zheng yuanyuan

15



16

Consulted by Beijing Friendship Hospital

Immunodeficiency related DLBCL (activated B-Cell subtype)

CD20, Mum-1, EBER and EBNA2 were diffusely positive.

PAX-5 and BCL-2 were partially positive.

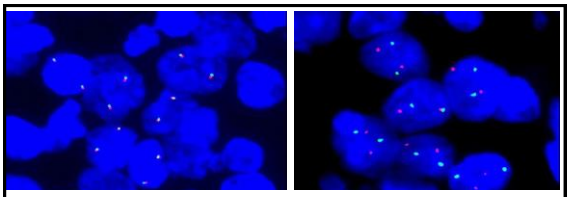
LMP1, CD68 and CD3 were scatteredly positive.

Ki67 index was about 80%.

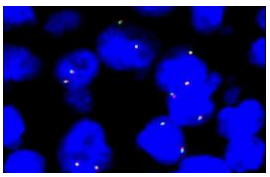
C-myc were about 60% positive.

CD30, CD79a, CD10, BCL-6, ALK, HHV-8,CD38, CD138 and CyclinD1 are negative.

17



No C-myc gene rearrangement No TP53 gene abnormalities



No BCL-2 gene abnormalities

18

Management and prognosis

- The patient rejected to receive immunochemotherapy
- He took oral anti-HIV drug and Chinese medicine with unknown ingredients
- No extrapulmonary lesions were found by PET-CT scan after 6 months follow up

19

Differential diagnosis

Negative staining of HHV-8*,CD38 and CD138.

✖

Plasmablastic lymphoma

HHV8-associated lymphoproliferative disorders

*HHV-8 staining is courtesy of professor Zheng yuanyuan

20

Differential diagnosis and discussion

Immunodeficiency-associated LPD

HIV-related DLBCL ? **LYG grade 3**

Chemotherapy and poor outcome*

*Song JY, Pittaluga S, Dunleavy K, et al, Jaffe ES. Am J Surg Pathol. 2015 Feb;39(2):141-56.

21

LYG Lymphomatoid granulomatosis (LYG) is an **angiocentric** and angiodestructive **lymphoproliferative disease** involving extranodal sites, composed of **EBV-positive B cells** admixed with **reactive T cells**, which usually **predominate.** The lesion has a spectrum of histological grade and clinical aggressiveness, which is related to **the proportion of large B cells**.

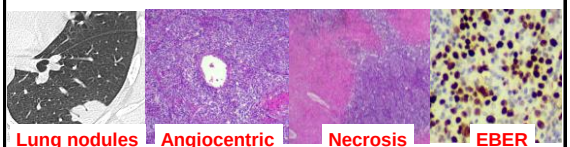
Grade 3 still show an **inflammatory background**, but contain **large atypical cells** that are readily identified by CD20 and can form **larger aggregates**. Markedly pleomorphic, and Hodgkin-like cells are often present, and **necrosis** is usually extensive. **EBER positive cells were numerous (>50 /HPF)**

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue, **2016**

22

LYG Grade 3 ICD-O codes 9766/3

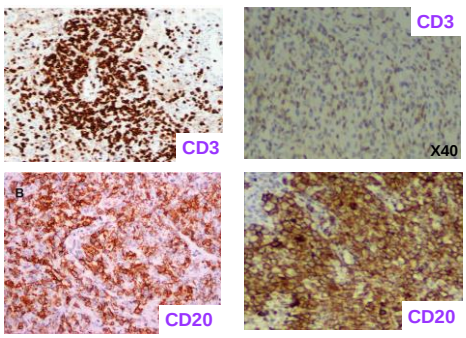
1. Most favorite site is lung
2. Angiocentric lymphoproliferative disease
3. Obvious necrosis
4. EBER positive cells were numerous (>50 /HPF)



Lung nodules | Angiocentric | Necrosis | EBER

23

LYG Grade 3 — Our case



Jaffe ES. Am J Surg Pathol. 2015 Feb;39(2):141-56.

24

Differential diagnosis and discussion

Immunodeficiency-associated LPD

How to define the "larger aggregates" (Cutoff number)?

HIV-related DLBCL = ? LYG grade 3

Chemotherapy and poor outcome

25

Acknowledgement

Dr. Zheng Yuanyuan,
Beijing friendship hospital,
Capital medical university

Dr. Pan Zenggang
Associate Professor,
Department of Pathology ,
Yale University School of Medicine

中日联谊医院
CHINA-JAPAN UNION HOSPITAL OF JILIN UNIVERSITY

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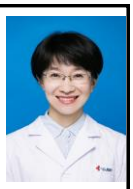

Thanks

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The 1st Joint CSHP/SH Workshop 2019

SESSION 3

HIV and HHV8-Related Lymphomas

CASE 4 (No 1455006)

Christos Masaoutis
Resident in Anatomical Pathology
Evangelismos General Hospital
Athens, Greece

1

59-year-old immunocompetent man with long-standing, mediastinal lymphadenopathy (localized)

2000: pretracheal lymphadenopathy → anti-TB → no response

2009: (chest CT) enlarged lymph nodes compared to 2000

2017: chest pain

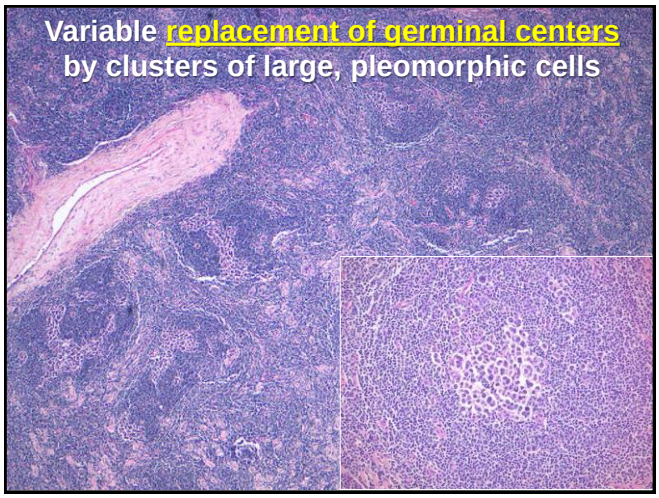
→ Excision of all hypermetabolic (PET/CT) lymph nodes

HIV (-) HBV (-) HCV (-)

work-up unremarkable (mild hypogammaglobulinemia)

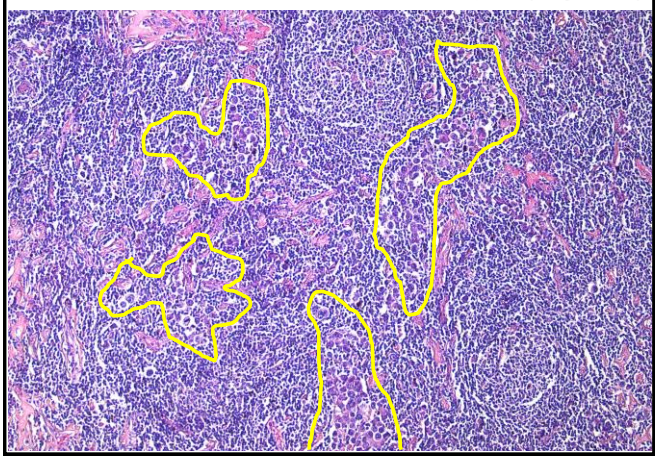
2

Variable replacement of germinal centers by clusters of large, pleomorphic cells



3

VERY RARE involvement of the interfollicular space (only focus)



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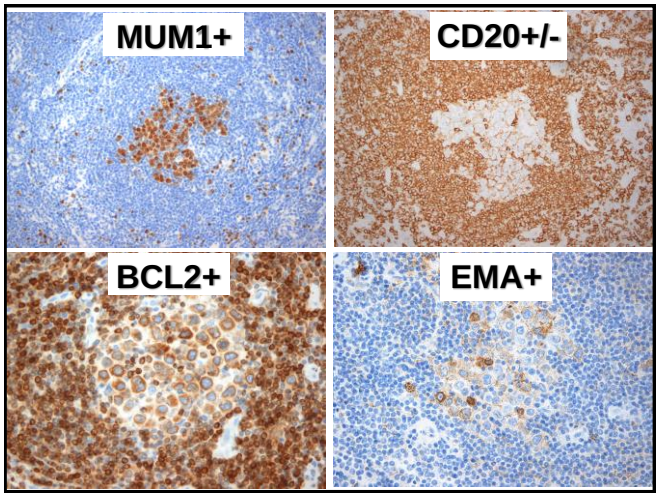

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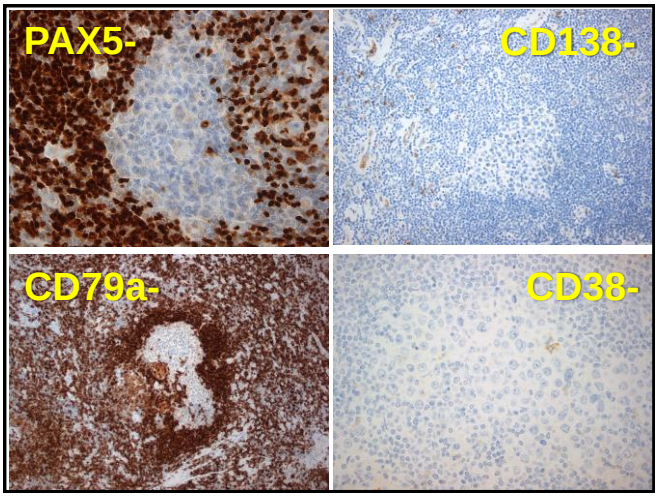

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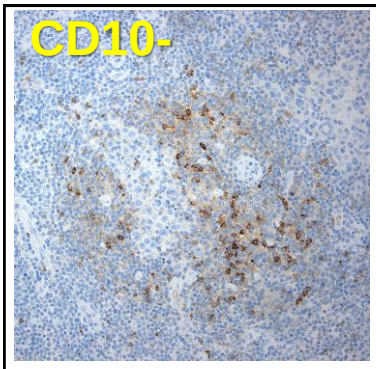
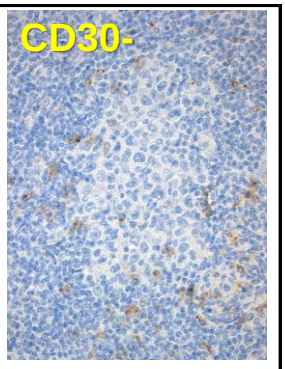




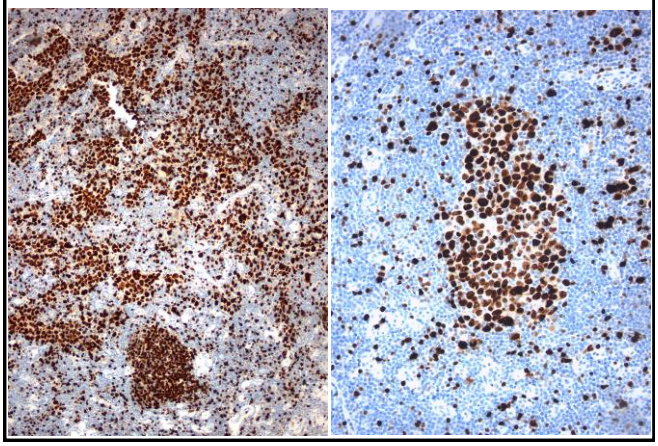
ALSO NEGATIVE:

CD3 CD15 BCL6 PGM1 CD56 p63

AE1/AE3 Melan-A

7

Ki67 ~100%



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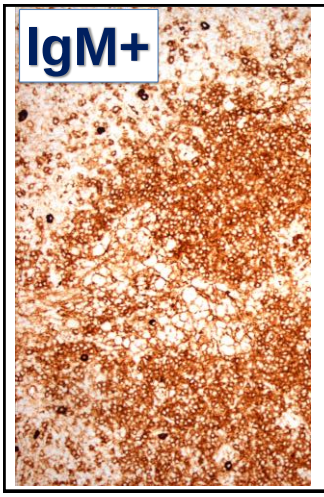
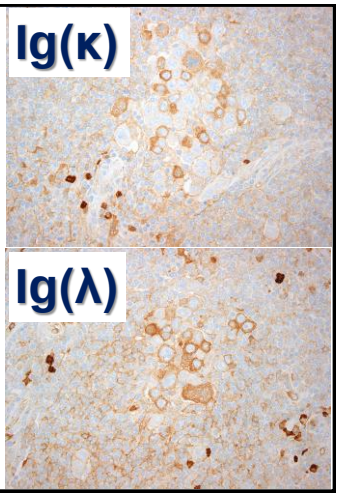
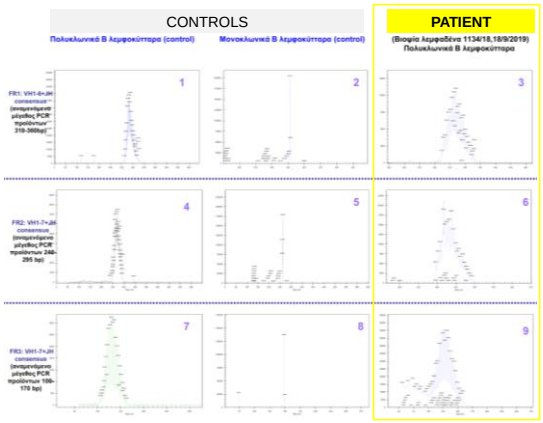




9

Polyclonal immunoglobulin rearrangements



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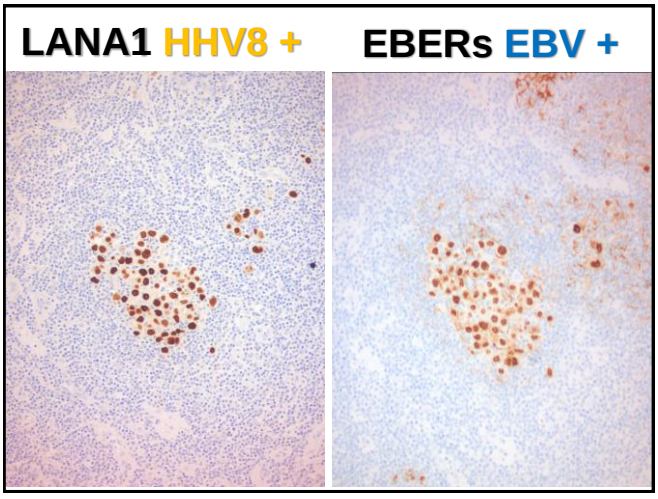


11

GERMINOTROPIC LYMPHO-PROLIFERATIVE DISORDER (GLPD)

=

large ("plasmablastic") cells

+

striking predilection for germinal centers
(*limited* interfollicular growth is allowed)

+

"null" immunophenotype, may only MUM1+
may express plasma- of B- or T-(**!**) cell markers

+

poly- or oligoclonal; Ig *may* be monotypic

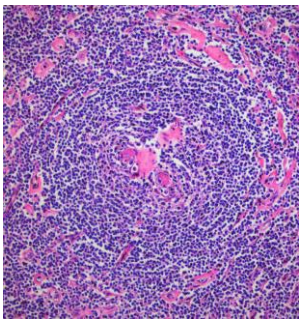
+

HHV8 & EBV (*latency type I*) co-infection

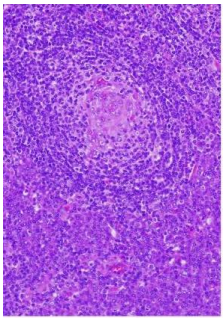
12

There may be **Castleman-like** areas in germinotropic LPD

REGRESSED FOLLICLES



OUR CASE



(Bhavsar et al. 2017)

13

DIFFERENTIAL DIAGNOSIS:
HHV8-associated "plasmablastic" lymphomas (WHO 2017)

	GERMINOTROPIC LPD	EXTRACAVITARY PEL	HHV8+ DLBCL, NOS (MCD-ASSOCIATED)
HHV8	+	+	+
EBV	+	+/-	-
GERMINOTROPIC LOCALIZATION	+	-	-
IMMUNO-COMPROMISED	-	+	+
PRESENTATION	localized nodal disease no systemic symptoms	nodal or extranodal	generalized, disease, systemic symptoms
BEHAVIOUR	INDOLENT	AGGRESSIVE	AGGRESSIVE
MONOCLONALITY	-	+	+
Ig EXPRESSION	+	-/+	+
SOMATIC HYPERMUTATIONS	+	+	-

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CLINICAL OUTCOME

NO OTHER TREATMENT BESIDES EXCISION

2 YEARS AFTER DIAGNOSIS:

PATIENT **WITHOUT DISEASE**

15

EXCEPTIONS

16

EXCEPTIONS

	GERMINOTROPIC LPD	EXTRACAVITARY PEL	HHV8+ DLBCL, NOS (MCD-ASSOCIATED)
HHV8	+	+	+
EBV	+	+/-	-
GERMINOTROPIC LOCALIZATION	+	-	-
IMMUNO-COMPROMISED	-	+	+
PRESENTATION	localized nodal disease no systemic symptoms	nodal or extranodal	generalized, disease, systemic symptoms
BEHAVIOUR	INDOLENT	AGGRESSIVE	AGGRESSIVE
MONOCLONALITY	-	+	+
Ig EXPRESSION	+	-/+	+
SOMATIC HYPERMUTATIONS	+	+	-

17

EXCEPTIONS: EBV

One case of EBV-negative germinotropic LPD

(Gonzalez-Farre et al. 2017)

18

EXCEPTIONS

	GERMINOTROPIC LPD	EXTRACAVITARY PEL	HHV8+ DLBCL, NOS (MCD-ASSOCIATED)
HHV8	+	+	+
EBV	+	+/-	-
GERMINOTROPIC LOCALIZATION	+	-	-
IMMUNO-COMPROMISED	-	+	+
PRESENTATION	localized nodal disease no systemic symptoms	nodal or extranodal	generalized, disease, systemic symptoms
BEHAVIOUR	INDOLENT	AGGRESSIVE	AGGRESSIVE
MONOCLONALITY	-	+	+
Ig EXPRESSION	+	-/+	+
SOMATIC HYPERMUTATIONS	+	+	-

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EXCEPTIONS: "GERMINOTROPIC" LOCALIZATION

(1) IN **HHV8+ MCD-ASSOCIATED LYMPHOMA** (EBV-)
(Gonzalez-Farre et al. 2017)

(2) IN **EBV+ DLBCL** (HHV8-)
(Lorenzi et al. 2016) (Asakrah et al. 2018)

(3) IN **EARLY PRIMARY EFFUSION LYMPHOMA (PEL)**
(Fend et al. 2012)

(4) IN **EBV IN HHV8+ MCD**
(Gonzalez-Farre et al. 2017)

20

EXCEPTIONS: "GERMINOTROPIC" LOCALIZATION

(1) IN **HHV8+ MCD-ASSOCIATED LYMPHOMA** (EBV-)
(Gonzalez-Farre et al. 2017)

(2) IN **EBV+ DLBCL** (HHV8-)
(Lorenzi et al. 2016) (Asakrah et al. 2018)

(3) IN **EARLY PRIMARY EFFUSION LYMPHOMA (PEL)**
(Fend et al. 2012)

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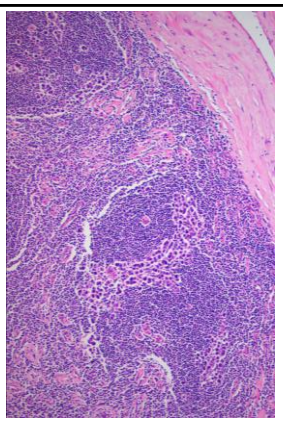
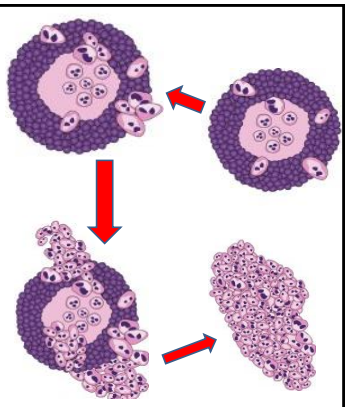
(Gonzalez-Farre et al. 2017)

21





OUR GERMINOTROPIC LPD

Hypermutated cells, EBV-positive

HHV8+ PLASMABLASTIC PROLIFERATION IN MCD by Dr J Goodlad (Chadburn et al. 2017)

Naïve cells, EBV-negative

22

EXCEPTIONS: "GERMINOTROPIC" LOCALIZATION

(1) IN **HHV8+ MCD-ASSOCIATED LYMPHOMA** (EBV-)
(Gonzalez-Farre et al. 2017)

(2) IN **EBV+ DLBCL** (HHV8-)
(Lorenzi et al. 2016) (Asakrah et al. 2018)

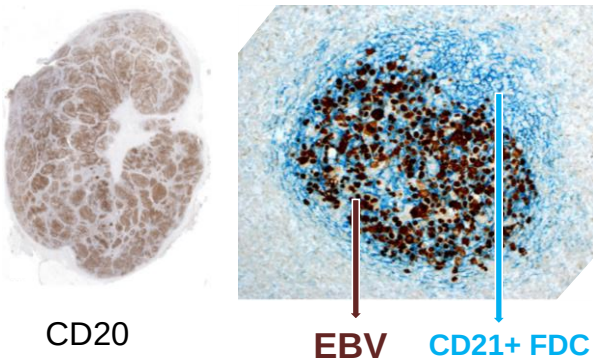
(3) IN **EARLY PRIMARY EFFUSION LYMPHOMA (PEL)**
(Fend et al. 2012)

(4) IN **EBV IN HHV8+ MCD**
(Gonzalez-Farre et al. 2017)

23

NODULAR INTRAFOLLICULAR EBV+ DLBCL
(CD20+, LMP1+, HHV8-)

images from (Lorenzi et al. 2016)



24

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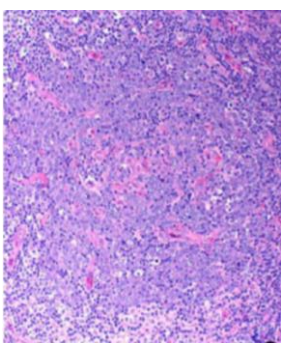
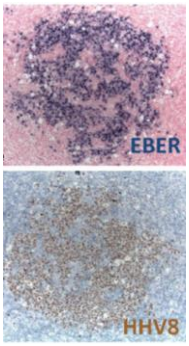
(Gonzalez-Farre et al. 2017)

25

"Early PEL" vs Germinotropic LPD





*"These cells were coinfected by EBV and HHV-8, but **lacked Ig expression**, favouring PEL over germinotropic LPD"*

(Fend et al. 2012)

subm. by Dr Peker, **Workshop of the XV. Meeting of the EAHP/SH.** Uppsala, Sweden (2012)

26

EXCEPTIONS: "GERMINOTROPIC" LOCALIZATION

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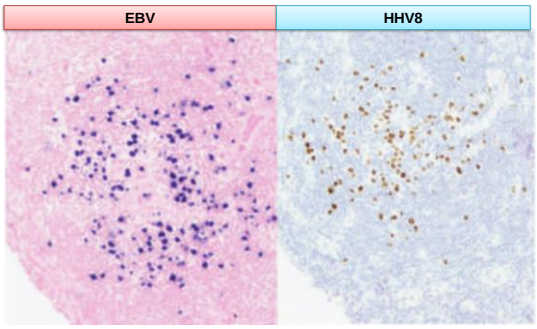
(Gonzalez-Farre et al. 2017)

(2) IN **EBV+ DLBCL** (HHV8-)
(Lorenzi et al. 2016) (Asakrah et al. 2018)

(3) IN **EARLY PRIMARY EFFUSION LYMPHOMA (PEL)**
(Fend et al. 2012)

(4) IN **EBV IN HHV8+ MCD**
(Gonzalez-Farre et al. 2017)

27



(Gonzalez-Farre et al. 2017)

SMALL EBV+ HHV8+ CELLS IN ISOLATED REGRESSIVE FOLLICLE IN **MULTICENTRIC CASTLEMAN DISEASE**

28

EXCEPTIONS

	GERMINOTROPIC LPD	EXTRACAVITARY PEL	HHV8+ DLBCL, NOS (MCD-ASSOCIATED)
HHV8	+	+	+
EBV	+	+/-	-
GERMINOTROPIC LOCALIZATION	+	-	-
IMMUNO-COMPROMISED	-	+	+
PRESENTATION	localized nodal disease no systemic symptoms	nodal or extranodal	generalized, disease, systemic symptoms
BEHAVIOUR	INDOLENT	AGGRESSIVE	AGGRESSIVE
MONOCLONALITY	-	+	+
Ig EXPRESSION	+	-/+	+
SOMATIC HYPERMUTATIONS	+	+	-

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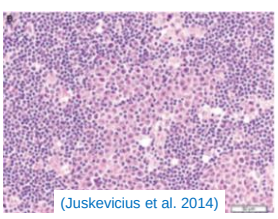
EXCEPTIONS: IMMUNE STATUS, PRESENTATION AND BEHAVIOUR

(1) **Germinotropic** LPD in **HIV-positive** patients
(Bhavsar et al. 2017)
(Gonzalez-Farre et al. 2017)

(2) Non-germinotropic nodal HHV8+ EBV+ proliferation ("**extracavitary PEL**") in **HIV-negative** patients with **favorable outcome** (>4years)

(Juskevicius et al. 2014)
(Courville et al. 2014)
(Ibrahim et al. 2017)



(Juskevicius et al. 2014)

30

GERMINOTROPIC LPD AND HIV → ATYPICAL FEATURES

5 REPORTED HIV+ CASES with:

• B-symptoms	in 4/5 cases
• generalized lymphadenopathy	in 4/5 cases
• splenomegaly	in 3/5 cases
• growth beyond germinal centers	in 4/5 cases
• monoclonality	in 2/4 tested cases
• CD79a expression	in 2/5 cases
• persistent disease despite treatment	in 2/5 cases
• subsequent EBV+ DLBCL	in 1 case

(Bhavsar et al. 2017)
(Gonzalez-Farre et al. 2017)

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EXCEPTIONS

	GERMINOTROPIC LPD	EXTRACAVITARY PEL	HHV8+ DLBCL, NOS (MCD-ASSOCIATED)
HHV8	+	+	+
EBV	+	+/-	-
GERMINOTROPIC LOCALIZATION	+	-	-
IMMUNO-COMPROMISED	-	+	+
PRESENTATION	localized nodal disease no systemic symptoms	nodal or extranodal	generalized, disease, systemic symptoms
BEHAVIOUR	INDOLENT	AGGRESSIVE	AGGRESSIVE
MONOCLONALITY	-	+	+
Ig EXPRESSION	+	-/+	+
SOMATIC HYPERMUTATIONS	+	+	-

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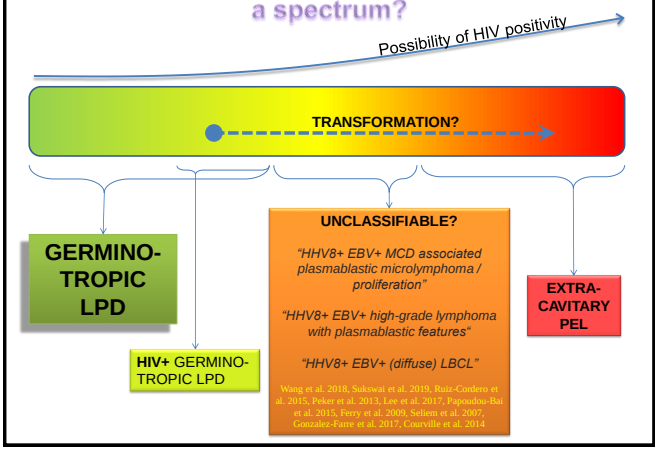
DOES GERMINOTROPIC LPD *TRANSFORM* ?

PRESUMED TRANSFORMATION	IMMUNE STATUS	
polyclonal GLPD → aggressive, widespread HHV8+ EBV+ lymphoma	HIV- HBV+ HCV+ but **concomitant nodal Kaposi**	(Courville et al. 2014)
10-year-long **mass-forming GLPD** → → EBV+ (HHV8-) DLBCL	**HIV+**	(Bhavsar et al. 2017)
GLPD-like & CD-like areas in aggressive, widespread HHV8+ EBV+ lymphoma	**HIV+**	(Seliem et al. 2007)
GLPD-like area in aggressive, widespread HHV8+ > EBV+ lymphoma	**HIV+**	(Gonzalez-Farre et al. 2017)

33

Nodal HHV8+ EBV+ (co-infection) proliferations: a spectrum?



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CONCLUSIONS

HHV8 & EBV CO-INFECTION:

- TWO WHO ENTITIES
  - **GERMINOTROPIC LYMPHOPROLIFERATIVE DISORDER** (17 HIV- and 5 HIV+ cases)
  - **PRIMARY EFFUSION LYMPHOMA**
- UNCLASSIFIABLE CASES, usually HIV+ (10-12 cases)

BE STRICT WITH DIAGNOSTIC CRITERIA OF GERMINOTROPIC LPD, BECAUSE OF:
- GLPD-like areas in aggressive disease
- tendency of unclassifiable cases to be aggressive

FEATURES - CRITERIA "REASSURING" OF AN INDOLENT NATURE:
- STRIKING **GERMINOTROPIC LOCALIZATION**
- AND - **LOCALIZED LYMPHADENOPATHY**, NO SYSTEMIC DISEASE
- AND - **IMMUNOCOMPETENCE**

GLPD *HAS BEEN* DIAGNOSED IN HIV+ PATIENTS, BUT THESE:
- tend to present with generalized, symptomatic disease
- may run the risk of transformation

35

THANK YOU


Evangelismos General Hospital of Athens

ACKNOWLEDGEMENTS TO

Dr. Dimitra Rontogianni
Head of Department of Pathology
Evangelismos General Hospital of Athens

Dr. Panagiotis Panagiotidis
Professor of Hematology
National and Kapodistrian University of Athens

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The 1st CSHP/SH Workshop and CSHP 2019 Oct.19-20, 2019

HIV-unrelated HHV-8 (+) EBV (-) extracavitary PEL

Kennosuke Karube
Dept. Pathol. and Cell Biol. University of the Ryukyus
UNIVERSITY OF THE RYUKYUS

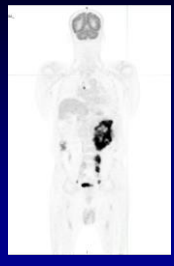
1

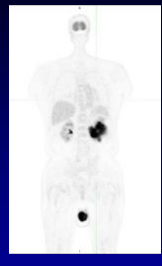
Case report

- An Okinawan resident
- 81-year old, male
- Weight loss and testicular mass
- LDH: 1911 U/l, soluble IL-2 R: 7388 U/l
- HTLV-1 Ab (-), HIV Ab (-)

2

FDG-PET

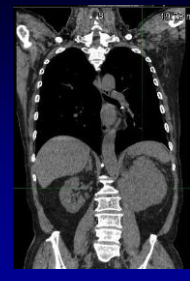


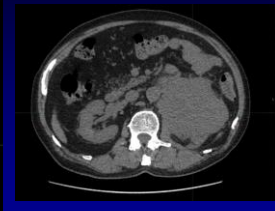


- 11cm mass in the right kidney→Biopsy
- 5cm testicular mass
- Multiple retroperitoneal lymphadenopathy

3

CT

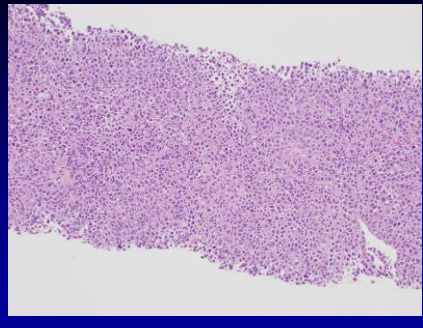




No pleural, pericardial, peritoneal effusions

4

Needle biopsy of the kidney



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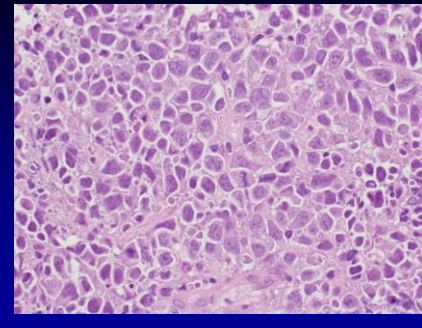
Needle biopsy of the kidney



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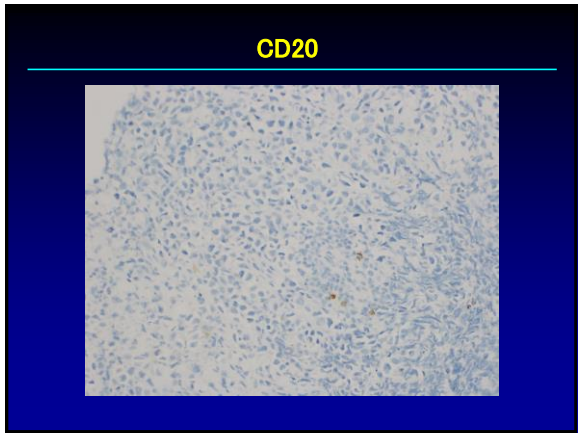

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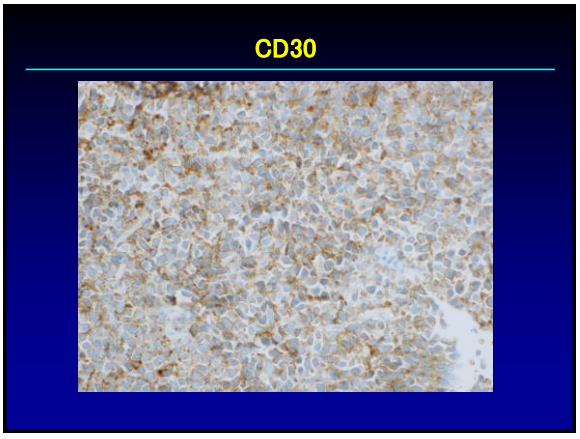

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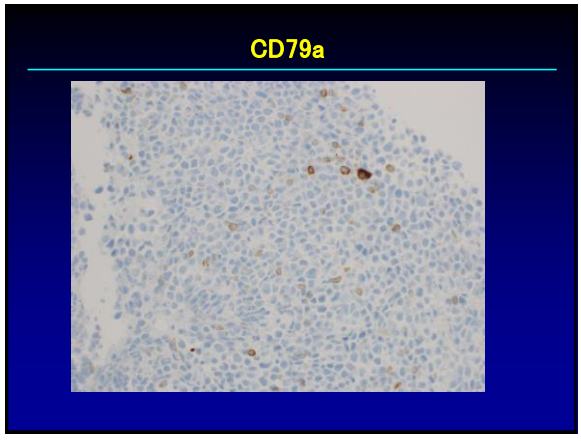

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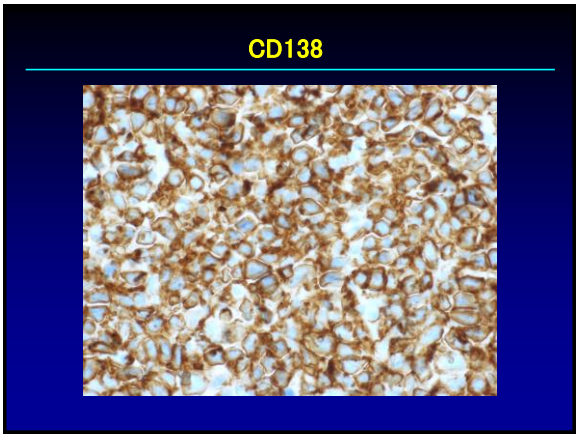

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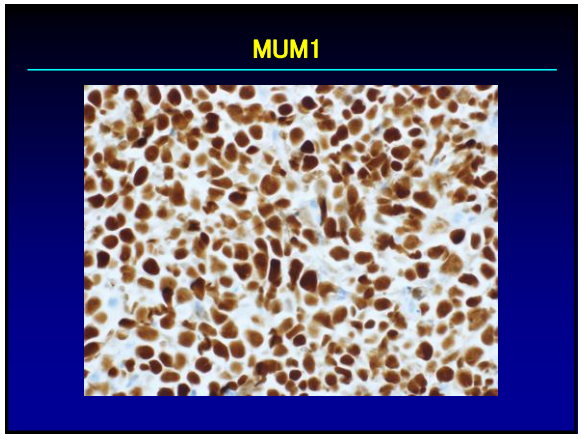

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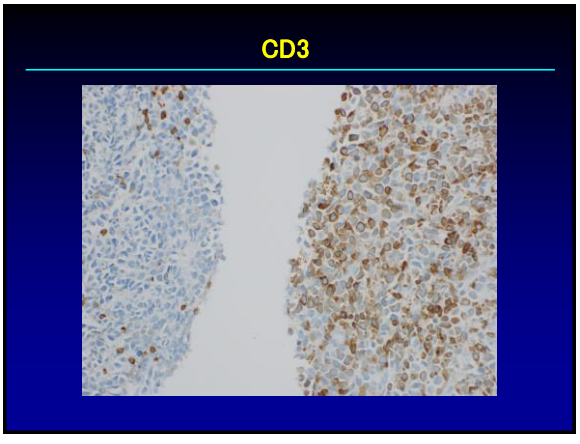
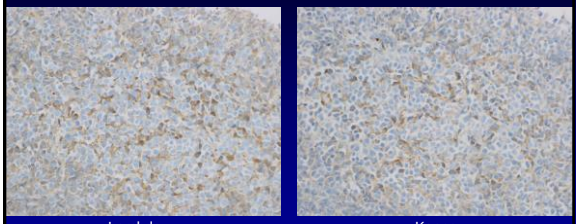


12

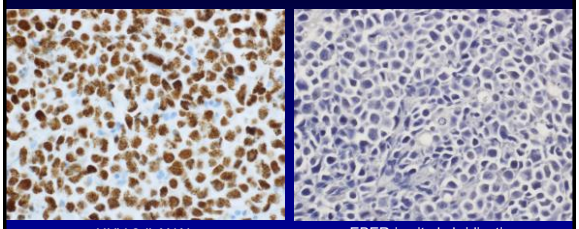
Immunoglobulin light chain



Lambda Kappa

13

HHV8 (+) but EBV (–)



HHV-8 (LANA) EBER-in situ hybridization

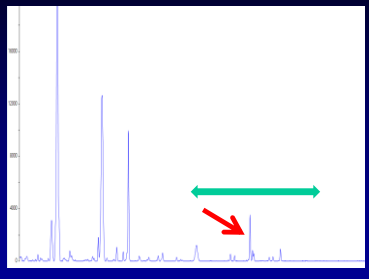
14

Summary of immunophenotype

Positive	Negative
CD30	CD20
CD138	CD79a
MUM1	PAX5
HHV-8 (LANA)	CD5
CD3 (partially)	Kappa
	Lambda
	EBER-ISH

15

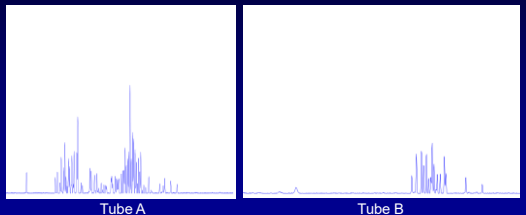
PCR (BIOMED2) for IGH-FR3



Clonality (+)

16

PCR (BIOMED2) for TCR-gamma



Tube A Tube B

Clonality (-)

17

Proposed diagnosis
Extracavitary primary effusion lymphoma
(HHV-8 (+), EBV(-))

Panel diagnosis
HIV-unrelated HHV8 (+) EBV (-) extra-cavitary PEL

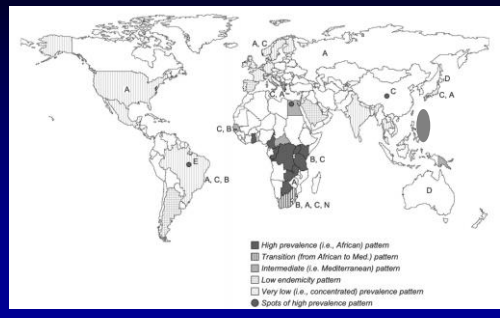
18

Primary effusion lymphoma (PEL)

- First demonstrated as HIV-related lymphomas
- A malignant effusion in body cavities
- Rarely, lymphomas identical to PEL may occur as solid mases without any effusion
- EBV-negative PEL occurs mainly in HIV-negative, but elderly patients especially originating in HHV-8 endemic regions

19

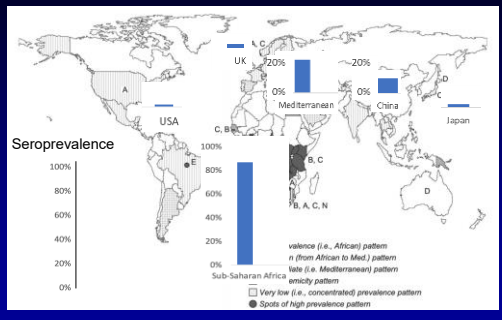
Prevalence of HHV-8 in the world



Whitby *JNCI* 1998, Dukers *AIDS* 2003, Awazawa *J Infect Dis* 2017

20

Prevalence of HHV-8 in the world



Whitby *JNCI* 1998, Dukers *AIDS* 2003, Awazawa *J Infect Dis* 2017

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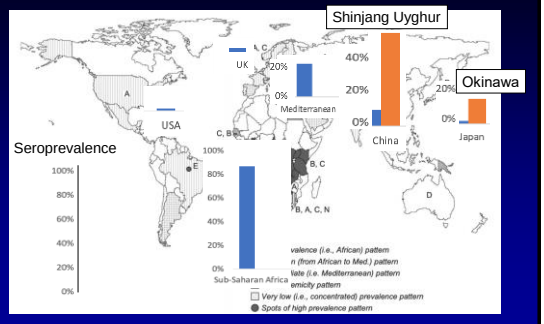
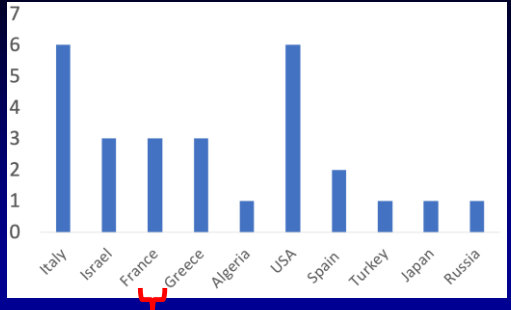
Prevalence of HHV-8 in the world



Whitby *JNCI* 1998, Dukers *AIDS* 2003, Awazawa *J Infect Dis* 2017

22

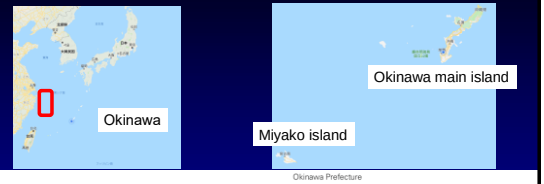
Reported case number of HIV (−) HHV8 (+) PEL



Ascoli *Haematologica* 2002 etc.

23

Existence of co-factors in the development of Kaposi sarcoma

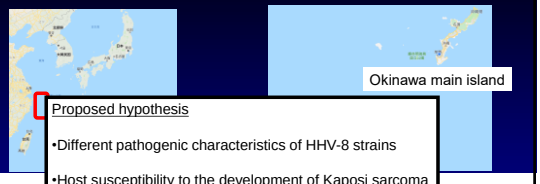


Statistical Data	Okinawa Prefecture: Miyako Islands	Okinawa Prefecture: Okinawa (Excluding Miyako Islands)	Japan (Excluding Okinawa Prefecture)
HHV8 infection rate (%) (≥50 years old)	18.0	1.43[d]	2.4[e]
Male	18.4	1.02[d]	2.4[e]
Female	17.5	2.38[d]	2.4[e]
KS annual rate among HHV8 carriers aged 50 years or older (%)	0.025	0.019	0.00023
Male	0.045	0.032	0.00034
Female	0.0080	0.0089	0.00013

Google Map, Awazawa *J Infect Dis* 2017

24

Existence of co-factors in the development of Kaposi sarcoma



Okinawa main island

Proposed hypothesis

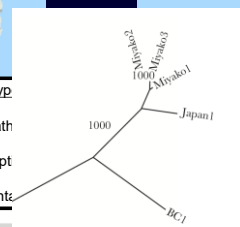
- Different pathogenic characteristics of HHV-8 strains
- Host susceptibility to the development of Kaposi sarcoma
- Environmental factors: e.g. high altitude

Statistical Data			Japan (Excluding Okinawa Prefecture)
HHV8 infection rate [			2.4[a]
Male	18.4	1.02[c]	2.4[a]
Female	175	2.38[c]	2.4[a]
KS annual rate among HHV8 carriers aged 50 years or older (%)	0.025	0.019	0.00023
Male	0.045	0.032	0.00034
Female	0.0080	0.0089	0.00013

Grossman Mayo Clinic Proc 2002, Awazawa *J Infect Dis* 2017

25

Existence of co-factors in the development of Kaposi sarcoma



Okinawa main island

Proposed hyp

- Different path
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ins

arcoma

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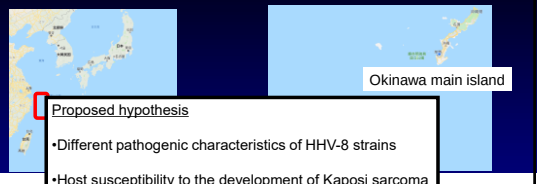
KS annual rate among HHV8 carriers aged 50 yea			0.00023
Male	0.045	0.032	0.00034
Female	0.0080	0.0089	0.00013

Grossman Mayo Clinic Proc 2002, Awazawa *J Infect Dis* 2017

26

Existence of co-factors in the development of Kaposi sarcoma



Okinawa main island

Proposed hypothesis

- Different pathogenic characteristics of HHV-8 strains
- Host susceptibility to the development of Kaposi sarcoma
- Environmental factors: e.g. high altitude
- ➡Similar mechanisms in HHV-8 associated lymphoma?

Statistical Data			Japan (Excluding Okinawa Prefecture)
HHV8 infection rate [			2.4[a]
Male			2.4[a]
Female			2.4[a]
KS annual rate among			0.00023
Male	0.045	0.032	0.00034
Female	0.0080	0.0089	0.00013

Grossman Mayo Clinic Proc 2002, Awazawa *J Infect Dis* 2017

27

Acknowledgement

University of the Ryukyus

Department of Radiology
Hitoshi Maemoto

Department of Pathology and Cell Biology
Mitsuyoshi Takatori
Shugo Sakihama

28

HIV-Associated Lymphomas in 2019: Spectrum of Disease and Treatment Approach

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1

Disclosure Information
Paul G. Rubinstein, MD

- I Have no financial relationships to disclose
- I will not discuss the off label use or investigational agents in this presentation.

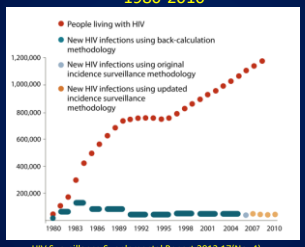
2

Acquired Immunodeficiency Syndrome

- 1981- AIDS was recognized
  Increase number of cases of Kaposi sarcoma affecting mainly males having sex with males
- 1982-Centers for Disease Control (CDC) broadened the definition of AIDS to patients dx with Kaposi sarcoma and primary CNS lymphoma
- 1987-CDC added non-Hodgkin lymphoma
- 1992-CDC added cervical cancer and CD4+ T-cell count
- 1996- Combined anti-retroviral therapy became standard

3

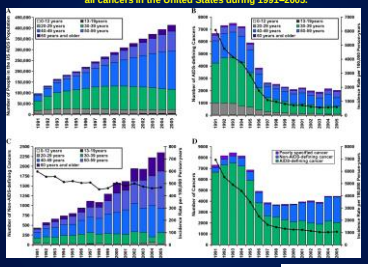
Prevalence of Patients Living With HIV/AIDS in the United States 1980-2010



HIV Surveillance Supplemental Report 2012;17(No. 4). http://www.cdc.gov/hiv/topics/surveillance/resources/reports/#supplemental.

4

Number of people living with AIDS, AIDS-defining cancers, non-AIDS-defining cancers, and all cancers in the United States during 1991–2005.

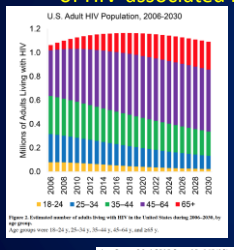


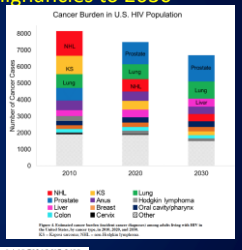
Shiels M S et al. JNCI J Natl Cancer Inst 2011;jnci.djr076

JNCI

5

Projection of Prevalence and Distribution of HIV-associated Malignancies to 2030





Ann Intern Med. 2018 June 19; 168(12): 866–873. doi:10.7326/M17-2499.

6

Standard Incidence Ratios of Malignancies in the Pre-HARRT and early HAART Era

Table 1. Standard incidence ratios for common AIDS-defining and non-AIDS-defining cancers in the early and later HAART era and in the context of tumor-associated oncogenic viruses.

HIV-associated malignancies	SIR pre-HAART (1990–1995)	SIR early-HAART era (1996–2002)
ADCs		
Kaposi sarcoma	22 100	3640
PCNSL	5000	>1020
Burkitt's lymphoma	52	49
DLBCL	64	29.6
All NHLs	79	22.6
Cervical carcinoma	4.2	5.3
NADCs		
Hodgkin lymphoma	8.1	14
Anal carcinoma	18.3	33
Lung carcinoma	2.3	2.2–6.6
Head and neck carcinoma	1.2	1–4
Prostate cancer	N/A	4
Hepatocellular carcinoma	19	7–35
Melanoma	N/A	3
All NADCs	1.8	1.7–2

ADCs, AIDS-defining cancers; DLBCL, diffuse large B-cell lymphoma; EBV Epstein–Barr virus; HPV, human papillomavirus; N/A, not available; NADCs, non-AIDS-defining cancers; NHL, non-Hodgkin lymphoma; PCNSL, primary central nervous system lymphoma; SIR, standard incidence ratio.

Rubinstein PG, Abulafia D, Zoloza A. AIDS. 2014 Feb 20;28(4):453-65.

7

RESEARCH ARTICLE

Spectrum of malignancies among the population of adults living with HIV infection in China: A nationwide follow-up study, 2008–2011

Weiming Zhu[1,2], Yurong Mao[1], Houlin Tang[1], Jennifer M. McGoogan[1], Zuo-Feng Zhang[2], Roger Detels[2], Na He[3], Zunyou Wu[1,2]*

1 National Center for AIDS/STD Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China, 2 Department of Epidemiology, Fielding School of Public Health, University of California–Los Angeles, Los Angeles, California, United States of America, 3 Department of Epidemiology, School of Public Health, Fudan University, Shanghai, China

PLOS ONE | https://doi.org/10.1371/journal.pone.0219766 July 25, 2019

8

Standard Incidence Ratio and Burden of Cancer in Patients living with HIV in China 2008-2011

Cancer	Number of Cases Observed Male/Female	SIR
Lung	713/140	4.8
Liver	539/84	3.9
Lymphoma	299/117	12.9
Brain/CNS	216/105	8.2
Stomach	137/60	1.1/2.1
Kaposi Sarcoma	132/39	1500
Leukemia	50/28	2.9/3.2

PLOS ONE | https://doi.org/10.1371/journal.pone.0219766 July 25, 2019

9

The Correlation Between HIV and Cancer

- Pts living with HIV/AIDS (PLWHA) in the United States have a 12 fold increase over the general population in being dx with NHL
- Since 2003 more Non-AIDS defining cancers than AIDS defining
- PLWHA in studies in North America, Europe, and Australia with clinical info and death certificate info… 7-15% deaths were due to AIDS Defining Cancers and 12-27% Non-AIDS Defining Cancers
- 20-42% of deaths depending was due to cancer.

CID 2017:65 (15 August) • Engels et al

10

Clinical Infectious Diseases

MAJOR ARTICLE

Cancer-Attributable Mortality Among People With Treated Human Immunodeficiency Virus Infection in North America

Eric A. Engels,[1] Elizabeth L. Yanik,[1] Willion Wheeler,[2] M. John Gill,[3] Meredith S. Shiels,[1] Robert Dubrow,[4] Keri N. Althoff,[5] Michael J. Silverberg,[6] John T. Brooks,[7] Mari M. Kitahata,[8] James J. Goedert,[1] Satbir Grover,[9] Angel M. Mayo,[10] Richard D. Moore,[11] Lesley S. Park,[12] Anita Rachlis,[13] Keith Sigel,[14] Timothy R. Sterling,[8] Jennifer E. Thorne,[15] and Ruth M. Pfeiffer[1]; for the North American AIDS Cohort Collaboration on Research and Design of the International Epidemiologic Databases to Evaluate AIDS

Table 2. Cancer-Attributable Mortality Among Human Immunodeficiency Virus–Infected Individuals in North America

Cancer	Total Cancer Cases, N	Deaths With Prior Cancer, N	PAF, %[a]	Adjusted Analysis[b] HR	PAF, %	(95% CI)	Adjusted Analysis[c] HR	PAF, %	(95% CI)
Total	1997	1069	11.9	6.27	10.0	(9.3–10.7)	5.54	9.8	(9.1–10.5)
AIDS-defining cancers	570	303	3.4	6.22	2.8	(2.5–3.2)	4.11	2.6	(2.2–2.9)
Kaposi sarcoma	252	95	1.1	3.45	0.8	(0.5–1.0)	2.06	0.5	(0.3–0.8)
Non-Hodgkin lymphoma	312	207	2.3	9.70	2.1	(1.8–2.4)	7.23	2.0	(1.7–2.3)
Cervix	6	1	0.0	—	0.0	—	—	0.0	—
Non-AIDS-defining cancers	1427	766	8.6	5.89	7.1	(6.5–7.7)	5.91	7.1	(6.5–7.7)
Lung	265	222	2.5	20.33	2.4	(2.0–2.7)	14.71	2.3	(2.0–2.6)
Anus	154	62	0.7	3.13	0.5	(0.3–0.6)	2.73	0.4	(0.3–0.6)
Liver	103	80	0.9	23.06	0.9	(0.7–1.1)	31.26	0.9	(0.7–1.1)
Other	905	402	4.5	3.81	3.3	(2.9–3.7)	4.04	3.4	(2.9–3.8)

Cancer-Attributable Mortality in HIV • CID 2017:65 (15 August)

11

Oncogenic Viruses Associated with HIV-associated Malignancies

Table 3. Common AIDS-defining and non-AIDS-defining and tumor-associated oncogenic viruses.

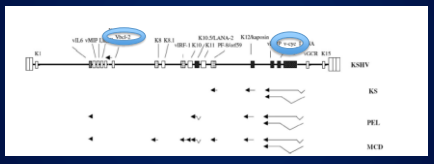
HIV-associated malignancies	Associated oncogenic virus
Kaposi sarcoma	100% HHV-8 [53]
PCNSL	100% EBV [26]
Burkitt's lymphoma	20–40% EBV [26]
DLBCL	Centroblastic 30% EBV [26] Immunoblastic 90% EBV [26]
PEL	100% HHV-8 [27] 100% EBV [25,27]
Plasmablastic	50% EBV [25,26]
Cervical	100% HPV [53]
Hodgkin lymphoma	80–100% EBV [25,26]
Anal carcinoma	100% HPV [52]
Head and neck carcinoma	HPV [53] EBV [53]
Hepatocellular Carcinoma	HBV [53] HCV [53]

DLBCL, diffuse large B-cell lymphoma; EBV, Epstein–Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HHV-8, human herpesvirus 8; HPV, human papillomavirus; NADC, non-AIDS-defining cancer; NHL, non-Hodgkin lymphoma; PCNSL, primary central nervous system lymphoma; PEL, primary effusion lymphoma.

Rubinstein PG, Abulafia D, Zoloza A. AIDS. 2014 Feb 20;28(4):453-65.

12

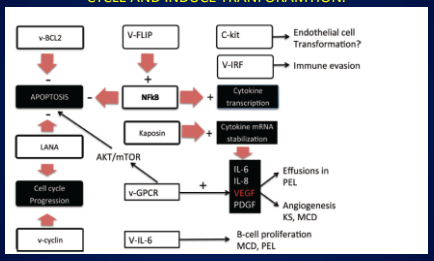
HHV8 Virus Expresses vBCL-2 and v-cyclin



Kaplan L, Hematology 2013 pg 103-107

13

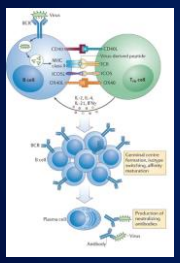
HHV8 VIRUS EXPRESSES MULTIPLE PROTEINS THAT ALTER CELL CYCLE AND INDUCE TRANFORAMTION.



Kaplan L, Hematology 2013 pg 103-107

14

The Role of CD4 Cells on Germinal Centers

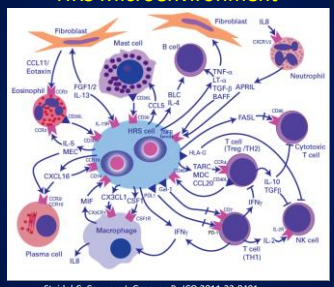


- CD4 T-Helper Cells Migrate to The Germinal Center
- CD4 T-Cells Prevent Germinal B-Cell Lymphocytes from Undergoing Apoptosis and help develop the germinal center.
  Nature Reviews Immunology **12**, 136-148 (February 2012)
- A Loss of gastrointestinal tract germinal centers have been demonstrated In patients with HIV
  Levesque MC et al. Plos Medicine
  DOI: 10.1371/journal.pmed.1000107

Swain SL et al. Nature Reviews Immunology **12**, 136-148 (February 2012)

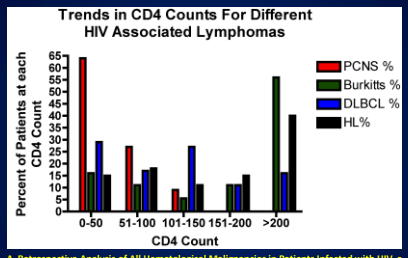
15

HRS Microenvironment



Steidel C, Connors J, Gascyne R. JCO 2011;32:8401

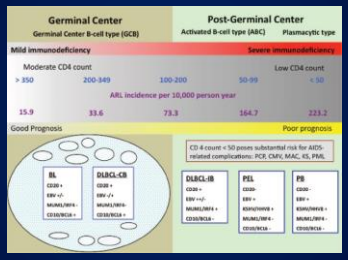
16



A Retrospective Analysis of All Hematological Malignancies in Patients Infected with HIV, a Subset Analysis of the CHAMP Study (Cook County Hospital (CCH) AIDS Malignancy Project)
S. Gupta et al. American Society of Hematology Abs#3693 12/2011

17

Model for Prognosis, Histology, Incidence and CD4 T-cell Count for AIDS-related Lymphoma



Dunleavy K et al. BLOOD, 5 APRIL 2012 VOLUME 119, NUMBER 14

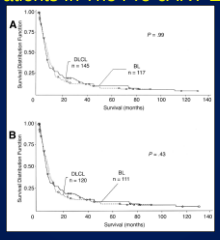
18

Treatment Approach

- Treatment should be done in conjunction with HIV specialist and pharmacologist
- An Accurate Risk Assessment of HIV Status
- Type of patient i.e low CD4 count, multiple co morbidities vs. healthy HIV patient vs. Lymphoma as presenting sx/sx of HIV (Cardiac)
- Occam's Razor does not apply in advanced AIDS Opportunistic Infection Risk/History/Prevention Co-Infection Status i.e. HEP B/C or HHV8 (KS) cART/Medication Assessment: Potential Adverse Events and Chemotherapy-cART/Medication Interactions

19

Survival of DLBCL and Burkitt Lymphoma Patients In The Pre-cART ERA



Lim, S. T. et al. J Clin Oncol; 23:4430-4438 2005

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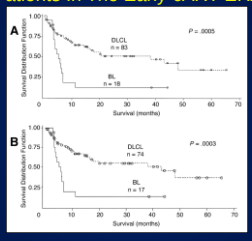
Survival of DLBCL and Burkitt Lymphoma Patients In The Early-cART ERA



Lim, S. T. et al. J Clin Oncol; 23:4430-4438 2005

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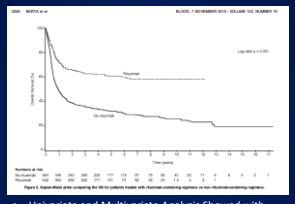
21

Overall Survival of Patients with Rituximab-based Regimens vs. Non-Rituximab Based Regimens

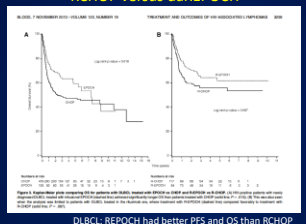


- Univariate and Multivariate Analysis Showed with Rituximab: Improved OS, PFS, CR rates
- Improved Outcomes were only noted when CD4 counts were above 50 cells/mm³

Barta et al. Blood 2013,122:3251-3262

22

Comparison of the Survival of Patients Receiving RCHOP versus daREPOCH

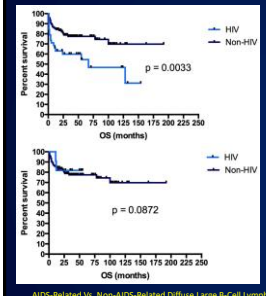


DLBCL: REPOCH had better PFS and OS than RCHOP
On multivariate analysis infusional RCDE and EPOCHR had improved OS and PFS vs. Non-infusional Regimens
Burkitt's Lymphoma: intensive regimens Better OS than Infusional Regimens

Barta et al. Blood 2013,122:3251-3262

23

Survival of AIDS-Related DLBCL at Cook County Hospital



51 HIV+ DLBCL Patients
vs. 240 HIV- DLBCL

240 HIV- DLBCL
vs. 14 AIDS-Related
DLBCL treated with daEPOCHR

AIDS-Related Vs. Non-AIDS-Related Diffuse Large B-Cell Lymphoma. Analysis of the County Hospital AIDS Malignancy Project (CHAMP)
Kelly A. Petrowski, Harsha Poola, Katherine Lee Chuy, Shweta Gupta, and Paul G. Rubinstein ASH ABS#2684, 2015, Orlando, Florida

24

Plasmablastic Lymphoma Survival

Study	Year	N	CD4+ T cell Count[1]	Treatment	Median Survival (Months)
Castillo et al.[9]	1997-2007	112	178	CHOP, EPOCH, CODOX-M/IMVAC	15 month
Castillo et al.[17]	2000-2010	50	206	CHOP/CHOP like (63%) Other (37%)	11 months
Castillo et al[16]	1997-2006	70	165	CHOP/CHOP like Intensive Regimens, ie CODOX-M/IMVAC	14months 14months
Noy et al.[18]	2013	19	110	daEPOCH,CDE,CHOP	12 months (67%)
Ibrahim IF[19]	2014	25	87	daEPOCH (n=14) CHOP/CHOP like (n=11)	17months 7 months
Lee et al.[20]	2014	12	152	daEPOCH (58%)	17months
Schommers et al. [6]	2013	18	85	CHOP (50%)	5 months

[1]Median CD4+ T cell count (cells/mm³) at diagnosis. Dose adjusted etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (daEPOCH). Cyclophosphamaide, doxorubicin, vincristine and prednisone (CHOP). Cyclophosphamide, Doxorubicin, Methotrexate, Ifosphamide, Etoposide, Cytarabine (CODOX-M/IVAC)

Rubinstein PG and Wyne C. AIDS Related Plasmablastic Lymphoma

25

HIV-associated ALCL

Table 1: Clinical Characteristics

Pt	AGE	SEX	HIV RISK	CD4 (#/μL)	ART*	KS/OI	SITE	OTHER SITES	TREATMENT	FOLLOW-UP
CC1	42	M	Unknown	60	Yes	No	Lymph node	Lung, spleen, abdominal/pelvic LN	CHOP, IT Chemotherapy	Died (5 mo)
CC2	45	M	Heterosexual	411	Yes	No	Neck mass	No	DA-EPOCH, CHOEP	Remission (48 mo)
NU1	47	M	Unknown	N/A	N/A	N/A	Eyelid	Not documented	Unknown	Lost to F/U (7 mo)
NU2	39	M	Heterosexual	79	Yes	KS/OI	Peri-rectal	Skin, lung, LN, bone, soft tissues	ICE, SCT, Brentuximab	Remission (42 mo)
WC1	33	M	Homosexual	42	Yes	No	Arm	Nasal, lung, LN, paraspinal region, bone, liver, spleen	CHOP, IT ARA-C, XRT	Died (10 mo)
WC2	63	M	Homosexual	285	Yes	KS	Skin	Lung, pleural fluid	CHOP	Died (6 mo)

*NU1, WC1, WC2 all diagnosed before 1996 and use of cART

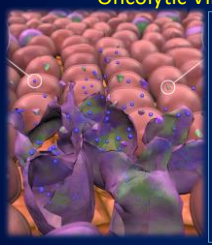
Clinicopathologic and Genetic Analysis of HIV-Associated Anaplastic Large Cell Lymphomas (ALCL)
Anna Nam, Susan Mathew, Paul Rubinstein, Yi-Hua Chen, Swarna Gogineni, Kelly Petrowski, Amy Chadburn
USCAP 2017

26

OTHER NEW APPROACHES

27

Oncolytic Viral Strategies



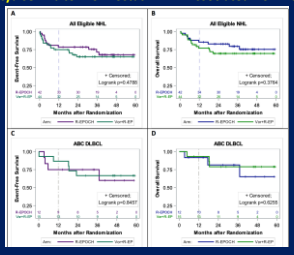
Lytic activation of viruses residing with cells:

- Direct cell lysis
- Expression of lytic phase viral proteins increases immuno-genicity

- Proteasome inhibitors
- HDAC inhibitors
- Azidothymidine (AZT)
- Taxol
- Cisplatin

28

Randomized Phase II Trial of EPOCH-based Chemotherapy Alone or in Combination with the Histone Deacetyalse Inibitor Vorinostat in HIV-Associated Non-Hodgkin Lymphomas



Ramos JC, Sparano JA, Reid EG, Ambinder RF, Siegel E, Moore PC, Chadburn A, Rubinstein PG et al.
ASCO 2018

29

Survival Data for Primary Effusion Lymphoma

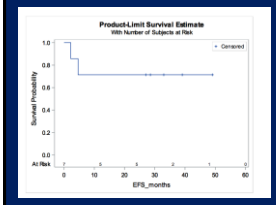
Table 1. Clinical series of PEL and extracavitary PEL

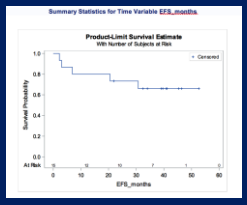
	El-Fattah[11]	Olszewski et al[24]	Guillet et al[7]	Boulanger et al[25]	Simonelli et al[12]	Chadburn et al[2]	Carbone et al[5]
No. of patients	105	106	51	28	11	8	4
Disease, n PEL Extracavitary PEL	105 —	106 —	34 17	28 —	11 —	— 8	— 4
Median age, y	41	44	45	44	41	40	40
Male, %	90	>95	92	96	90	100	75
HIV+, %	NA	100	100	100	100	100	75
EBV coinfection, %	NA	NA	66	72	NA	NA	NA
History of KS, %	NA	NA	49	67	27	25	25
HAART, %	NA	NA	69	78	NA	20 (1 of 5)	NA
Receiving chemotherapy, %	NA	61	88	79	73	75	100
CR rate, %	NA	NA	56	41	42	NA	NA
Median follow-up duration	4 mo	NA	10 y	3.8 y	NA	11 mo	1.1 mo
Median OS	NA	0.4 y	10.2 mo	4.2 mo	6 mo	11 mo	0.8 mo
y OS rate, %	30	NA	NA	39.3	NA	40	0
y OS rate, %	18	NA	NA	NA	NA	40	0
y OS rate, %	17	28	NA	NA	NA	40	0

blood® 1 NOVEMBER 2018 | VOLUME 132, NUMBER 18

30

Randomized Phase II Trial of EPOCH-based Chemotherapy Alone or in Combination with the Histone Deacetyalse Inibitor Vorinostat in HIV-Associated Non-Hodgkin Lymphomas





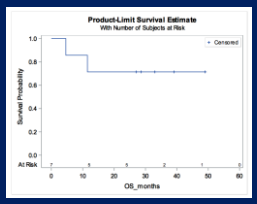
PEL n=7

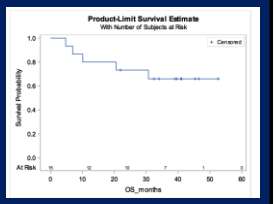
PBL n=15

Ramos JC, Sparano JA, Reid EG, Ambinder RF, Siegel E, Moore PC, Chadburn A, Rubinstein PG et al. ASCO 2018 ABS#

31

OS for 075 data for PEL in Plasmablastic and Primary Effusion Lymphoma





PEL n=7

PBL n=15

Ramos JC, Sparano JA, Reid EG, Ambinder RF, Siegel E, Moore PC, Chadburn A, Rubinstein PG et al. ASCO 2018 ABS#

32

New Therapies Cont.

1. PBL/PEL/CD30+ NHL: Brentuximab Vedotin
2. Plasmablastic lymphoma: daEPOCH+ Daratumumab (anti CD38)
3. NHL: daEPOCHR+ ibrutinib
4. Relapsed NHL: RICE+ Ibrutinib
5. Relapsed cHL: Nivolumab

33

CD30 Expression aside from cHL

- PEL 80% EBV and 100% HHV8
- PBL 60-80% EBV Positive
- Commonly seen in EBV driven lymphoproliferative disorders
- ALTCL and other T-cell lymphoma
- Expressed in DLBCL…about 20% in non-HIV DLBCL

Thus utilize the CD30 Antibody Drug Conjugate with Mono-Methyl Aurastain E as a means to improve survival by targeting CD30 + Malignant Cells.

BLOOD, 4 APRIL 2013 x VOLUME 121, NUMBER 14

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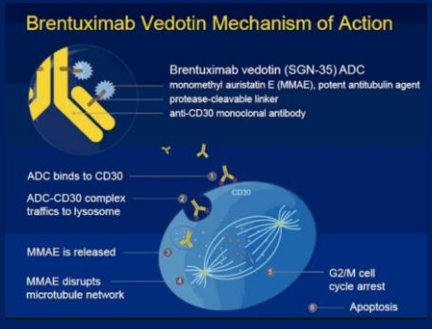
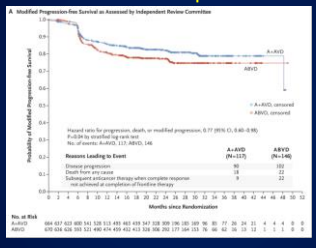
Brentuximab Vedotin Mechanism of Action



35

Brentuximab Vedotin upfront in non-HIV cHL



2 yr mPFS 82% For AVD+BV

2 yr mPFS 77% For ABVD

N Engl J Med 2018; 378:331-344 DOI: 10.1056/NEJMoa1708984

36

AVD-BV in HIV-associated cHL

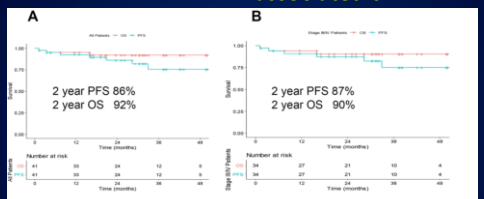


Figure 1: **A:** Kaplan-Meier curves of progression free survival (PFS) and overall survival (OS) of all subjects and **B:** of subjects with advanced stage HIV-associated cHL.

Safety and Efficacy of Brentuximab Vedotin in Combination with AVD in Stage II-IV HIV-Associated Classical Hodgkin Lymphoma: Results of the Phase 2 Study, AMC 085
Rubinstein PG, Moore PC, Bimali M, Chadburn A, Cesarman E et al. ASH 2019; Abstract # 130

37

Brentuximab Vedotin + CHP vs CHOP in Previously Untreated CD30+ PTCL (ECHELON-2): Background

- PTCL is a rare form of aggressive NHL with heterogeneous biology[1]
- CHOP/CHOP-like regimens are standard frontline therapy for PTCL, but response is typically poor and new therapies are needed[1-4]
  - ALK+ sALCL with better outcomes than other subtypes, depending on age/IPI score[2]
- CD30 expression varies across PTCL subtypes with universal expression in sALCL[5,6]
- BV: CD30-directed antibody–drug conjugate[7,8]
  - In phase I trial, 50% of patients treated with BV + CHP in frontline achieved durable remissions[7]
  - Approved as monotherapy for R/R sALCL, with recent expansion of indication based on the current data to include untreated sALCL and other CD30+ PTCLs in combination with CHP[8]
- Current study compared efficacy, safety of BV + CHP vs SoC CHOP in untreated CD30+ PTCL[9,10]

1. Khan. Best Pract Res Clin Haematol. 2018;31:306-314. 2. Savage. Ann Oncol. 2004;15:1467. 3. Savage. Blood. 2008;111:5496. 4. Simon. Br J Haematol. 2010;151:159. 5. Bossard. Blood. 2014;124:2983. 6. Sabattini. Haematologica. 2013. 7. Brentuximab vedotin PI. 8. Fanale. Blood. 2018;131:2120. 9. Horwitz. ASH 2018. Abstr 997. 10. Horwitz. Lancet. 2018;[Epub].

Slide credit: clinicaloptions.com

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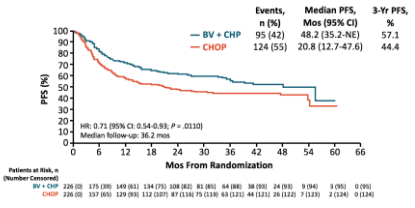
ECHELON-2: PFS by BICR in ITT Population (Primary Endpoint)



Horwitz. Lancet. 2018;[Epub].

Slide credit: clinicaloptions.com

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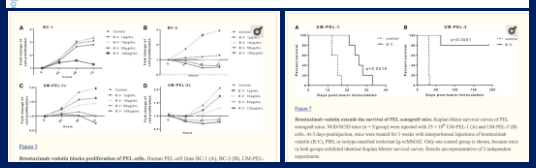
Blood. 2013 Aug 15; 122(7): 1233–1242.
Prepublished online 2013 Jul 9. doi: 10.1182/blood-2013-01-481713
PMCID: PMC3744990
PMID: 23838350

CD30 targeting with brentuximab vedotin: a novel therapeutic approach to primary effusion lymphoma

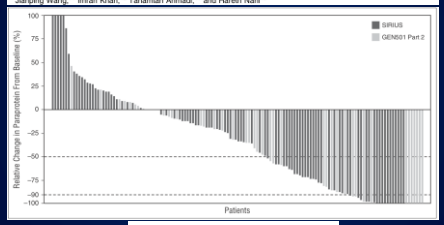
Shruti Bhatt,[1,2] Brittany M. Ashlock,[3] Yasodha Natkunam,[4] Victoria Sujoy,[5] Jennifer Rose Chapman,[5] Juan Carlos Ramos,[2] Enrique A. Mesri,[3] and Izidore S. Lossos[1,2]



40

Clinical efficacy of daratumumab monotherapy in patients with heavily pretreated relapsed or refractory multiple myeloma

Saad Z. Usmani,[1] Brendan M. Weiss,[2] Torben Plesner,[3] Nizar J. Bahlis,[4] Andrew Belch,[5] Sagar Lonial,[6] Henk M. Lokhorst,[7] Peter M. Voorhees,[8] Paul G. Richardson,[9] Ajai Chari,[10] A. Kate Sasser,[11] Amy Axel,[11] Huaibao Feng,[12] Clarissa M. Uhlar,[11] Jianping Wang,[11] Imran Khan,[12] Tahamtan Ahmadi,[11] and Hareth Nahi[13]



BLOOD, 7 JULY 2016 • VOLUME 128, NUMBER 1

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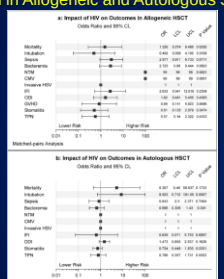
HIV survival in Allogeneic and Autologous Stem Cell Transplantation



42

letters to nature

Nature 382, 722 - 725 (22 August 1996); doi:10.1038/382722a0

Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene

MICHEL SAMSON*, FRÉDÉRICK LIBERT*, BENJAMIN J. DORANZ†, JOSEPH RUCKER†, CORINNE LIESNARD*, CLAIRE-MICHELE FARBER‡, SENTOB SARAGOSTI*, CLAUDINE LAPOUMEROULIE§, JACQUELINE COGNIAUX§, CHRISTINE FORCELLE§, GAETAN MUYLDERMANS§, CHRIS VERHOFSTEDE§, GUY BURTONBOY§, MICHEL GEORGES‖, TSUNEO IMAI¶, SHALINI RANA††, YANJI YI††, ROBERT J. SMYTH††, RONALD G. COLLMAN††, ROBERT W. DOMS†, GILBERT VASSART*‡‡ & MARC PARMENTIER*

Figure 1. HIV Entry: HIV binds to its receptor, CD4, on the cell surface through the virus' gp120 protein (left). The virus then binds to its co-receptor, either CCR5 or CXCR4 (center). Binding to the co-receptor allows the virus to fuse with the cell surface, and enter the interior of the cell to cause infection (right). (Image credit: National Institute of Allergy and Infectious Diseases)

At least 4-16% of patients of European Decent Have at least 1 Copy of the CCR5 delta 32 Gene

43

BRIEF REPORT

Long-Term Control of HIV by CCR5 Delta32/Delta32 Stem-Cell Transplantation

THE LANCET

Second person reported in remission from HIV infection

Patient No More

Timothy Brown—a.k.a. "the Berlin Patient"—is the Man Who Once Had HIV.

'The London Patient'	Timothy Brown
• Homozygous for wild type CCR5	• Heterozygous for Δ32
• Infection with R5 using virus	• Infection with R5 using virus
• Hodgkin Lymphoma	• Acute Myelogenous Leukemia
• Single HSCT	• Two HSCT
• No irradiation	• Total Body Irradiation
• Reduced intensity conditioning	• Full intensity conditioning
• T cell depletion with aCD52	• T cell depletion with ATG
• Mild GVH	• Mild GVH
• 100% T cell donor chimerism	• 100% T cell donor chimerism

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Conclusions

- HIV-associated malignancies in north america represent 20-25% mortality in the HIV community
- Transformation to cancer is multifactorial and depends in part on CD4+ T-cell count, microenvironment, and oncogenic Viruses
- Treatment can be well tolerated but care must be taken in managing comorbidities
- Outcomes are becoming similar to the non-HIV population
- New treatments taking advantage of lymphoma biology are improving outcomes.
- Stem cell transplant are well tolerated in patients infected with HIV
- Stem cell manipulation deleting CCR5 may lead to cure

45

Thanks

AMC AIDS Malignancy Consortium

46

Richard Longnecker

Viral lymphomagenesis, host susceptibility, and update on vaccines against lymphomagenic viruses (e.g., EBV and HIV vaccines).

Xi'an

October 2019

1

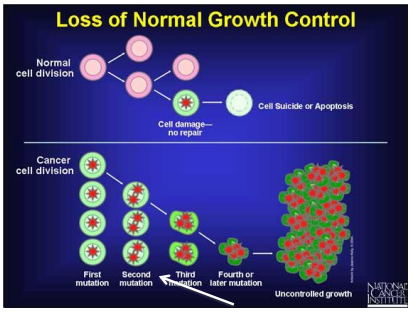
Worldwide Incidence of Cancers Attributable to Viruses

Virus	Cancer	Major regions affected	Refs
Epstein–Barr virus	• 40% of Hodgkin lymphoma • >95% of endemic Burkitt lymphoma • 10% gastric carcinoma • Most (type II and III) nasopharyngeal carcinoma • Kaposi sarcoma • Other lymphomas	• East Asia • East Africa • Regions of the Americas	140, 141
Hepatitis B virus	• 53% of hepatocellular carcinoma	• Asia • Sub-Saharan Africa • Regions of South America	142
Human T-lymphotropic virus 1	• >99% of adult T cell leukaemia	• Japan • Australia • Regions of Africa, South America and the Middle East	143, 144
Human papillomavirus	• >95% of cervical carcinoma • 70% of oropharyngeal carcinoma • Other anogenital carcinomas	• Central America • South America • Sub-Saharan Africa • Regions of Asia	145, 146
Hepatitis C virus	• 25% of hepatocellular carcinoma • Non-Hodgkin B cell lymphomas	• Regions of Asia, the Americas, North Africa and the Mediterranean	147, 148
Kaposi sarcoma-associated herpesvirus	• >99% of Kaposi sarcoma • >99% of primary effusion lymphoma	• Regions of Europe and sub-Saharan Africa	149
Merkel cell polyomavirus	• 80% of Merkel cell carcinoma	• North America • Australia • Europe	15, 150

Nathan A. Krump & Jianxin You, Nature Reviews Microbiology (2018)

2

Cancer is a Multistep Process



Viruses can participate in this process – replacing a requirement for mutation or enhancing cellular gene mutation.

3

Viruses and human cancers

- More people infected by viruses than come down with cancer.
- Viral sequences typically detected in tumors.
- Viral infection is necessary but not sufficient.
- Latency can vary before appearance of cancer.
  - Can be several decades between infection and development of cancer (HBV and liver cancer) or quite short (EBV and BL).

4

Mechanisms of Viral Transformation

- Activation of cellular signal transduction pathways.

Mimics of cellular signaling molecules or activators of cellular signaling molecules. Oncogenes and Tumor suppressors.

- Cell cycle control pathways.

Abrogation of normal cell cycle restriction points (inhibition of p53 or pRb, production of virus-specific cyclins, cell cycle changes.

- Mutation of Cellular DNA.

Mutagenic retroviruses, HBV, HCV, Inflamation etc

5

Human Herpesviruses

- Alpha-
  - Herpes Simplex Virus (HSV)
  - Varicella-Zoster Virus (VZV)
- Beta-
  - Cytomegalovirus (CMV)
  - Human Herpesvirus 6 (HHV-6)
  - Human Herpesvirus 7 (HHV-7)
- Gamma-
  - Epstein-Barr virus (EBV)
  - Human Herpesvirus 8 (HHV-8) or (KSHV)

6

Epstein-Barr Virus

•Causes infectious mononucleosis.

•Co-factor in Burkitt Lymphoma, Hodgkin Lymphoma, diffuse B cell lymphoma, nasopharyngeal carcinoma, gastric carcinoma, etc

•Causes oral hairy leukoplakia and lymphoproliferative disease in the immunocompromised (HIV/AIDS patients and transplant patients).

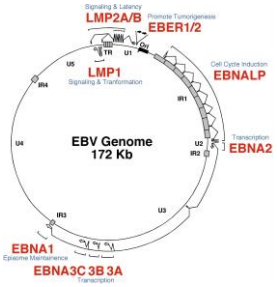
•Links to other disease – but controversial.

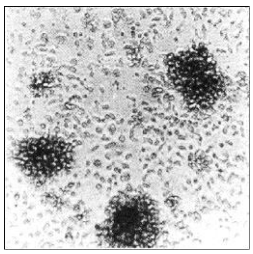
•No virus-specific treatments.

•Vaccine?

7

EBV Transformed B Cells - LCLs

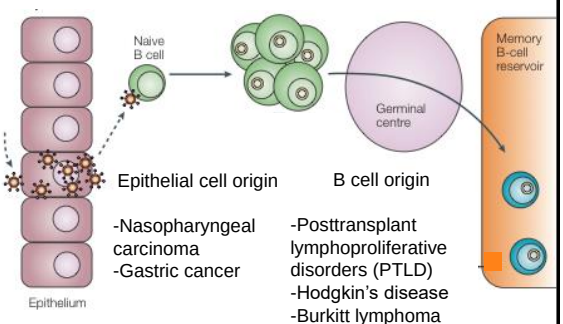




Latently infected - no virus production.

8

EBV latency and cancers



Epithelial cell origin

-Nasopharyngeal carcinoma
-Gastric cancer

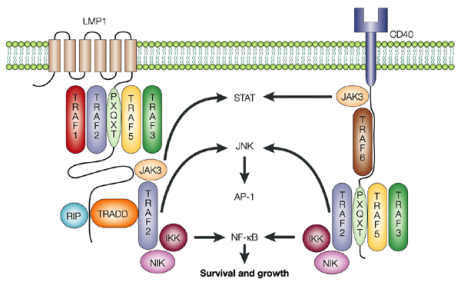
B cell origin

-Posttransplant lymphoproliferative disorders (PTLD)
-Hodgkin's disease
-Burkitt lymphoma

Adapted from Young LS and Rickinson AB. Nat Rev Cancer (2004)

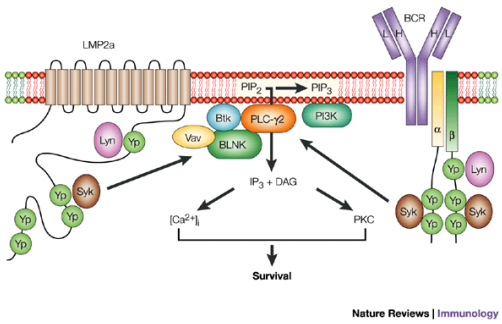
9

LMP1 Constitutively Mimics CD40 Signaling



10

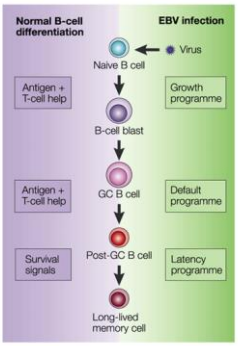
LMP2A Constitutively Mimics BCR Signaling



11

EBV Drives B Cell Differentiation and Growth

LMP1 and LMP2A provide T cell help (CD40) and antigen (BCR) signals - other viral proteins insure genome maintenance and viral gene expression.

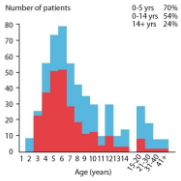


12

Identification of EBV: Burkitt's lymphoma

1964: Anthony Epstein and Yvonne Barr identify a novel herpesvirus in electron micrographs of cultured lymphoma cells

Age distribution of African BL



Magrath, I. *ecancermedicalscience* **vol 3**, no 159 (2009)

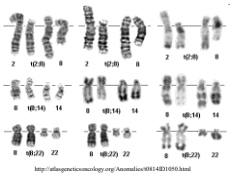
3 Forms of BL are now recognized:

- **Endemic**- 98% EBV association, incidence of 5-10/100,000 children in equatorial belt of Africa
- **Sporadic**- 15%-80% EBV association, varying by region
- **HIV-associated**- 30-40% EBV association

13

Burkitt's lymphoma and *MYC*

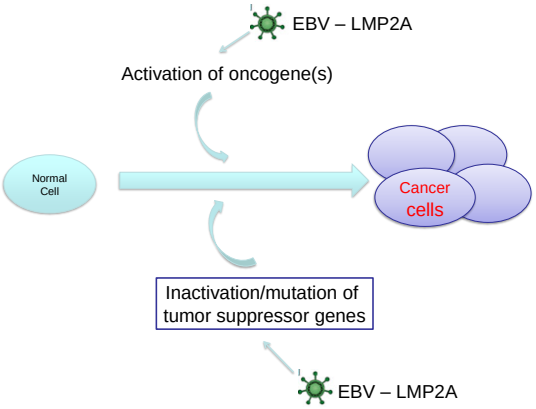
All Burkitt's tumors are characterized by a translocation between the *MYC* gene and the immunoglobulin heavy or light chain loci that result in high Myc expression.



VanGogh - *Starry Night Over Rhone*

- Tumors are thought to be derived from germinal center B cells.
  - *MYC* translocation may occur during chromosomal rearrangements that occur in GCs
- Histologically, tumors show "starry sky" pattern– caused by phagocytosis of apoptotic debris by macrophages.

14



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LMP2A is expressed in NPC, Hodgkin Lymphoma, and Burkitt Lymphoma

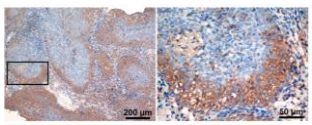
EBV Genome is found NPC cells

Few viral genes are expressed

LMP2A promotes invasiveness of epithelial cancers

-Epithelial-mesenchymal transition

-Matrix metalloprotease-9 upregulation



Kong QL,…, Zeng MS, PLoS Pathog. 2010

16

LMP2A/Myc Transgenic Mice

LMP2A and Myc Transgene Expressed in B cells

How Might LMP2A Contribute to Burkitt Lymphoma?

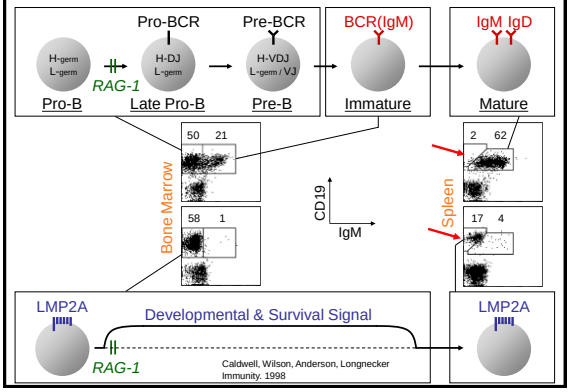
Tumor

Bone Marrow (BM)
Developing B Cells

Spleen (SP)
Mature, Peripheral B Cells

17

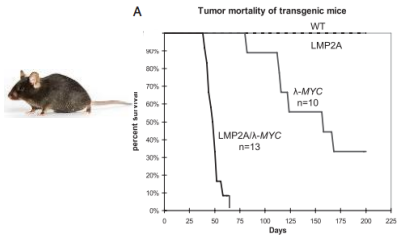
LMP2A Alters B Cell Development and Enhances Survival



Caldwell, Wilson, Anderson, Longnecker Immunity. 1998

18

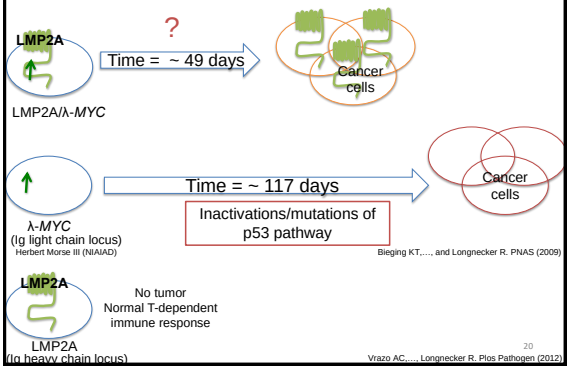
LMP2A/MYC Mice Develop Tumors Faster than MYC Mice



Bieging, K.T., Amick, A.C., and Longnecker, R. (2009). *PNAS*

19

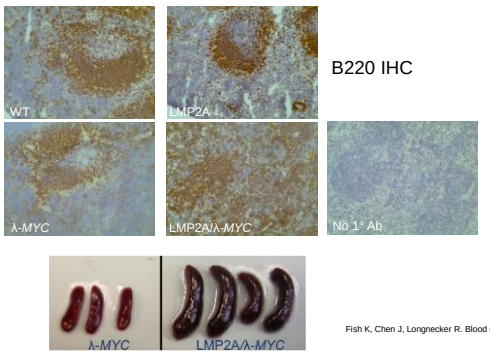
Roles of LMP2A and MYC in cancer development using transgenic mouse models



20

20

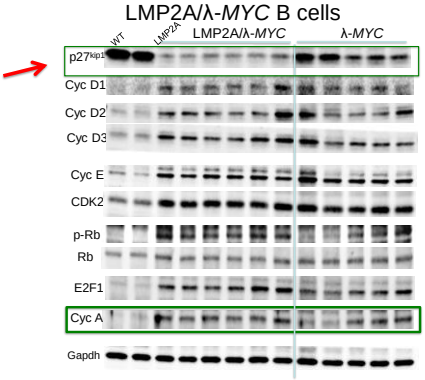
Splenomegaly and disorganized B cell follicles in LMP2A/λ-*MYC* mice



Fish K, Chen J, Longnecker R. Blood (2013)

21

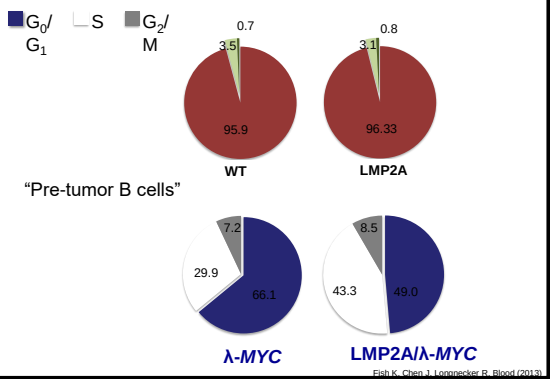
P27^{kip1} is downregulated in pre-tumor LMP2A/λ-*MYC* B cells



Fish K, Chen J, Longnecker R. Blood (2013)

22

LMP2A couples with MYC to promote G_1-S transition



Fish K, Chen J, Longnecker R. Blood (2013)

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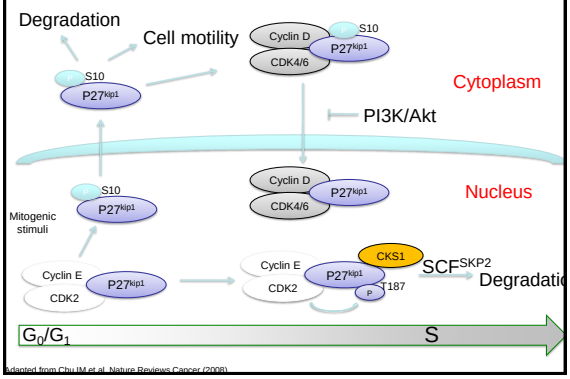
Regulation of p27^{kip1} at the protein level



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Regular Article

blood

LYMPHOID NEOPLASIA

EBV latent membrane protein 2A orchestrates $p27^{kip1}$ degradation via Cks1 to accelerate MYC-driven lymphoma in mice

Kamonwan Fish, Richard P. Sora, Samantha J. Schaller, Richard Longnecker, and Masato Ikeda

Department of Microbiology and Immunology, Feinberg School of Medicine, Northwestern University, Chicago, IL

Key Points

- LMP2A enhances MYC expression, resulting in the rapid degradation of the $p27^{kip1}$ tumor suppressor via Cks1, a member of the SCF^{Skp2} complex.
- Loss of Cks1 restores levels of the $p27^{kip1}$ tumor suppressor and prolongs LMP2A-mediated lymphomagenesis.

Epstein-Barr virus (EBV) establishes lifelong infection in B lymphocytes of most human hosts and is associated with several B lymphomas. During latent infection, EBV encodes latent membrane protein 2A (LMP2A) to promote the survival of B cells by mimicking host B-cell receptor signaling. By studying the roles of LMP2A during lymphoma development in vivo, we found that LMP2A mediates rapid MYC-driven lymphoma onset by allowing B cells to bypass MYC-induced apoptosis mediated by the p53 pathway in our transgenic mouse model. However, the mechanisms used by LMP2A to facilitate transformation remain elusive. In this study, we demonstrate a key role of LMP2A in promoting hyperproliferation of B cells by enhancing MYC expression and MYC-dependent degradation of the $p27^{kip1}$ tumor suppressor. Loss of the adaptor protein cyclin-dependent kinase regulatory subunit 1 (Cks1), a cofactor of the SCF^{Skp2} ubiquitin ligase complex and a downstream target of MYC, increases $p27^{kip1}$ expression during a premalignant stage. In mice that express LMP2A, Cks1 deficiency reduces spleen weights, restores B-cell follicle formation, impedes cell cycle progression of pretumor B cells, and eventually prolongs MYC-driven tumor onset. This study demonstrates that LMP2A uses the role of MYC in the cell cycle, particularly in the $p27^{kip1}$ degradation process, to accelerate lymphomagenesis in vivo. Thus, our results reveal a novel mechanism of EBV in diverting the functions of MYC in malignant transformation and provide a rationale for targeting EBV's roles in cell cycle modulation. (*Blood*. 2017;130(23):2516-2526)

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RESEARCH ARTICLE
Host-Microbe Biology

Two Pathways of $p27^{Kip1}$ Degradation Are Required for Murine Lymphoma Driven by Myc and EBV Latent Membrane Protein 2A

Richard P. Sora,ª Masato Ikeda,ª Richard Longneckerª

ªDepartment of Microbiology and Immunology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA

ABSTRACT Epstein-Barr virus (EBV) latent membrane protein 2A (LMP2A), expressed in EBV latency, contributes to Burkitt lymphoma (BL) development in a murine model by acting as a constitutively active B cell receptor (BCR) mimic. Mice expressing both LMP2A and *MYC* transgenes (LMP2A/λ-*MYC*) develop tumors significantly faster than mice only expressing *MYC* (λ-*MYC*). Previously, we demonstrated the cell cycle inhibitor $p27^{Kip1}$ is present at significantly lower levels in LMP2A/λ-*MYC* mice due to increased posttranslational degradation. $P27^{Kip1}$ degradation can occur in the cytoplasm following phosphorylation on serine 10 (S10) or in the nucleus via the SCF^{Skp2} complex, which depends on Cks1. We previously demonstrated an S10A knock-in of $p27^{Kip1}$ ($p27^{S10A/S10A}$) that prevented S10 phosphorylation failed to significantly delay tumor onset in LMP2A/λ-*MYC* mice. We also previously demonstrated that a Cks1 knockout partially delayed tumor onset in LMP2A/λ-*MYC* mice, but onset was still significantly faster than that in λ-*MYC* mice. Here, we have combined both genetic manipulations in what we call $p27^{Super}$ mice. LMP2A/λ-*MYC*/$p27^{Super}$ mice and λ-*MYC*/$p27^{Super}$ mice both displayed dramatic delays in tumor onset. Strikingly, tumor development in LMP2A/λ-*MYC*/$p27^{Super}$ mice was later than that in λ-*MYC* mice and not significantly different from that in λ-*MYC*/$p27^{Super}$ mice. The $p27^{Super}$ genotype also normalized G_1-S-phase cell cycle progression, spleen size, and splenic architecture in LMP2A/λ-*MYC* mice. Our results reveal both major pathways of $p27^{Kip1}$ degradation are required for the accelerated BL development driven by LMP2A in our BL model and that blocking both degradation pathways is sufficient to delay Myc-driven tumor development with or without LMP2A.

March/April 2019 Volume 10 Issue 2 e00548-19 mbio.asm.org 1

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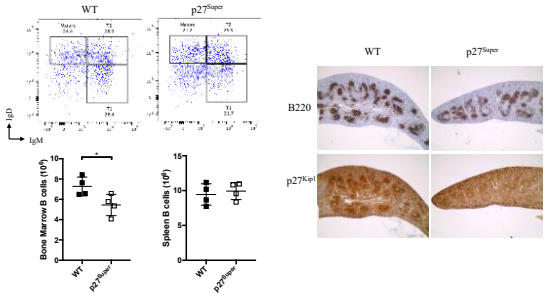
$p27^{Super}$ Mice Exhibit Normal B Cell Development



Sora, Ikeda, and Longnecker.

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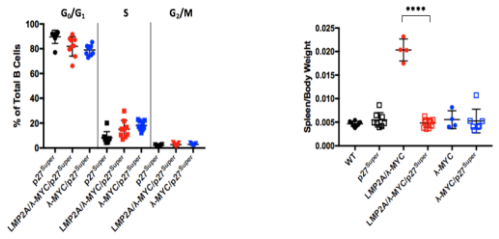
Cell Cycle and Spleen Size is Normalized by $p27^{Super}$



Sora, Ikeda, and Longnecker.

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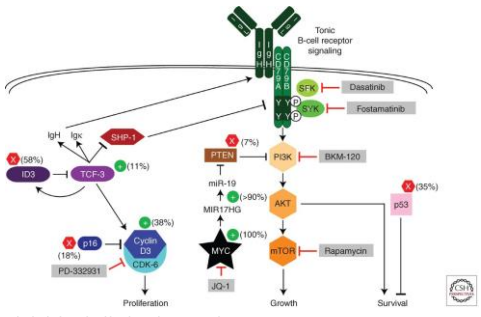
Schematic of recurrent oncogenic pathways in Burkitt lymphoma.



Roland Schmitz et al. Cold Spring Harb Perspect Med 2014;4:a014282

©2014 by Cold Spring Harbor Laboratory Press

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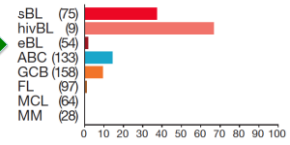
EBV infection may result in distinct genetic lesions in human cancer

Frequencies of *cell cycle* mutations in BL biopsies

sBL= Low % EBV+
hivBL = Intermediate % EBV+
eBL = >90% EBV+



Schmitz R,……, and Staudt LM. Nature (2012)

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Hepatitis C Virus

- HCV belongs to the genus Hepacivirus a member of the family Flaviviridae.
- Contains a positive sense single-stranded RNA genome. The genome consists of a single open reading frame that is about 9,600 nucleotides long. This single open reading frame is translated to produce a single protein product, which is then further processed to produce smaller active proteins.

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Hepatitis C Virus

- The infection is often asymptomatic, but chronic infection can lead to scarring of the liver and ultimately to cirrhosis, which is generally apparent after many years. In some cases, those with cirrhosis will go on to develop liver failure, liver cancer, or life-threatening esophageal and gastric varices.
- HCV is spread primarily by blood-to-blood contact associated with intravenous drug use, poorly sterilized medical equipment, and transfusions. An estimated 150–200 million people worldwide are infected with hepatitis C. No vaccine against hepatitis C is available.

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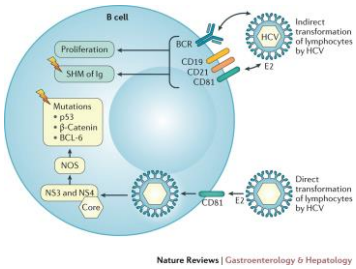
Putative non-exclusive mechanisms of HCV-related lymphomagenesis



Nature Reviews | Gastroenterology & Hepatology

Pol, S. *et al.* (2018) Extrahepatic cancers and chronic HCV infection
Nat. Rev. Gastroenterol. Hepatol. doi:10.1038/nrgastro.2017.172

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Variety of NEW Direct-acting antivirals

Protease inhibitor antiviral medications (NS3/4A inhibitors)

paritaprevir, for genotype 1
simeprevir (Olysio), for genotypes 1 and 4
grazoprevir, for genotypes 1 and 4

Protease inhibitors (NS5A inhibitors) all HCV genotypes. used alone or in combination with:

ledipasvir (a component of the combination drug Harvoni)
ombitasvir (a component of the combination drug Viekira Pak)
elbasvir (a component of the combination drug Zepatier)
daclatasvir (Daklinza)

Nucleotide/nucleoside and nonnucleoside polymerase inhibitors (NS5B inhibitors) blocking a protein called NS5B. The hepatitis C virus needs this protein to replicate itself and survive. Examples of these drugs include:

sofosbuvir (Sovaldi)
dasabuvir

Combinations pills to make taking the medication easier. Brand names of current combination therapies include:

Harvoni
Viekira Pak
Zepatier
Technivie
Epclusa
Vosevi
Mavyret

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Gilead Announces Generic Licensing Agreements to Increase Access to Hepatitis C Treatments in Developing Countries

PDF Download

-- Indian companies granted license to produce generic sofosbuvir and investigational single tablet regimen of ledipasvir/sofosbuvir for treatment of chronic hepatitis C --

NEW DELHI--(BUSINESS WIRE)--Sep. 15, 2014-- Gilead Sciences, Inc. (Nasdaq:GILD) today announced that the company has signed non-exclusive licensing agreements with seven India-based generic pharmaceutical manufacturers to expand access to its chronic hepatitis C medicines in developing countries. The agreements allow the companies – Cadila Healthcare Ltd., Cipla Ltd., Hetero Labs Ltd., Mylan Laboratories Ltd., Ranbaxy Laboratories Ltd., Sequent Scientific Ltd. and Strides Arcolab Ltd. – to manufacture sofosbuvir and the investigational single tablet regimen of ledipasvir/sofosbuvir for distribution in 91 developing countries.

The countries within the agreement account for more than 100 million people living with hepatitis C, representing 54% of the total global infected population.

"Hepatitis C is a significant public health issue worldwide, and Gilead is working to make its chronic hepatitis C medicines accessible to as many patients, in as many places, as quickly as possible. In developing countries, large-volume generic manufacturing and distribution is widely regarded as a key component in expanding access to medicines. These agreements are essential to advancing the goals of our humanitarian program in these countries," commented Gregg H. Alton, Executive Vice President, Corporate and Medical Affairs, Gilead Sciences.

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HTLV-1

- Naturally infects CD4+ T lymphocytes and can be transmitted between close contacts through blood transfusion or from mother to infant through cells in breast milk.
  - In most cases the infection is harmless. However, as many as 1 in 20 infected individuals eventually develop a type of adult T cell leukemia in which every tumor cell carries a clonally integrated HTLV1 provirus.
- HTLV-1 differs from RSV in its mechanism of action.
  - Drives cell growth through expression of the viral protein (Tax) in latently-infected cells.

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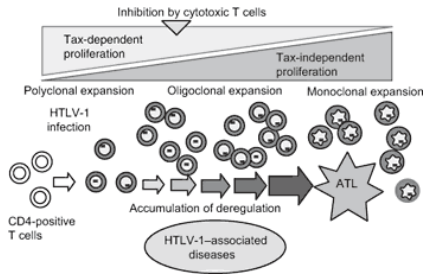
HTLV-1



Tax transactivates expression of a number of key cellular genes that enhance cell growth.
No virus specific treatments.

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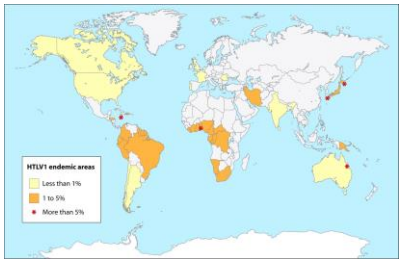
HTLV-1



HTLV-1 has an interesting geographical distribution pattern with a virtually exclusive concentration in southwestern Japan, the Caribbean islands, regions of South America, and tropical Africa.

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Conclusions

- Carcinogenesis is a multi-step process.
- The process involves mutations of cellular proto-oncogenes and tumor suppressor genes – virus can function in this process.
- Mechanisms of viral transformation include:
  - Activation of cellular signal transduction pathways.
  - Alteration of Cell cycle control pathways.
  - Cellular DNA repair impairment – mutation or increased of cellular proteins.
  - Inflammation

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Vaccine Basics for EBV, HIV, and HCV

- HIV and HCV are very different compared to EBV

- EBV is a DNA Virus – little to no sequence variation – high fidelity DNA polymerase used to replicate virus

- HIV – lots of variation – integrated DNA genome – RNA intermediate – polymerase used to replicate genome is error prone – generates diversity

- HCV – lots of variation similar to HIV - RNA genome with error prone polymerase

*For HIV and HCV a variety of very effective drugs exist that target different aspects of the virus life cycle

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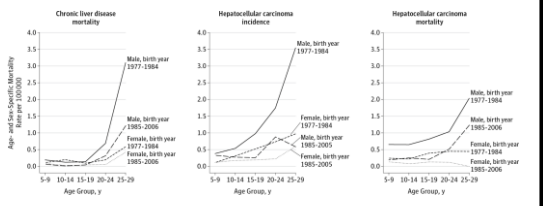
The JAMA Network

From: **Thirty-Year Outcomes of the National Hepatitis B Immunization Program in Taiwan**

JAMA. 2013;310(9):974-976. doi:10.1001/jama.2013.276701



The decline was greatest from 1981-1984 to 1985-1988 and from 1989-1992 to 1993-1996, coincident with the launch of the national immunization program in 1984 and the change to recombinant vaccines in 1992.

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EBV Prophylactic Vaccine Approaches

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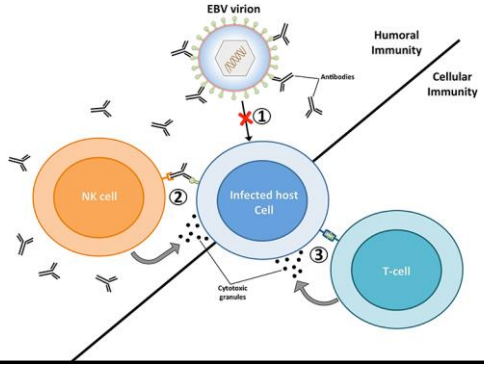
Immune Response to EBV Infection



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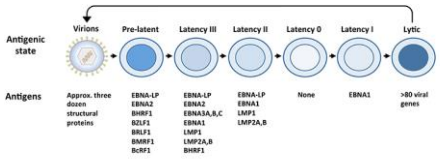
Large Variety EBV Antigens are Expressed in Latent and/or Lytic Infection.



Henri-Jacques Delecluse, 2019

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TABLE 2: Summary of prophylactic and therapeutic EBV vaccine candidates that have been developed.

Vaccine category	EBV antigens included	Results
Epitope vaccine	EBNA3A (23)	Vaccination induced EBNA3A-specific T-cell responses, but the vaccine did not protect against EBV infection. Vaccinated individuals had a lower incidence of IM, but sample sizes were to small to enable statistical analysis.
Antigen-antibody conjugates	EBNA1 (101)	Targeting of dendritic cells enabled the induction of EBNA1-specific $CD4^+$ and $CD8^+$ T cells and vaccination of humanized mice generated EBNA1-specific T cells.
	Several latent antigens (102, 103)	Multiple Antibody-antigen conjugates were several orders of magnitude more efficient than peptide at stimulating cytotoxic $CD4^+$ T cells.
Monomeric	gp350 (21)	Vaccination induced neutralizing gp350-specific antibodies, reduced the incidence of IM but did not protect against EBV infection.
Multimeric	Tetrameric gp350 (104)	Rabbits vaccinated with tetrameric gp350 had neutralizing titers 19-fold higher than rabbits vaccinated with monomeric gp350.
	Trimeric gH/gL and trimeric gB (105)	Serum neutralizing titers were respectively >100-fold and 18-fold higher in rabbits vaccinated with trimeric gH/gL and trimeric gB compared to monomeric gp350.
Nanoparticles	gp350 (106)	Mice and monkeys that were vaccinated with gp350-containing nanoparticles generated potent gp350-specific neutralizing antibodies compared to animals vaccinated with monomeric gp350.
Chimeric NDV VLPs	gp350 (107)	NDV-VLPs containing gp350 elicited superior neutralizing antibodies in vaccinated mice compared to monomeric gp350.
	gH/gL, gp42, LMP2, EBNA1 (108)	The NDV VLP platform was utilized to incorporate various EBV latent and lytic antigens.
EBV VLPs	More than three dozen structural proteins (109)	EBV VLPs elicited neutralizing EBV-specific antibodies and T-cell responses in vaccinated mice
	More than three dozen structural proteins (63)	EBV VLPs stimulated structural protein-specific T cells to the same degree as wtEBV.
	More than three dozen structural proteins and EBNA1 (110)	Modified EBV VLPs stimulated structural protein- and latent protein-specific T cells and afforded increased protection in humanized mice.

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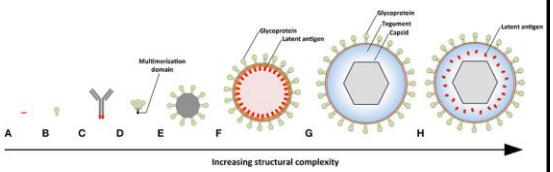
EBV vaccine candidates vary in their structural complexity and antigenic content.



(A) An epitope peptide from EBNA3A.
(B) Recombinant gp350 expressed as a monomeric protein.
(C) EBV latent antigens (red) conjugated to dendritic cell- or B-cell-specific antibodies.
(D) Multimeric forms of EBV structural proteins
(E) Self-assembling ferritin nanoparticles presenting gp350 or gp42 and gH/gL.
(F) Chimeric NDV VLPs containing lytic and latent antigens.
(G) EBV VLPs comprise numerous envelope (green), tegument (blue), and capsid (gray) proteins.
(H) The tegument of EBV VLPs can be modified to contain latent antigens (red).

Henri-Jacques Delecluse, 2019

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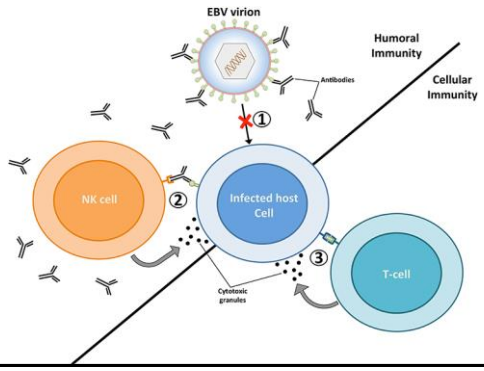
EBV Target Proteins to Block Infection



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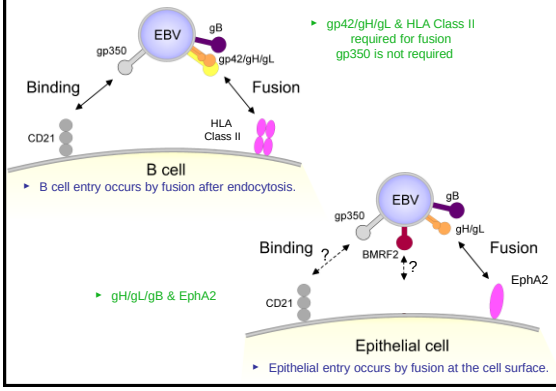
EBV Infects Two Cell Types Through Two Pathways



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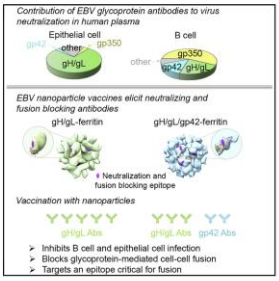
Immunity

Immunization with Components of the Viral Fusion Apparatus Elicits Antibodies That Neutralize Epstein-Barr Virus in B Cells and Epithelial Cells

Graphical Abstract



Authors

Wei Bu, M. Gordon Joyce, Hanh Nguyen, ..., Gary J. Nabel, Masaru Kanekiyo, Jeffrey I. Cohen

Correspondence

jcohen@niaid.nih.gov

In Brief

EBV is associated with B cell and epithelial-cell malignancies. Bu et al. showed that an EBV nanoparticle vaccine elicits antibodies to EBV gH/gL and gp42 in mice and non-human primates that inhibit the viral-fusion apparatus and block infection of B cells and epithelial cells. This approach may be important for developing an effective EBV vaccine.

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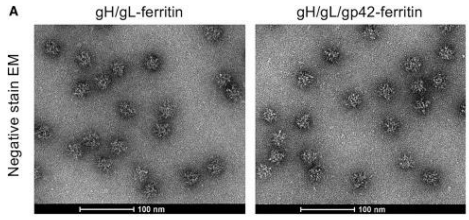
EBV Nanoparticle vaccine



Bu, Joyce, . . Cohen - Immunity. 2019 May

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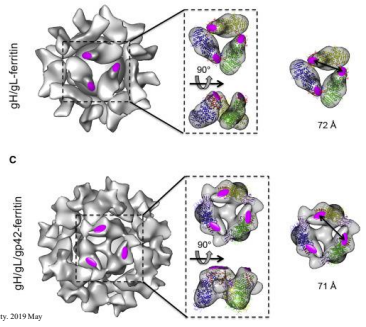
Cryo-EM reconstruction models of gH/gL-ferritin (B) and gH/gL/gp42-ferritin (C) nanoparticles.



Bu . . . Cohen - Immunity. 2019 May

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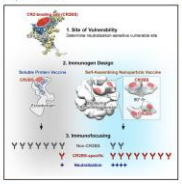
Cell

Rational Design of an Epstein-Barr Virus Vaccine Targeting the Receptor-Binding Site

Graphical Abstract



Authors

Masaru Kanekiyo, Wei Bu, M. Gordon Joyce, ..., Barney S. Graham, Jeffrey I. Cohen, Gary J. Nabel

Correspondence

jcohen@niaid.nih.gov (J.I.C.), gary.nabel@sanofi.com (G.J.N.)

In Brief

Structurally designed EBV vaccine candidates based on self-assembling nanoparticles elicit potent and durable virus-neutralizing antibodies that target the receptor-binding site on the viral envelope protein gp350, a site of vulnerability, serving as a template to develop an EBV vaccine and providing a basis for immunofocusing through rational vaccine design.

Highlights

- Self-assembling nanoparticles present the conserved gp350 receptor-binding domain
- The nanoparticles elicit more potent neutralizing antibodies than soluble gp350
- These neutralizing antibodies predominantly target the CR2-binding site on gp350
- The nanoparticles elicit potent neutralizing antibodies in mice and non-human primates

CellPress

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FIRST IN 10 YEARS FDA-APPROVED

THE SHINGRIX DIFFERENCE

SHINGRIX is the first shingles vaccine approved by the FDA in more than 10 years.

SHINGRIX is the only shingles vaccine proven to be up to 90% effective in clinical trials.

HOW SHINGRIX WORKS

SHINGRIX works to boost your body's protection against shingles. Your immune system declines as you age, and that puts you at an increased risk for shingles. For those who are 50 years and older, SHINGRIX helps your immune system defend against shingles regardless of age.

Consists of Glycoprotein E (gE) – important for viral entry - essential for replication and T cell entry with an extract of the Quillaja Saponaria, a saponin, from a tree

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The Journal of Infectious Diseases

IDSA hivma

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Modified mRNA-Based Vaccines Elicit Robust Immune Responses and Protect Guinea Pigs From Ebola Virus Disease

Michelle Meyer, Eric Huang, Olga Yuzhakov, Palaniappan Ramanathan, Giuseppe Ciaramella, Alexander Bukreyev

The Journal of Infectious Diseases, Volume 217, Issue 3, 1 February 2018, Pages 451–455, https://doi.org/10.1093/infdis/jix592

Published: 21 December 2017 Article history

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Article Contents

Abstract

METHODS

RESULTS

DISCUSSION

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References

Abstract

Most current Ebola virus (EBOV) vaccine candidates are based on viral vectors, some of which cause side effects or require complex manufacturing. Modified mRNA vaccines are easily produced, safe, and are highly immunogenic. We developed 2 mRNA vaccines based on the EBOV envelope glycoprotein, which differed by the nature of signal peptide for improved glycoprotein post-translational translocation. The mRNAs were formulated with lipid nanoparticles to facilitate delivery. Vaccination of guinea pigs induced EBOV-specific IgG1 and neutralizing antibody responses and 100% survival after EBOV infection. The efficacy of our mRNA vaccine combined with preclinical safety data supports testing in clinical studies.

The Journal of Infectious Diseases

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EBV Therapeutic Vaccine Approaches

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EBV-Associated NPC – an Ideal Cancer for a Therapeutic Vaccine

In EBV-related NPC EBV target antigens are expressed that are potential therapeutic targets for immune modulation or cytotoxic T cell therapies.

Adoptive autologous cytotoxic T-cell therapy and therapeutic cancer vaccines have shown success in inducing tumor responses in a small subset of treatment-refractory patients.

Phase I/II single-arm studies have confirmed the efficacy and safety anti-PD-1 immune checkpoint inhibitors (pembrolizumab, nivolumab, and camrelizuma) in recurrent or metastatic NPC.

An immunosuppressive environment is present in NPC which facilitates tumor persistence and progression and complicates treatment.

Immunotherapeutic approaches in nasopharyngeal carcinoma
James CH Chow, Roger KC Ngan, KM Cheung & William CS, July, 2019

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Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation

Cliona M Rooney, Colton A Smith, Catherine Y C Ng, Susan Loftin, Congfen Li, Robert A Krance, Malcolm K Brenner, Helen E Heslop

Summary

Reactivation of Epstein-Barr virus (EBV) after bone-marrow transplantation leads in many cases to lymphoproliferative disease that responds poorly to standard therapy and is usually fatal. To prevent or control this complication, we prepared EBV-specific cytotoxic T-lymphocyte (CTL) lines from donor leucocytes and infused them into ten allograft recipients.

Three of the patients had shown signs of EBV reactivation, with or without overt lymphoproliferation, and the others received CTL infusions as prophylaxis. No patient developed any complication that could be attributed to the CTL infusions. In the three patients with EBV reactivation, EBV DNA concentrations (measured by semiquantitative polymerase chain reaction [PCR]), which had increased 1000-fold or more, returned to the control range within 3–4 weeks of immunotherapy. The most striking consequence was the resolution of immunoblastic lymphoma in a 17-year-old patient who received four CTL infusions (two $1\times10^7/m^2$ and two $5\times10^7/m^2$). Because the CTL had been genetically marked before infusion, we were able to show by PCR analysis that they persisted for 10 weeks after administration.

EBV-specific donor-type T-cell lines seem to offer safe and effective therapy for control of EBV-associated lymphoproliferation.

Lancet 1995; **345:** 9–13

Introduction

Epstein-Barr virus (EBV) has been linked with a growing number of malignant diseases, especially in patients with compromised immunity. The virus has been found in immunoblastic lymphomas arising in allograft recipients and HIV-infected patients, in the oral leucoplakia of AIDS, and in Reed-Sternberg cells from some patients with Hodgkin's disease.[1-4] Although it infects about 90% of the world's population, EBV is believed to be controlled in the normal host by virus-specific cytotoxic T lymphocytes (CTL), which lyse EBV-infected B cells when they recognise fragments of viral proteins that have been degraded to peptides within the B cell then presented on the cell surface by MHC class I molecules.[5] Although unable to eliminate EBV from the body, CTL seem to be essential in maintaining control of latently infected cells.

If these assumptions about virus-specific T lymphocytes are correct, infusions of such cells should offer a safe and effective form of treatment for immunodeficient patients with EBV-related lymphoproliferation. Papadopoulos et al[6] used unseparated leucocytes from EBV-seropositive donors to treat five patients with EBV-associated immunoblastic lymphomas that arose after transplantation of T-cell-depleted bone-marrow allografts. All patients had complete responses judged by clinical or laboratory criteria; these responses were attributed to the activity of donor-type EBV-reactive T cells. Although promising, this approach was marred by the development of fatal

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CLINICAL TRIALS AND OBSERVATIONS

Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial

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We present the results of a multicenter clinical trial using Epstein-Barr virus (EBV)–specific cytotoxic T lymphocytes (CTLs) generated from EBV-seropositive blood donors to treat patients with EBV-positive posttransplantation lymphoproliferative disease (PTLD) on the basis of the best HLA match and specific in vitro cytotoxicity. Thirty-three PTLD patients who had failed on conventional therapy were enrolled. No adverse effects of CTL infusions were observed and the response rate (complete or partial) in 33 patients was 64% at 5 weeks and 52% at 6 months. Fourteen patients achieved a complete remission, 3 showed a partial response, and 16 had no response at 6 months (5 died before completing treatment). At 5 weeks, there was a significant trend toward better responses with higher numbers of CD4+ cells in infused CTL lines ($P = .001$) that were maintained at 6 months ($P = .001$). Patients receiving CTLs with closer HLA matching responded better at 6 months ($P = .048$). Female patients responded better than male patients, but the differences were not statistically significant. Our results show that allogeneic CTLs are a safe and rapid therapy for PTLD, bypassing the need to grow CTLs for individual patients. The response rate in this poor prognosis patient group is encouraging. (Blood. 2007;110:1123-1131)

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Bone Marrow Transplantation (2019) 54:759–764
https://doi.org/10.1038/s41409-019-0614-1

EBMT

REVIEW ARTICLE

Therapeutic advantages provided by banked virus-specific T-cells of defined HLA-restriction

Richard J. O'Reilly[1] · Susan Prockop[1] · Aisha Hasan[1] · Ekaterina Doubrovina[1]

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Abstract

We have developed banks of EBV and CMV-specific T-cell lines generated from healthy seropositive third party donors and characterized them as to their HLA type, virus specificity, lack of alloreactivity, and HLA restriction. We here summarize results of studies employing these immediately accessible, broadly-applicable third party virus-specific T-cells for adoptive therapy of EBV lymphomas and CMV infections in allo-HCT recipients. We describe the characteristics contributing to their safety. We also discuss several distinctive advantages of banked third party virus-specific T-cells selected on the basis of their HLA restriction, particularly in the treatment of Rituximab-non-responsive EBV+ lymphomas and drug refractory CMV infections complicating HLA non-identical transplants.

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Table 1. Therapeutic vaccine strategies against EBV-associated diseases.

Disease setting	Source of T cells	Cohort size	Clinical outcome	Reference
PTLD (HSCT)	HSCT donor-derived	10	3 CR and 7 DF	[107]
PTLD (HSCT)	HSCT donor-derived	14	14 DF	[105]
PTLD (HSCT)	HSCT donor-derived	39	33 DF and 6 CR	[106]
PTLD (SOT)	Autologous	1	1 CR	[111]
PTLD (SOT)	Autologous	7	2 CR and 5 PR	[113]
PTLD (SOT)	Autologous	1	1 CR	[68]
PTLD (SOT)	Autologous	12	11 NP and 1 CR	[112]
PTLD (HSCT)	HSCT donor-derived	4	3 CR and 1 PD	[109]
PTLD (HSCT)	HSCT donor-derived	10	3 CR	[69]
PTLD (HSCT)	HSCT donor-derived	6	3 CR and 3 PD	[134]
PTLD (HSCT)	HSCT donor-derived	114	101 DF, 11 CR and 2 PD	[108]
PTLD (HSCT)	HSCT donor-derived	14	13 CR, 4 PD and 1 NE	[110]
PTLD (HSCT)	HSCT donor-derived	3	3 CR	[135]
PTLD	Off-the-shelf T cell therapy	33	14 CR, 3 PR and 16 PD	[139]
PTLD (SOT)	Off-the-shelf T cell therapy	3	2 CR	[70]
PTLD	Off-the-shelf T cell therapy	2	2 CR	[71]
PTLD	Off-the-shelf T cell therapy	8	3 CR and 2 PD (3 DNC)	[138]
PTLD	Off-the-shelf T cell therapy	2	2 CR	[142]
PTLD	Off-the-shelf T cell therapy	5	4 CR and 1 PD	[110]
PTLD	Off-the-shelf T cell therapy	1	1 CR	[143]
PTLD	Off-the-shelf T cell therapy	35	22 CR, 3 PR and 10 PD	
PTLD	Off-the-shelf T cell therapy	10	8 CR	[140]
primary CNS B-cell lymphoma	Off-the-shelf T cell therapy	1	Steady neurological improvement	[72]
NPC	Autologous	4	4 PD	[73]
NPC	Autologous	10	2 PR, 4 SD and 4 PD	[116]
NPC	Autologous	10	4 NP, 2 CR, 1 PR, 1 SD and 2 PD	[119]
NPC	Autologous	8	1 CR, 2 SD and 5 PD	[118]
NPC	Autologous	23	5 NP, 3 CR, 2 PR, 5 SD and 8 PD	[117]
NPC	Autologous	11	2 PR, 4 SD and 5 PD	[123]
NPC	Autologous	14	10 SD AND 4 PD	[74]
NPC	Autologous	35	3 CR*, 22 PR* and 10 SD*	[124]
NPC	Autologous	1	1 PR	[75]
NPC	Autologous	29	6 CR, 12 SD and 1 PD	[123]
HL	Autologous	3	2 CR*, 1 SD	[121]
HL	Autologous	14	1 NP, 4 CR, 1 PR, 5 SD and 3 PD	[76]
EBV+ NKTL	Sibling derived or autologous	3	2 NP and 1 PD	[120]
NKTL	Autologous	10	9 NP and 1 PD	[77]
NKTL, HL, PTLD and DLBCL	Autologous	50	29 NP, 11 CR, 2 PR and 9 PD	[136]
MS	Autologous	1	Clinical improvement with reduced disease activity	[133]
MS	Autologous	10	7 withneurological improvement	[132]

Abbreviations: CR: complete response; DF: disease free; DLBCL: diffuse large B-cell lymphoma; HL: Hodgkin lymphoma; HSCT: hematopoietic stem cell transplantation; NE: not evaluable; NKTL: NK/T cell lymphoma; NP: no progression; NPC: nasopharyngeal carcinoma; PD: progressive disease; PR: partial response; PTLD: post-transplant lymphoma; SD: stable disease; SOT: solid-organ transplant.
*Clinical response determined following combination of chemotherapy and cytotoxic T lymphocytes.

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Table 2. Human clinical trials using adoptive T-cell therapy against EBV-associated diseases.

Disease setting	Vaccine formulation	Cohort size	Clinical outcome	Reference
NPC	Autologous DCs pulsed with HLA-A1101-, A2402-, or B40011-restricted epitope peptides from LMP2	16	$CD8^+$ T-cell responses boosted in 9 patients	[90]
NPC	Autologous DCs pulsed with HLA-restricted epitope peptides from LMP2	16	9 patients responded to LMP2A peptides, and serum EBV-DNA level significantly decreased	[91]
NPC	DCs transduced with adenovirus encoding a truncated LMP1 (DeltaLMP1) and full-length LMP2 (Ad-DeltaLMP1-LMP2)	16	No increase detected in the frequency of peripheral LMP1/2-specific T cells	[92]
NPC	Recombinant vaccinia virus, MVA-EL, which encodes an EBNA1/LMP2 fusion protein	18	T-cell responses to EBNA1 and/or LMP2 increased in 15 patients	[94]
NPC	MVA-EL	16	T-cell responses to EBNA1 and/or LMP2 increased in 8 patients	[95]
NPC	Recombinant adenoviral vaccine expressing EBV-LMP2 protein (rAd5-EBV-LMP2)	24	Proportion of $CD3^+$ $CD4^+$ cells in peripheral blood significantly increased	[96]

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Table 3. Immunotherapies in ongoing clinical trials.

Disease setting	Name of the study	Phase	Source of T cells	Clinical trial ID
Transplantation	Using Multi-virus Cytotoxic T-Cells Following T-Cell Depleted Allogeneic HPCT for Prophylaxis Against Epstein Barr Virus, Adenovirus, And Cytomegalovirus (ACE)	Phase I	Off-the-shelf T cell	NCT01535885
Transplantation (HSCT)	Tabelecleucel for Allogeneic Hematopoietic Cell Transplant Subjects With EBV+ PTLD After Failure of Rituximab	Phase III	Off-the-shelf T cell	NCT03392142
Transplantation (SOT)	Tabelecleucel for Solid Organ Transplant Subjects With EBV+ PTLD After Failure of Rituximab or Rituximab and Chemotherapy	Phase III	Off-the-shelf T cell	NCT03394365
EBV^+ PTLD	A Study of the Safety and Efficacy of EBV Specific T-cell Lines	Phase I/II	Autologous; Allogeneic donor; Matched or partially matched related donor	NCT02580539
Acute Lymphocytic Leukaemia; Lymphoma	In Vitro Expanded Allogeneic Epstein-Barr Virus Specific Cytotoxic T-Lymphocytes (EBV-CTLs) Genetically Targeted to the CD19 Antigen in B-cell Malignancies	Phase I	Donor derived	NCT01430390
NPC	A Phase III Trial Evaluating Chemotherapy and Immunotherapy for Advanced Nasopharyngeal Carcinoma (NPC) Patients	Phase III	Autologous	NCT02578641
EBV^+ Lymphomas; EBV+ Malignancies	Biological Therapy in Treating Patients at High-Risk or With Lymphoma, Lymphoproliferative Disease, or Malignancies	Phase I/II	Off-the-shelf T cell	NCT00002663
Hodgkin Disease; Non-Hodgkin Lymphoma; Severe Chronic Active EBV; T/NK-Lymphoproliferative Disease	Most Closely Matched 3rd Party Rapidly Generated LMP, BARF1 And EBNA1 Specific CTL, EBV-Positive Lymphoma (MABEL)	Phase I	Off-the-shelf T cell	NCT02287311
EBV^+ PTLD	Rituximab and LMP-Specific T-Cells in Treating Pediatric Solid Organ Recipients With EBV-Positive, CD20-Positive Post-Transplant Lymphoproliferati [italic: order	Phase II	Off-the-shelf T cell	NCT02900976
Diffuse Large B-Cell Lymphoma; B Acute Lymphoblastic Leukaemia; Burkitt Lymphoma	Evaluation of CD19/CD22 chimeric antigen receptor T cells efficacy in various clinical trials	Phase I/II	CD19 CAR T cells	NCT03233854 NCT03671460 NCT03614858 NCT03599375 NCT03574168 NCT03559439 NCT03467256 NCT03289455 NCT02443831
Relapsed B-cell Acute Lymphoblastic Leukaemia after CD19 CAR-T adoptive cellular immunotherapy	A Feasibility and Safety Study of CD38 CAR-T Cell Immunotherapy for Relapsed B-cell Acute Lymphoblastic Leukaemia After CD19 CAR-T Adoptive Cellular Immunotherapy	Phase I/II	CD38 CAR T cells	NCT03754764

Dasari, Sinha, Neller, Smith and Khannam EXPERT REVIEW OF VACCINES, 2019

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HIV Vaccine Approaches

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History of HIV Vaccine Research

1981
HIV was identified as the cause of AIDS. U.S. HHS Secretary Margaret Heckler declared that an AIDS vaccine will be ready for testing within two years.

1987
The first HIV vaccine clinical trial opened at the National Institutes of Health (NIH) Clinical Center in Bethesda, Maryland. This Phase 1 trial enrolled 138 healthy, HIV-negative volunteers. The gp160 subunit vaccine showed no serious adverse effects.

1988
The NIAID AIDS Vaccine Evaluation Group (AVEG), the first U.S. cooperative HIV vaccine clinical trials group, began enrolling volunteers in its first trial.

1992
NIAID launched the first Phase 2 HIV vaccine clinical trial. This trial included HIV-negative volunteers with a history of high-risk behavior – injection drug use, multiple sex partners, or sexually transmitted infections. Participants were counseled repeatedly to avoid any behaviors that put them at risk of HIV infection.

1998
The first annual HIV Vaccine Awareness Day to honor vaccine study volunteers was observed.

The first large-scale HIV vaccine trial began. VaxGen initiated a Phase 3 trial of AIDSVAX (VAX004) in North America and the Netherlands involving more than 5,400 volunteers.

1999
NIAID began the first African-preventive HIV vaccine trial in Uganda.

The first large-scale HIV vaccine trial in a developing country began. VaxGen initiated a Phase 3 trial of AIDSVAX (VAX004) involving over 2,500 volunteers in Thailand.

The newly established Vaccine Re

2000
NIAID formed the HIV Vaccine Trial
more than 25 sites in the United St

The first African HIV vaccine trial w

2003
The U.S. and Royal Thai governme

Formation of the Global HIV Vaccir

2004
Both VaxGen candidates failed to confer protection against HIV in Phase 3 trials.

2007
NIAID halted the Phase 2 Step and Phambili studies due to safety concerns.

2009
The Phase 2 HVTN 505 study was initiated to evaluate a "prime-boost" vaccine regimen developed by the VRC.

Results of the Phase 3 Thai Trial (RV144) revealed that the vaccine combination demonstrated a modest preventive effect in humans. The trial, which enrolled more than 16,000 volunteers, was the first, and to date only, large clinical study to demonstrate efficacy for an investigational HIV vaccine.

2010
VRC scientists identified two potent antibodies that neutralize most strains of HIV in the laboratory (VRC01 and VRC02).

The Pox-Protein Public-Private Partnership (P5), an international collaborative team committed to building on the modest success of RV144, was formed.

2011
HVTN 505 was expanded to include protection from HIV as primary endpoint.

2012
Additional analyses of samples from RV144 provided insight into what types of immune responses may be needed for an effective vaccine.

2013
HVTN 505 immunizations were stopped due to lack of efficacy.

2015
The Phase 1/2 HVTN 100 study, part of the P5 research endeavor, launched to test the safety of an experimental HIV vaccine regimen based upon the RV144 findings, as well as its ability to generate an immune response.

2016
NIAID launched the AMP Studies to test whether intravenous infusions of the antibody VRC01 are safe, tolerable and effective at preventing HIV infection. The trials were also designed to answer fundamental scientific questions for HIV prevention and vaccine research.

HVTN 702, part of the P5 research endeavor, launched to test whether a new version of the RV144 HIV vaccine candidate safely prevents HIV infection among adults in South Africa.

2017
NIAID and partners launched Imbokodo or HVTN 705/HPX2008, a Phase 2b proof-of-concept study evaluating the safety and efficacy of an experimental regimen based on a "mosaic" vaccine designed to induce immune responses against a wide variety of global

RV144 trial in Thailand moderate protective effect of 31% with respect to placebo administration. Over six months, volunteers received a prime-boost vaccination including six injections, four injections of a vaccine called ALVAC HIV (vCP1521) with the last two being at the same time as two injections of another vaccine called AIDSVAX B/E (gp120).

https://www.niaid.nih.gov/diseases-conditions/hiv-vaccine-research-history

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FOCUS ON RESEARCH

Broadly Neutralizing Antibodies for HIV-1 Prevention or Immunotherapy

Marina Caskey, M.D., Florian Klein, M.D., and Michel C. Nussenzweig, M.D., Ph.D.

Despite tremendous efforts to prevent transmission of human immunodeficiency virus type 1 (HIV-1), new infections and AIDS-related deaths continue to be a global problem. Antiretroviral therapy (ART) can effectively suppress HIV-1 replication and limit disease progression. However, ART fails to eradicate the virus, and suppression requires lifelong therapy, which may have side effects and poses a risk of the development of resistance. New approaches to preventing and treating HIV-1 infection are therefore necessary to contain the epidemic and to bolster nascent efforts to find a cure.

A new generation of highly potent broadly neutralizing antibodies (bNAbs) may represent a promising approach to combating HIV-1 infection.[1] Although antibodies that neutralize HIV-1 were discovered shortly after the disease was first described, most HIV-1–infected people produce nonneutralizing antibodies or antibodies that neutralize only a limited number of different HIV-1 strains. However, high serum titers of HIV-1 neutralizing activity develop in a small fraction of infected persons. In contrast to the typical antibody response, bNAbs can take years to develop, and it has not yet proved possible to elicit them by standard immunization strategies.

Isolation of bNAbs from infected persons with high levels of HIV-1–neutralizing serum activity (so-called elite neutralizers) was not easily achieved until the advent of single-cell–based anti-HIV-1 antibody cloning techniques.[2] The anti–HIV-1 antibodies obtained

The New England Journal of Medicine
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ARTICLE

https://doi.org/10.1038/s41586-018-0531-2

Combination therapy with anti-HIV-1 antibodies maintains viral suppression

Pilar Mendoza[1,19], Henning Gruell[2,3,4,19], Lilian Nogueira[1], Joy A. Pai[1], Allison L. Butler[1], Katrina Millard[1], Clara Lehmann[3,4,5], Isabelle Suárez[3,4,5], Thiago Y. Oliveira[1], Julio C. C. Lorenzi[1], Yehuda Z. Cohen[1], Christoph Wyen[3,6], Tim Kümmerle[3,6], Theodora Karagounis[1], Ching-Lan Lu[1], Lisa Handl[2], Cecilia Unson-O'Brien[1], Roshni Patel[1], Carola Ruping[2], Malke Schlotz[2], Maggi Witmer-Pack[1], Irina Shimeliovich[1], Gisela Kremer[1], Eleonore Thomas[1], Kelly E. Seaton[8], Bill Horowitz[1], Anthony P. West Jr[9], Pamela J. Bjorkman[9], Georgia D. Tomaras[8,10,11,12], Roy M. Gulick[13], Nico Pfeifer[7,14,15,16], Gerd Fätkenheuer[3,4], Michael S. Seaman[17], Florian Klein[2,4,5,20]*, Marina Caskey[1,20]* & Michel C. Nussenzweig[1,18,20]*

Individuals infected with HIV-1 require lifelong antiretroviral therapy, because interruption of treatment leads to rapid rebound viraemia. Here we report on a phase 1b clinical trial in which a combination of 3BNC117 and 10-1074, two potent monoclonal anti-HIV-1 broadly neutralizing antibodies that target independent sites on the HIV-1 envelope spike, was administered during analytical treatment interruption. Participants received three infusions of 30 mg kg^{-1} of each antibody at 0, 3 and 6 weeks. Infusions of the two antibodies were generally well-tolerated. The nine enrolled individuals with antibody-sensitive latent viral reservoirs maintained suppression for between 15 and more than 30 weeks (median of 21 weeks), and none developed viruses that were resistant to both antibodies. We conclude that the combination of the anti-HIV-1 monoclonal antibodies 3BNC117 and 10-1074 can maintain long-term suppression in the absence of antiretroviral therapy in individuals with antibody-sensitive viral reservoirs.

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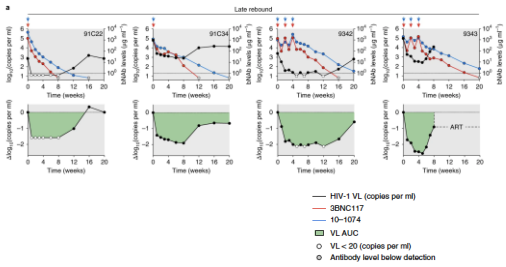
Fig. 2: Viral load following 3BNC117/10-1074 infusions in HIV-1-infected participants.

From: Safety and antiviral activity of combination HIV-1 broadly neutralizing antibodies in viremic individuals



Safety and antiviral activity of combination HIV-1 broadly neutralizing antibodies in viremic individuals
Yotam Bar-On, Henning Gruell, … Michel C. Nussenzweig
Nature Medicine, pages1701–1707 (2018)

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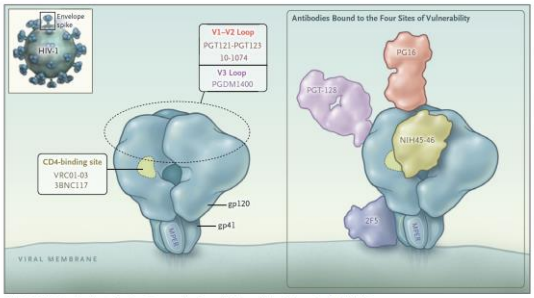

HIV-1 Spike Protein, Showing Sites Targeted by Broadly Neutralizing Monoclonal Antibodies.
The inset shows the virus with its surface spikes. The left panel shows target sites of monoclonal antibodies in clinical development. The right panel illustrates the binding of four different broadly neutralizing antibodies.

Dashti A et al. Trends Mol Med. Trends in Molecular Medicine, March 2019

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SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

HIV

A live-attenuated RhCMV/SIV vaccine shows long-term efficacy against heterologous SIV challenge

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Scott G. Hansen[1], Emily E. Marshall[1]*, Daniel Malouli[1], Abigail B. Ventura[1], Colette M. Hughes[1], Emily Ainslie[1], Julia C. Ford[1], David Morrow[1], Roxanne M. Gilbride[1], Jin Y. Bae[1], Alfred W. Legasse[1], Kelli Oswald[2], Rebecca Shoemaker[2], Brian Berkemeier[2], William J. Bosche[2], Michael Hull[2], Jennie Womack[1], Jason Shao[3], Paul T. Edlefsen[3], Jason S. Reed[1], Ben J. Burwitz[1], Jonah B. Sacha[1], Michael K. Axthelm[1], Klaus Früh[1], Jeffrey D. Lifson[2], Louis J. Picker[1†]

Previous studies have established that strain 68-1–derived rhesus cytomegalovirus (RhCMV) vectors expressing simian immunodeficiency virus (SIV) proteins (RhCMV/SIV) are able to elicit and maintain cellular immune responses that provide protection against mucosal challenge of highly pathogenic SIV in rhesus monkeys (RMs). However, these efficacious RhCMV/SIV vectors were replication and spread competent and therefore have the potential to cause disease in immunocompromised subjects. To develop a safer CMV-based vaccine for clinical use, we attenuated 68-1 RhCMV/SIV vectors by deletion of the Rh110 gene encoding the pp71 tegument protein (ΔRh110), allowing for suppression of lytic gene expression. ΔRh110 RhCMV/SIV vectors are highly spread deficient in vivo (~1000-fold compared to the parent vector) yet are still able to superinfect RhCMV$^+$ RMs and generate high-frequency effector-memory–biased T cell responses. Here, we demonstrate that ΔRh110 68-1 RhCMV/SIV–expressing homologous or heterologous SIV antigens are highly efficacious against intravaginal (IVag) SIV$_{mac239}$ challenge, providing control and progressive clearance of SIV infection in 59% of vaccinated RMs. Moreover, among 12 ΔRh110 RhCMV/SIV–vaccinated RMs that controlled and progressively cleared an initial SIV challenge, 9 were able to stringently control a second SIV challenge ~3 years after last vaccination, demonstrating the durability of this vaccine. Thus, ΔRh110 RhCMV/SIV vectors have a safety and efficacy profile that warrants adaptation and clinical evaluation of corresponding HCMV vectors as a prophylactic HIV/AIDS vaccine.

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Published Online: 11 April, 2019 | Supp Info: http://doi.org/10.1084/jem.20190287
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JEM Journal of Experimental Medicine

TECHNICAL ADVANCES AND RESOURCES

HIV-specific humoral immune responses by CRISPR/Cas9-edited B cells

Harald Hartweger[1], Andrew T. McGuire[2,3], Marcel Horning[1], Justin J. Taylor[2,3,4], Pia Dosenovic[1], Daniel Yost[1], Anna Gazumyan[1], Michael S. Seaman[5], Leonidas Stamatatos[2,3], Mila Jankovic[1], and Michel C. Nussenzweig[1,6]

A small number of HIV-1–infected individuals develop broadly neutralizing antibodies to the virus (bNAbs). These antibodies are protective against infection in animal models. However, they only emerge 1–3 yr after infection, and show a number of highly unusual features including exceedingly high levels of somatic mutations. It is therefore not surprising that elicitation of protective immunity to HIV-1 has not yet been possible. Here we show that mature, primary mouse and human B cells can be edited in vitro using CRISPR/Cas9 to express mature bNAbs from the endogenous *Igh* locus. Moreover, edited B cells retain the ability to participate in humoral immune responses. Immunization with cognate antigen in wild-type mouse recipients of edited B cells elicits bNAb titers that neutralize HIV-1 at levels associated with protection against infection. This approach enables humoral immune responses that may be difficult to elicit by traditional immunization.

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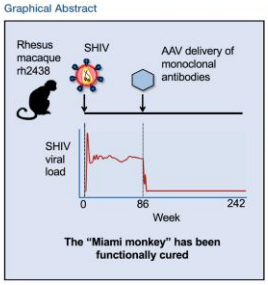
Immunity

Report

Adeno-Associated Virus Delivery of Anti-HIV Monoclonal Antibodies Can Drive Long-Term Virologic Suppression

Graphical Abstract



Authors
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In Brief

Although anti-retroviral drug therapy can suppress HIV viral replication, it is not a cure. Martinez-Navio et al. report the functional cure of the "Miami monkey," in which long-term SHIV suppression was achieved after a single administration of adeno-associated viruses (AAVs) encoding broadly neutralizing antibodies. They also report that host-generated anti-antibodies can hamper the applicability of this approach.

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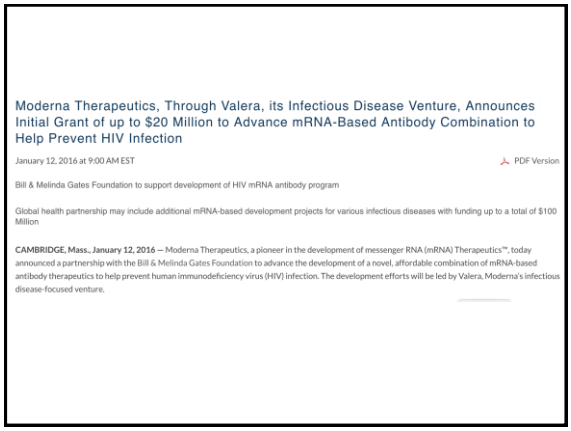

Moderna Therapeutics, Through Valera, its Infectious Disease Venture, Announces Initial Grant of up to $20 Million to Advance mRNA-Based Antibody Combination to Help Prevent HIV Infection

January 12, 2016 at 9:00 AM EST

PDF Version

Bill & Melinda Gates Foundation to support development of HIV mRNA antibody program

Global health partnership may include additional mRNA-based development projects for various infectious diseases with funding up to a total of $100 Million

CAMBRIDGE, Mass., January 12, 2016 — Moderna Therapeutics, a pioneer in the development of messenger RNA (mRNA) Therapeutics™, today announced a partnership with the Bill & Melinda Gates Foundation to advance the development of a novel, affordable combination of mRNA-based antibody therapeutics to help prevent human immunodeficiency virus (HIV) infection. The development efforts will be led by Valera, Moderna's infectious disease-focused venture.

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The 1st CSHP/SH Workshop and CSHP 2019 Oct.19-20, 2019

Adult T-cell leukemia/lymphoma with dermatopathic reaction

Kennosuke Karube[1], Takuya Miyagi[2], Kenzo Takahashi[2]

[1]Dept. Pathol. and Cell Biol. University of the Ryukyus
[2]Dept. Dermatol. University of the Ryukyus

University of the Ryukyus

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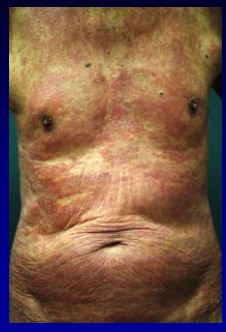
Case report

- 79-year old, male
- Pruritic erythema in trunk and extremities
- Right axillary lymph node enlargement subsequently appeared
- WBC: 10600/μl (Neu 81.7%, no atypical cells), Hb: 10.6 g/dl, Plt: 19.7 x 10^3 /μl, LDH: 326 U/l, soluble IL-2 R: 2990 U/l
- HTLV-1 Ab (+)

2

Dermatologic findings



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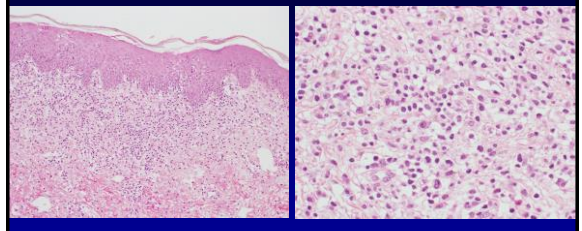
Skin biopsy



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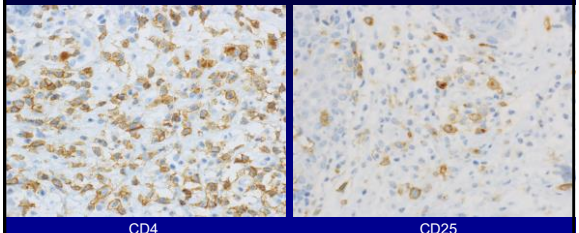
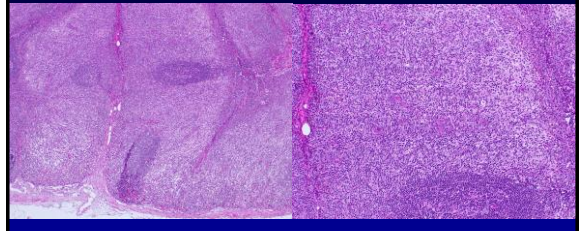
Skin biopsy



CD4 CD25

5

Lymph node



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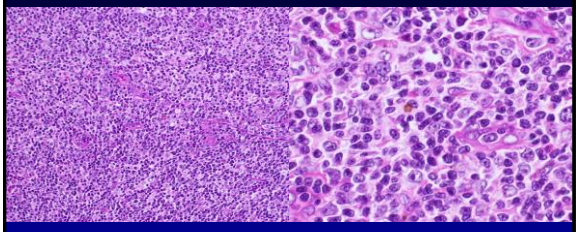
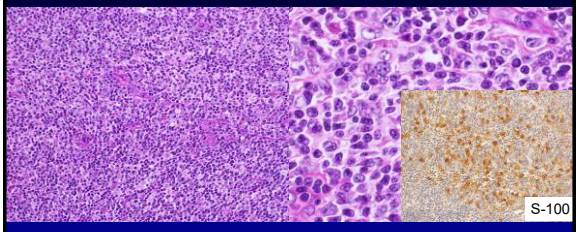
Lymph node



- Proliferation of dendritic cells with abundant clear cytoplasm
- Pigment deposition
- Medium-sized to large atypical lymphoid cells

7

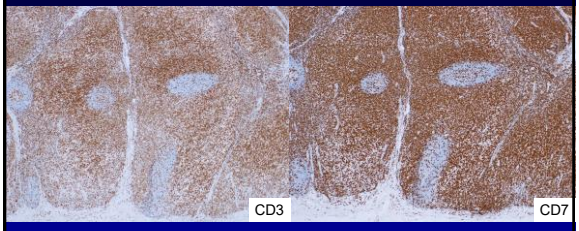
Lymph node



- Proliferation of dendritic cells with abundant clear cytoplasm
- Pigment deposition
- Medium-sized to large atypical lymphoid cells

8

Lymph node



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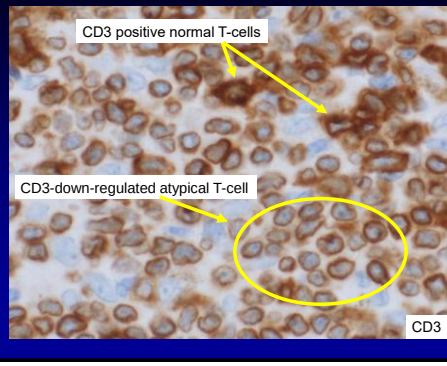
Lymph node



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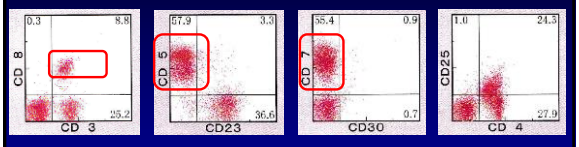
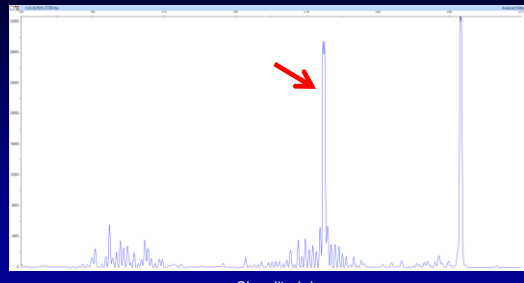
Lymph node: Flowcytometry



CD3 positive cells occupy 34% of gated population whereas 55-58% of them were positive for CD5 and CD7, suggesting aberrant T-cell phenotype.

11

PCR (BIOMED2) for TCR-gamma



Clonality (+)

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Other findings

- CT: Bilateral axillary lymphadenopathies, but no other lymphadenopathy
- Bone marrow: not evaluated

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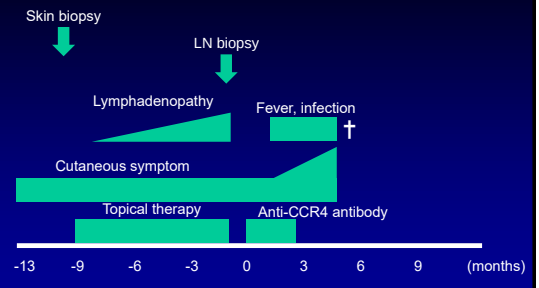
Clinical course



14

Proposed diagnosis:
Adult T-cell leukemia/lymphoma with dermatopathic reaction

Panel diagnosis:
Adult T-cell leukemia/lymphoma, lymphomatous variant

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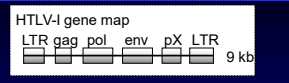
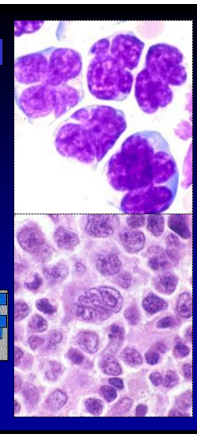
ATLL (adult T-cell leukemia/lymphoma)



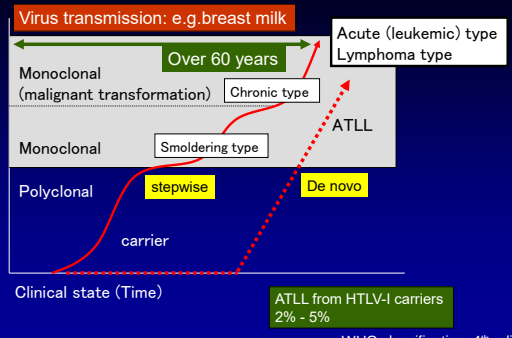
- Human T-cell malignancy associated with HTLV-I
- Flower cells in PB
- Lymphadenopathy: frequent
- Diagnosis: Identification of the monoclonal integration of HTLV-1 provirus



Courtesy of Dr. Ohshima (Kurume University)

16

Progression model of ATLL

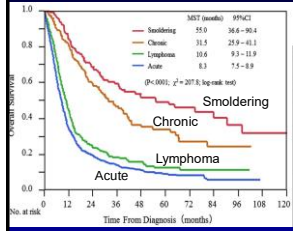


WHO classification, 4th edition

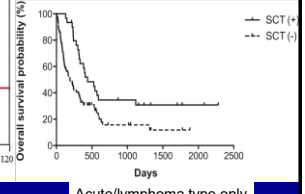
17

Clinical types, treatment and prognosis

Combination chemotherapy (e.g.CHOP) for acute/lymphoma type



Allogeneic stem cell transplantation improves overall survival

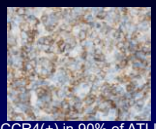


Acute/lymphoma type only

Katsuya *J Clin Oncol* 2015, Iyama *Clin Lymph Myel Leuk* 2017

18

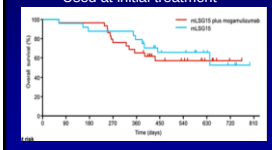
Mogamulizumab: Anti CCR4 antibody



CCR4(+) in 90% of ATLL

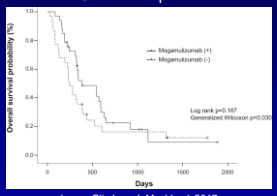
- Elevates CR rate
- Slight improve of OS when used at relapse
- Biomarkers for the effectiveness prediction?

Used at initial treatment



Ishida *Br J Hematol* 2015

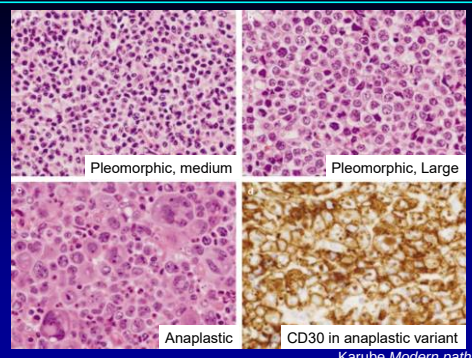
Used at relapse



Iyama *Clin Lymph Myel Leuk* 2017

19

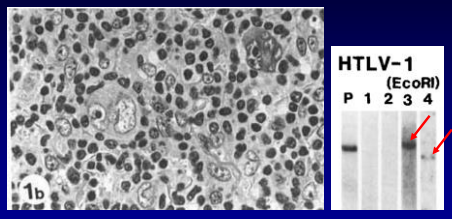
Morphological diversity of ATLL



Karube *Modern pathol* 2008

20

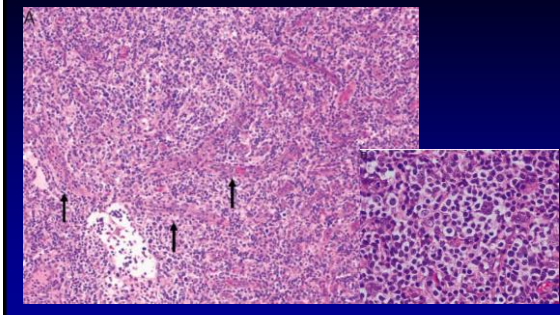
Hodgkin-like ATLL



Ohshima *Cancer* 1991

21

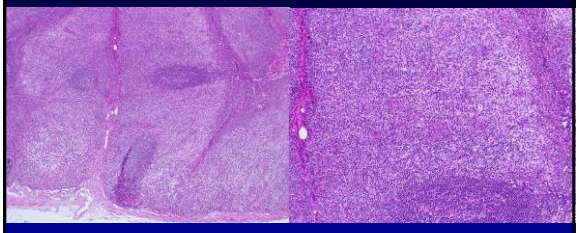
Angioimmunoblastic T-cell lymphoma-like ATLL



Karube *Am J Surg Pathol* 2007

22

Lymph node



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Lymphadenopathy with dermatopathic reaction in HTLV-1 carriers: Case series in Okinawa

Age/sex	Diagnosis	Immunohistochemistry	Clonality	Clinical type	Transformation	Status at the last follow-up
69/F	HAL-D	CD3=CD7	polyclonal	Smoldering type	NO	7 months, Alive without transformation
75/M	HAL-D	CD3=CD7	monoclonal	Smoldering type	NO	40 months, Alive without transformation
64/M	HAL-D	CD3=CD7	polyclonal	Smoldering type	NO	36months, Alive without transformation
90/F	HAL-D	CD3=CD7	polyclonal	HTLV-1 carrier	NO	21months, Alive without transformation
74/M	HAL-D	CD3=CD7	polyclonal	HTLV-1 carrier	NO	72 months, Dead
86/M	HAL-D	CD3=CD7	polyclonal	HTLV-1 carrier	NO	2 months, Alive without transformation
85/M	HAL-D	CD3=CD7	polyclonal	Smoldering type	NO	2 months, Alive without transformation
80/F	HAL-D	CD3=CD7	polyclonal	Smoldering type	NO	27 months, Alive without transformation
81/M	HAL-D	CD3=CD7	polyclonal	HTLV-1 carrier	NO	4 months, Alive without transformation
82/M	HAL-D	CD3=CD7	polyclonal	Smoldering type	NO	1 months, Alive without transformation
76/F	HAL-D	CD3=CD7	n.e.	Smoldering type	NO	2 months, Dead
76/M	HAL-D	CD3=CD7	polyclonal	HTLV-1 carrier	YES	31 months, Dead
74/M	HAL-D	CD3=CD7	n.e.	HTLV-1 carrier	NO	31 months, Alive without transformation
83/M	HAL-D	CD3=CD7	polyclonal	Smoldering type	NO	10 months, Alive without transformation
68/M	HAL-D	CD3=CD7	polyclonal	HTLV-1 carrier	NO	2 months, Alive without transformation
74/M	ATLL-D	CD3>CD7	n.e.	Lymphoma type?	***	14 months, Dead
79/M	ATLL-D	CD3<CD7	monoclonal	Lymphoma type?	***	7 months, Dead
64/M	ATLL-D	CD3>CD7	monoclonal	Chronic type?	***	2 months, Alive

Present case

- HAL-D: HTLV-1 associated lymphadenitis with dermatopathic reaction (Ohshima *Int J Cancer* 1996)
- ATLL-D: ATLL with dermatopathic reaction

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ATLL with dermatopathic reaction (ATLL-D)

- ATLL-D: A new morphological pattern of lymphomatous ATLL.
- Medium-sized lymphoma cells are predominant, accompanied by prominent dermatopathic reaction.
- The distinction from HTLV-1 associated lymphadenitis with dermatopathic reaction (HAL-D, non-tumorous status) is important.
- Based on the case series, abnormal T-cell phenotype accompanied by TCR clonality would help to distinguish HAL-D and ATLL-D.

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Acknowledgement

University of the Ryukyus

Department of Pathology and Cell Biology
Mitsuyoshi Takatori
Shugo Sakihama

26

Follicular lymphoma, grade 1-2, in the setting of chronic hepatitis C

Yukiko Kitagawa, MD, PhD
Magdalena Czader, MD, PhD

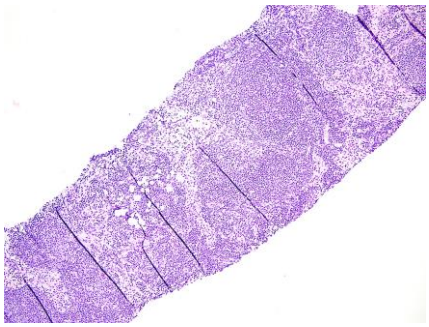
Indiana University School of Medicine,
Indianapolis, Indiana, USA

1

Clinical History

- 55 year-old female with a long standing hepatitis C (genotype 3, contracted through RBC transfusion post delivery) and cirrhosis, treated intermittently with PEG-Interferon and ribavirin
- In 2013, the patient presented with retroperitoneal, mesenteric and mediastinal lymphadenopathy
- A core needle biopsy of retroperitoneal lymph node was performed

2



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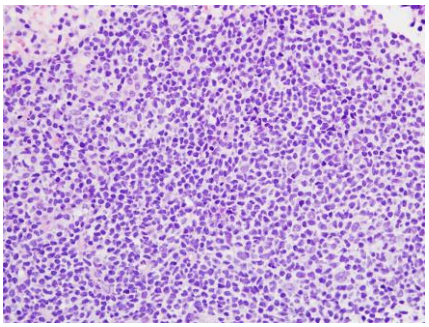

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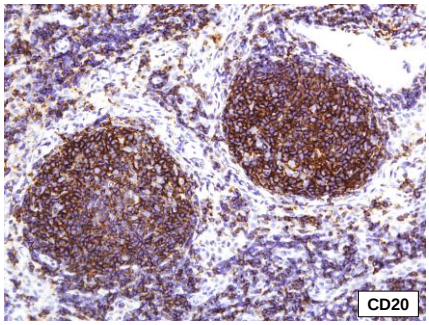

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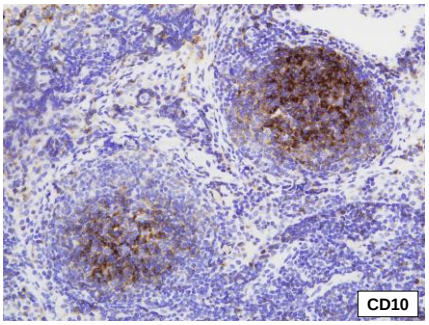

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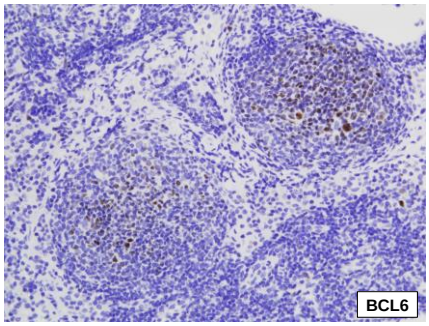

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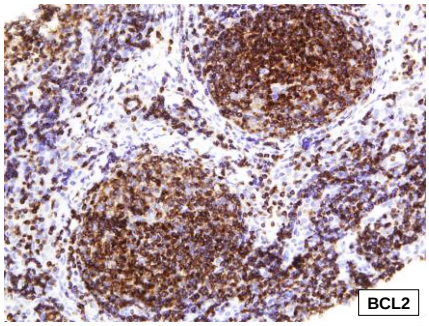

8

Additional studies

- Flow cytometry: small lymphoid cells, positive for CD19, CD20, CD10, partial CD23, HLA-DR and dim kappa light chain
- Next generation sequencing (targeted 596 gene panel): failed analysis due to insufficient material quantity

9

Proposed Diagnosis

Follicular lymphoma, grade 1-2,
in the setting of hepatitis C

10

Follow-up Clinical History

- Patient achieved sustained clearance of HCV after 24 weeks of therapy with daclatasvir and sofosbuvir
- Patient did not receive chemotherapy or immunotherapy for follicular lymphoma
- Interval imaging showed no abdominal lymphadenopathy and patient is considered to be in a complete remission (last follow-up in 2019)

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Lymphoproliferative disorders associated with HCV infection

- Mixed cryoglobulinemia type II
- Marginal zone lymphoma (spleen, lymph nodes, salivary glands, skin)
- Diffuse large B-cell lymphoma
- Follicular lymphoma
- Lymphoplasmacytic lymphoma
- Monoclonal B-cell proliferations with no clinical or histological evidence of lymphoma (peripheral blood, bone marrow, other tissues, +/- cryoglobulinemia)

Mollejo et al Mod Pathol 2014, De Vita et al Arthritis Rheum 2000, Magalini et al J Pathol 1998, Racanelli et al J Immunol 2001, Franzin et al Br J Haematol 1995, Tucci et al Blood 2018

12

Clonal B-cell proliferations associated with HCV

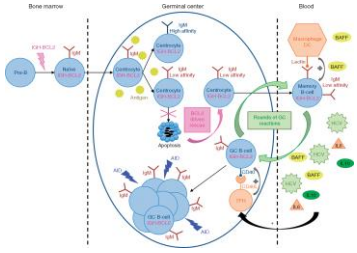
- Peripheral blood
- Tissue-based: bone marrow, liver, spleen, lymph nodes
- Incidental finding with a low probability of developing lymphoma
- Include clonal expansions of *IGH-BCL2* rearranged clones

- Increased frequency of class-switched memory B-cells, decreased transitional and naïve B-cells, as compared to healthy individuals
- Increased usage of specific IGHV genes, including IGHV1-69 and IGHV4-59 (linked to mixed cryoglobulinemia and HCV-associated lymphomas)
- Expanded clones in IgM+ memory B-cells in patients with chronic HCV infection

Franzin et al Br J Haematol 1995, Vallat et al Arthritis Rheum 2004, Tucci et al Blood 2018, Rawstron AC. Histopathology 2011

13

Hypothetical pathogenesis of follicular lymphoma in the setting of HCV infection



Couronne et al Ann Oncol 2018

14

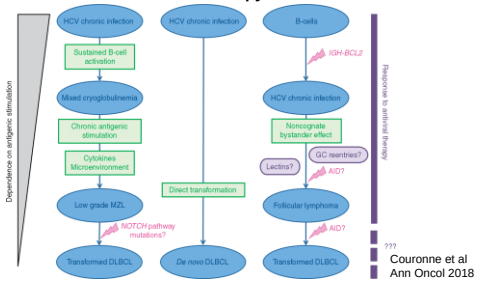
Antiviral therapy as treatment of HCV-associated indolent B-cell lymphoma

- Currently recommended by international guidelines (ESMO, NCCN, EASL)
- Supportive data from both interferon+-ribavirin, and direct-acting antiviral agents
- Sustained virological response (SVR) in >95% patients
- Overall response rate of 44-75% in indolent B-cell lymphomas
- Correlation between SVR and hematological remission
- Early antiviral therapy reduces risk of lymphoma
- Disappearance of *IGH-BCL2* positive clones

Merli et al World J Gastroenterol 2016, Ioannou et al Hepatol Comm 2019, Marsone and Persico Cancer 2019, Su et al Aliment Pharmacol Ther 2019

15

Models of HCV-associated lymphomagenesis and response to antiviral therapy



Couronne et al Ann Oncol 2018

16

Case No. 1456578: Systemic lymphoproliferative disorder with a fulminant course

Session Five, 20-October 2019
CSHP/SH Workshop, Xi'an, P.R., China
Dr. Sarah Ondrejka – Cleveland Clinic, Ohio, USA

1

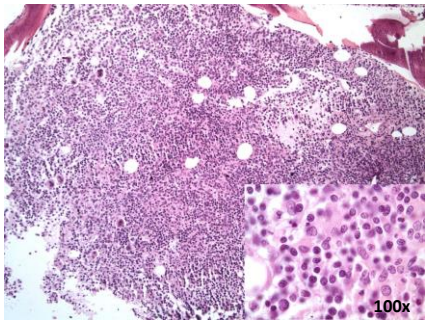
Case presentation

- 51 year old healthy man taking no medications
- Skin rash, fever for several days, and pancytopenia
- Resolved

- One month later – fever, fatigue, night sweats
- WBC 0.6 x 10^9/L; hemoglobin 117 g/L; platelets 77 x 10^9/L
- Slightly elevated liver enzymes, CT abdomen showed a prominent spleen

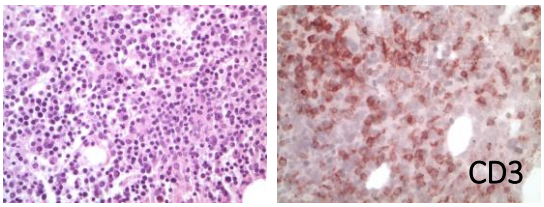
2

Bone marrow biopsy, 10x



3

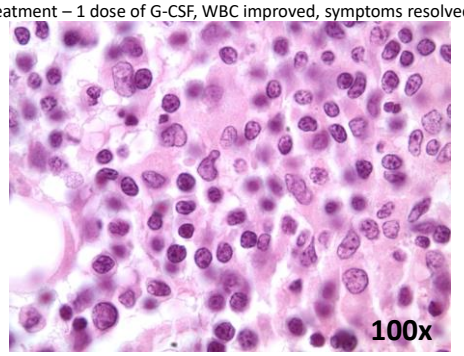
Bone marrow biopsy, 40x



T-cell immunophenotype: CD3+, CD2+, CD4+, CD5+, CD7-, CD8-
PCR for T-cell receptor gene rearrangement – Unsatisfactory due to poor DNA preservation
Cytogenetic karyotype: Normal
HTLV1/2 Ab screen: Non-reactive

4

Diagnosis – Hypercellular bone marrow suspicious for a T-cell lymphoproliferative process
Treatment – 1 dose of G-CSF, WBC improved, symptoms resolved



5

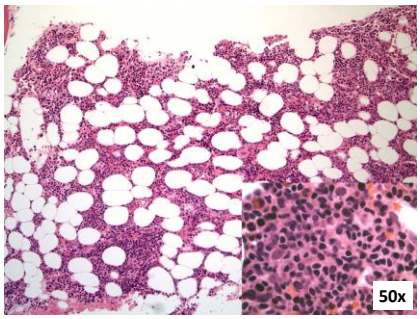
Six months later

- Asymptomatic
- CBC: WBC 4.04 x 10^9/L; hemoglobin 143 g/L; platelets 113 x 10^9/L

- Bone marrow biopsy:
  - Normocellular with trilineage hematopoiesis and low-level involvement by an abnormal T-cell lymphoproliferation (CD3+, CD4-, CD8-, CD7-, CD26-)
  - T cell receptor gamma PCR: Positive
  - Normal karyotype

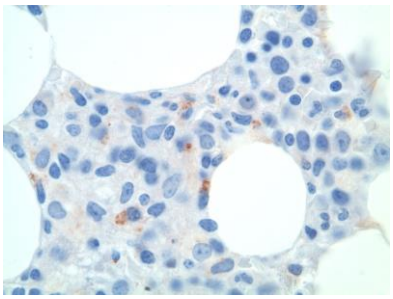
6

Bone marrow biopsy – six months after presentation (H&E, 10x)



7

Bone marrow biopsy – six months after presentation (granzyme B, 100x)



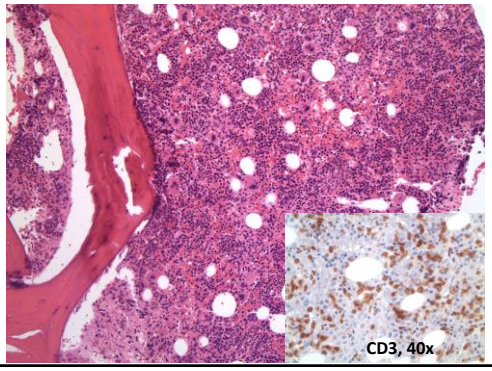
8

After 18 months of observation

- WBC 2.3 x 10^9/L; Hgb 140 g/L; platelets 72 x 10^9/L
- Became acutely ill and hospitalized for 3 weeks with unexplained febrile illness and severe neutropenia
- WBC 0.27 x 10^9/L; Hgb 102 g/L; platelets 40 x 10^9/L
- ALT 62 U/L; AST 58 U/L
- Epstein-Barr viremia (6,000 copies/mL)
- A third bone marrow biopsy was taken
- Treated with immunoglobulin and G-CSF

9

Third bone marrow biopsy, H&E, 10x



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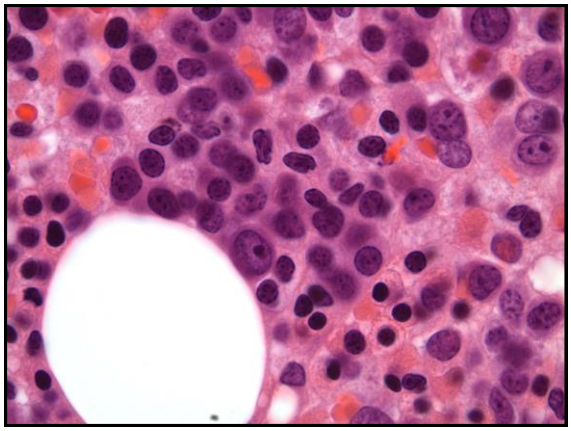


11

1 week after hospitalization

- Asymptomatic and feeling well, finishing courses of antibiotics and antifungals
- WBC 3.1 x 10^9/L; (N74, L10, M14, E1, B1), ANC 2.3 x 10^9/L, hemoglobin 102 g/L, platelets 159 x 10^9/L, AST 20 U/L, ALT 34 U/L
- Clinical assessment: Poorly defined T-cell lymphoproliferative disorder, possibly a compromised immune system, and a unexplained recent febrile illness. Perhaps he had an acute or recurrent Epstein Barr virus infection resulting in neutropenia and hepatitis, which is spontaneously improving and/or a fungal infection, which is responding to amphotericin and now fluconazole and/or a tooth abscess, which developed or progressed while neutropenic and is responding to extraction

12

1 month follow up visit

- Feeling well except for periodic left abdominal discomfort
- WBC 2.2 x 10^9/L, (N74, L15, M9, E1, B1), ANC 1.6, hemoglobin 119 g/L, platelets 81 x 10^9/L, AST 25 U/L, ALT 25 U/L
- Quantitative EBV DNA (6/15/04) - 6131 copies/ml
- Given immunoglobulin (1 gm/kg) 65 gm IV for bicytopenia
- No response – WBC count dropped to 1.0 x 10^9/L
- Given G-CSF with response – WBC 3.0 x 10^9/L (ANC 2.3), and left shift
- Plan for splenectomy for relief from "possible immune-mediated cyclical cytopenias" and poorly defined T-cell LPD on observation

13

After splenectomy

- 2 weeks post: WBC 3.5 x 10^9/L (ANC 1.4), hemoglobin 129 g/L, platelets 114 x 10^9/L
- Received G-CSF
- 4 weeks post: WBC 5.0 x 10^9/L (ANC 1.4), hemoglobin 142 g/L, platelets 100 x 10^9/L
- AST 153 U/L, ALT 158 U/L, Epstein-Barr virus quantitative DNA – 14,490 copy/mL
- Spleen pathology – Small amount of extramedually hematopoiesis, normal cytogenetics, TCR gamma PCR negative

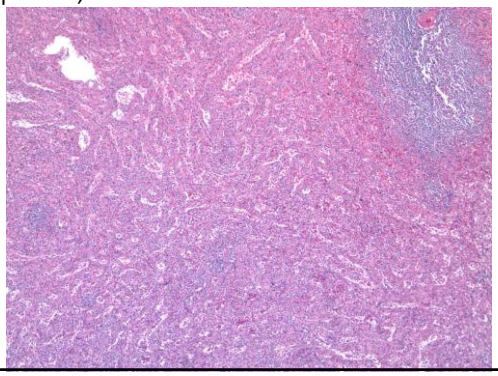
14

Three months after splenectomy

- Admitted to hospital with fever of unknown origin
- Rapidly developed multisystem organ failure, pleural effusions, and disseminated intravascular coagulation
- WBC 1.04 x 10^9/L, hemoglobin 84 g/L, platelets 27 x 10^9/L
- EBV DNA quantitative test 161,200 copies/mL
- LDH 19,215 U/L
- ALT 1,200 U/L; AST 11,382 U/L

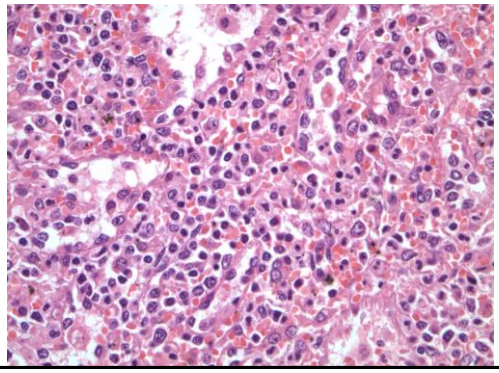
15

Spleen, 20x



16

Spleen, 40x



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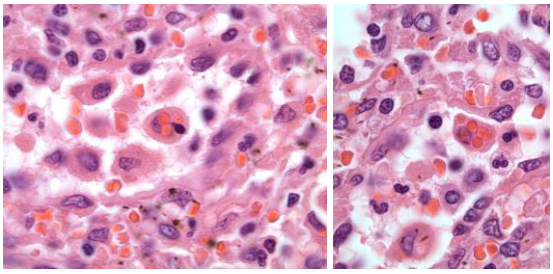
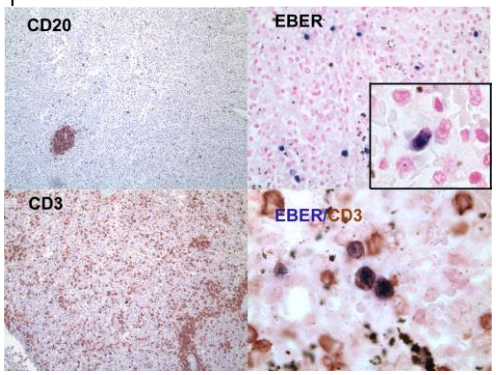
Spleen, 100x



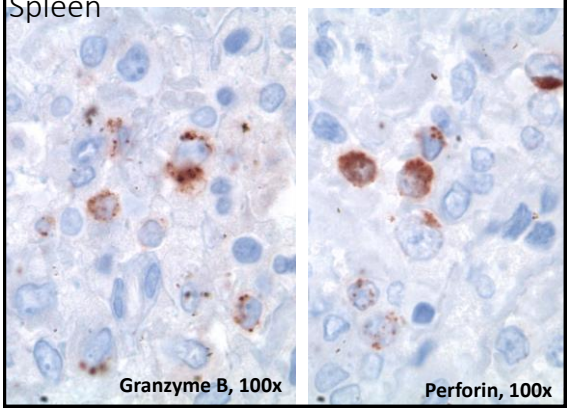
18

Spleen



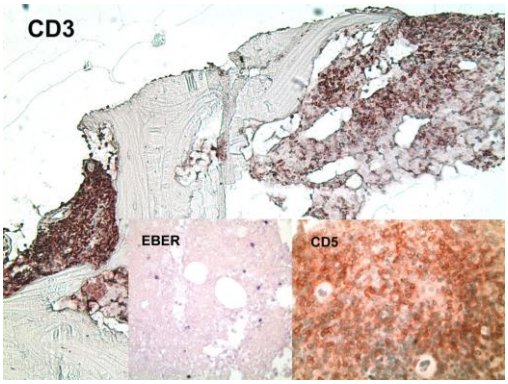
19

Spleen



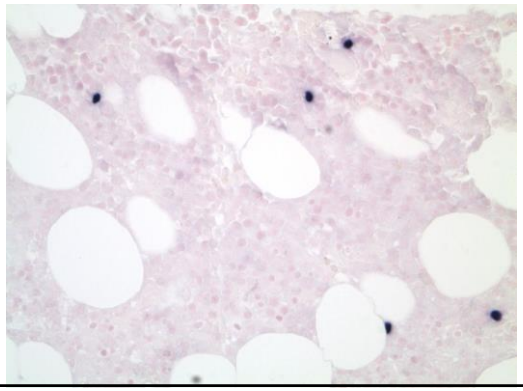
20

Bone marrow biopsy #1



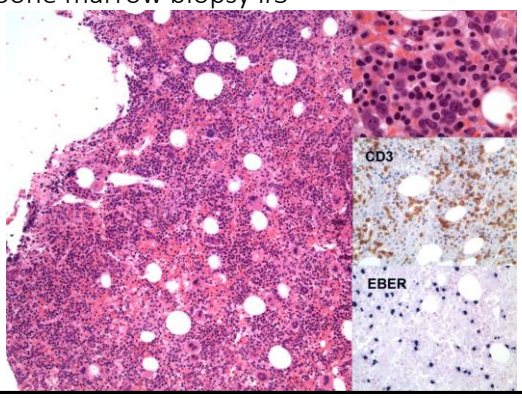
21

Bone marrow biopsy #2



22

Bone marrow biopsy #3



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Workshop Panel Diagnosis

- Chronic active EBV infection with associated hemophagocytic lymphohistiocytosis

24

Features of CAEBV, systemic form

- Fever, lymphadenopathy, persistent hepatitis, splenomegaly, typically follows primary EBV infection
- Most common in children and young adults
- Most common in patients of Asian or Hispanic descent
- EBV in T/NK cells in Japan, B cells in US
- Follows a chronic, relapsing clinical course with a risk of progression to systemic EBV+ T- or NK-cell lymphoma
- CAEBV of T-cell origin has high IgG titers against EBV viral capsid and early antigens and a worse prognosis
- Adult-onset CAEBV is associated with a worse prognosis

Fernandez-Pol S. et al, Haematologica 2018
Kimura H. et al, Blood 2001
Cohen JI et al, Blood 2011

25

Diagnostic criteria of CAEBV

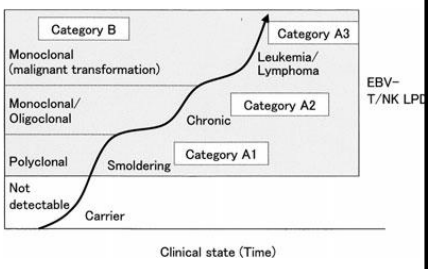
- Infectious mononucleosis-type symptoms persisting for >3 months
- Increased EBV DNA (>$10^{2.5}$ copies/mg) in peripheral blood
- Histologic evidence of organ disease
- Demonstration of EBV RNA or viral protein in affected tissues in patients without known immunodeficiency, malignancy, or autoimmune disease

WHO blue book, 2017

26

Complications of CAEBV

- Infection
- Immune deficiency
- Multi-organ failure
- EBV-positive lymphomas



- Hemophagocytosis – CD4+ T-cells most often

Kimura H and Cohen JI, Frontiers in immunology 2017
Cohen JI, et al, Blood 2011

27

HLH: criteria for diagnosis HLH-2004… 5 of 8 criteria*

- 1. Fever
- 2. Splenomegaly
- 3. Bicytopenia
- 4. Hypertriglyceridemia
- 5. Hemophagocytosis
- 6. Low/absent NK activity
- 7. Hyperferritinemia
- 8. High soluble interleukin-2 receptor levels

*Exception for patients with + molecular testing

Henter JI, et al. Pediatr Blood Cancer 2007

28

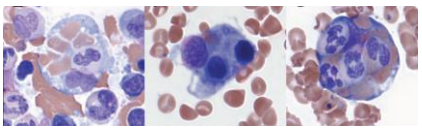
HLH – criteria for diagnosis (secondary subtypes)

- H-score: online scoring system, based on 9 clinical, laboratory and histological variables, cutoff of 169 accurately predicts 90% of secondary HLH
  - (Fardet et al, 2014)
- Malignancy associated HLH: the Histiocyte Society reported consensus recommendations for the diagnosis in flow-chart format, with a subdivision between HLH occurring at the presentation or relapse of malignancy and HLH occurring during chemotherapeutic treatment
  - (Lehmberg et al, 2015)

29

HLH – criteria for diagnosis

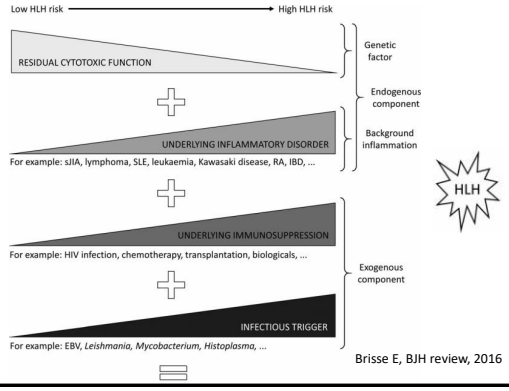
- Morphology – Recent retrospective study developed a predictive model for discriminating patients with HLH based on quantitative analysis and hemophagocytes with nucleated cell lineages (erythroid, granulocyte, lymphocyte)



- E Gars et al, Haematologica, 2018

30

HLH: clinical implications



Brisse E, BJH review, 2016

31

Case summary

- This was a Taiwanese man living in Cleveland who developed intermittent, self-resolving fever and pancytopenia, with an a indolent T-cell lymphoproliferation at the start.
- For two years, he was followed by the infectious disease service for relapsing fevers of unknown origin and by hematology for cyclical and immune-mediated cytopenias
- It was recognized too late that this was a rare systemic chronic active EBV, just prior to development of HLH, multi-system organ failure, and death

32

Systemic EBV-positive T-cell lymphoma of childhood

mimicking

Anaplastic large cell lymphoma

Zihang Chen, Sha Zhao, Weiping Liu

Department of Pathology, West China Hospital, SCU

2019.10 Xi'an

1

Medical History

- 24/M
- **Chief complain**

High fever with detected lung nodules, hepatosplenomegaly and multiple enlarged lymph nodes for 7 days

- **Laboratory test**

Hb 83 g/L; Plt 69×10^9/L WBC: 3.27×10^9/L

EBV-DNA loads :1.42×10^5 copies/mL

- **Past History**

The patient had a 5-years history of CAEBV presenting with recurrent fever, lymphadenopathy and elevated EBV-DNA loads

2

Clinical Diagnosis

- **Clinical Feature**

Fever ①

Hepatosplenomegaly ②

- **Laboratory Test**

Cytopenias ③

Ferritin:2842.0 ng/mL ④

Fibrinogen: 78 mg/dL ⑤

sCD25:12865.0 U/ml ⑥

Diagnosis：HLH

Histiocyte Society HLH-2004 diagnostic criteria.

The diagnosis HLH requires that either 1 or 2 below are fulfilled:
(1) A molecular diagnosis consistent with HLH
(2) Diagnostic criteria for HLH fulfilled (5 out of the 8 criteria below)
(A) Initial diagnostic criteria
- Fever
- Splenomegaly
- Cytopenias (affecting ≥2 of 3 lineages in the peripheral blood):
  - Hemoglobin <90 g/L (in infants < 4 weeks: hemoglobin < 100 g/L)
  - Platelets < 100 × 109/L
  - Neutrophils < 1.0 × 109/L
- Hypertriglyceridemia and/or hypofibrinogenemia:
  - Fasting triglycerides ≥ 3.0 mmol/L (i.e., ≥265 mg/dL)
  - Fibrinogen ≤ 1.5 g/L
- Hemophagocytosis in bone marrow or spleen or lymph nodes

(B) New diagnostic criteria
- Low or absent NK-cell activity
- Ferritin ≥ 500 mg/L
- Soluble CD25 (i.e., soluble IL-2 receptor) ≥2400 U/mL

Pediatr Blood Cancer. 2007;48:124–131

3

Medical History

PET-CT

- **Lymph node**

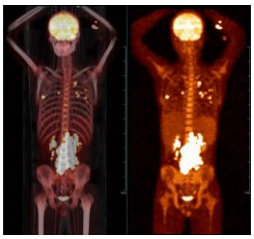
multiple enlarged lymph nodes in bilateral lung hilum, retroperitoneal region and mesenteric region (maximum cross section 112 mm × 48mm; maximum SUV 17.79)

- **Chest**

multiple nodules in bilateral superior lobe of the lung(maximum cross section 31 mm × 20mm; maximum SUV 9.97)

- **Abdomen**

hepatosplenomegaly



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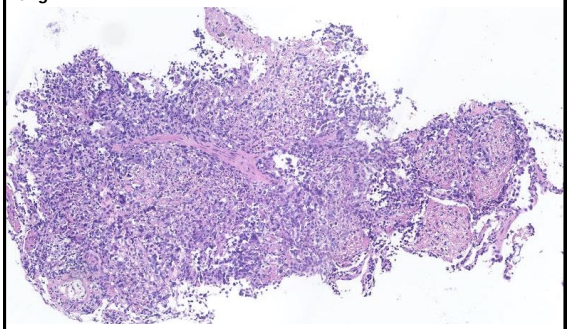
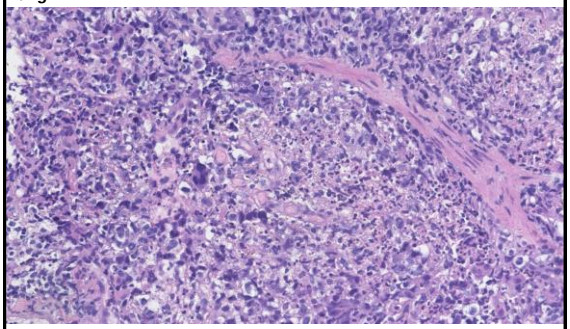
Lung



Core needle biopsy HE 40X

5

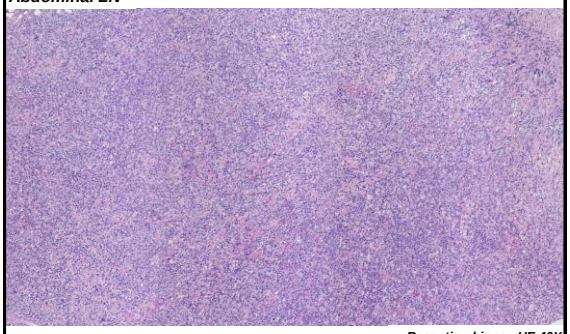
Lung



Core needle biopsy HE 100X

6

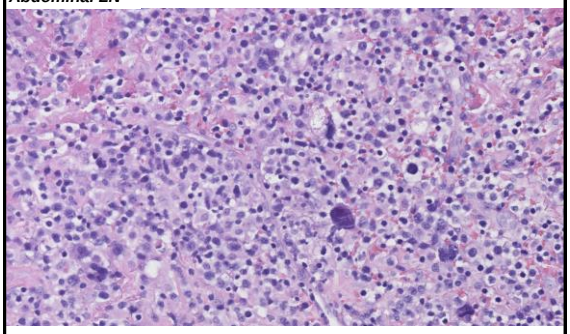
Abdominal LN



Resection biopsy HE 40X

7

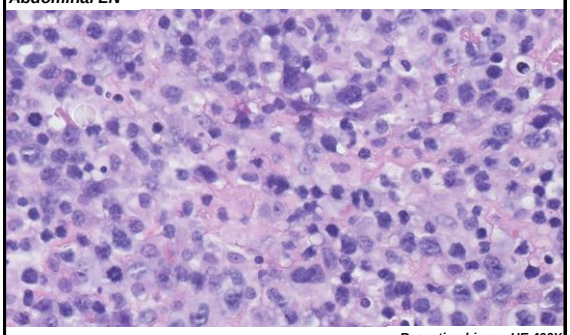
Abdominal LN



Resection biopsy HE 200X

8

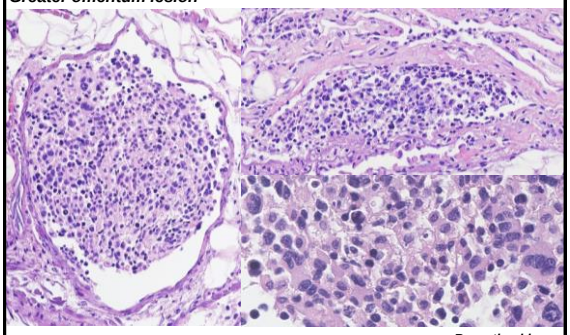
Abdominal LN



Resection biopsy HE 400X

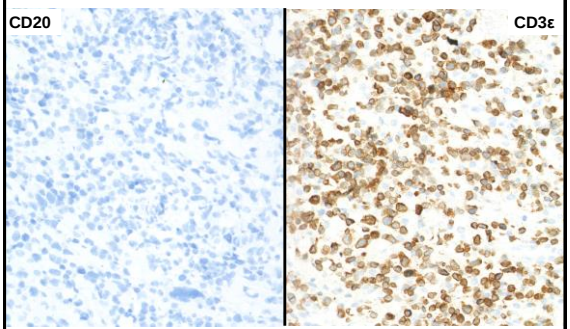
9

Greater omentum lesion



Resection biopsy

10



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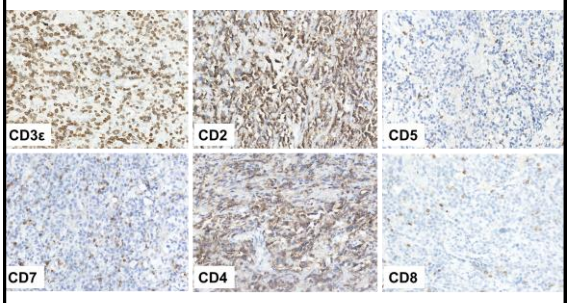

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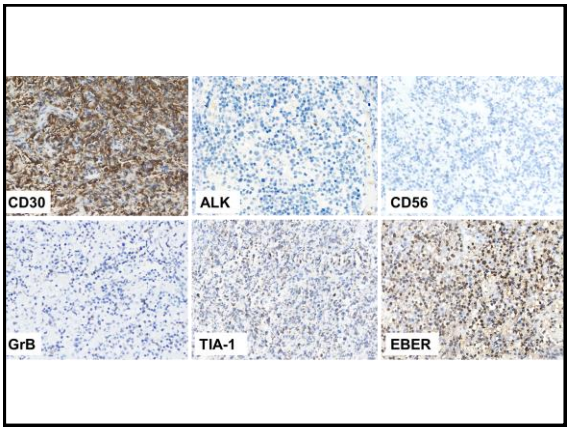

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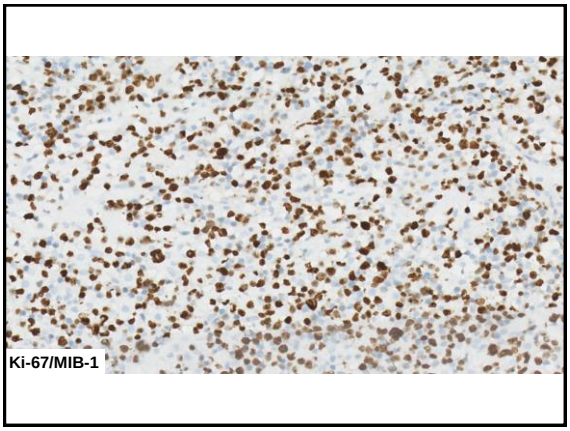

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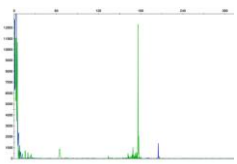
Molecular Analysis

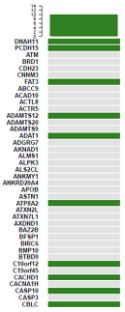
TCR-γ Rearrangement

Monoclonal



WES (Mutated genes)

DNAH11, PCDH15, FAT3, ADAMTS12, ADAT1, ATP8A2, C10orf12, CACHD1, CASP10, CBLC



15

Diagnosis

Systemic EBV-positive T-cell lymphoma of childhood

Clinical Features

- **Symptoms**

  Fever, pancytopenia

- **PET-CT**

  Lung nodules

  Multiple LN enlargement,

  Hepatosplenomegaly

- **Past History**

  A long history of CAEBV （5 years）

Pathological Features

- **Morphology**

  The biopsied samples showed either a diffuse or an intravascular pattern of infiltration with cohesive growth of large atypical cells with irregular nuclei and abundant cytoplasm resembling the hallmark cells of anaplastic large cell lymphoma

- **IHC and ISH**

  Positive: CD2, CD3, CD4, CD30, TIA-1,GrB, Ki-67(80%)

  Negative: CD5, CD8, CD20, CD56, ALK

  EBER-ISH: (+,80%)

- **TCR-γ Rearrangement**

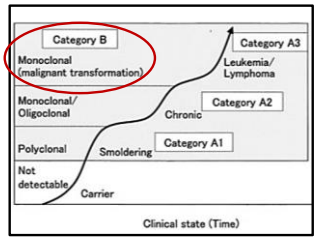
  Monoclonal

16

Progression & Transformation

CAEBV-T/NK-LPD ➡ STLC

- **Long history of CAEBV**
- **Monoclonal**
- **Monomorphic**
- **Fulminant clinical course**



1. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues Revised 4th edition
2. Koichi Ohshima, et al. Pathology International 2008; 58: 209–217
3. Jaffe ES. Hematopathology. 2nd ed, 2016.

17

Differential Diagnosis

1. Primary EBV-positive nodal T-cell or NK-cell lymphoma

Items	The current case	Primary EBV-positive nodal T-cell or NK-cell lymphoma
Age	Young (24y)	Old
Immune status	Immunocompetent	Immunocompromised (often)
Cell origin	CD4+cytotoxic T	Mostly CD8+cytotoxic T
Extranodal involvement	Multiple involvement (lung, greater omentum and spleen)	Limited extranodal involvement

1. Jeon YK, et al. Hum Pathol. 2015;46(7):981–990.
2. Kato S, et al. Am J Surg Pathol. 2015;39(4):462–471.

18

Differential Diagnosis

2. Extranodal NK/T-cell lymphoma, nasal type

Items	The current case	Extranodal NK/T-cell lymphoma, nasal type
Site	Multiple lymphadenopathy with extranodal involvement	Extranodal presentation (almost) Lymph node involvement (rare)
Necrosis	Not obvious	Extensive necrosis
Cell origin	CD4+cytotoxic T	NK-cell (mostly) γδT-cell (CD4-,CD8-) αβT-cell (CD8+)

1. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues Revised 4th edition*
2. *Jhuang JY, et al. Hum Pathol (2015) 46, 313-321.*

19

Differential Diagnosis

3. EBV-HLH

Items	The current case	EBV-HLH
EBV statues	Past infection	Initial infection (common)
Site	Multiple lymphadenopathy with extranodal involvement	Bone marrow, Spleen and Liver
Erythrophagocytosis	Not obvious	Present
Cell atypia	Anaplastic	Without atypia

1. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues Revised 4th edition*
2. *Jaffe ES. Hematopathology. 2nd ed, 2016.*

20

Follow-up

- **The patient underwent intensive chemotherapy but showed no response.**

  (Regimen: GLIDE, gemcitabine+ L-asparaginase + ifosfamide+ dexamethasone+ etoposide)

- **The patient died of disease within 2 months of diagnosis.**

21

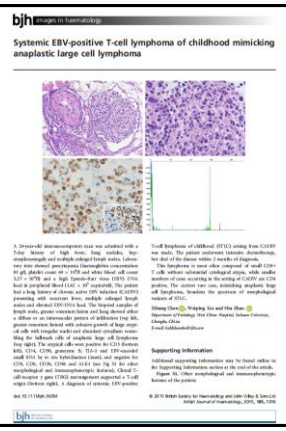
Summary

- Systemic EBV-positive T-cell lymphoma of childhood is most often composed of small CD8+ T cells without substantial cytological atypia, while smaller numbers of cases occurring in the setting of CAEBV are CD4 positive.
- This a typical case of systemic EBV-positive T-cell lymphoma of childhood arising from chronic active EBV infection.
- The current case showed uncommon growth pattern (intravascular infiltration)
- The atypical cells with cohesive growth of large atypical cells of irregular nuclei (horseshoe-shaped, or kidney-shaped) and abundant cytoplasm mimicking hallmark cells of anaplastic large cell lymphoma.

This case broadens the spectrum of morphological variants of systemic EBV-positive T-cell lymphoma of childhood

22

Summary


bjh Images in haematology

Systemic EBV-positive T-cell lymphoma of childhood mimicking anaplastic large cell lymphoma

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THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

HHV8-positive multicentric Castleman disease with concurrent Kaposi sarcoma

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Dept. of Hematopathology

The University of Texas MD Anderson Cancer Center

1

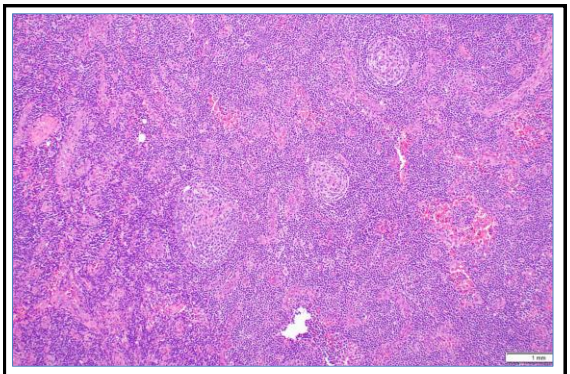
Clinical History

1) A 63-year-old woman has a history of Kaposi's sarcoma (HIV negative) involving lymph nodes and skin initially diagnosed at the age of 40.

2) Recent PET-CT scan showed multiple lymphadenopathy with hypermetabolic activity.

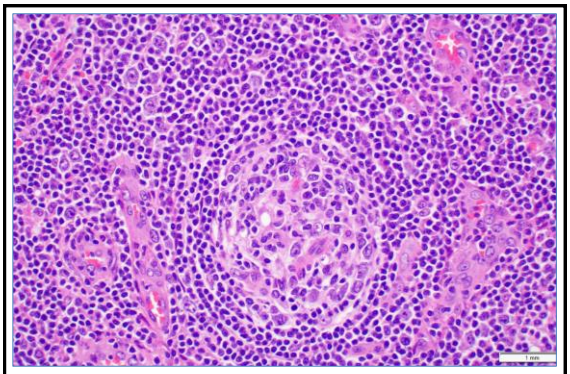
3) An excisional biopsy of left axillary lymph node was performed.

2



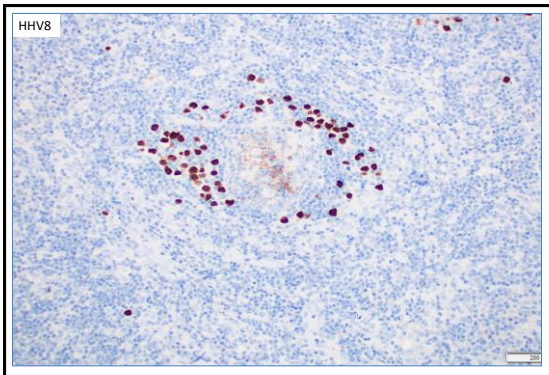
In most areas, the lymph node is composed of scattered follicles with expanded interfollicular zones. Many follicles show hyaline-vascular changes including lymphocyte depleted germinal centers with onion-skin like mantle zones.

3



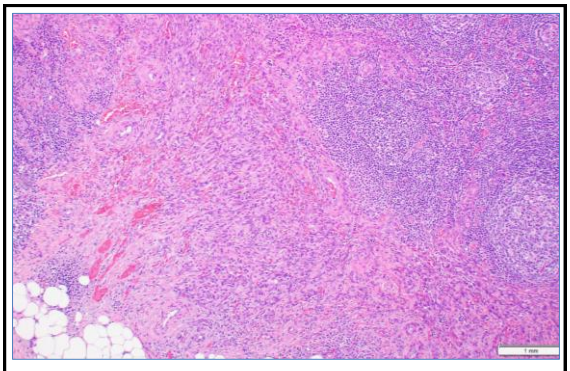
Scattered large cells are present in mantle zones and some have distinct nucleolus, consistent with immunoblasts/plasmablasts.

4



HHV8 immunostain highlights large cells in mantle zones. Scattered large cells in interfollicular areas are also positive.

5



In focal areas, there is a spindle cell proliferation with extravasated red blood cells

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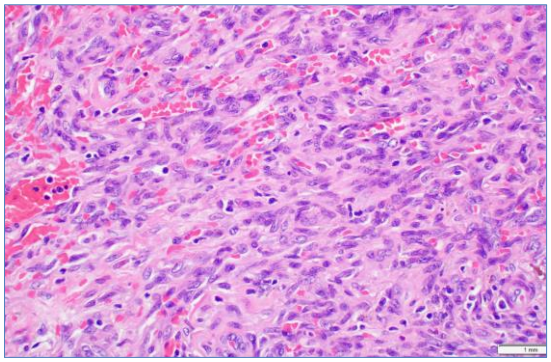
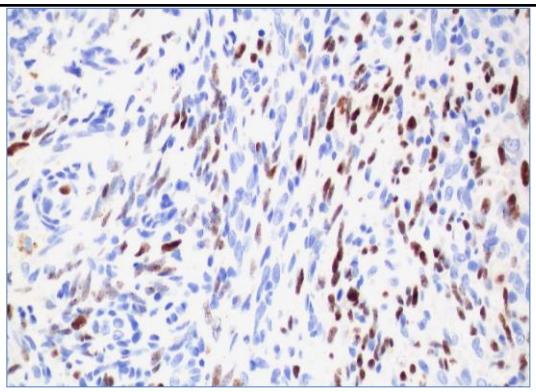


High-power view of spindle cell proliferation: cells show a bland morphology and no mitotic figures.

7



HHV8 immunostain highlights spindle cell proliferation

8

Our diagnosis:

HHV8-positive multicentric Castleman disease with concurrent Kaposi sarcoma

Panel diagnosis:

HHV8-positive multicentric Castleman disease and concurrent Kaposi sarcoma

9

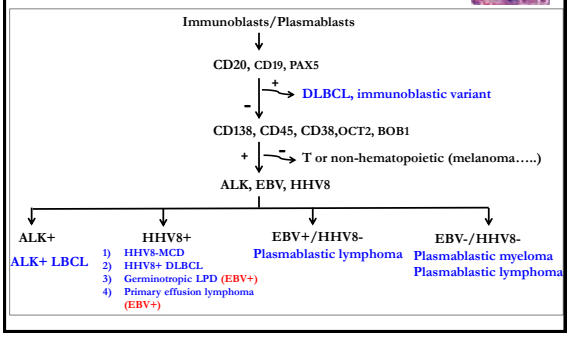
The panel asks us to discuss:

1. The differential diagnosis between HHV8-positive **MCD** and **EC-PEL** particularly when there are aggregates of HHV8+ cells
2. Anti-IL6 and anti-IL6R therapy in MCD

MCD: multicentric Castleman disease
EC-PEL: Extracavitary primary effusion lymphoma

10

LPDs with immunoblast/plasmablast morphology



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The differential diagnosis of HHV8+LPDs

	MCD	DLBCL	Germinotrophic LPD	PEL
Immunosuppression	yes	yes	no	yes
Presentation	Generalized	Generalized	Localized	Generalized
Prognosis	Poor	Poor	Good	Poor
Morphology	PB/IB in mantle zones	PB/IB, diffuse	PB/IB within germinal centers	PB/IB in fluid or extracavitary sites
Castleman features	+	+ or -	-	-
HIV status	+/-	+/-	-	+/-
HHV8	+	+	+	+
EBV	-	-	+	+
CD138	+/-	+	-/+	+
CD20	-/+	-	-	-
Ig	IgM, λ	monotypic	monotypic	-
Molecular (IgH)	Polyclonal	Clonal	Polyclonal	Clonal

MCD: multicentric Castleman disease; PEL: Primary effusion lymphoma, PB: plasmablast; IB: immunoblast

W Wang et al. Histopathology 2018, 72, 855–861

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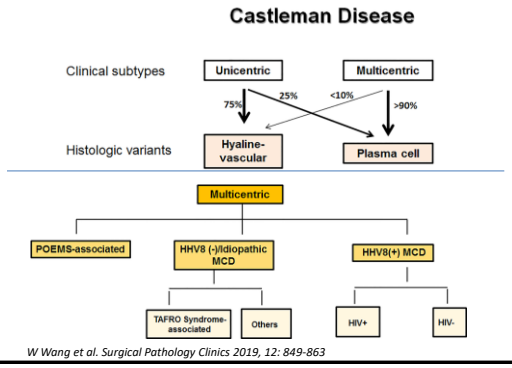
Anti-IL6 and anti-IL6R therapy in MCD

1. What types of CD can be treated with IL-6 targeted therapy?
2. Why targeting IL-6?
3. What options do we have to target IL-6?

13

What types of CD can be treated with IL-6 targeted therapy? Answer: Multicentric CD



W Wang et al. Surgical Pathology Clinics 2019, 12: 849-863

14

Why targeting IL-6 in MCD?

1. IL-6 level is elevated in MCD, not only in HHV8-positive MCD, but also in idiopathic MCD.
2. IL-6 plays a critical role in the pathogenesis of MCD

1. *SM Hsu et al, Hum Pathol 1993;24:833–9*
2. *SJ Brandt et al, J Clin Invest 1990;86:592–9.*

15

What options do we have to target IL-6?

1. Anti-IL-6 antibody: Siltuximab, which was approved by FDA in 2014 for the treatment of patients with MCD who are HIV negative and HHV8 negative.
2. Anti-IL-6 receptor antibody: tocilizumab, approved in Japan in 2005.

16

Extranodal NK/T cell Lymphoma Arising in a Patient with Concurrent Hepatitis B Viremia

Minh Yen T. Mays MD
Julia Geyer, MD
Wayne Tam, MD PhD
Amy Chadburn, MD
Genevieve M. Crane, MD PhD

1

57 Year Old Asian Female

- End of 2016:
  - Presented with prolonged dental infections, oral pain, dysphagia
- February 2017:
  - Hepatitis B virus positive serology; began treatment with tenofovir disoproxil fumarate
- Progressively worsening dysphagia and oral pain requiring hospitalization
- August 2017:
  - PET/CT with FDG-avid, destructive soft tissue mass in right nasal cavity; also involving oropharynx, nasopharynx, subcutaneous tissue, right bilateral cervical lymph nodes, spleen, bone marrow and mediastinal nodes
  - Lymph node core biopsy, lymph node excisional biopsy, bone marrow biopsy

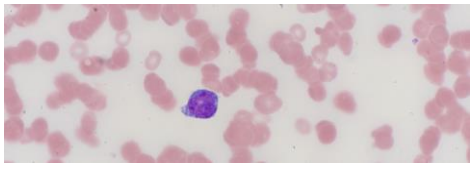
2

Complete Blood Count

	WBC K/µL	Hb g/dL	Platelets K/µL	Absolute Lymphocyte Count K/µL
7/31/2017	1.8	6.5	70	0.4
8/11/2017	0.7	6.9	41	0.3
9/4/2017	22.6	7.1	159	0.8
9/27/2017	3	10.1	87	0.4

3

Peripheral Blood Smear



Rare atypical circulating cells, no abnormal population identified by flow cytometry

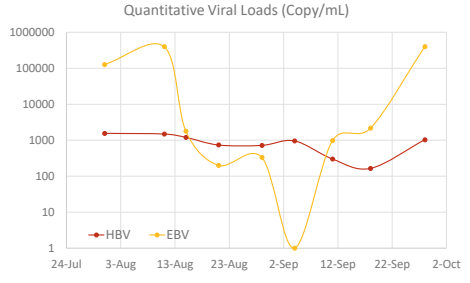
4

Laboratory Values

7/30/2017		
	Total protein	6.3 g/dL
	Albumin	2.3 g/dL
	Globulin	4 g/dL
	AST	63 U/L
	ALT	32 U/L
	Alkaline phosphatase	174 U/L
7/31/2017		
	HB surface antibody	Non Reactive
	HB surface antigen	Reactive
	HB core Antibody	Reactive
	HB core IgM	Non - Reactive
	HBV viral load	1,550 copy/mL
	HBe antigen	Positive
8/1/2017		
	EBV DNA, quantitative	127,240 copy/mL
	EBV (Log)	5.10
	LDH	525 U/L
	Total bilirubin	1.1 mg/dL
	Direct bilirubin	0.5 mg/dL
	Indirect bilirubin	0.6 mg/dL

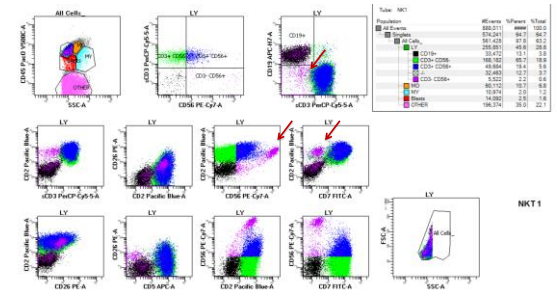
5

EBV and HBV



6

Flow Cytometry (Resected Mass): Atypical CD56+, CD2+, sCD3(-),CD7(-) Population



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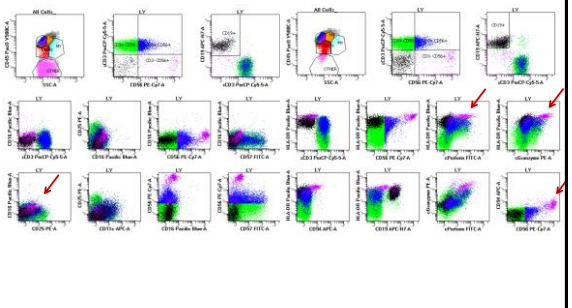
Atypical NK Cells Co-Express CD94 (dim), Perforin, Granzyme, CD25 (very dim)



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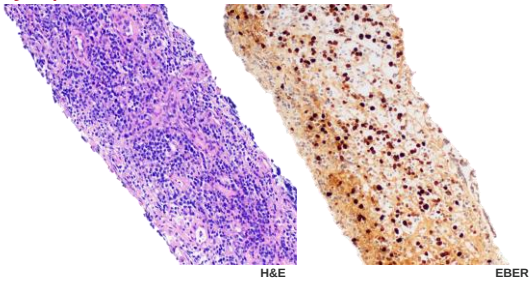
8

Core Biopsy, Right Neck Level 1B Lymph Node



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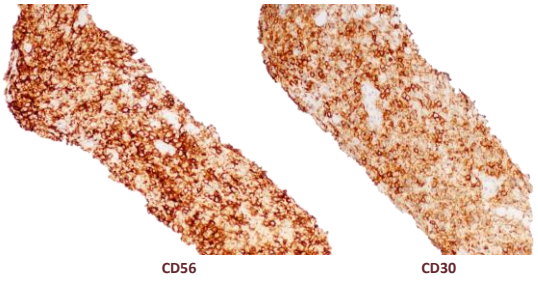
Consistent with EBV+ Extranodal NK/T Cell Lymphoma, Nasal Type



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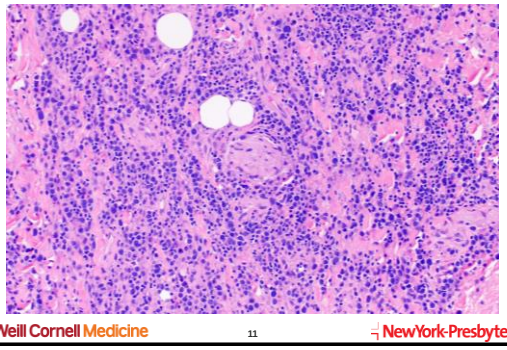
Subsequent Excisional Biopsy, Right Facial Lymph Node and Soft Tissue Lesion



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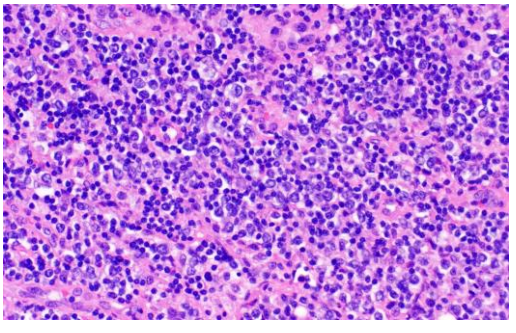
Other Areas of the Excision



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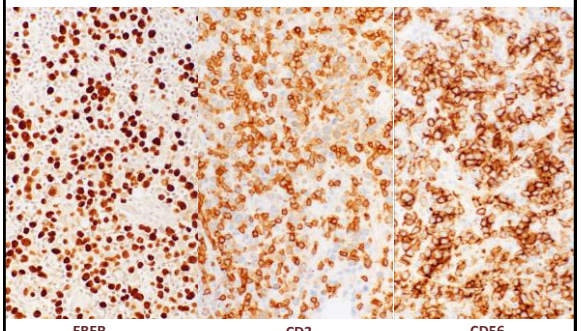

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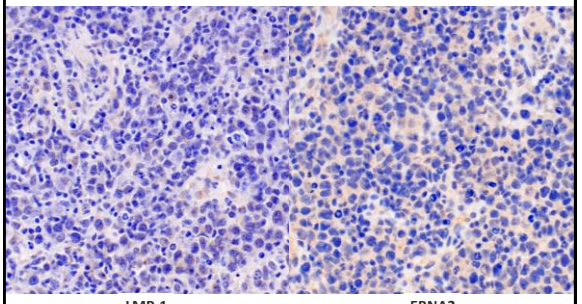
Negative for EBV LMP-1 and EBNA2



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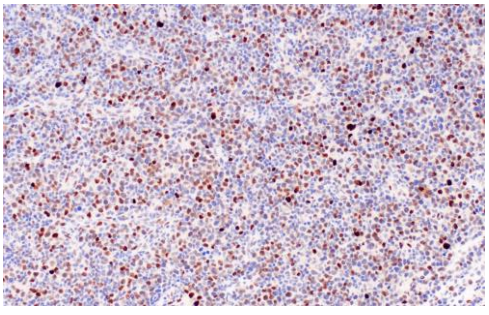
Positive for TP53



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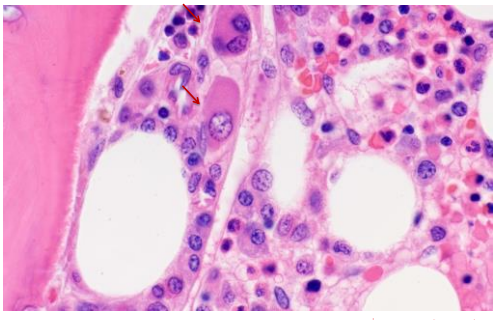
Megakaryocytes with Viral Cytopathic Effect



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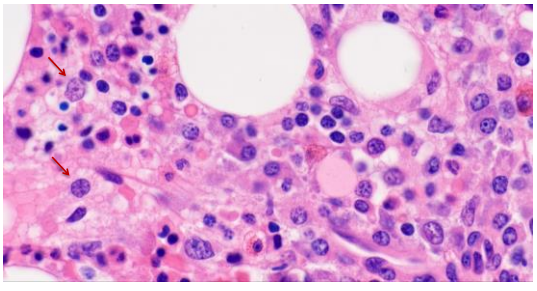
Rare Atypical Cells



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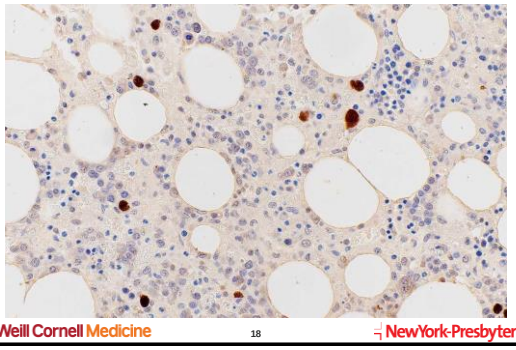
Rare Atypical Cells EBER+



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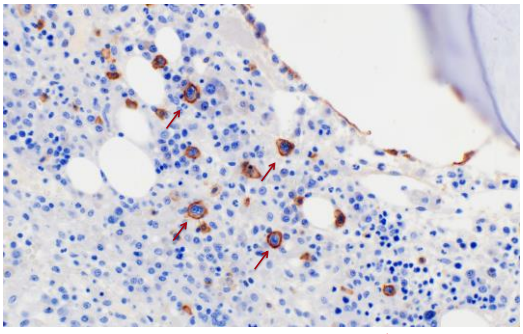
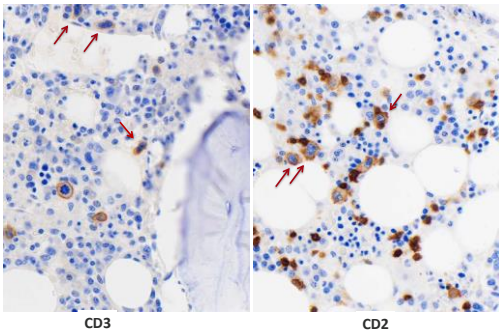
Atypical CD56+ cells



19

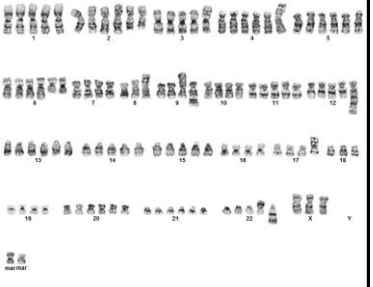
Atypical CD3 and CD2 cells



20

Cytogenetics and Molecular Analysis

- August 2017: Lymph node excisional biopsy failed to yield metaphase cells
- August 2017: Bone marrow biopsy demonstrated complex karyotype
- August 2017: TCR gamma chain gene rearrangement analysis of bone marrow aspirate was clonal in a polyclonal background



21

Morphology

- Diffuse and permeative lymphoid infiltrate
- Broad cytologic spectrum
  - Cells may be small, medium, large or anaplastic
  - May contain mixed small and large cells
  - Inflammatory background may be prominent, particularly with small-cell or mixed-cell populations
- Ulceration common, nearly always necrosis
  - Angiocentric/angiodestructive lesions
  - Fibrinoid necrosis in blood vessels
  - Coagulative necrosis and apoptosis

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Phenotypic Features

- Immunophenotype (NK type):
  - Positive: CD2, CD56, cCD3-epsilon, granzyme B, TIA1, perforin, EBER, HLA-DR, CD30 (30%), CD7 (variable)
  - Often: CD43, CD45RO, CD25
  - Negative: CD5, sCD3, CD4, CD8, CD16, CD57
- T cell lineage: CD5, CD8, T Cell Receptor (alpha/beta or gamma/delta) may be expressed, cytotoxic markers are positive
- CD56(-) cases: CD3-epsilon+, must have both cytotoxic markers and EBV

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Differential Diagnosis

- Non-specific inflammatory process
- Lymphomatoid granulomatosis
- Aggressive NK cell leukemia
- Peripheral T cell lymphoma, NOS
  - Fails to meet criteria for more specific T cell lymphoma
- Primary EBV positive nodal T cell or NK cell lymphoma
  - Appear distinct from extranodal presentation
  - Elderly patients/immune deficiency
  - Monomorphic infiltration
  - Lack angiodestruction and necrosis
  - Considered variant of peripheral T cell lymphoma, NOS

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Hepatitis B, EBV Co-Infection and Lymphoma Risk

- **HBV infection implicated in hepatocellular carcinoma, cholangiocarcinoma, B-cell non-Hodgkin lymphoma**
- **HBV co-infection may increase risk of EBV-related nasopharyngeal carcinoma (Ye et al. 2015)**
- **Diffuse large B cell lymphoma with HBV co-infection:**
  - **Chen et al. (2008): 68 out of 271 (25%) cases were HBsAg+, showing a higher carrier rate**
  - **Marcucci et al. (2006): 20 of 230 cases of aggressive B-NHL with HbsAg, OR 3.75, pointing toward a positive association**
  - **Taborelli et al. (2016): 15 of 290 DLBCL cases with HBsAg, OR 2.69**

Ye et al. Cancer Epidemiology, Biomarkers & Prevention (2015) 24:1766-73.
Chen et al. Ann Hematol (2008) 87: 475.
Marcucci et al. Haematologica 2006; 91(4):554-557.
Taborelli et al. Infect Agent Cancer 2016; 11(27):1-6.

25

Molecular Studies and Pathogenesis

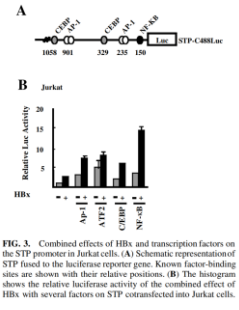

FIG. 3. Combined effects of HBx and transcription factors on the STP promoter in Jurkat cells. (**A**) Schematic representation of STP fused to the luciferase reporter gene. Known factor-binding sites are shown with their relative positions. (**B**) The histogram shows the relative luciferase activity of the combined effect of HBx with several factors on STP cotransfected into Jurkat cells.

- **Assogba et al. (2004):**
- **Investigated HBV and EBV co-infection in cell lines**
- **HBV oncoprotein, HBx, interacts with the transcription factor, NFκB, to activate the EBV related-*LMP1* promoter**
- ***LMP1* gene was consistently upregulated by HBx at the molecular level**

Figure from Assogba B, Paik N, Rho H. DNA and Cell Biology 2004; 23(3): 141-148

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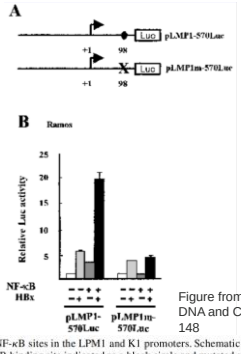

Figure from Assogba B, Paik N, Rho H. DNA and Cell Biology 2004; 23(3): 141-148

FIG. 5. Site-directed mutation of NF-κB sites in the LPM1 and K1 promoters. Schematic diagrams of pLMP1-570Luc (**A**) and pK1-886Luc (**C**), showing one NF-κB binding site indicated as a black circle and mutated sites labeled "X." Plasmids pLMP1m-570Luc and pK1m-886Luc have a mutated NF-κB binding site. These plasmids were transfected into Ramos cells to examine the effects of HBx and NF-κB (**B** and **D**).

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Molecular Studies and Pathogenesis

Table 2. The associations of HBV infection with the risk of NK/T cell lymphoma.

	Cases (N=417)		Controls (N=488)		Univariable			Multivariable	
Variable	**No.**	**%**	**No.**	**%**	**OR**	**95%CI**	***p***	**AOR***	**95%CI**	***p***
HBV markers									
HBsAg(-)	367	88.01	432	88.5		1(reference)			1(reference)	
HBsAg(+)	50	11.99	56	11.5	1.051	0.700-1.577	0.810	1.004	0.662-1.525	0.984
Anti-HBs(-)	166	39.81	187	38.3		1(reference)			1(reference)	
Anti-HBs(+)	251	60.19	301	61.7	0.939	0.719-1.228	0.647	0.936	0.712-1.231	0.637
HBeAg(-)	406	97.36	476	97.7		1(reference)			1(reference)	
HBeAg(+)	11	2.64	11	2.3	1.172	0.503-2.733	0.713	1.096	0.457-2.628	0.838
Anti-HBe(-)	288	69.06	365	74.8		1(reference)			1(reference)	
Anti-HBe(+)	129	30.93	123	25.2	1.329	0.993-1.779	0.056	1.343	0.998-1.809	0.052
Anti-HBc(-)	243	58.27	320	65.6		1(reference)			1(reference)	
Anti-HBc(+)	174	41.73	168	34.4	1.364	1.041-1.786	0.024	1.352	1.025-1.782	0.033
HBV infection status										
HBsAg(-)/anti-HBc(-)†	241	58.1	320	65.6		1(reference)			1(reference)	
HBsAg(+)/anti-HBc(+)‡	48	11.6	56	11.5	1.138	0.748-1.733	0.546	1.092	0.708-1.685	0.690
HBsAg(-)/anti-HBc(+)§	126	30.4	112	23.0	1.494	1.102-2.026	0.010	1.497	1.098-2.042	0.011
Immune response for HBV										
anti-HBs(+)/anti-HBc(-)¶	147	35.3	216	44.3		1(reference)			1(reference)	
anti-HBs(+)/anti-HBc(+)#	104	24.9	85	17.4	1.798	1.261-2.564	0.001	1.880	1.308-2.701	0.001
anti-HBs(-)/anti-HBc(+)&	70	16.8	82	16.8	1.254	0.856-1.837	0.244	1.213	0.815-1.804	0.341
anti-HBs(-)/anti-HBc(-)	96	23.0	105	21.5	1.343	0.949-1.901	0.096	1.436	1.006-2.050	0.047

The AOR* was adjusted by sex, age (as a continuous variable), ABO blood type, smoking status, alcohol drinking and a family history of cancers.
Abbreviations: OR, odds ratio; AOR, adjusted odds ratio; 95% CI, 95% confidence interval. HBsAg, hepatitis B surface antigen; anti-HBs, hepatitis B surface antibody; HBeAg, hepatitis B e antigen; anti-HBeAb, hepatitis B e antibody; anti-HBc, hepatitis B core antibody.
† Never infected with HBV; ‡ Chronic carrier of HBV; § Previously infected with HBV (resolved hepatitis B); ¶ Immune via vaccine; # Naturally immune to HBV infection; & Lack of immune response to HBV infection.

Table from Wang K, Yang H, He W, et al. J Cancer 2017; 8(14):2676-2683.

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Treating Lymphomas in Patients with Hepatitis B or Hepatitis C

- **Antiviral therapy for at least 6 months**
  - **Goal: HBV DNA levels <3 $\log_{10}$ IU/mL**
  - **Entecavir and tenofovir first line agents**
  - **Lamivudine for short term (<6 month) therapy in treatment naïve patients, HBV DNA levels <2000 IU/mL, or if unable to obtain first line agents**
- **Immunosuppressive agents**
  - **Rituximab (recommend anti-viral therapy for at least 12 months prior to administering)**

29

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- **Dalia S, Suleiman Y, Croy D, Sokol L. Association of Lymphomagenesis and the Reactivation of Hepatitis B Virus in Non-Hodgkin Lymphoma. Cancer Control 2015; 22(3): 360-365.**
- **Kelling M, Sokol L, Dalia S. Hepatitis B Reactivation in the Treatment of Non-Hodgkin Lymphoma. Cancer Control 2018; 25(1): 1-6.**
- **Marcucci F, Mele A, Spada E, et al. High prevalence of hepatitis B virus infection in B-cell non-Hodgkin's lymphoma. Haematologica 2006; 91(4):554-557.**
- **Ozoya O, Chavez J, Sokol L, Dalia S. Optimizing antiviral agents for hepatitis B management in malignant lymphomas. Ann Transl Med 2017; 5(3): 1-10.**
- **Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H. *WHO classification of tumours of haematopoietic and lymphoid tissues.* 4th ed. Lyon, France: IARC; 2008.**
- **Taborelli M, Polesel J, Montella M, et al. Hepatitis B and C viruses and risk of non-Hodgkin lymphoma: a case-control study in Italy. Infect Agent Cancer 2016; 11(27):1-6.**
- **Wang K, Yang H, He W, et al. Association between extranodal natural killer/T-cell lymphoma and hepatitis B viral infection: a case-control study. J Cancer 2017; 8(14):2676-2683. doi:10.7150/jca.19665.**
- **Ye YF, Xiang YQ, Fang F, et al. Hepatitis B virus infection and risk of nasopharyngeal carcinoma in Southern China. Cancer Epidemiology, Biomarkers & Prevention: A publication of the American Association for Cancer Research 2015; 24:1766-73.**

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Case Submission Form-1

Submitter(s) and Titles (MD or MD PhD): Lisa Rimsza, M.D. and Alanna Maguire, PhD.

Affiliation: Mayo Clinic Arizona

E-mail: rimsza.lisa@mayo.edu

Clinical History: The patient is a 41 year old male, with history of HIV infection, currently on highly active anti-retroviral therapy, and new lymphadenopathy.

Biopsy Fixation Details: Unknown

Description of Clinical Image if Any: None

Details of Microscopic Findings: Low power demonstrates complete effacement of the lymph node by large centroblastic and some Burkitt-like cells, frequent mitoses, and tingible body macrophages.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
IHC demonstrates a B cell phenotype with CD10, weak BCL2, and high Ki67.

Special Stains: EBER negative.

Case Submission Form-2

Cytogenetics: Array CGH demonstrates an unusually stable genome with few genetic alterations. The BCL2 locus shows only deletions (no translocations or gains/amplifications).

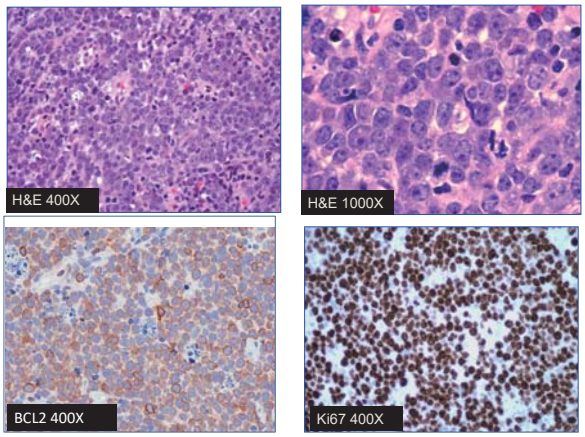
Molecular Analysis: Lymph2Cx assay on NanoString platform indicates Germinal Center B Cell gene expression profile. Gene expression profiling shows an increase in genes associated with the DNA damage response, proliferation, and apoptotic pathways.

Interesting Feature(s) of Submitted Case: This case falls under category 5 as an "Exemplary demonstration of various subtypes of Infectious agent-association lymphoproliferation using multidisciplinary correlations". It is one of a case series of 19 HIV(+) DLBCL and demonstrates a previously undescribed possible lymphomogenic pathway and cell of origin that is distinct from Germinal Center B Cell DLBCL in HIV(-) patients.

Proposed Diagnosis:

HIV-associated Diffuse Large B Cell Lymphoma, Germinal Center subtype.

Comments: Many additional H&E and IHC images are available if needed, not included at this time to save space.



Theory of immunocompromised-related lymphomagenesis:

- Immunosuppression→ proliferation of a polyclonal B cell population (due to increased IL6 and other cytokines).
- During the uncontrolled proliferation, mutations or translocations can occur causing transformation to malignancy.

Is this the whole story?

Our study: Cell of origin of HIV(+) versus (-) cases with DLBCL morphology

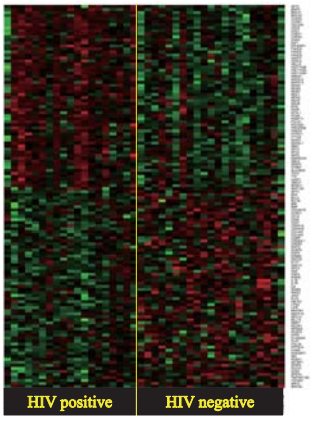
	HIV(-) DLBCL (n = 39)	HIV(+) DLBCL (n = 27)	P-value
Collected	1982 - 2012	1989 - 2007	
% males	49 (19/39)	93 (25/27)	<0.0001
Median Age	70 yrs (20-89)	41 yrs (28-66)	<0.0001
GCB	21 (54%)	19 (70%)	0.2080
UNC	5 (13%)	5 (19%)	0.7286
ABC	13 (33%)	3 (11%)	0.0455

PMID 2439 8326
Scott et al 2014, Lymph2Cx assay on NanoString instrument
Determining cell-of-origin subtypes of DLBCL using gene expression in FFPE tissues

Pan-Cancer gene expression panel (740 genes) in GCB DLBCL

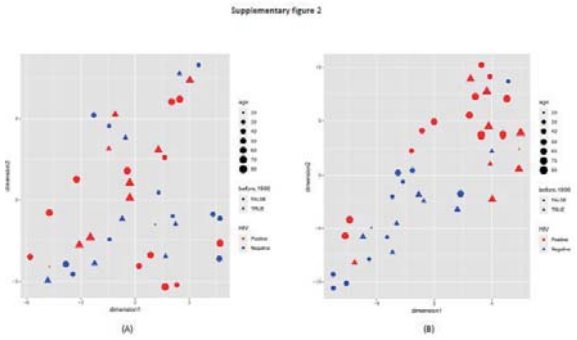
126 genes differentially expressed @ p<0.05 48% (61/126) were increased

52% (65/126) were decreased



Courtesy of Dr. Alanna Maguire

Regarding differences in treatment eras for the HIV(+) patients: No significant differences in gene expression patterns pre- and post-HAART or by Age



Pathway analysis

* Indicates pathway identified by both MSigDB overlap analysis & GSEA enrichment analysis

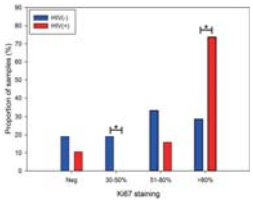
Cell cycle*
DNA replication*
DNA repair*
Fanconi Anemia pathway*
ATRBRCA pathway
Mismatch repair
E2F cell cycle targets*

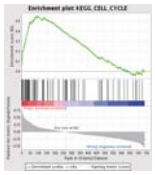
Pathways enhanced in **upregulated** genes

B-cell receptor (BCR) signaling
Pi3K-ERBB2 signaling
MAPK signaling*
Intrinsic apoptotic pathway

Pathways enhanced in **downregulated** genes

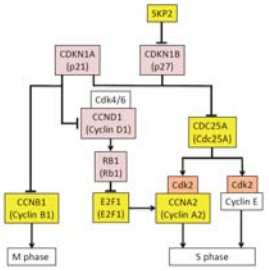
Pathway analysis: Proliferation





CCNA2, CCNB1, CCND1, **CDC25A, CDC6, CDC7**, CDKN1A, CDKN1B, **CHEK1, CHEK2, E2F1, HDAC2, MAD2L2, MCM2, MCM4, MCM7, PCNA, PKMYT1, PRKDC, PTTG2**, RB1, **RBX1, SKP2, SMC3, TTK**

P-value <0.0001, FDR-value 1.107E-5

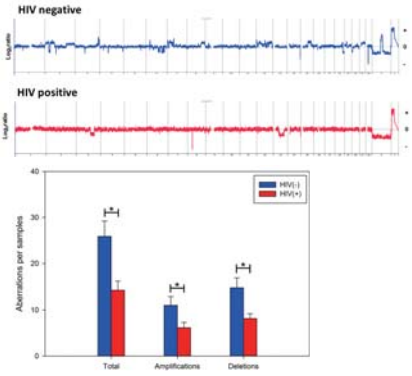


Conclusion: HIV(+) GCB-DLBCL are more proliferative than HIV(-) tumors

Pathway analysis

Cell cycle*
DNA replication*
DNA repair*
Fanconi Anemia pathway*
ATRBRCA pathway
Mismatch repair
E2F cell cycle targets*

B-cell receptor (BCR) signaling
PI3K-ERBB2 signaling
MAPK signaling*
Intrinsic apoptotic pathway



HIV(+) tumors have…

Enhanced DNA repair gene expression

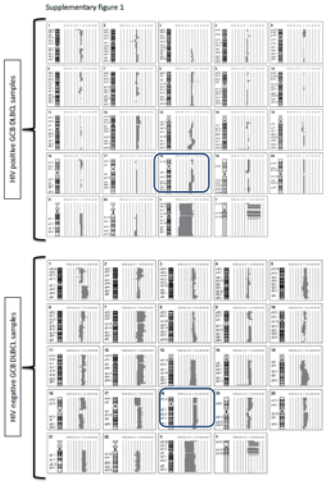
Enhanced genomic stability

BCL2, located at 18q21.33, was similarly diploid (2N) in the HIV(-) and HIV(+) cohorts; 85% and 78% respectively

When aberrations did occur, they were exclusively gains/amplifications in the HIV(-) and exclusively losses/deletions in the HIV(+) cohort.

All HIV(-) samples with *BCL2* amplifications stained 100% positive for BCL2 protein expression and low Ki67 ≤40%.

All HIV(+) samples with *BCL2* gene deletions were negative for, or poorly expressed ≤ 10% BCL2 protein with high Ki67 ≥ 80%.



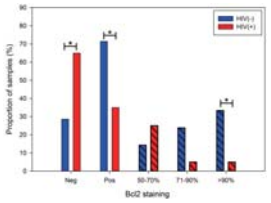
Pathway analysis

Intrinsic apoptotic pathway

5 Bcl2 family members identified…

4 Pro apoptotic (BAX BIM BMF PUMA)

1 Anti apoptotic (BCL2)



	HIV neg, n=21 n (%)	HIV pos, n=19 n (%)	P-value
Diploid	18 (86)	15 (79)	0.6889
Deletion	0 (0)	4 (21)	0.0424
Amplified	3 (14)	0 (0)	0.2327

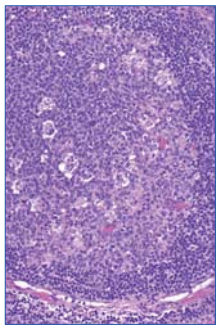
HIV(+) tumors…

- similarly aberrant @ 18q21.33 (BCL2 locus)
- reduced expression of pro-survival Bcl2
- reduced expression of anti-apoptotic genes

Results suggest an alternative pro-survival mechanism

Germinal Center Light and Dark Zones

- Centrocytes versus Centroblasts
- Changes in activation and proliferation
- Alternating single cell type rather than distinct differentiation stage



P. Milpied et al, Nature Immunol 2018

https://library.med.utah.edu/WebPath/HEMEHTML/HEME120.html

Cell-of-Origin – Germinal Center Reaction

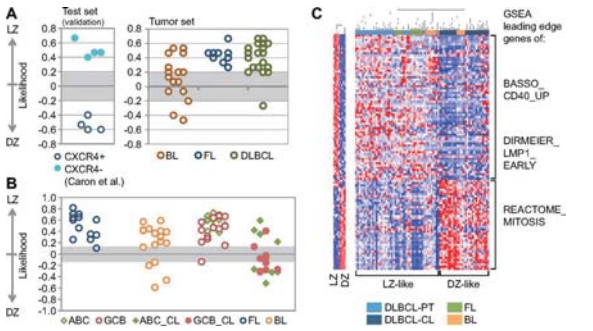
Interaction with TFH and FDC cells that dictates cell fate - memoryB/plasma cells; apoptosis; recycling for more SHM and replication.

In lymphoma, the TFH and FDC interactions are altered likely leading to continuous cycling of cells between light and dark zones.
Most lymphomas are derived from "light zone"

P. Milpied et al, Nature Immunol 2018

Figures Compliments of David Scott

Most GC-derived B-NHLs share an LZ-related phenotype.



Gabriel D. Victora et al. Blood 2012;120:2240-2248

©2012 by American Society of Hematology

blood

Germinal Center Zones related gene expression:

Light Zone

- CD83 high
- CXCR4 low
- Higher SIg
- CXCR5
- CD40
- NFKB
- MYC engagement

- 10% in S/G2M/M (almost none in G2M)

Dark Zone

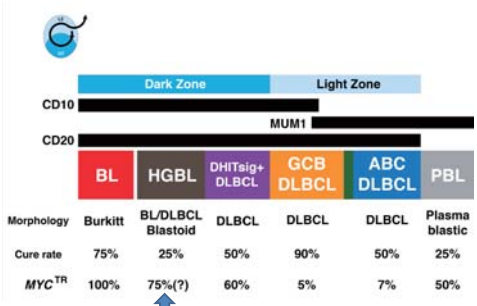
- CD83 low
- CDCR4 high
- CD27 high
- CCND2, CCND3, CCNA2
- DNA editing genes
- FOXP1
- TCF3
- BCL2

- 30% in S/G2M/M

GD Victora et al, Blood 2012

Red=over expressed in HIV(+) GCB-DLBCL
(Check tables in paper for other genes)

Speculation on cell of origin of HIV(+) DLBCL



HIV(+) GCB-DLBCL?
BLL morphology,
BCL2(-), ↑MMR

Figure Compliments of David Scott

Summary:

HIV+ DLBCL not solely defined by immune deficiency

- Enhanced proliferation
- Enhanced DNA repair and Genome Stability
- Alternative pro-survival mechanism

- Possible distinct cell of origin from usual GCB-DLBCL

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SESSION FOUR: Other Infectious Agent-Associated Lymphomas

Diffuse large B-cell lymphoma, BCL2+ with low proliferation arising in patient with HCV genotype 4-related hepatitis

Dr. Girish Venkataraman MD, MBBS
Reva Goldberg
Dr. Pari Jafari, MD

1

Case History

- The patient was a 72-year-old man with a 24-year history of treatment-naïve hepatitis C resulting in genotype IVa cirrhosis.
- He presented with two **hypoechoic hepatic lesions** on surveillance abdominal **ultrasound.**
- On **CT**: **hypoattenuating, "HCC-atypical" hepatic lesions** measuring 3.6 x 3.1 cm & 2.8 x 2.7 cm
- The patient denied fevers, chills, or night sweats. There is no known history of malignancy.

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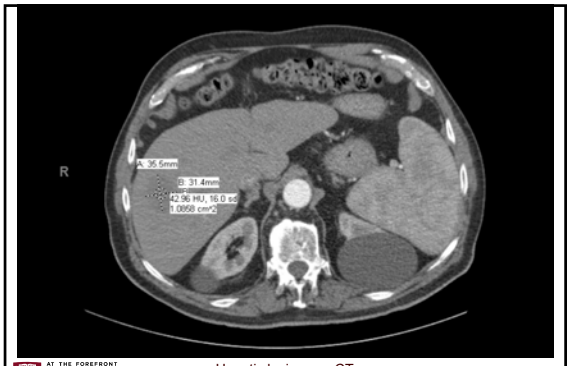
2



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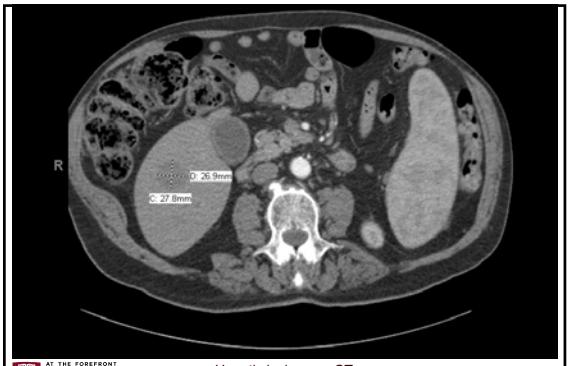
Hepatic lesions on CT

3

3



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Hepatic lesions on CT

4

4

Case History, Continued

- **Labs:** normal, with the exception of:
  - HCV viral load: 3071 IU/mL
  - Platelets: 53 K/uL (patient had multi-year history of thrombocytopenia, thought to be due to cirrhosis)

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5

5

Case History, Continued

- The core biopsy showed areas of DLBCL, non-GCB + cirrhosis with HCV-associated changes.
- There was no bone marrow involvement.

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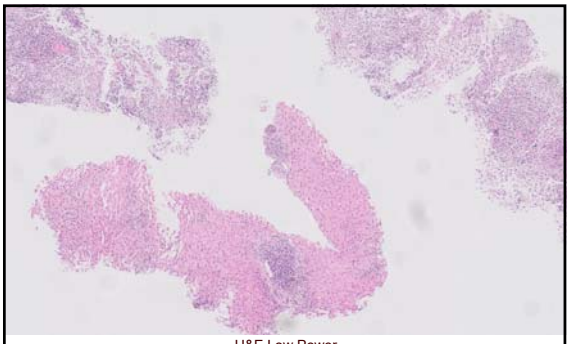
6

6

Case History, Continued

- The patient received R-CHOP resulting in a 2 year remission.
- Subsequently, the patient developed therapy-related AML.
- Died 4 years after initial DLBCL diagnosis.

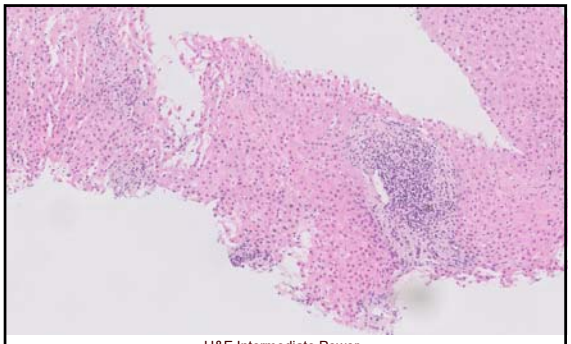
7



H&E Low Power

Low power image showing fragments of hepatic parenchyma with nodular portal lymphoid aggregates (HCV-associated changes) associated with separate fragments of atypical lymphoid infiltrate.

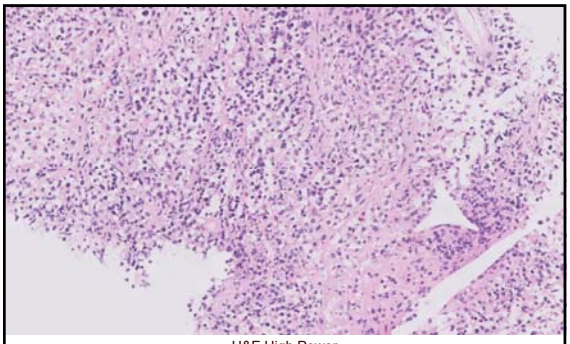
8



H&E Intermediate Power

Intermediate power H&E showing nodular portal lymphoid aggregate consistent with hepatitis C virus infection.

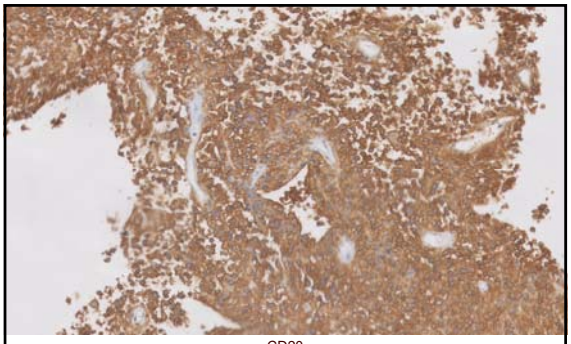
9



H&E High Power

High power H&E of the lymphoma fragment demonstrating diffuse infiltrate of large lymphoma cells with focal apoptosis.

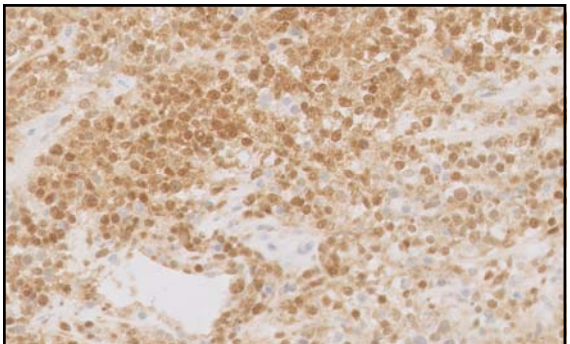
10



CD20

The lymphoma cells are positive for CD20.

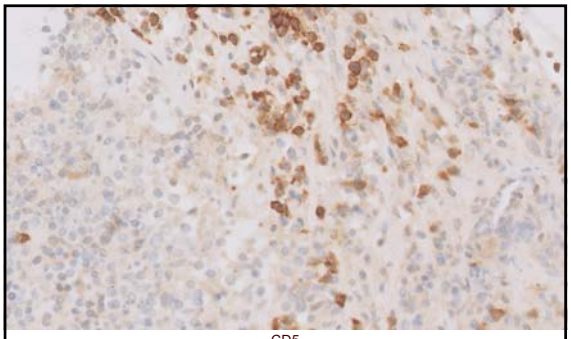
11



PAX5

The lymphoma cells are diffusely positive for PAX5.

12

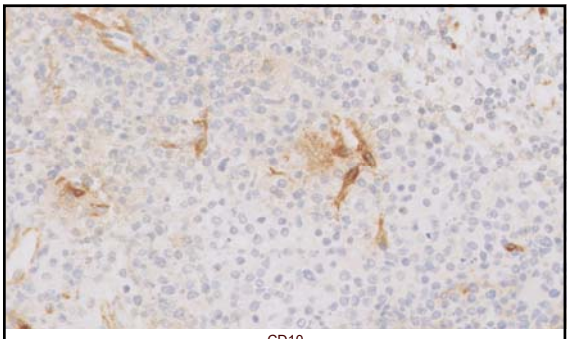


CD5

The lymphoma cells are negative for CD5 which highlights background T-cells.

13

13

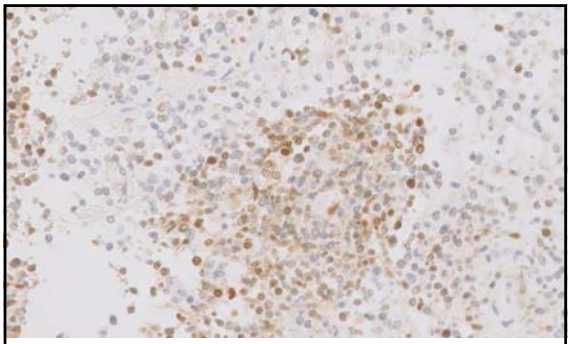


CD10

The lymphoma cells are negative for CD10.

14

14

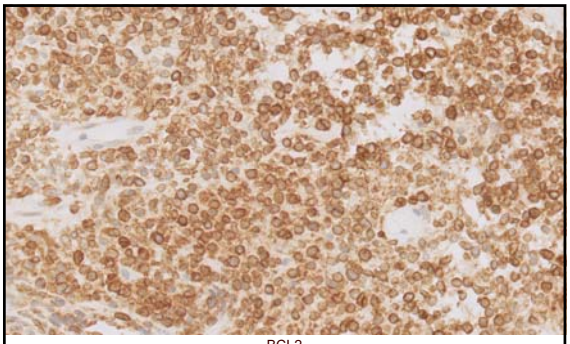


MUM1

The lymphoma cells are positive for MUM1 consistent with non-germinal center B-cell origin (non-GCB).

15

15

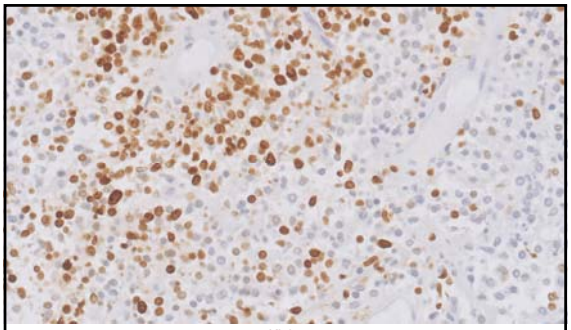


BCL2

The lymphoma cells are diffusely positive for BCL-2.

16

16



Ki-67

The proliferation index on Ki-67 is variable and ranges from 20 to 40%

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Final Diagnosis

Proposed and panel diagnosis:

Diffuse large B-cell lymphoma, non-GCB subtype BCL2+ with low proliferation in setting of chronic untreated HCV (genotype 4).

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Follow up

- The patient was given R-CHOP for his DLBCL and completed chemotherapy.
- Three years after the diagnosis, he re-presented with thrombocytopenia.
- A bone marrow biopsy revealed therapy-related myeloid neoplasm.
- Although he was in CR for the lymphoma, extensive cirrhosis and decompensation with ascites related to the untreated HCV precluded definitive treatment, and he passed away shortly thereafter.

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Unique Features of This Case

- Occurrence of low-proliferative BCL2+, DLBCL in the setting genotype 4 HCV.
- Based on literature, most HCV+DLBCLs exhibit high proliferation with low BCL2 expression and with HCV genotype 1 [1] predominating in most instances.[2]

1. Visco et al, Hepatitis C virus positive diffuse large B-cell lymphomas have distinct molecular features and lack BCL2 translocations British J Hematol, 2017
2. Torres HA, Mahale P Most patients with HCV-associated lymphoma present with mild liver disease: a call to revise antiviral treatment prioritization. *Liver Int* 2015; **35**: 1661-1664

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Discussion requested

1. Overview of Hep B and Hep C-related lymphomas.
2. Current understanding of pathogenesis.

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HCV-positive NHL: Overview

- Well-recognized association between B-cell NHLs and chronic HCV infection
  - Prevalence is difficult to assess accurately.
    - Lack of routine HCV screening at time of NHL diagnosis
    - Many HCV patients are unaware of their status.

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HCV-positive NHL: Subtypes

- HCV-positive lymphoproliferations include:
  - Marginal zone lymphoma (MZL)
  - Diffuse large B-cell lymphoma (DLBCL)
  - Follicular lymphoma (FL)
- MZL → DLBCL transformation is more frequent in HCV-positive patients (32%) than in HCV-negative patients (6%).

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Besson, JCO, 2006
Pozatto et al, Clin Liv Disease, 2017

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HCV-positive NHL: Distinctive Features

- HCV-positive NHLs typically develop 15 years after initial HCV infection.
- Compared to HCV- NHLs, HCV+NHLs are
  - more often found in extranodal locations (liver, spleen, stomach).
  - associated with poorer 2-year survival (56% vs. 80%).
  - associated with higher IPIs (likely due to elevated LDH 2/2 chronic HCV).

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HCV-positive NHLs: Pathogenesis

- Mechanisms of lymphomagenesis remain unclear.
- Visco et al. (2017) described differential expression of 23 genes between HCV-positive and HCV-negative DLBCL.
  - HCV-positive DLBCL associated with reduced *BCL2* expression and absence of *BCL2* translocations.
  - Findings support presence of distinct lymphomagenesis pathway in HCV-associated DLBCL.

Visco et al, Hepatitis C virus positive diffuse large B-cell lymphomas have distinct molecular features and lack BCL2 translocations British J Hematol, 2017

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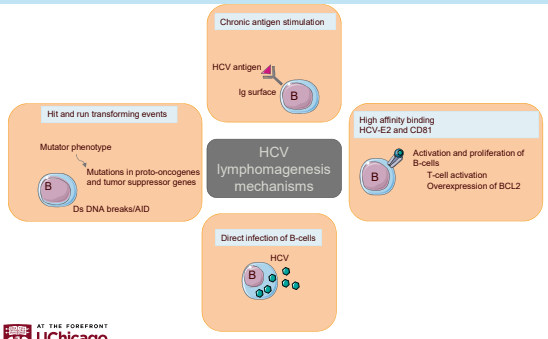
HCV-positive NHLs: Pathogenesis



Mazzaro, Cancer 2002; Marcucci, Blood 2011

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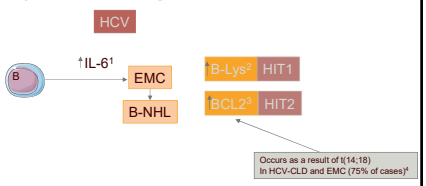
HCV and lymphomagenesis



1. Tanaka, Cancel Immunol Res, 2014
2. Fabris, Rhematology, 2007
3. Zuckerman, BJH, 2001
4. Zignego, Hepatology, 2000

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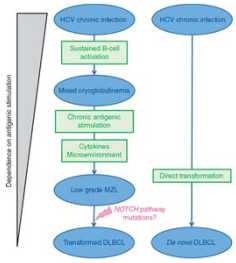
HCV and DLBCL pathegenesis



Couronne et al Annals of Oncology 2018

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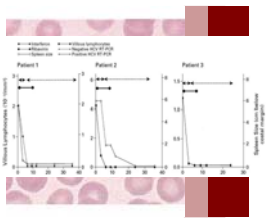
HCV-positive NHLs: treatment

Is there benefit in treating HCV concurrently (or even in treating it before addressing lymphoma)?

9 HCV-positive patients with splenic lymphoma with villous lymphocytes received interferon +/- ribavirin.

- All 9 entered partial/full remission.
- No remission in HCV-negative controls.



1: Hermine O, Lefrère F, Bronowicki JP, Mariette X, Jondeau K, Eclache-Saudreau V, Delmas B, Valensi F, Cacoub P, Brechot C, Varet B, Troussard X. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. N Engl J Med. 2002 Jul 11;347(2):89-94.

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29

HCV-positive NHLs: treatment

- Is there benefit in treating HCV concurrently (or even in treating it before addressing lymphoma)?
  - Hosry et al. (2016): improved survival in HCV-positive patients receiving R-CHOP/R-ICE and interferon.
  - Arcaini et al. (2016): regression of lymphoproliferative disease with direct-acting antiviral therapy for HCV.

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Overview of HBV-related lymphomas

- Most published studies evaluating associations (not causality) are based on retrospective population based case-control studies
- Methodology: Cohort of HBsAg+ vs. Cohort negative for HBsAg from meta-analysis.

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Choropleth map of Chronic HBV

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The Lancet 2014 384, 2053-2063DOI: (10.1016/S0140-6736(14)60220-8)

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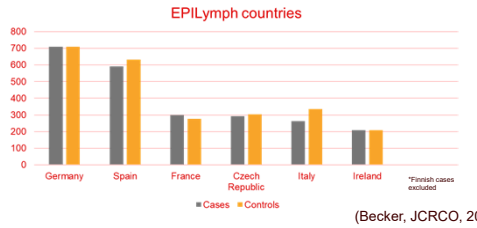
Illustrative Case-control study European Epilymph study



(Becker, JCRCO, 2009)

Cases: Consecutive lymphoma cases
Controls: No lymphoma, transplant, systemic Infection, HIV

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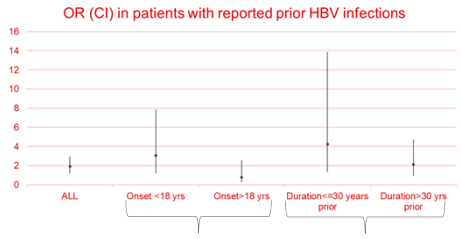
HBV infection and lymphoma risk



(Significant despite excluding HCV+ patients)
(Becker, JCRCO, 2009)

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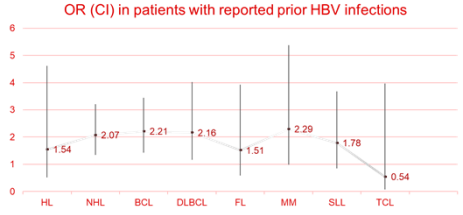
Lymphoma subtypes in HBV infection



(Becker, JCRCO, 2009)

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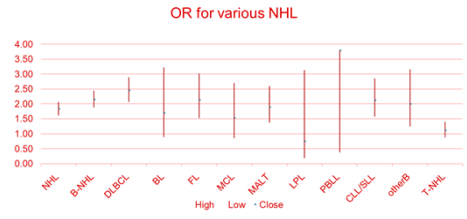
Zhou et al, BMC Cancer 2019

1:2 case-control study enrolling 3502 NHL cases and 7004 controls



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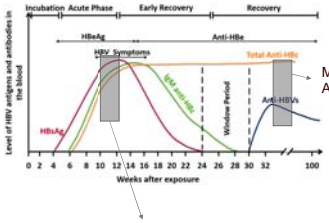
Zhou et al, BMC Cancer 2019



Moderate risk of B-NHL
AOR 2.25 (1.96–2.57)

High risk of B-NHL
AOR 6.23 (3.95–9.82)

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Zhou X et al (conclusions)

- Increased risk of B-NHL and not T-NHL
- Patients with active antigen-positive state have higher risk compared to immune patients with Anti-HBS and Anti-HBc

Association is definitive, yet what is the underlying mechanism?

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Genetic landscape of hepatitis B virus–associated diffuse large B-cell lymphoma. Ren W, Blood 2019

Chinese DLBCL Cohort (n=275)
HBSAG+ (n=56)

- De novo (N=266)
- Possible transformed (n=9)

WGS (60/60)
WES (47/47)

Targeted capture Reseq

1. Mutated in >= 3 cases
2. Recurrently mutated in >=3 cases in other BCL
3. Import for DNA repair
4. Important for targeted therapy

Transcriptome reseq/GSEA (n=108)

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Genetic landscape of hepatitis B virus–associated diffuse large B-cell lymphoma. Ren W, Blood 2019

Demographics

Contrasts with HCV+DLBCL older

- HBSAg+ DLBCL patients are younger and at higher stage and have splenomegaly
- No difference in GC vs. non-GCB proportions.
- High viral loads at diagnosis correlated with younger age at presentation

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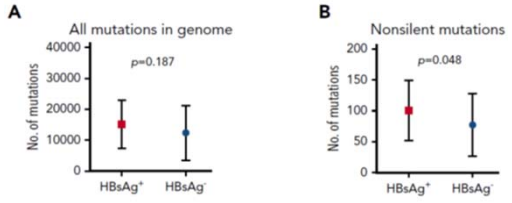
Genetic landscape of hepatitis B virus–associated diffuse large B-cell lymphoma. Ren W, Blood 2019

WGS- no differences in mutations | Coding WES significant



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Genetic landscape of hepatitis B virus–associated diffuse large B-cell lymphoma. Ren W, Blood 2019

DLBCL Discovery cohort
65+ previous 31 matched tumor/PB pairs
20/96 were HBsAG+

WGS	WES	
10835	162	Somatic mutations
79	67	Non-silent Somatic

131 genes affected >5% of cases; 67 significant

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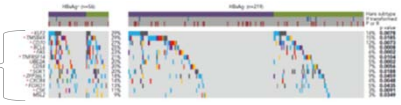
Genetic landscape of hepatitis B virus–associated diffuse large B-cell lymphoma. Ren W, Blood 2019

Summary



Significantly different genes mutated in HBSAG+ cases

-11/14* are off-targets of AID

- Distinct several key pathways in lymphomagenesis (histone, NFKB, P53, immune evasion)
- BCL6 alterations rather frequent (79% vs 28%)
- KLF2 mutation (recurrent in SMZL but not DLBCLs)
- Aberrant SHM due to AID

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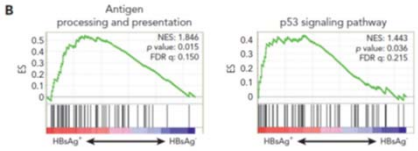
Genetic landscape of hepatitis B virus–associated diffuse large B-cell lymphoma. Ren W, Blood 2019

Transcriptome analysis indicates genes related to Antigen presentation and P53 signaling pathways are upregulated in HBV+ DLBCLs



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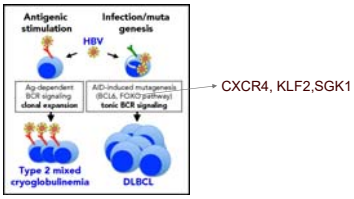
HBV messing with the B-cell genome leads to DLBCL



Marcella Visentini,Milvia Casato, HBV messing with the B-cell genome leads to DLBCL, Blood, 2018,

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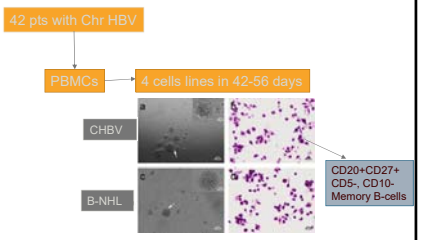
Pathogenesis data and mechanisms are still unknown



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Qi X, PLOS 2019

46

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Summary and take home points

- HCV is associated with increased lymphoma risk
  - EMC, MZL, DLBCL
  - Multiple mechanisms, B-cell stimulation/t(14;18)
  - Chronic antigenic stimulation and EMC association with progression to DLBCL is much more common
- HBV also associated with increased risk
  - DLBCL, SLL, FL (epidemiologic data mostly)
  - Much less likely to have antigen driven EMC
- Genomic studies
  - Distinct several key pathways in lymphomagenesis (histone, NFKB, P53, immune evasion)
  - BCL6 alterations

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ACKNOWLEDGEMENT

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西安中原

Group Discussion Cases

Type 1

Chronic EBV infections and borderline lymphoproliferations

Case presentation

Department of Pathology, People's Hospital of Guangxi Province

Mo Xianglan

1

Clinical History

- A 35-year-old male was admitted to People's Hospital of Guangxi Province because he had been suffering from skin and oral multiple pain ulcers for 6 months. He was no system symptoms such as fever, weight loss, night sweat. The skin lesions could self-healing and recurrence alternately.

2

Clinical History2

- The physical examination revealed there were multiple ulcers with maximum diameter of 0.8cm to 2.5cm in his trunk and limbs skin. The ulcers with intumescent edge and the long axis parallel to dermatoglyph(Figure 1A). Some ulcers had been healed with scar(Figure 1B) . No enlargement of superficial lymph nodes were detected. Laboratory examination were normal. A biopsy of the skin ulcer in the right lower abdomen was done in order to get accurate diagnosis.

3

Clinical History3

- The patient received 6 cycle of CHOP chemotherapy and got completely remission. There was no evidence of recurrence after a 35 months follow up.

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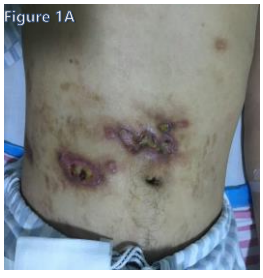

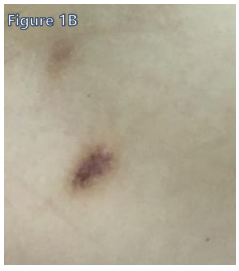

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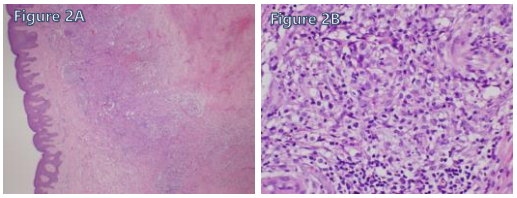
Biopsy Fixation Details

- The tissue was fixed with 10% neutral buffered formalin, paraffin imbedding, hematoxylin and eosin staining. Morphologically, the lesion was localized in the dermis with clear boundary and focal necrosis(Figure 2A). The cells were heterogeneous and diffusely distribution, immunoblastic-like cells, Reed-stemberg-like cells and apoptotic cells could be seen(Figure 2B). There many inflammatory cells in the background(Figure 2C).

6



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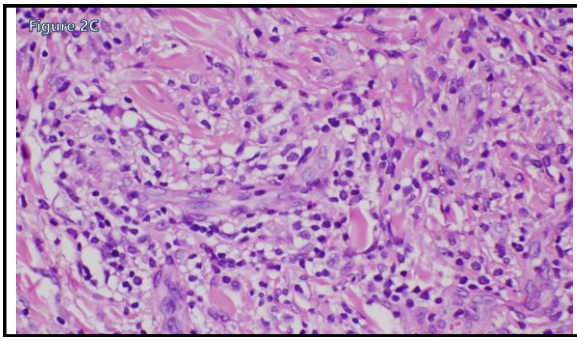


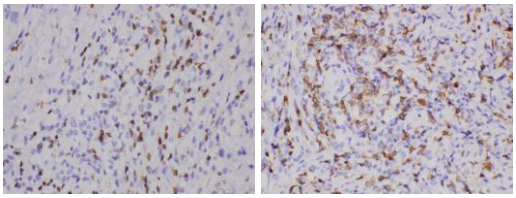
8

Immunophenotyping

- The proliferative lymphoid cells were positive to CD20, PAX-5, MUM1, CD10 and CD30; while negative to CD3, CD56, GranzymeB, TIA-1, CD15, CK, ALK, EMA and Desmin. Some of the cells were positive to EBER.
- The small lymphoid cell of background were positive to CD3.

9

CD3



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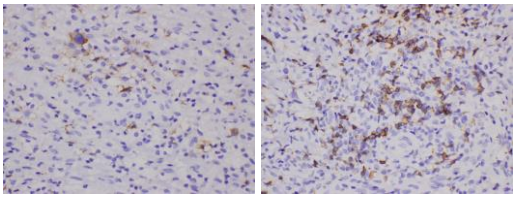
CD20



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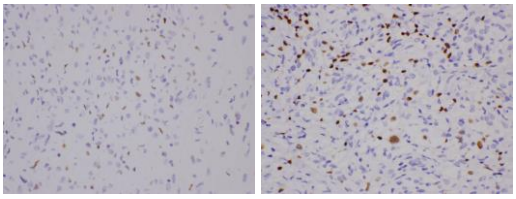
PAX-5



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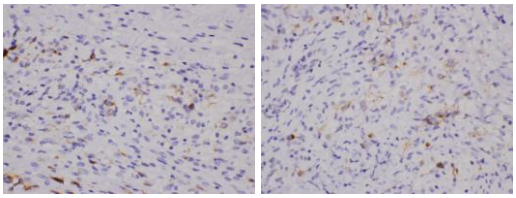
CD30



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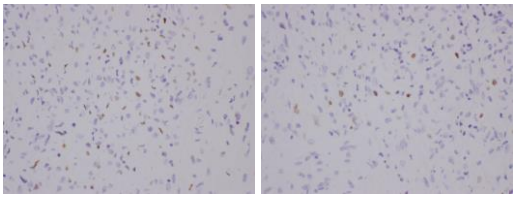
EBER



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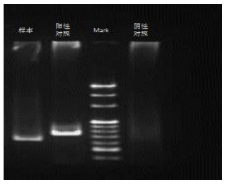
Molecular Analysis

- PCR-electrophoresis testing showed clonal rearrangement of IgH gene was positive, while clonal rearrangement of IgK and TCRY genes were negative.

IgH gene clonal rearrangement was positive



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Proposed Diagnosis: EBV positive diffuse large B cell lymphoma, NOS

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Comments

- It is uncommon that EBV-positive lymphoid proliferative disease between EBV-positive mucocutaneous ulcer and EBV- positive diffuse large B cell lymphoma. The clinical process and prognosis of the disease are under-investigated.

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CASE SUBMISSION FORM

Title:
Multiple Synchronous Primary EBV-Associated Polymorphic Posttransplant Lymphoproliferative Disorders of Different Clonal Compositions Occurring in a Single Patient Following Bone Marrow Transplantation

Submitter(s) and Titles (MD or MD PhD):
Miguel Dario Cantu, MD
Amy Chadburn, MD

Affiliation:
New York Presbyterian-Weill Cornell Medical Center

E-mail:
mdc9012@nyp.org

Clinical History:
The patient was a 59-year-old male with history of T cell acute lymphoblastic leukemia who received chemotherapy and haploidentical-cord allogeneic stem cell transplant from a female donor, 8 months following his initial diagnosis of leukemia. His conditioning regimen consisted of fludarabine, melphalan, and 400 cGY total body irradiation. Graft-versus-host disease (GVHD) prophylaxis consisted of mycophenolate mofetil, tacrolimus, and anti-thymocyte globulin.

His course was complicated by GVHD, HHV-6 viremia (peak 6,879cpy/mL), EBV viremia (peak 27,620 cpy/mL), and EBV encephalitis (peak >400,000 cpy/mL). Six months after transplant, whole body PET CT revealed multiple synchronous FDG avid lesions within the left frontal lobe, the left palatine tonsil, and gastric fundus. All lesions were biopsied and submitted to pathology to examination. Concurrent bone marrow aspiration and needle core biopsies were negative for a T cell neoplasm.

Following biopsy, the patient received intravenous and intrathecal rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP), whole brain radiation, and EBV-specific adoptive T cell therapy. He ultimately developed multifocal pneumonia, ten months after transplant and died.

A full autopsy was performed 24 hours following death and revealed a necrotic 0.8 x 0.5 x 0.5 cm left frontal lobe lesion. Gross examination of the gastrointestinal tract and oropharynx was unremarkable. Microscopic examination of lung consolidations revealed *Pneumocystis jirovecii* pneumonia, confirmed by direct immunofluorescence and GMS staining.

Biopsy Fixation Details:

The left frontal lobe biopsy was submitted as a 0.8 x 0.5 x 0.2 cm aggregate of tissue for frozen section diagnosis. A portion was submitted for flow cytometric analysis. The frozen tissue was then formalin fixed, and paraffin embedded. The left frontal lobe lesion was again sampled at autopsy, 24 hours following death. The autopsy specimen measured 0.8 x 0.8 x 0.4 cm, was formalin fixed, and paraffin embedded.

The left tonsil lesion biopsy was submitted as a 0.9 x 0.5 x 0.1 cm aggregate of tissue. A portion was submitted for flow cytometric analysis. The remaining tissue was submitted in formalin and paraffin embedded.

The gastric fundus ulcer biopsy was submitted as five pieces of tissue measuring from 0.1 x 0.1 x 0.1 cm to 0.3 x 0.2 x 0.1 cm. The specimen was submitted in formalin and paraffin embedded.

Description of Clinical Image if Any:
PET CT of the head reveals a focus of FDG avidity in the left palatine.
Upper GI endoscopy images show a 3 cm oozing, cratered gastric ulcer with adherent clot in the gastric fundus.
MRI brain with contrasts shows an enhancing lesion in the left frontal lobe.

Details of Microscopic Findings:
Histologic sections of the left tonsil reveal benign squamous mucosa, extensive necrosis, and infiltration by a polymorphous subepithelial population of small lymphocytes, immunoblast-like cells, occasional plasma cells, and few larger lymphoid forms.

Histologic sections of the gastric fundus ulcer show a dense, polymorphous infiltrate consisting of numerous large, atypical lymphocytes with associated necrosis.

Histologic sections of the left frontal lobe lesion show infiltration of brain parenchyma by an atypical, heterogenous population of lymphoid cells that range from small to large in size with irregular nuclear contours, and necrosis. Scattered mitotic figures are seen. A subset of cells are plasmacytoid, multinucleated, and Reed-Sternberg-like. These cells are morphologically similar to those seen in the tonsil and fundus. Sections of the left frontal lobe lesion at autopsy reveal predominantly necrosis with rare scattered lymphoid cells at the periphery of the lesion.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
Left tonsil lesion
IHC: CD20 highlights many scattered small to medium cells. CD10 is negative. MUM1 is positive. In situ hybridization for EBER is positive. LMP1, and EBNA2 stains many EBER positive cells. CD163 highlights many histiocytes. p53 stains 20% of cells. Ki67 proliferation fraction is 40%. MYC stains 5-10% of cells. CD3 reveals a small subset of positive small lymphocytes that are negative for CD34 and TdT.

Flow cytometry: A large B cell population was identified by CD19 expression, accounting for ~70% of viable cells analyzed. The CD19 positive cells are monotypic, expressing surface immunoglobulin kappa.

Gastric fundic ulcer
IHC: Large atypical lymphoid cells are positive for CD19, CD20, PAX5, and show monotypic cytoplasmic staining for immunoglobulin kappa light chain. Many of these cells stain for EBER, LMP1, and EBNA2.

Left frontal lobe lesion
IHC: Atypical lymphoid cells are negative for CD3 and TdT. These cells are positive for CD20, CD10 negative, BCL6 negative, and MUM1 positive. These cells are positive for EBER (80-90%), LMP1, and EBNA2. The proliferation rate by Ki-67 is moderate (30-40%). These cells are negative for TdT. A subset of cells are positive for p53. The autopsy specimen of the necrotic brain mass reveals scattered residual cells that are positive or CD20, EBER, LMP1, and EBNA2.

Flow cytometry: A large B cell population is identified by CD19 expression, accounting for 70% of viable cells analyzed. These CD19 positive cells are monotypic and express surface immunoglobulin kappa.

Special Stains:
Not performed on these specimens.

Cytogenetics:
Not performed on the brain, tonsil, or gastric specimens.

Concurrent posterior iliac crest bone marrow aspirates were submitted for conventional cytogenetics and fluorescence in situ hybridization (FISH) using multiplex CEP X and Yp12 probes to evaluate donor status following transplantation. A normal male karyotype was observed in 20 analyzed metaphase cells. No numerical or structural abnormalities were found. Donor (XX, female) cells were observed in 3.2% of 500 interphase nuclei evaluated by FISH analysis. The remaining 96.8% of cells showed host (XY, male) cells.

Molecular Analysis:
Four unstained slides, cut at 5 microns each, were submitted from each of the frontal lobe, tonsil, and fundic lesions. Cells from the unstained slides were lysed and DNA was extracted for analysis of Ig heavy chain and Ig kappa light chain gene rearrangements using the BIOMED-2 IgH Tube (FR3) assay. To increase the sensitivity of clonality detection, additional PCR reactions were performed to assess the common rearrangements in immunoglobulin kappa light gene (IGK) using the BIOMED 2 assay. These two multiplex PCR reactions were conducted using six sets of primers in each reaction that were hybridized to the IGH locus. PCR products were analyzed by capillary electrophoresis on the ABI 3500 Genetic Analyzer.

The gastric fundus lesion revealed an *IGH* monoclonal rearrangement (139 bp) and *IGK*

monoclonal rearrangements (152 bp and 201 bp, tube a). The tonsil lesion revealed an *IGH* monoclonal rearrangement (114 bp), as well as *IGK* clonal peaks (144 bp, tube a; 237 bp and 284 bp, tube b). In the frontal lobe lesion, an *IGH* monoclonal peak was detected (142 bp), as well as *IGK* clonal peaks (152 bp, tube a; 242 bp, tube b).

Concurrent posterior iliac crest bone marrow aspirates were submitted to assess for the presence of *TCRG* chain gene rearrangements using BIOMED-2 assay. No molecular evidence of a neoplastic T cell process was identified.

Interesting Feature(s) of Submitted Case:
This case demonstrates an interesting phenomenon rarely described in the literature, as multiple morphologically similar synchronous monoclonal primary PTLDs, each of distinct clonal composition arising in a single patient. Each of these PTLD lesions represents a distinct tumor clone, rather than multiple metastatic foci derived from a primary tumor clone as in the conventional theory of neoplastic spread. This phenomenon is in contrast to non-Hodgkin lymphomas in immunocompetent patients, which tend to be uniclonal, multifocal processes based on immunoglobulin and T cell receptor rearrangement studies.

It is thought that PTLDs develop via a multistep process in which lack of normal immune surveillance allows for the expansion of multiple EBV infected immortalized B cell clones. Therefore, it is possible for the expansion of more than one EBV-driven B cell proliferation to develop at any given time, which can account for the rapid development of large tumor burden seen in some patients with PTLD. Some studies have suggested that the presence of multiple clonally distinct PTLDs confers a worse prognosis as these lesions may not respond to standard therapy, possibly through the acquisition of diverse genetic alterations. These lesions may also account for the unpredictable clinical behavior of some polymorphic PTLDs, as previous reports have suggested that clonally distinct lesions are more likely to be classified as polymorphic and EBV positive with a latency III pattern, based on positive expression of EBER, LMP1 and EBNA2.

The presence of clonally distinct monoclonal PTLD lesions in individual patients is possibly under reported due to current standards of biopsy sampling practices. It is not common practice to sample multiple PTLD lesions, but rather a single biopsy is often taken to represent the systemic disease process. It is possible that patients that are found to have multiple clonally diverse lesions may potential benefit from earlier aggressive clinical intervention if clonal diversity is proven, as in this case.

Proposed Diagnosis:
Multiple synchronous primary EBV-driven polymorphic post-transplant lymphoproliferative disorders (PTLD) comprising of three independent clonal B cell expansions.

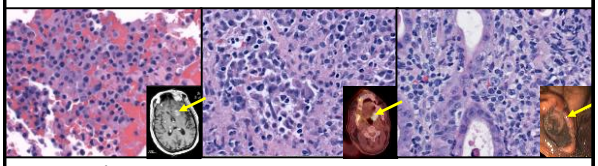
Multiple Synchronous Primary EBV-Associated Polymorphic Posttransplant Lymphoproliferative Disorders of Different Clonal Compositions Occurring in a Single Patient Following Bone Marrow Transplantation

Miguel D. Cantu, MD, Amy Chadburn, MD

Weill Cornell Medicine
Pathology & Laboratory Medicine

1

Synchronous Polymorphic Lesions, Different Anatomic Sites



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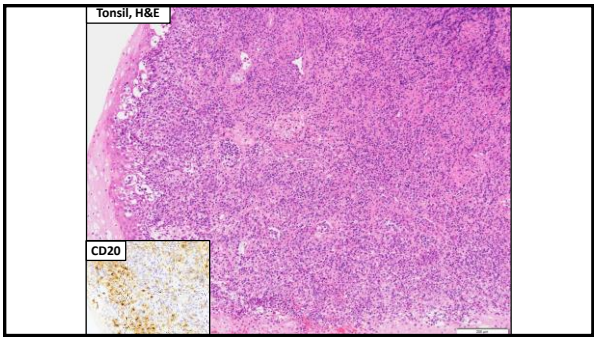

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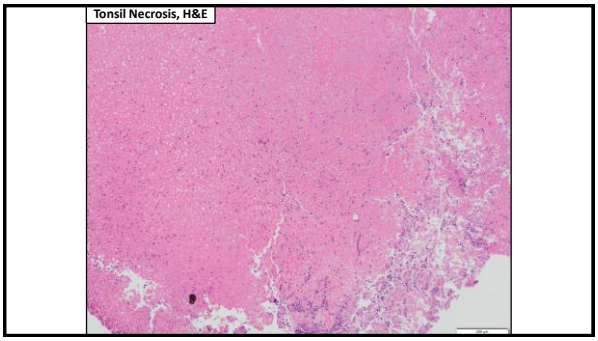
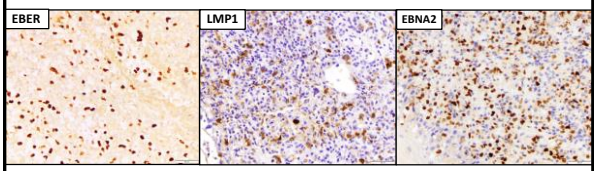


4

Tonsil Lesion EBV Latency



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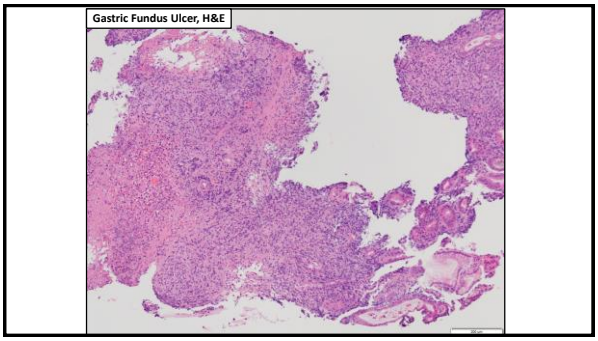

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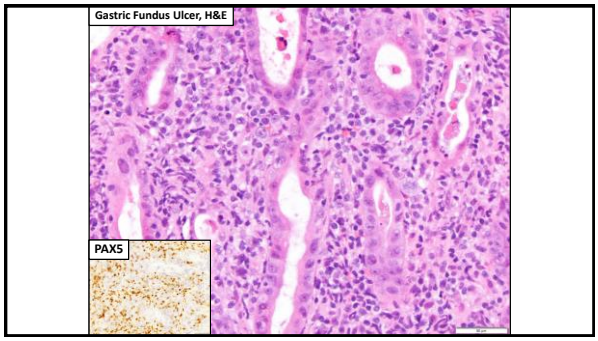
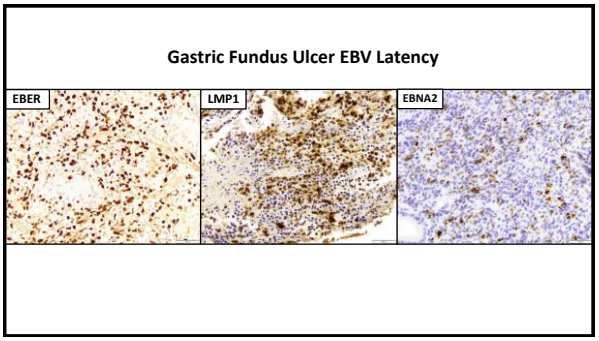
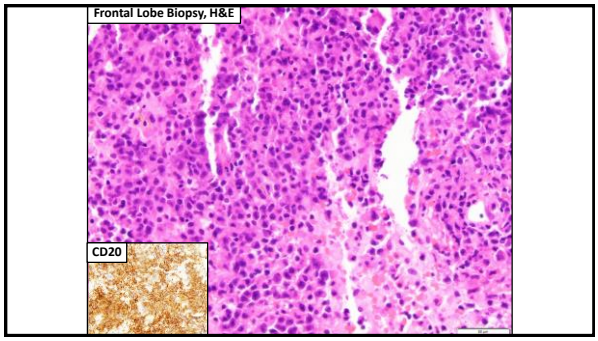

Gastric Fundus Ulcer, H&E
PAX5

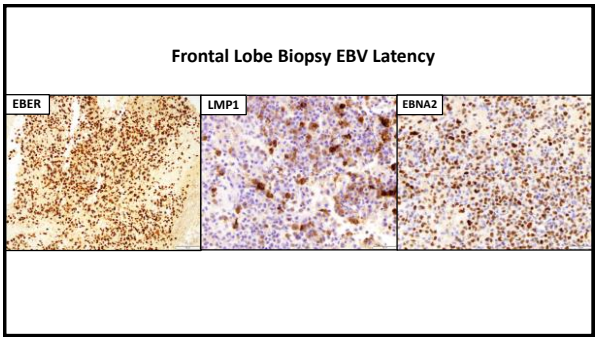
7


Gastric Fundus Ulcer EBV Latency
EBER
LMP1
EBNA2

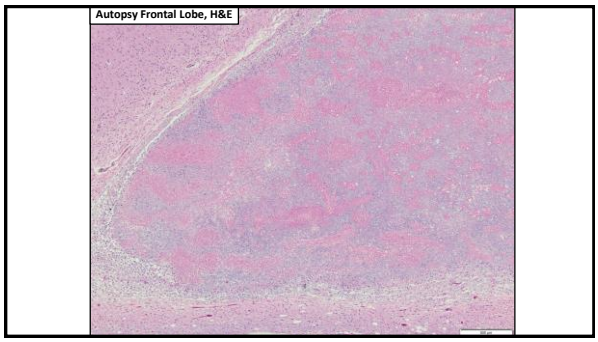
8


Frontal Lobe Biopsy, H&E
CD20

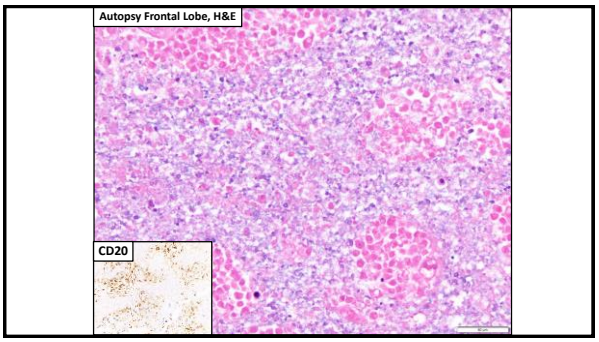
9


Frontal Lobe Biopsy EBV Latency
EBER
LMP1
EBNA2

10

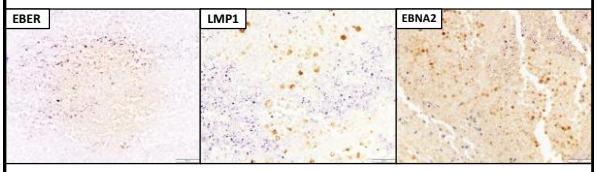

Autopsy Frontal Lobe, H&E

11


Autopsy Frontal Lobe, H&E
CD20

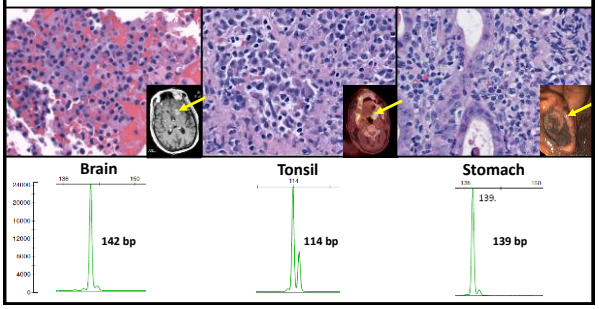
12

Autopsy Frontal Lobe Lesion EBV Latency



13

Clonally Diverse, Synchronous Polymorphic Lesions, Different Anatomic Sites



14

EBV-Positive Mucocutaneous Ulcer with Bone Invasion

Benjamin Kaumeyer, MD[1]
Shiraz Fidai, MD[2]
Girish Venkataraman, MD[3]

1. Pathology Resident, University of Chicago Department of Pathology
2. Hematopathology Fellow, University of Chicago Department of Pathology
3. Associate Professor, University of Chicago Department of Pathology

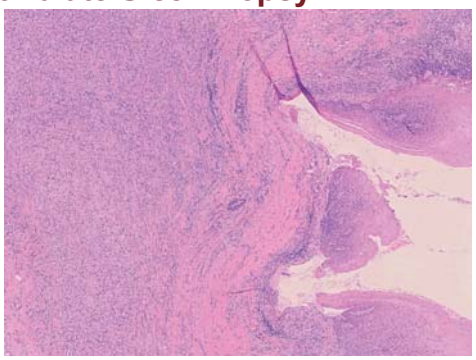
Clinical History

- 72 year old male with a past medical history of coronary artery disease, indolent prostate cancer, and CLL/SLL discovered on routine laboratory work-up (initial work up of CLL/SLL from 2017 shown below)
  - WBC: 11.9 *10^3/uL
  - Flow: 41% kappa restricted B-cells (CD19+, CD20+, CD23+, CD5+, CD10-, CD38+)
  - IGH translocation, trisomy for chromosome 12, chromosome 13 deletion
  - NOTCH1 p.Q2440* (NM_017617.5) mutation
  - Stable disease not requiring treatment

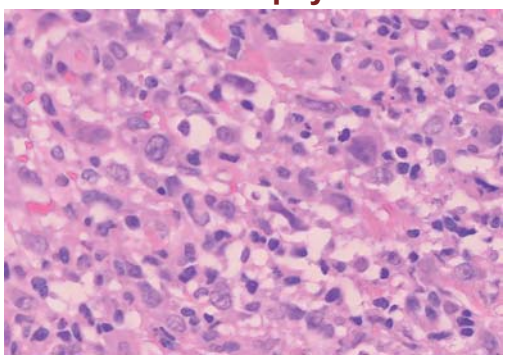
Clinical History

- Two years after the diagnosis of CLL/SLL, the patient developed a **painful oral ulceration of the left soft palate**
- The ulceration progressed over multiple months and a biopsy was performed

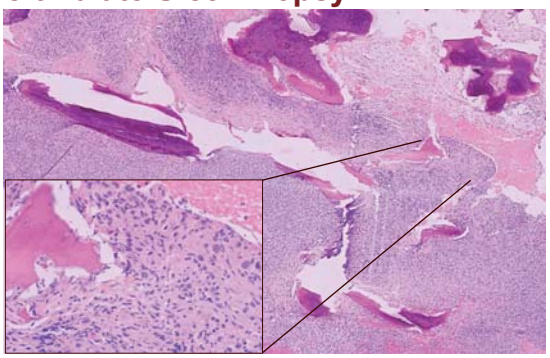
Left Palate Ulcer Biopsy



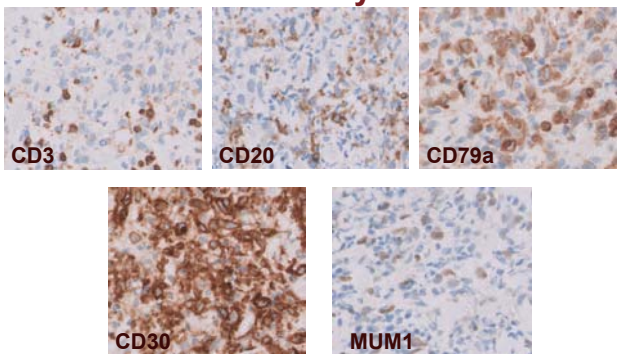
Left Palate Ulcer Biopsy



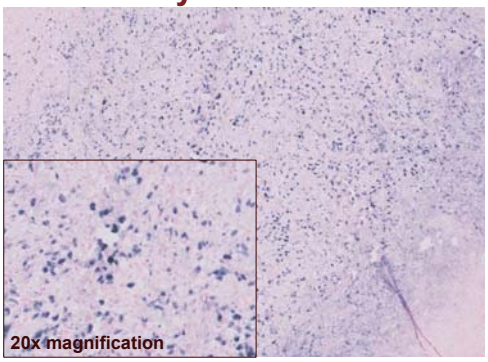
Left Palate Ulcer Biopsy



Immunohistochemistry



EBER in-situ hybridization



Summary of Biopsy Findings

- Ulceration of squamous mucosa with a polymorphic, dense lymphoid infiltrate composed of medium to large atypical lymphoid cells (including Hodgkin/Reed-Sternberg-like cells), and mature lymphocytes
- Lymphoid infiltrate invades through the submucosa into underlying bony trabeculae.
- Medium to large atypical lymphoid cells positive for CD79a, MUM1, CD30 and negative for CD3, CD5, and CD20
- EBER in-situ hybridization positive in full spectrum of lymphoid cells

EBV-Positive Mucocutaneous Ulcer

- Clinical presentation, morphologic findings, and EBV positive lymphocytes is consistent with **EBV-Positive Mucocutaneous Ulcer (WHO 2016)**[2]

EBV-Positive Mucocutaneous Ulcer

- Clinicopathological entity with mucosal (most often oral) or cutaneous ulceration and an outgrowth of EBV positive cells[2]
- Occurs in a variety of settings where there is decreased EBV surveillance, including old age, iatrogenic immunosuppression, solid organ transplant, and HIV[1,2]
- Indolent course with good prognosis[1,2]
  - Spontaneous remission is common and most cases respond to reduction of immunosuppression
  - Rituximab, radiation and chemotherapy have been successfully used in cases refractory to immunosuppression reduction
  - Rare cases have progressed to more aggressive disease[6]

EBV-Positive Mucocutaneous Ulcer

- Microscopic findings most commonly include[2]:
  - Polymorphic infiltrate with variable number of plasma cells, histiocytes, and eosinophils
  - Substantial number of large transformed cells resembling atypical immunoblasts or Hodgkin/Reed-Sternberg-like cells
  - Band of mature lymphocytes at the deep margin
- Large transformed immunoblasts and Hodgkin-like cells are B cells[2]
  - Most often express CD20 (weak to strong expression)
  - Pax5, OCT2, and often CD79a positive
  - IRF4/MUM1 positive
- **EBER positivity in range of cells** (from lymphocytes to Hodgkin-like cells) [1,2]

Follow up

- One month after oral biopsy, the patient underwent CLL/SLL restaging CT/PET scan and bone marrow biopsy
- Bone marrow biopsy:
  - Hypercellular bone marrow (70%) with small atypical B-cell infiltrate (~69%, CD19+, CD20+, CD5+, CD10-, EBER-) and no increase in blasts
  - Peripheral blood with marked atypical lymphocytosis
    - WBC: 22.1 10^3/uL; 67% lymphocytes
- EBV viral load (blood quantitative PCR): <1000 IU/mL

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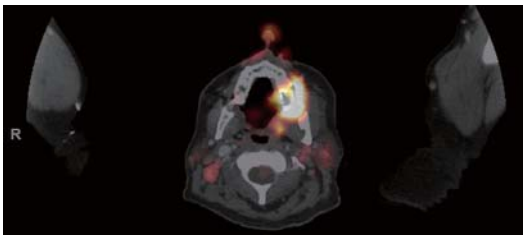
Post-Biopsy PET/CT scan



- Markedly hypermetabolic left maxillary lesion (shown above)
- Mildly hypermetabolic and bulky lymph nodes in the neck, mediastinum, mesentery, and retroperitoneum (images not shown)
  - Enlarged lymph nodes most likely represent CLL/SLL (SUV max 4.1)

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Follow up

- Patient started on ibrutinib for CLL/SLL while the oral ulcer will be monitored
- Plan to monitor lymphadenopathy with serially CT scans
- Ulcer had no change in size two months after starting ibrutinib

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Unique features of the case

- To the best of our knowledge, there have been no reported cases of EBV-positive mucocutaneous ulcer with bone invasion[1-6]
  - Bone invasion is worrisome for a more aggressive disease, nevertheless, the clinical presentation, morphology, immunohistochemical findings and EBV positivity are all most consistent with EBV positive mucocutaneous ulcer
- Development of EBV-positive mucocutaneous ulcer in setting of clinically worsening CLL/SLL
  - Possible that decreased immune function resulting from CLL/SLL contributed to the development of EBV-MCU
  - Additionally, age related immunosenesence may have contributed to disease development

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Case Summary

- 72 year old male with a history of well controlled CLL/SLL not requiring therapy developed an oral ulceration
- Biopsy of ulceration demonstrates dense, polymorphous infiltrate with large atypical cells and Hodgkin/Reed-Sternberg-like cells
  - Lesion focally invades bone
  - lymphoid cells are positive for EBER in-situ hybridization
- Oral ulceration most consistent with EBV Positive Mucocutaneous Ulcer
- Bone invasion has never been reported in this entity
- Patient was placed on ibrutinib for CLL/SLL and ulcer has remained stable after 2 months of treatment

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References

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4. Dojcinov S, Venkataraman G, et al. Age-related EBV-associated lymphoproliferative disorders in the Western population: a spectrum of reactive lymphoid hyperplasia and lymphoma. *Blood*. 2011;18:4726-4735.
5. Prieto-Torres L, Erana I, et al. The spectrum of EBV-Positive Mucocutaneous Ulcer: A Study of 9 Cases. *Am J Surg Pathol*. 2019; 43:201-210.
6. Moran N, Webster B, et al. Epstein Barr virus-positive mucocutaneous ulcer of the colon associated Hodgkin lymphoma in Crohn's disease. *World J Gastroenterol*. 2015; 21: 6072-6076.

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CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
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Clinical History:
On July 18th, 2017, a 31-year-old Chinese man was admitted to the Zhongshan Hospital for haematemesis and fever for 22 days. The patient presented intermittent haematemesis of fresh blood about 10 ml each time, 10 times a day. On June 27th the patient went to the Sixth People's Hospital of Shanghai for blood tests: red blood cell: 4.77X10^12/L; hemoglobin: 138g/L; platelet: 122X10^9/L; white blood cell: 5.3X10^9/L; upper abdomen contrast-enhanced computed tomography (CT) showed 1. Fatty liver. 2. Mild mesenteric panniculitis. 3. The stomach wall is slightly thicker. Gastroscopy reported diffuse gastric mucosal lesions, gastric Ca (Borrmann IV)? Gastric lymphoma? Gastroscopic biopsies showed: (Gastric Body) hyperplasia of lymphoid tissue, with local atypical hyperplasia. Immunohistochemistry results: CK (epithelial +), CK19 (epithelial +), CD3 (T lymphocyte +), CD20 (B lymphocyte +), MUM1 (plasma cell +), CD38 (plasma cell +), Ki67 (20 %+). In our hospital, the patient experienced 6 times of upper GI endoscopy from July, 2017 to January, 2019. The first GI endoscopy revealed an ulceroproliferative tumor extending from the cardia to the pylorus. Then this case presented an indolent course without any progression. The GI endoscopy revealed the lesion of stomach shrank each time. The most recent GI endoscopy showed no remarkable lesions in stomach. Pathological analysis of gastroscopic biopsies showed the same trend. Bone marrow aspiration and biopsy revealed no abnormalities.The patient's body weight dropped by about 4kg from the very beginning. The patient has no night sweats.

Biopsy Fixation Details:
10% formalin for 24 hours

Description of Clinical Image if Any:
PET/CT demonstrated: 1. Thickening of the stomach wall below cardia with elevated FDG uptake; 2. Enlargement of numerous lymph nodes (bilateral supraclavicular, bilateral hilar and tracheal esophageal groove) with elevated FDG uptake; 3. Probably mesenteric panniculitis.

Details of Microscopic Findings:
Histologic sections from the stomach showed prominent expansion of the mucosa by a diffuse, small-medium-sized lymphocytic infiltrate. The cells had an irregular nuclear contour, open chromatin. Mitosis was easily detectable. Lymphoepithelial lesion could be easily found.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
CK7(epithelium+),CD3(+),CD5(+),CD10(a few cells +),CD20(a few cells +),CD21(-),Bcl2(+),Bcl6(+),MUM-1(a few cells +),c-Myc(sporadic cells +),Cyclin-D1(sporadic cells +),HP(-),Ki-67(20%+),CD56(-),GranB(-),EBER(+)

Special Stains:
N/A

Cytogenetics:
Karyotype analysis: 46,XY[17]

Molecular Analysis:
基因重排检测结果：

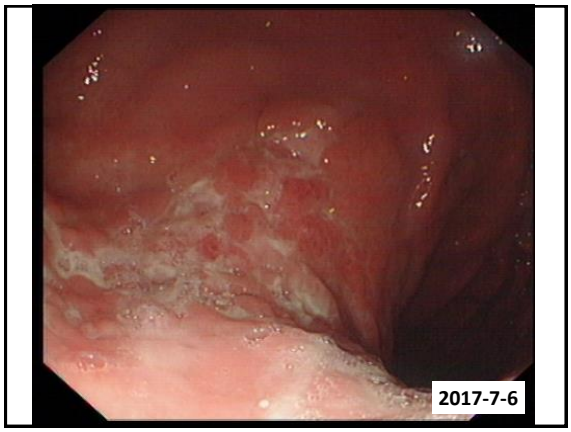
IG基因重排检测		1	2	3	4	5	6
	Master Mix	Control Gene Tube	IGH Tube A	IGH Tube B	IGH Tube C	IGK Tube A	IGK Tube B
	Target		FR1-JH	FR2-JH	FR3-JH	Vk-Jk	Vk-Kde+intron-Kde
	有效检测范围	101-200,300-395	310-360	250-295	110-170	120-160,190-210,260-300	210-250,270-300,350-390
		仅出现100,200,300bp	-	-	-	-	-
TCR基因重排检测		1	2	3	4	5	6
	Master Mix	Control Gene Tube	TCRB Tube A	TCRB Tube B	TCRB Tube C	TCRG Tube A	TCRG Tube B
	Target		VB-JB	VB-JB	DB-JB	Vγ1-Jγ、Vγ10-Jγ	Vγ9、Vγ11-Jγ
	有效检测范围	101-200,300-395	240-285	240-285	170-210,285-325	145-255	80-220
		仅出现100,200,300bp	+	-	+	+	-

备注：该样本Control管仅出现100,200,300bp条带，不排除300bp条带以后的假阴性现象。

Interesting Feature(s) of Submitted Case:
This case took an indolent clinical course without evidence of systemic dissemination. The lesion of stomach trended to be heal. To date, he is still followed at our institution, and he has remained in clinical remission for almost 2 years.

Proposed Diagnosis:
Gastric EBV-associated Peripheral T-Cell Lymphoproliferative Disorders (LPD)

Comments:



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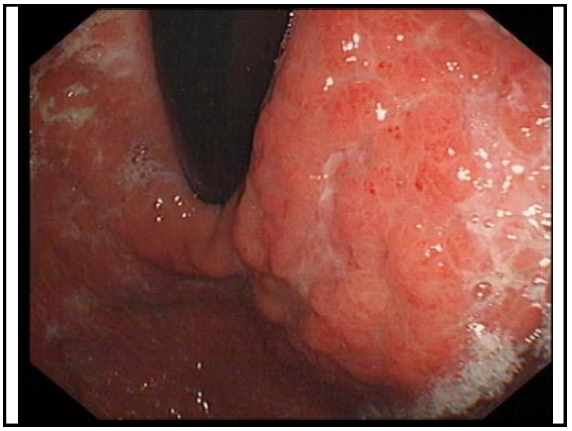

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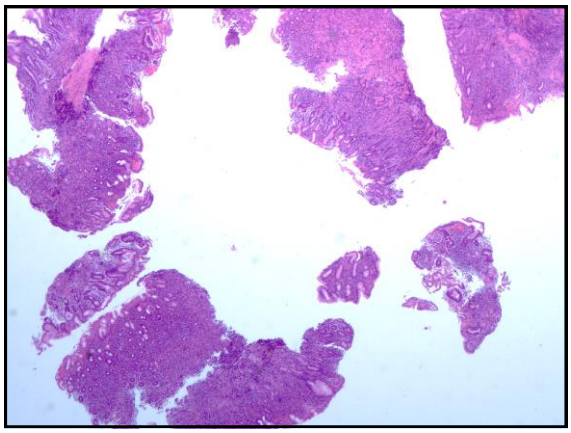

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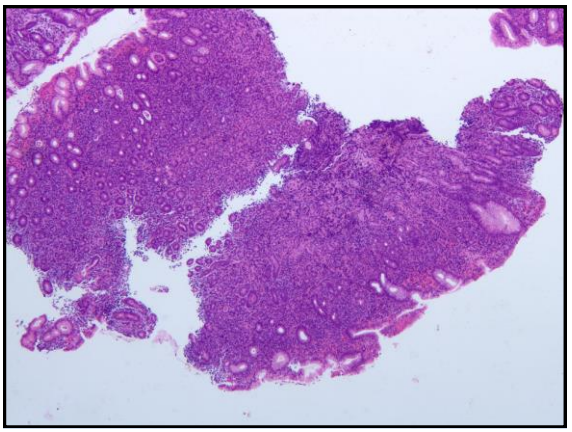

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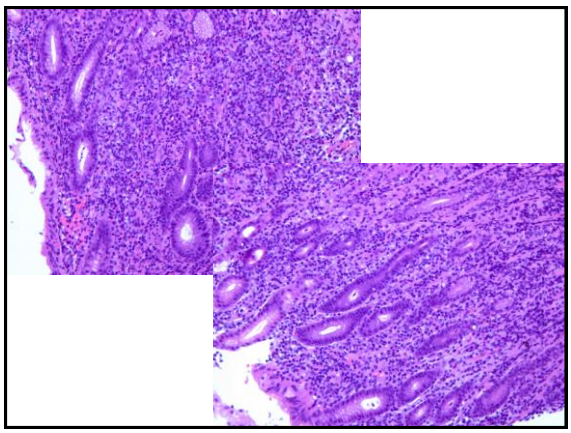

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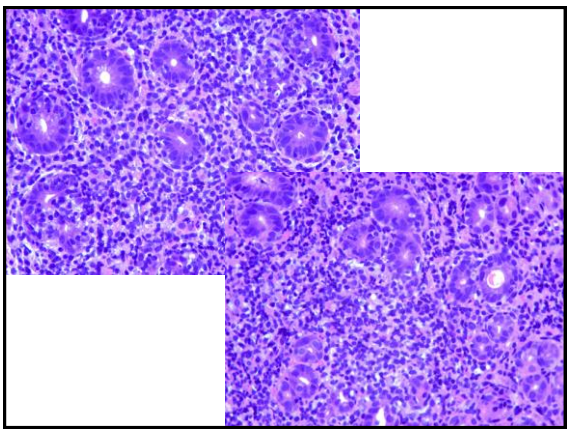

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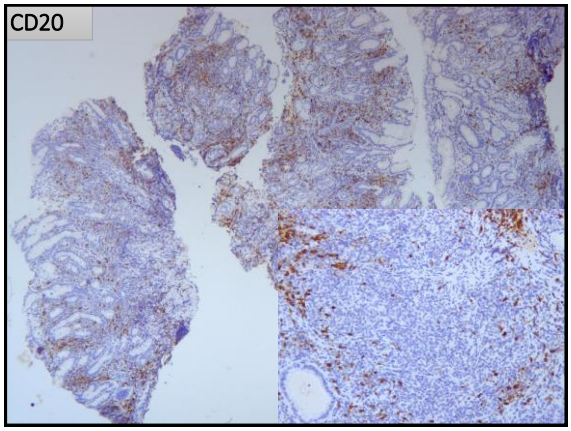

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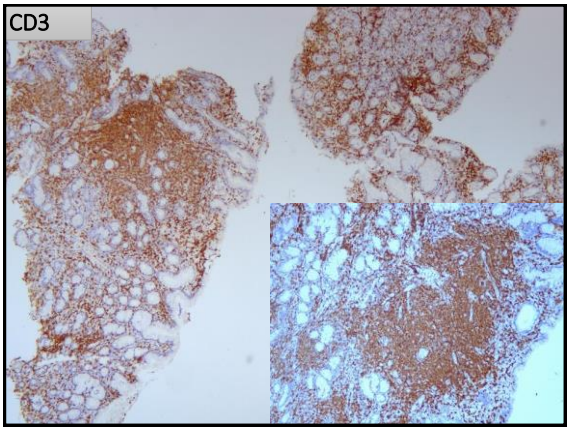

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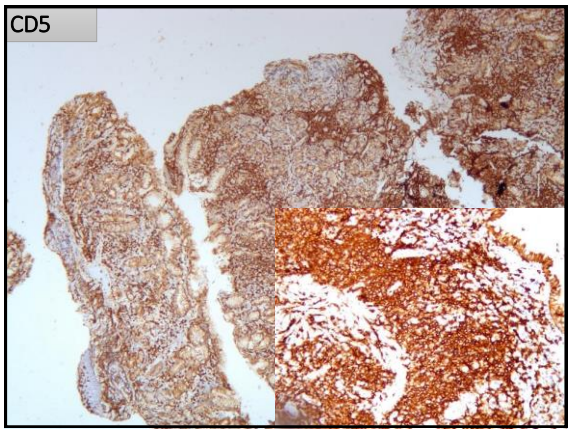

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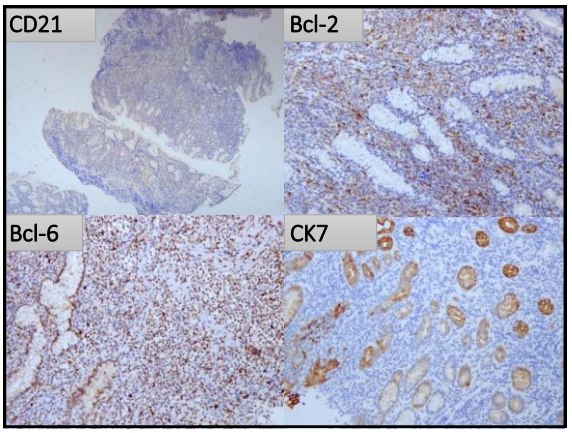

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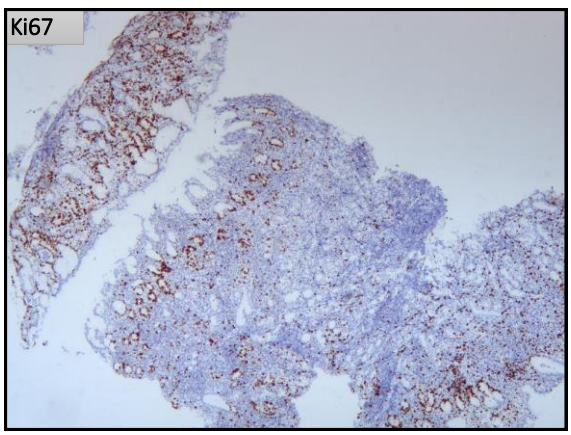

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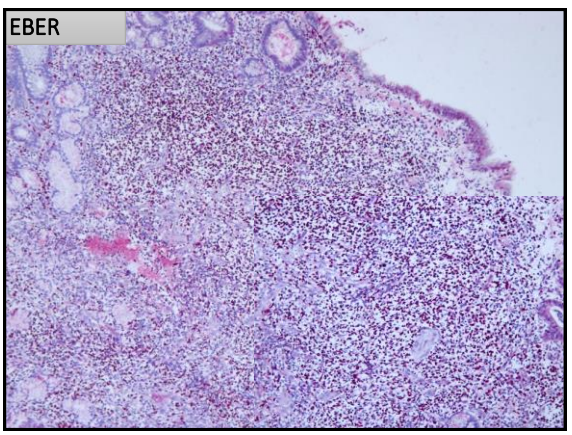

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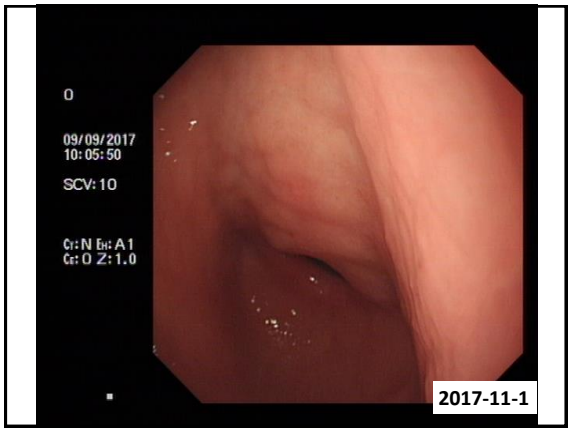

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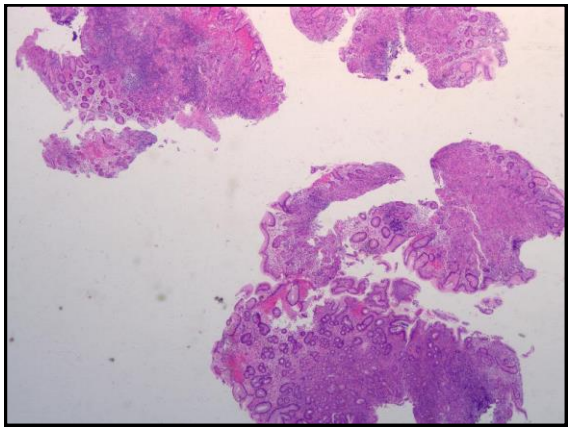

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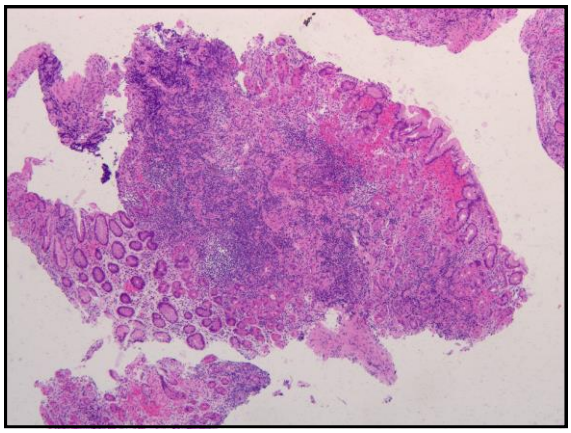

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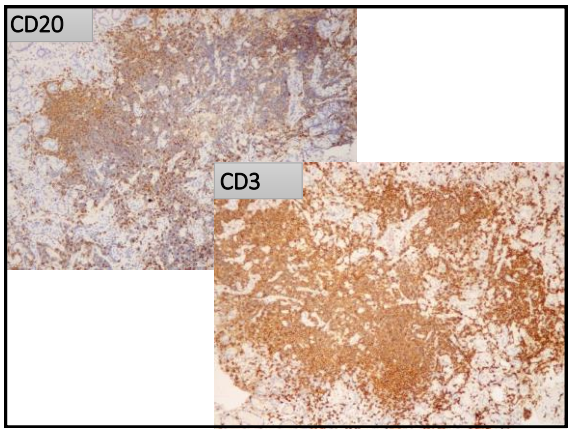

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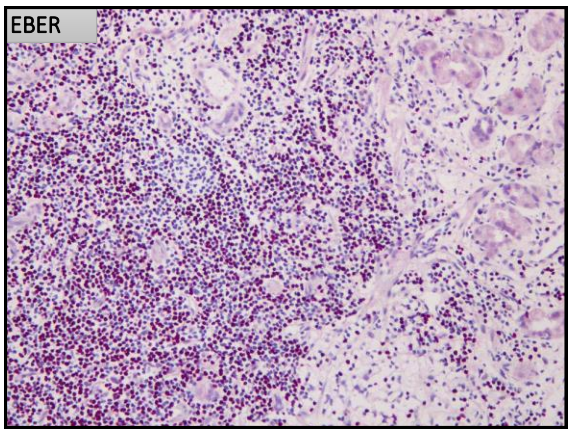

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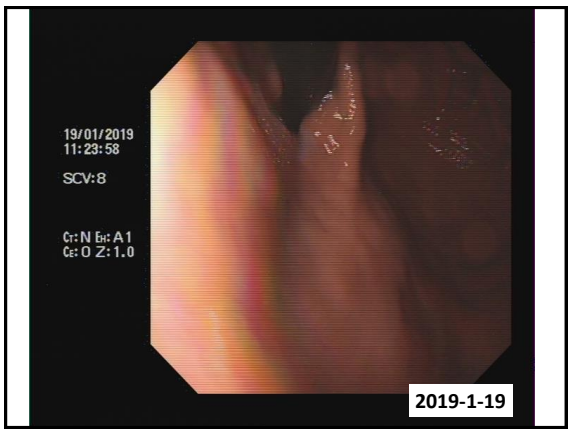

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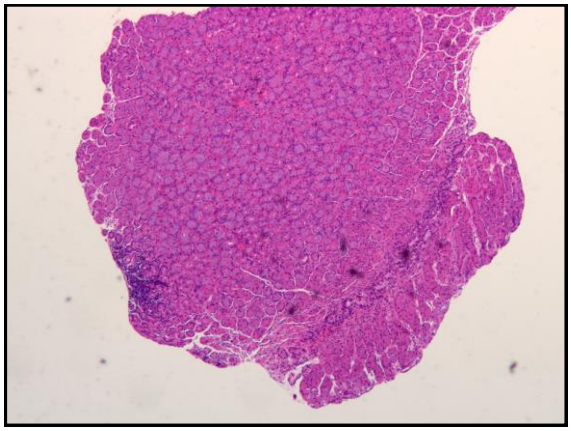

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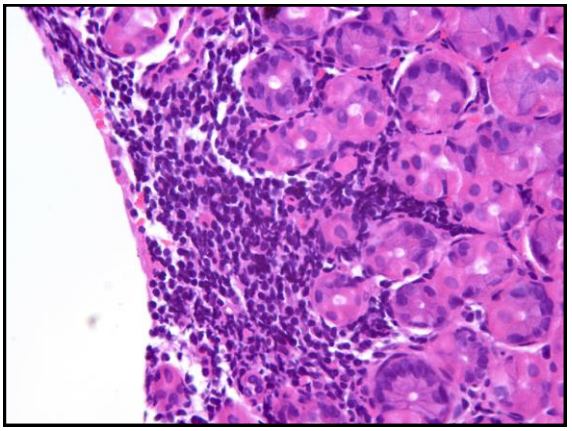

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CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Sha Zhao，MD PhD

Affiliation:
Department of Pathology, West China Hospital, Sichuan University, Chengdu, 610041, China

E-mail:
azhsh@126.com

Clinical History:
Male, 41 years old, complained of fever and sore-throat for one month, aggravated for 2 weeks. The patient had fever, sore-throat and dizziness after catching a cold one month ago. Temperature was high at night with night sweats. About 5kg weight was lost in one month. He was immunocompetent and had no history of other disease.

Lab examination

- Blood routine test： pancytopenia (Hg 105g/L, PLT $31x0^9$/L, WBC 0.90 $x10^9$/L);
- Coagulative examination: thrombin time 24.4 seconds, fibrinogen 0.84 g/L;
- Biochemical examination: alanine aminotransferase 385 IU/L, aspartate aminotransferase 480 IU/L, LDH 671 IU/L;
- EB virus DNA loading： $5.18x10^4$ copies/mL;
- Urine routine test: the occult blood 33(1+) cell/μL, qualitative urine protein 0.2 (+/-) g/L;

Treatment and prognosis
Clinical diagnosis: Hemophagocytic lymphohistiocytosis (HLH)

The patient had recurrent fever and sore-throat, persistent elevated EBV-DNA loads in PB and splenomegaly for more than one year. He had underwent chemotherapy(GLIDE) and symptomatic therapy for 6 times and died of the disease after 20 months of diagnosis.

Biopsy Fixation Details:
The cervical lymph node biopsy had been performed for diagnosis and it was formalin fixed and paraffin embedded.
Gross appearance: gray and brown with tender, the size was 1.0cmx0.8cmx0.4cm

Description of Clinical Image if Any:
CT:
(1) Multiple lymphadenopathy;
(2) Increased lung marking and a little patchy shadow in bilateral lung. Fibrous induration lesions had be considered;
(3) Splenomegaly with multiple low density lesions was shown;
(4) A small amount of pericardial effusion was detected.

Details of Microscopic Findings:
The lymph node architecture was preserved with paracortical expansion. Variable numbers of HRS-like cells were scattered in the complex background （small to medium-sized lymphocytes, histiocytes, and a few plasma cells）. Most HRS-like cells were large with irregular, single or lobulated nuclei, and moderate eosinophilic or amphophilic cytoplasm. The huge nuclei showed thick membranes, pale chromatin, and variably prominent eosinophilic nucleoli that often resembled a nuclear inclusion. Mummified cells were observed. Moreover, histiocytic hyperplasia in cluster or sheet often appeared even with erythrophagocytosis. No eosinophils and necrosis were observed.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

HRS-like cells
The HRS-like cells were positive for CD3p(partial), CD30, CD56（variable）, CD7, CD2, mum-1, TIA-1 and GranB and negative for LCA, CD20, PAX-5,CD5, CD4, CD8, CD15 and ALK-1. Index of Ki-67 was about 50%。

Small to medium-sized surrounding T-cells
Most of the surrounding cells were T-cells, which expressed CD3p, CD2, CD4, CD5, CD7 and CD8 as well as cytotoxic molecules (TIA-1 and/or GranB), and CD8+ T-cells outnumbered CD4+ T-cells,

EBV status
EBER-ISH was positive in all HRS-like cells and some surrounding T-cells(30%), but LMP-1 was negative.

Special Stains:
Not done

Cytogenetics:
Not done

Molecular Analysis:
TCRγ rearrangement: polyclonal
NGS(112 targeted gene): missense mutation of SETD2,STAT3,MAP2K1,NOTCH1

Interesting Feature(s) of Submitted Case:
1. The clinical manifestation of this case met the diagnostic criteria of CAEBV and experienced hemophagocytic lymphohistiocytosis
2. The morphologic characteristics of this case was scattered HRS-like cells surrounded by a mixture of small-sized lymphocytes, plasma cells, and macrophages, which masqueraded cHL. Beside, erythrophagocytosis was detected.

3. The IHC and ISH results demonstrated HRS-like cells were of NK-cell origin and EBER1/2 positive
4. The harbored mutations of this case involved in epigenetic modifiers, JAK-STAT signaling pathway, and notch pathway, which were similar to other EBV-associated T/NK-cell lymphoproliferative disorders.

Proposed Diagnosis:
Systemic chronic active Epstein-Barr virus infection of T/NK-cell type with NK-phenotype HRS-like cells

Comments:
Hodgkin/Reed-Sternberg (HRS) cells are large, abnormal, mononuclear, or multinuclear/multilobed cells that were described as hallmark cells in classical Hodgkin lymphoma (cHL) in many years ago. Nowadays, HRS-like cells have also been detected in a spectrum of lymphoproliferative disorders from B-cell malignancies to T-cell malignancies, and the cell origin is including B-cell lineage and T-cell lineage. Thus, the similar morphology and phenotype implies that HRS-like cells are not only a diagnostic clue but also a diagnostic pitfall.

This case has been identified as systemic chronic active Epstein-Barr virus infection of T/NK-cell type with NK-phenotype HRS-like cells according to the incorporation of clinical features, morphology, immunophenotype, and genetic findings, which broadens not only the spectrum of morphological variants of CAEBV-T/NK-S. but also the spectrum of cHL mimics. We should pay more attention to the significance of HRS-like cells in different disease and avoid misdiagnosis.

Systemic chronic active Epstein-Barr virus infection of T/NK-cell type with NK-phenotype HRS-like cells

Sha Zhao, Zihang Chen, Weiping Liu

Department of Pathology, West China Hospital, Sichuan University

2019-10, Xi'an

1

Clinical History

- Male, 41 years old, complained of fever and sore-throat for one month, aggravated for 2 weeks.

The patient had fever, sore-throat and dizziness after catching a cold one month ago. Temperature was high at night with night sweats. About 5kg weight was lost in one month .

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- **EB virus DNA load：** 5.18x10^4 copies/mL;
- **Urine routine test:** the occult blood 33(1+) cell/μL, qualitative urine protein 0.2 (+/-) g/L;

3

Imaging examination

CT

- Multiple lymphadenopathy
- Increased lung marking and a little patchy shadow in bilateral lung
- Splenomegaly with multiple low density lesions
- A small amount of pericardial effusion.

4

Treatment and prognosis

- Clinical diagnosis: Hemophagocytic lymphohistiocytosis (HLH)
- Recurrent fever and sore-throat, persistent elevated EBV-DNA loads in PB and splenomegaly for more than one year
- Underwent chemotherapy(GLIDE) and symptomatic therapy for 6 times
- Died of the disease after 20 months of diagnosis

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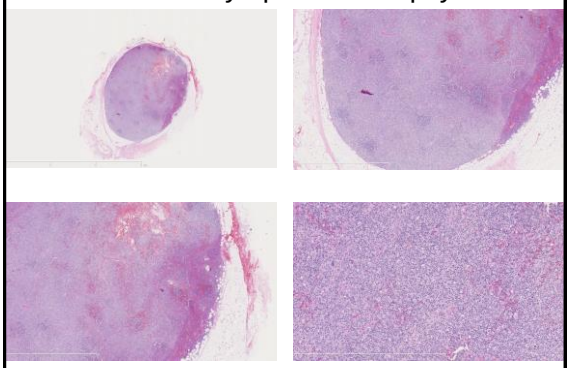
Cervical lymph node biopsy



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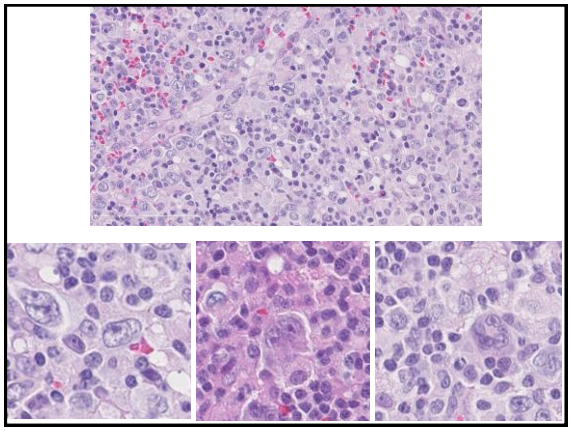

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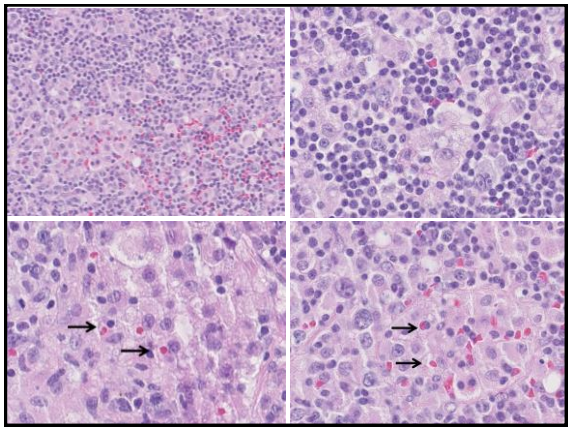

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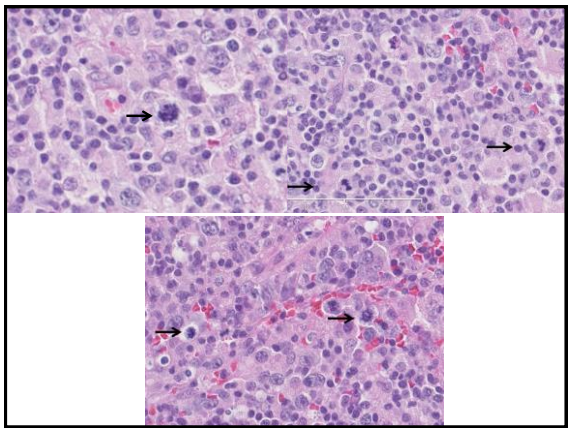

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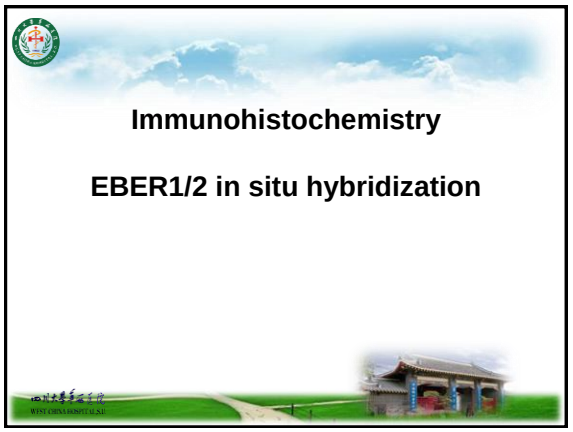
Immunohistochemistry
EBER1/2 in situ hybridization

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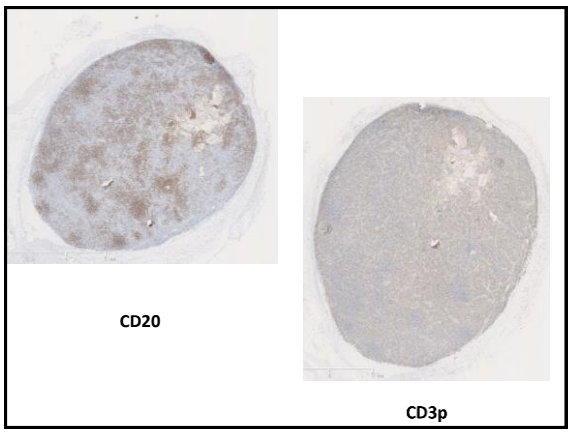
CD20
CD3p

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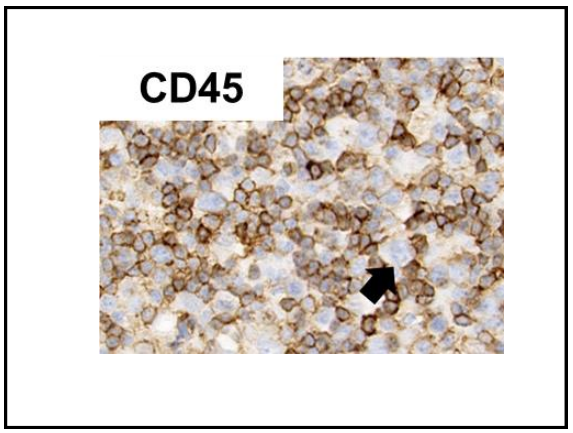
CD45

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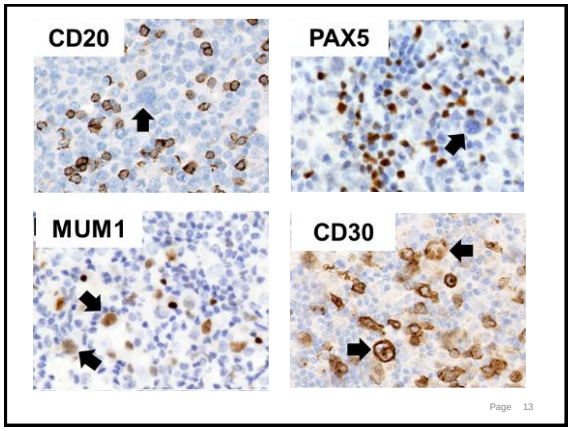

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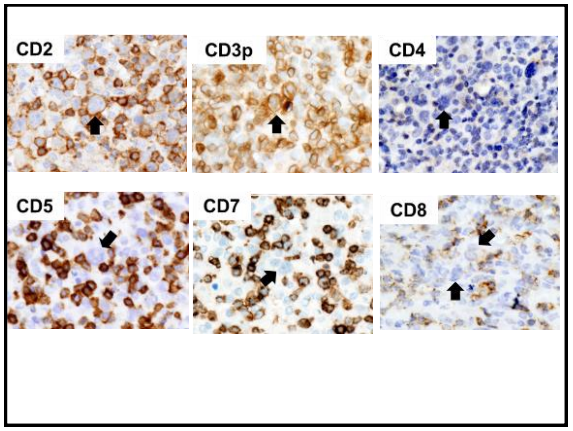

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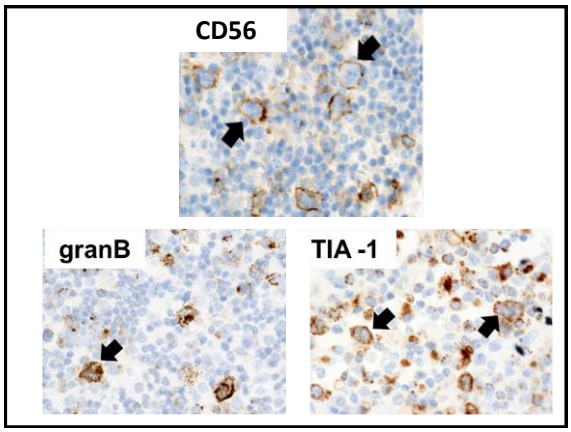

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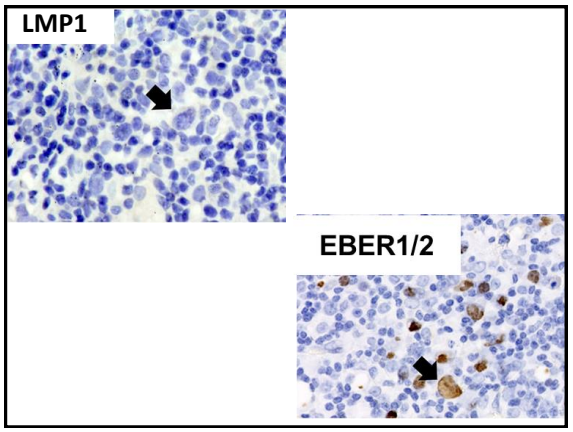

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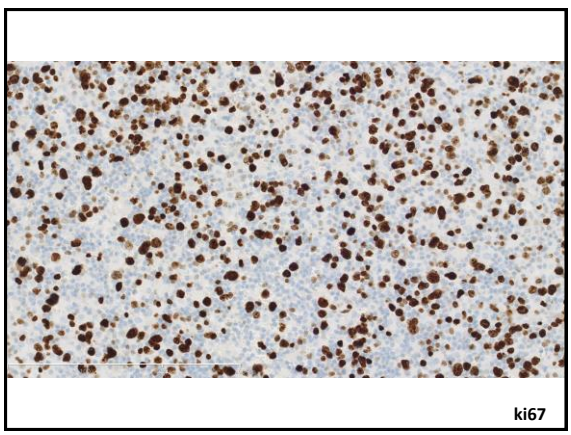

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HRS-like cells

- ✓ **Positive: CD3p(p), CD30, CD56（v）, CD2, mum-1, TIA-1, GranB**
- ✓ **Negative: LCA, CD20, PAX-5,CD5, CD7, CD4, CD8, CD15 and ALK-1.**

Small to medium-sized surrounding T-cells

- ✓ Most of the surrounding cells were T-cells
- ✓ Expressed CD3p, CD2, CD4, CD5, CD7 and CD8 as well as cytotoxic molecules (TIA-1 and/or GranB)
- ✓ CD8+ T-cells outnumbered CD4+ T-cells

EBV status

- ✓ EBER-ISH was positive in all HRS-like cells
- ✓ EBER-ISH was positive in some surrounding T-cells(30%)
- ✓ LMP-1 was negative.

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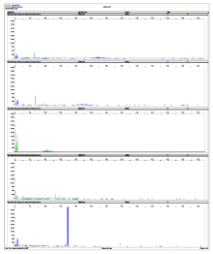
Molecular Analysis



polyclonal in TCRγ rearrangement

NGS(112 targeted gene)

Missense mutation

SETD2

STAT3

MAP2K1

NOTCH1

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Characteristics of this case

Clinical manifestation	Morphology	IH&ISH	Genetics
✓ Meeting the diagnostic criteria of CAEBV ✓ HLH	✓ Scattered HRS-like cells surrounded by a mixture of cells ✓ Erythrophago-cytosis	✓ HRS-like cells were of NK-cell origin ✓ EBER1/2(+)	Mutations involving in epigenetic modifiers, JAK-STAT signaling pathway, and notch pathway

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Pathologic diagnosis

Systemic chronic active Epstein-Barr virus infection of T/NK-cell type
with NK-phenotype HRS-like cells

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Conclusion

- HRS-like cells are not only a diagnostic clue but also a diagnostic pitfall. More attention should be paid to the significance of HRS-like cells in different disease and avoid misdiagnosis.
- The diagnosis of systemic CAEBV, T/NK-cell type with NK-phenotype HRS-like cells was based on the incorporation of clinical features, morphology, immunophenotype, and genetic findings.
  - ✓ broadening the spectrum of morphological variants of CAEBV-T/NK-S
  - ✓ broadening the spectrum of cHL mimics.

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CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Sarah Ondrejka, DO

Affiliation: Cleveland Clinic

E-mail: ondrejs@ccf.org

Clinical History: The patient is a 59-year-old female with an isolated right neck mass. She is otherwise healthy, without any defined source(s) of immunosuppression and without B symptoms.

Biopsy Fixation Details: 10% neutral buffered formalin

Description of Clinical Image if Any: None

Details of Microscopic Findings: The lymph node architecture is distorted by an abnormal interfollicular expansion composed of polymorphic plasmacytic cells ranging from small plasma cell forms to immunoblasts, scattered histiocytes, and increased mitotic activity. There are scattered plasmablasts and no definite Reed-Sternberg-like cells. The residual follicles are inconspicuous, with hyperplastic germinal centers that frequently lack mantle zones. Sinuses are compressed.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry: Flow cytometry does not identify a monotypic B-cell population or abnormal T-cell population.

Immunohistochemistry demonstrates CD20 positive B-cell follicles with slightly increased numbers of interfollicular B-cells, including some larger forms. There are numerous CD3 positive T-cells with a normal CD4:CD8 ratio and no loss of CD7. Kappa and lambda are positive in the CD138- and CD79a-plasmacytic cells and demonstrate a polytypic expression pattern. CD30 is positive in increased numbers of immunoblasts. The germinal centers are positive for CD10 and BCL6 and are associated with CD21 positive follicular dendritic cell meshworks, which are not significantly

disrupted. The Ki-67 proliferative index in the interfollicular area is moderate (50-60%). HHV8 and ALK1 are negative.
EBV by in situ hybridization is positive in a moderately high number of variably sized cells in the interfollicular area.

Special Stains: None

Cytogenetics: None

Molecular Analysis: A clonal rearrangement is not detected by PCR using primer sets targeting the immunoglobulin heavy chain (IGH) and light chain (IGK) loci. A clonal rearrangement is not detected by PCR using primer sets targeting the T-cell receptor beta (TCRB) or gamma (TCRG) chain loci.

Interesting Feature(s) of Submitted Case: This is an EBV+ polymorphic B-cell lymphoproliferation occurring in a healthy patient without a defined source of immunosuppression such as post-transplantation, HIV, or immunomodulatory/biologic medication. She lacks symptoms of lymphoma and symptoms of active EBV infection. Her monospot test is negative and quantitative EBV is less than 2.7 copies per microgram.

This proliferation lacks diagnostic features of lymphoma but does not easily fall into a typical pattern of reactive or infectious mononucleosis lymphadenitis. It has features of a polymorphic lymphoproliferative disorder that can be seen in immunosuppressed patients due to transplant or other causes. The presence of architectural effacement by this proliferation containing a full range of B-cell maturation stages and high mitotic index allows consideration as a "polymorphic B-cell lymphoproliferative disorder or polymorphic PTLD-like lesion" as discussed by Natkunam Y. et al, in their manuscript "EBV-positive B-cell proliferations of varied malignant potential" that followed the 2015 SH/EAHP workshop. It is similar to the EBV-associated polymorphic nodal cases described by Dojcinov SD. et al, with architectural effacement by an infiltrate reminiscient of polymorphic PTLD with inconspicuous follicles and cytologic atypia. This case is unique in that it lacks the Hodgkin/Reed-Sternberg-like cells that are often a feature of this proliferation, but rather contains a higher proportion of plasmablasts.

Of the 30 polymorphic B-cell lymphoproliferative disorders submitted to the 2015 SH/EAHP workshop, all but four occurred in patients with defined sources of immunosuppression. The four cases were presumably due to lapses in immune repertoire due to aging (ages 64-90 years). One case had

a clonal B-cell receptor gene rearrangement by PCR. Follow-up of those four cases was either unknown (1/4) or alive with no evidence of disease (3/4). The EBV-associated polymorphic nodal cases described by Dojcinov et al. had a higher rate of clonal immunoglobulin gene rearrangements (63%), variable treatment regimens and highly variable outcomes ranging from spontaneous resolution of lymphadenopathy without treatment, to clinical progression despite treatment.

Proposed Diagnosis: Polymorphic B-cell proliferation, EBV+, related to immune senescence

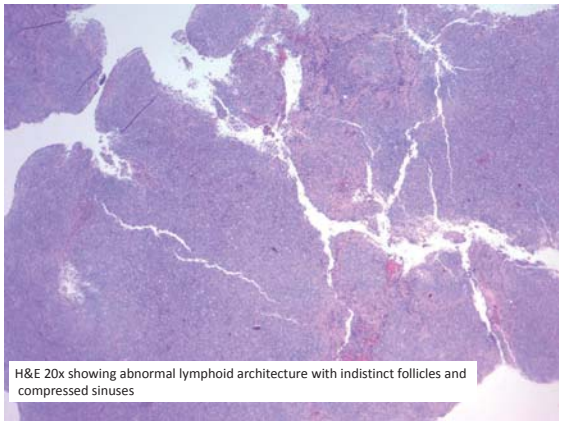
Comments: This patient had neck/chest/abdominal/pelvis tomography scans which showed lymphadenopathy restricted to the cervical lymph nodes. She was followed for 5 months without treatment and has showed no signs of clinical progression.

Natkunam Y, Goodlad JR, Chadburn A. et al. Am J Clin Pathol 2017;147:129-152.

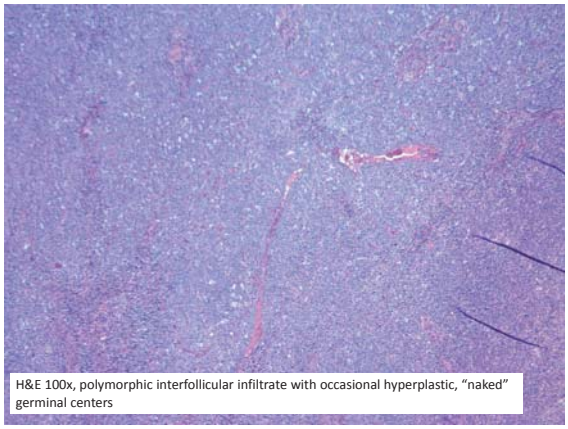
Dojcinov SD, Venkataraman G, Pittaluga S. et al. Blood 2011;117(18):4726-4735.

CSHP case

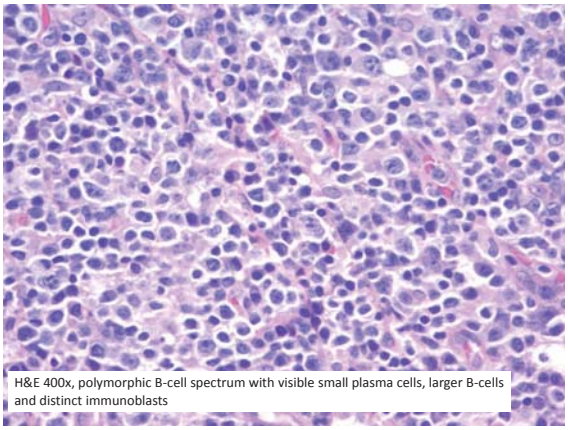
Submitter: Ondrejka, SL, Cleveland Clinic



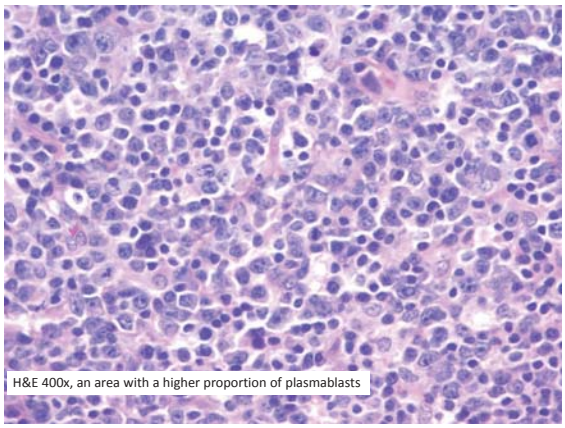
H&E 20x showing abnormal lymphoid architecture with indistinct follicles and compressed sinuses



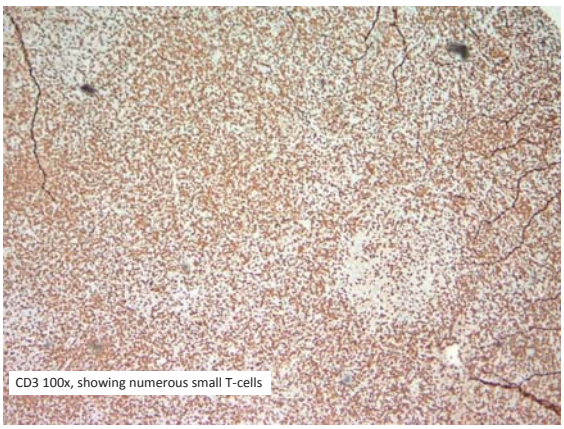
H&E 100x, polymorphic interfollicular infiltrate with occasional hyperplastic, "naked" germinal centers



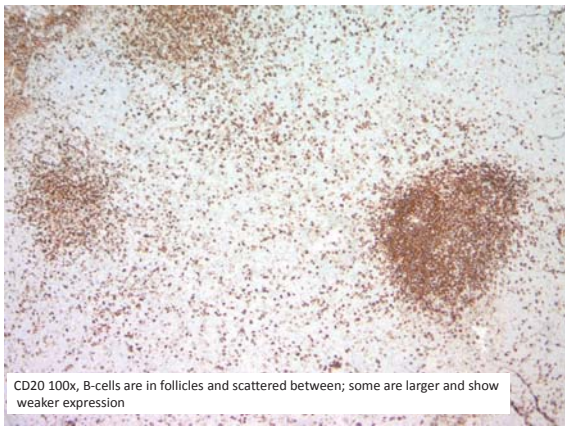
H&E 400x, polymorphic B-cell spectrum with visible small plasma cells, larger B-cells and distinct immunoblasts



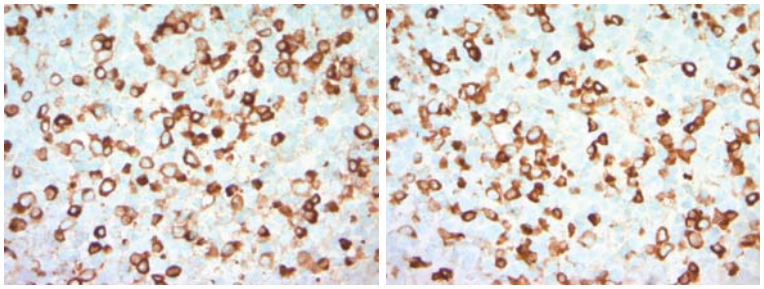
H&E 400x, an area with a higher proportion of plasmablasts



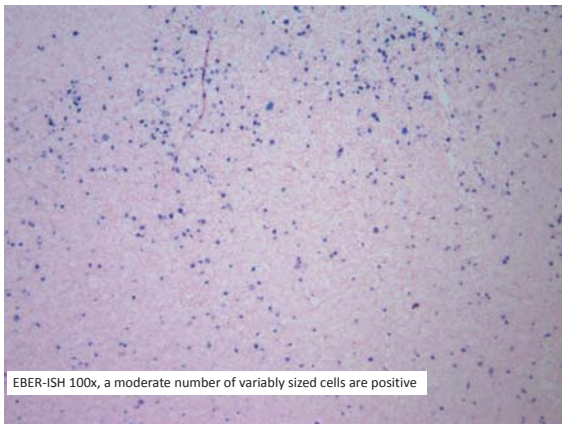
CD3 100x, showing numerous small T-cells



CD20 100x, B-cells are in follicles and scattered between; some are larger and show weaker expression



Kappa (left) and lambda (right), 400x, showing polytypic light chain expression



EBER-ISH 100x, a moderate number of variably sized cells are positive

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Huan-Ge Li M.D., Xiang-Nan Jiang M.D., Xiao-Qiu Li M.D.,PhD.

Affiliation:
Department of Pathology, Fudan University Shanghai Cancer Center, Shanghai 200032, China

E-mail:
lesleyjiang29@163.com

Clinical History:
The patient, a 18-year-old girl, presented with a left palatine ulcer

Biopsy Fixation Details:
None

Description of Clinical Image if Any:

Left palatine ulcer measuring1.0 cm in diameter

Details of Microscopic Findings:
Intraepidermal spongiotic vesiculation, infiltrates of small to medium-sized atypical lymphoid cells, distributed mainly in dermis

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
CD20, PAX5, CD79a, Oct-2, CD3, CD4, CD7, CD8, CD56, TIA-1, GrB, CD30, ALK1, EMA, LMP1,, Ki-67

Special Stains:
EBER in situ hybridization

Cytogenetics:
None

Molecular Analysis:
TCR IG rearrangement

Interesting Feature(s) of Submitted Case:
Rare special type of Infectious agent-association lymphoproliferation with unusual clinical, morphologic, immunophenotypic, or genetic features

Proposed Diagnosis:
Chronic active EBV infection (CAEBV) / hydroa vacciniforme -like lymphoproliferative disorder

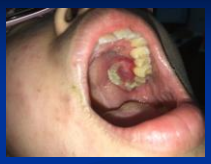
Comments:
Not all CD30+ T-cell LPDs are ALCL or primary cutaneous (mucosal) CD30+ T-cell LPD， we should always keep EBV-associated T- or B-cell LPDs in mind, particularly to those cutaneous (mucosal) lesions arising in an immunocompromised setting.

Chronic active EBV infection (CAEBV) / hydroa vacciniforme -like lymphoproliferative disorder

Huan-Ge Li, M.D., Xiang-Nan Jiang, M.D., Xiao-Qiu Li, M.D., Ph.D.
Fudan University Shanghai Cancer Center

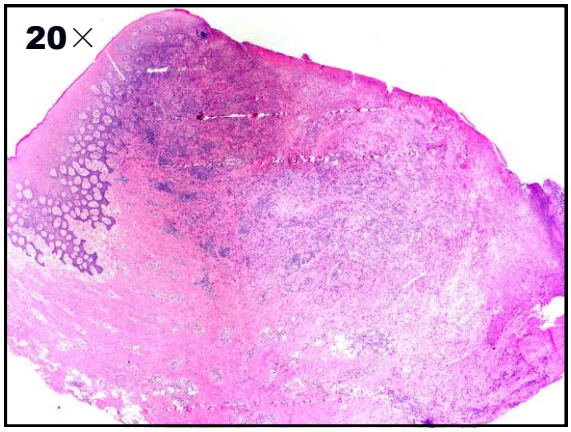
1

Clinical information



- The patient, a 18-year-old girl, presented with a left palatine ulcer measuring 1.0 cm in diameter
- She has no history of autoimmune disease or immunosuppressive usage
- Biopsy of the lesion revealed a diagnosis of “primary mucosal CD30+ T-cell lymphoproliferative disorder of the head and neck” by the referring pathologist

2



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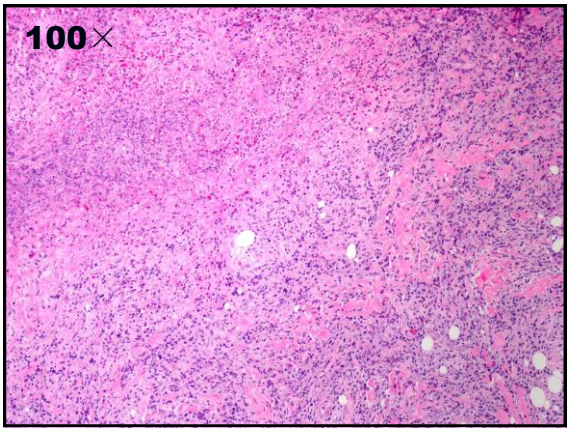

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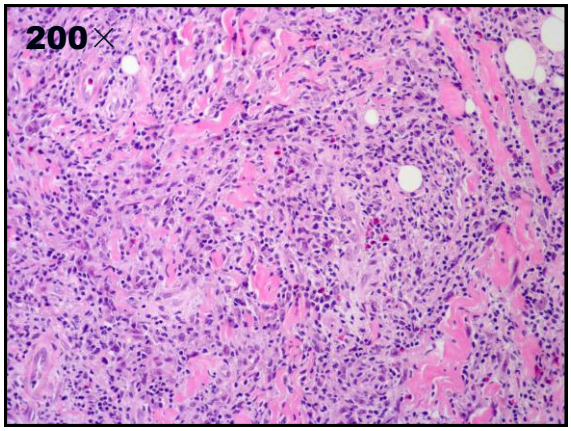

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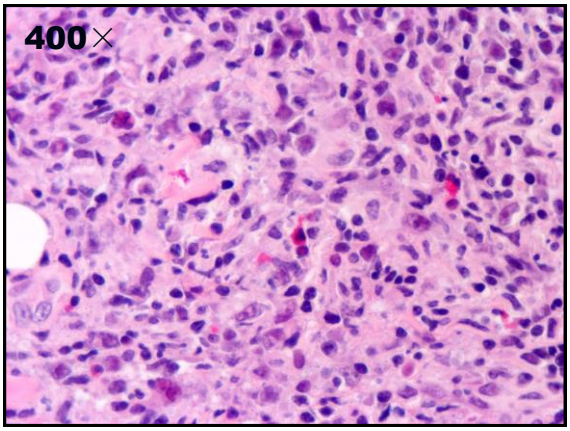

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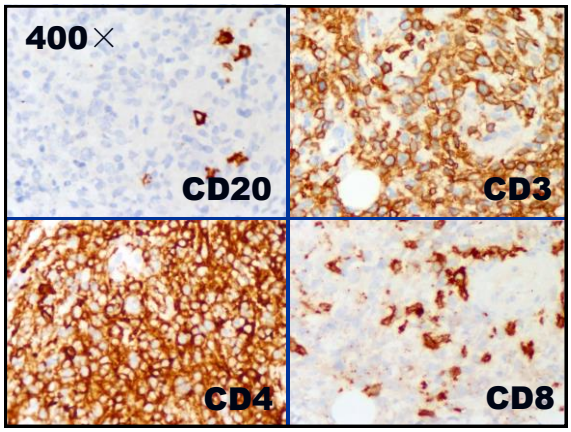

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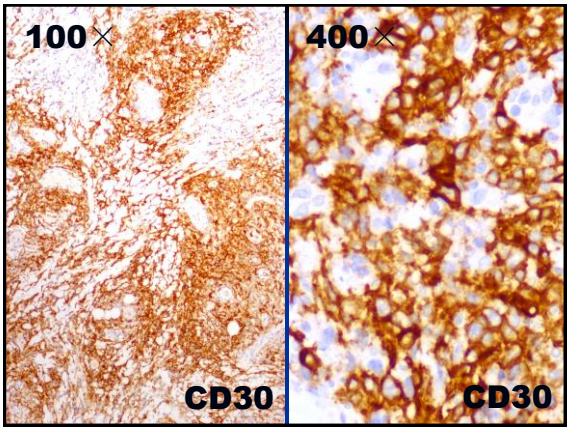

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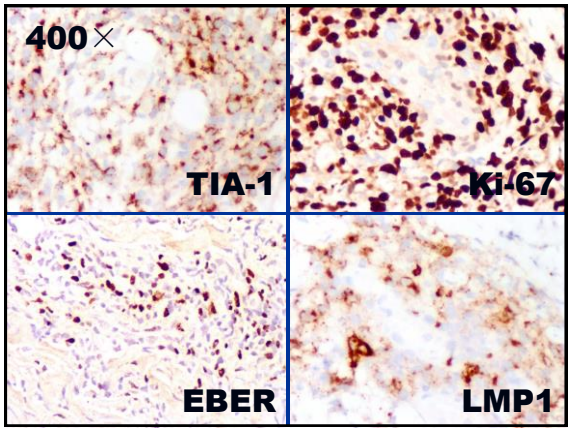

9

Phenotypic findings

- **Atypical lymphoid cells**
  CD20-, PAX5-, CD79a-, Oct-2-, CD3+, CD4+, CD7-/+, CD8-, CD56-, TIA-1+, GrB+, CD30+, ALK1-, EMA-, CD15-, LMP1+, EBER+, Ki-67+ (70%)

10

Clonality analysis

- ***TCRG*, *TCRB*, and *TCRD* genes were clonally rearranged**
- **No clonal rearrangements of *IGH*, *IGK*, or *IGL* genes were detected**

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Your diagnosis ?

A. Primary mucosal CD30+ T-cell lymphoproliferative disorder of the head and neck

B. Chronic active EBV infection (CAEBV)/hydroa vacciniforme-like lymphoproliferative disorder

C. Extranodal NK/T-cell lymphoma, nasal type

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Revised criteria for chronic active EBV infection (CAEBV)

- Chronic EBV infection of B-, T- or NK cells
  - Primarily associated with T-cells and less often NK-cells
  - Most common in pediatric population in Asia and Latin America and rare in Caucasians and African Americans
- Clinically presents with IM-like symptoms, skin rash, or other manifestations (HPS, DIC, hepatic failure, gastric perforation, CNS complications, myocarditis and interstitial pneumonitis)
- IM-like symptoms persisting for > 3 months
- Increased EBV DNA in peripheral blood (> $10^{2.5}$ copies/mg)
- Demonstration of EBV RNA or viral protein in affected tissues

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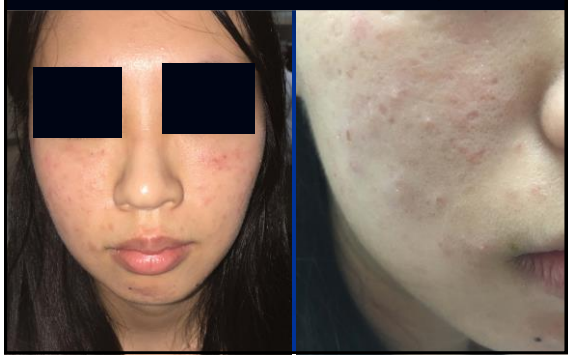
Hydroa vacciniforme-like LPD

- Mainly affects children and adolescents from Asia and in Native Americans from Central and South America
- Be associated or not associated with light hypersensitivity
- Skin lesions (oedema and erythema → papulovesicular eruption → ulceration → scarring) predominantly in sun-exposed and non-exposed areas, ± systemic symptoms
- Intraepidermal spongiotic vesiculation, infiltrates of small to medium-sized atypical lymphoid cells, distributed mainly in dermis (often show a periadnexal growth pattern), but may involve subcutis and epidermis as well; angioinvasion/ulceration commonly seen
- Mainly CD8+ T-cell and rarely NK-cell phenotype; monoclonal or polyclonal; express EBER, CCR4 and CD30
- A continuous spectrum of disease (classic HV, severe HV, and HV-like lymphoma) and variable clinical course (recurrent skin lesions up to 10 -15 yrs, progression to systemic disease with much more aggressiveness may occur)

14

The current patient had skin lesions characteristic of HVLLPD, too



15

Pathological diagnosis

Left palate:

Chronic active EBV infection (CAEBV) / hydroa vacciniforme -like lymphoproliferative disorder

16

Take home message

- Not all CD30+ T-cell LPDs are ALCL or primary cutaneous (mucosal) CD30+ T-cell LPD
- Always keep EBV-associated T- or B-cell LPDs in mind, particularly to those cutaneous (mucosal) lesions arising in an immunocompromised setting
- Clinicopathological features and biological nature of EBV+ T/NK-cell LPDs remain to be better defined

17

18

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Yang Yang , MD

Affiliation:
Department of Hematology, Tongji Hospital, Tongji Medical College,
Huazhong University of Science and Technology

E-mail:
eliteyoung@126.com

Clinical History:
The 51-year-old Han Chinese male patient was admitted to our hospital due to symptoms of fatigue and recurrent high-grade fever (> 39 °C) with a 4-month duration. One month ago, the patient developed bilateral low back pain symptom, then went to the township hospital, it was diagnosed as urinary infection, received treatment of anti-infection for 14 days, and the disease situation was not under control. When he went to our hospital, he was noted to have Epstein Barr virus (EBV) infection, plasma EBV-DNA 5.42×10^3copies/L, EBV-DNA in peripheral blood mononuclear cells (PBMCs) of more than 1×10^7 copies/L. First, we determined the haemophagocytic lymphohistiocytosis related laboratory indexes such as whole blood cell count， fibrinogen，ferritin, and soluble interleukin-2 receptor (sIL-2R). All the indexes were within normal range. His bone marrow cytology identified few abnormal lymphocytes with cytologic atypia **(PPT, slide 1)**. Bone marrow biopsy had no positive finding **(PPT, slide 2).** Flow cytometry showed that the T/NK/B lymphocytes were polyclonal **(PPT, slide 3)**. Peripheral blood cell sorting and EBV-FISH suggested predominant EBV infection with CD3+ T lymphocytes **(PPT, slide 4).** NK cell killing activity decreased to 8.50% (normally ⩾15.11%), and the expression levels of activated CD107a (for assessing NK cell degranulation) decreased to 27.82% (normally ⩾ 40%). Then PET-CT scan present that bilateral renal parenchyma showed high metabolic imaging features, the SUV-max was 8.3. Other organs and lymph nodes didn't show enlargement or abnormal intense high metabolism **(PPT, slide 5 and 6)**. So next, percutaneous renal biopsy with gun-biopsy under the guidance of ultrasound were performed. The pathologic diagnosis was EBV-associated T/NK-LPDs **(PPT, slide 7 and 8)**. Next Generation Sequencing demonstrated the presence of novel heterozygous UNC13D mutation (c.1228A>C) **(PPT, slide 9 and 10)**.

Following diagnosis, the patient was treated with P-GEMOX regimen (pegaspargase, 2500 IU/m^2 intramuscularly, on day 1; gemcitabine, 1000 mg/m^2 intravenously, on days 1 and 8; oxaliplatin, 130 mg/m^2 intravenously, on day 1). After two cycles of chemotherapy, the fever of this patient had been well-

controlled. To prevent the resistant to chemotherapy, we switched his therapy to the MEDA regimen(methotrexate, 3.0g/m^2/d intravenously, on day 1; etoposide, 100mg/m^2 intravenously, on day 2 to day 4;dexamethasone, 40mg/d intravenously, day 1 to day 4;pegaspargase, 2500IU/m^2/d intramuscularly, on day 4) for one cycle. One month after the third cycle treatment, we evaluated the curative effect using PET-CT again. PET-CT scan showed that the original high metabolic imaging of bilateral renal parenchyma disappeared **(PPT, slide 11 and 12)**. As the hematopoietic stem cell transplantation has been introduced as a curative therapy, the patient was persuaded to receive treatment of sequential allo-HSCT. But unfortunately, the patient and his family refused to accept it, and refused further chemotherapy. The unregular follow-up was performed. Amazingly, the patient has remained alive with disease without aggressive treatment.

Biopsy Fixation Details:

Under the microscope of renal puncture tissue, the skin and medulla of the kidney were clearly demarcated, and infiltrating lesions with lymphoid cells scattered in the renal cortex. Some lymphoid cells were moderately heterotypic, with some eosinophilic granulocytes. Glomerular fibrosis was occasionally seen.

Description of Clinical Image if Any:
PET-CT scan present that bilateral renal parenchyma showed high metabolic imaging features; the SUV-max was 8.3. Other organs and lymph nodes didn't show enlargement or abnormal intense high metabolism

Details of Microscopic Findings:
-.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
CD2,CD3,CD5,CD43 diffuse+; Ki-67（LI 5%）; CD7，CD4 strongly+；CD8,TIA-1 scattered+；CD20，CD79，PAX-5 few+；MPO-, CD56-, GrB-.

Special Stains:
Scattered T lymphocytes are EBER positive (EBER CISH+).

Cytogenetics:
No abnormal alteration found

Molecular Analysis:
Heterozygous UNC13D mutation (c.1228A>C)

Interesting Feature(s) of Submitted Case:

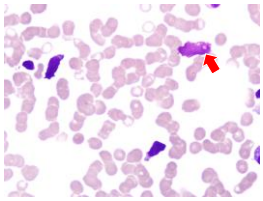
- A case of chronic active EBV infection of T-cell type, systemic form
- It is characterized by fever, renal involvement alone, and high copies of EBV-DNA.
- It is sensitive to anti- metabolism chemotherapy
- The patient carried heterozygous point mutation in UNC13D gene which represents the most common cause of primary HLH.

Proposed Diagnosis:
EBV-associated T and NK-cell lymphoproliferative diseases (EBV-T/NK LPDs): Chronic active EBV infection of T-cell type, Systemic form (Renal)

Comments:
EBV infection is the most frequent factor that triggers subsequent HLH. For people with diagnosed immune deficiencies which increase their EBV susceptibility, it would be particularly difficult to achieve full virus elimination. As a result, the persistence of EBV infection would contribute to HLH and NK/T lymphoma occurrence. In this case, unknown mechanisms that are able to inhibit EBV reactivation were probably involved in the current status of the patient.

To combat difficulties in treatment, hematopoietic stem cell transplantation has been introduced as a curative therapy. Although recent efforts at introducing reduced-intensity conditioning resulted in excellent transplantation outcome, the rates of complications associated with transplantation are high. In the future, customized gene therapy may provide solutions for the treatment of active EBV infection.

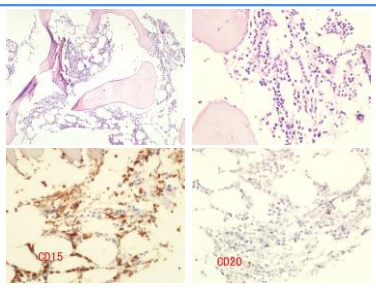
Bone Marrow Cytology



The N/C ratio decreased, the cytoplasm of the cells is pale blue with azurophilic granules and the nuclei is round or oval with moderately condensed chromatin and rare nucleoli.

1

Bone Marrow Biopsy



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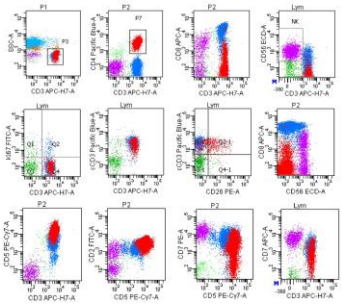
Flow Cytometry



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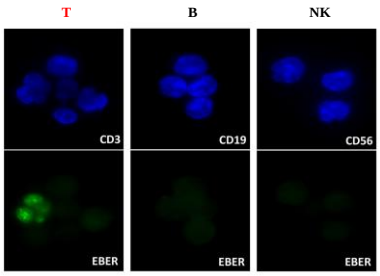
Sorting-Fish



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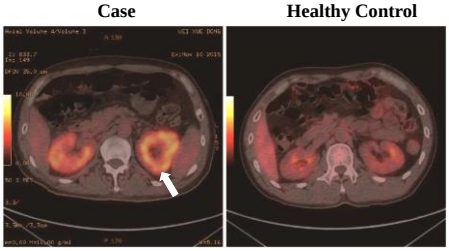
PET-CT

Case | Healthy Control



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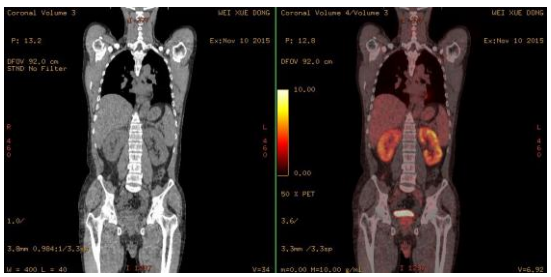
PET-CT



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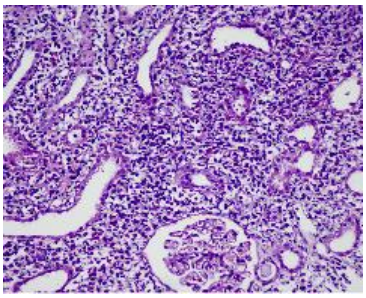
Pathologic Diagnosis



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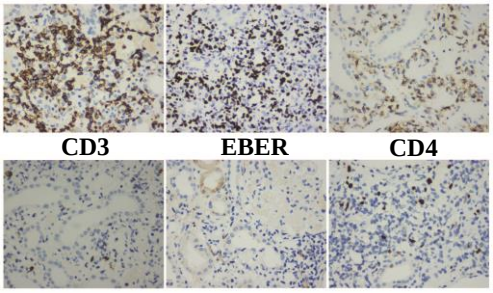
Pathologic Diagnosis

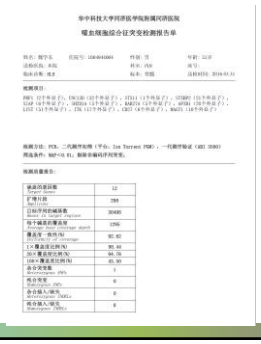


CD3 EBER CD4

CD56 CD20 Ki-67

8

Next Generation Sequencing



华中科技大学同济医学院附属同济医院

噬血细胞综合征突变检测报告单

9

Next Generation Sequencing

标本突变报告

染色体位置	基因	编码区位置	突变类型	杂合/纯合	突变比例	覆盖度	索引
chr17：72032723	UNC13D	c.1228A>C p.Ile410Leu	错义突变	杂合	50.48%	252	rs137221419

结论：

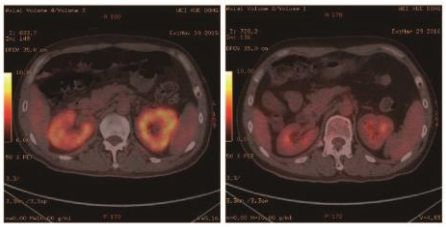
1. 检测到UNC13D基因第14号外显子存在错义突变，c.1228A>C，p.Ile410Leu（p.I410L），根据数据库及相关文献检索，该型突变为噬血细胞性淋巴组织细胞增生症（HLH）患者的热点突变。根据UniProt数据库检索，UNC13D编码蛋白Munc13-4，该蛋白参与效应淋巴细胞对靶细胞的输定以及细胞毒颗粒的分泌等过程，本例突变位点（I410）位于UNC13D的与RAB27A相互作用结构域（AA：240-543），而UNC13D和RAB27A的相互作用是细胞毒性颗粒输靠靶细胞所必须，该位点的突变可能引起细胞毒性T淋巴细胞（CTL）及自然杀伤细胞（NK）的细胞毒作用缺陷，进而引发HLH。目前，国内已有多例伴I410L杂合突变的HLH的报道（病例报告【1】，学位论文【2】，期刊【3】），国际会议中亦有该型突变的报道【4】。

2. 请结合临床综合考虑。

10

Evaluating Curative Effect

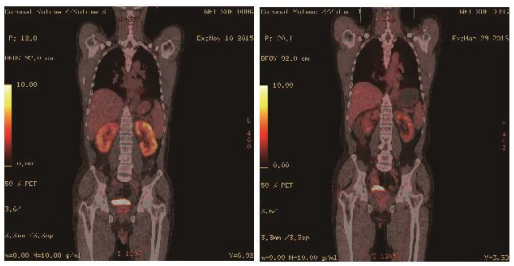
2015-11-10 2016-03-29



11

Evaluating Curative Effect

2015-11-10 2016-03-29



12

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Lu Sun. Primary LYG of CNS after treatment of CLPPERS.

Affiliation:
Department of Pathology, General Hospital of PLA

E-mail:
738873919@qq.com

Clinical History:
A 21 years old young man complained repeated rash for more than 10 months, found lymph node enlargement for 3 weeks.
In May 2018, the patient developed a facial rash without obvious incentives, accompanied by itching and pain. After treatment (specifically unknown), he improved.
In September 2018, his rash was widespread, and it appeared in batches with itching and pain. It was more common in the head and face and trunk. In October, he was diagnosed as chickenpox and was treated with cephalosporin in a hospital. After 2 weeks, the effect was poor.
In January 2019, he was considered "chickenpox; eczema" in another hospital and was treated with antiviral and antihistamine for 2 weeks. The effect was not good.
From 2019-1, there was repeated fever, with no fear of cold or cold, and the highest body temperature was 40.2 °C.
During the course of the disease, the patient had repeated fever and no night sweats, and the weight dropped by 10 kg in one month.
2019-2, Local hospital: Biopsies from skin, lymph node and BM were performed.
2019-3(in our hospital), EBV-DNA(blood) 2.369×10^5 copies/mL.
Blood regular test: erythopenia, leukopenia, thrombopenia. Clinical Diagnosis: Hemophagocytic syndrome.
Performed LN puncture biopsy, and treated according to HLH-1994 program induction treatment plan.

Biopsy Fixation Details:
FFPE

Description of Clinical Image if Any:
PET-CT(2019-3-29)1. multi-region lymph node enlargement with high metabolism, multiple bone high metabolism, spleen with high metabolic lesions, in summary, consider the blood system malignant disease, lymphoma may be, it is recommended that bone penetration or high metabolic site puncture clear.

2. hilar, bilateral inguinal lymph nodes mild high metabolism, considering inflammatory, it is recommended to follow up.
3. There was no obvious abnormal metabolic sign in the PET/CT examination of the brain.
Cervical lymph node SUVmax: 6.9; intraspinal lesion SUVmax: 8.3; spinal multiple radioactive uptake increased SUVmax: 7.6; left tibia large nodule increased radioactivity intake SUVmax: 9.8

PET-CT(2019-6) 1. The original multi-region lymph node abnormal high metabolic signs decreased or disappeared, and the spleen and bone high metabolizing lesions disappeared.
2. New high-metabolism lymph nodes in the posterior wall of the nasopharyngeal and bilateral high-metabolic lesions, retroperitoneal and mesenteric spaces
The posterior wall and double wall of the nasopharynx were thickened and radioactively concentrated. SUVmax: 7.5 The lymph nodes of different sizes in the retroperitoneal and mesenteric spaces were significantly increased compared with the previous volume. The radioactive intake was significantly higher than before, and some were newly added. The larger size is about 2.1×1.2cm, this time SUVmax: 29.0

Details of Microscopic Findings:
The normal structure of the lymph nodes disappeared and replaced with diffusely infiltrating lymphocytes and histiocytes, see focal hemorrhage. Lymphocytes are small, medium-sized and large, and some cells are heteromorphic. See the rod-shaped nucleus, which is easy to see. Suspicious macrophages phagocytose red blood cells.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
Histiocyte:CD68(+), CD163(+)
Lymphocyte: almost T cells, including atypical large cells and rod-shaped cells, CD3(+), CD2(+), CD5(+), CD7(+), large cells and rod-shaped cells CD8(+),granzymeB(+); numerous LC and HS were CD4(+)

Special Stains:
EBER(＜50 个/HPF).

Cytogenetics:

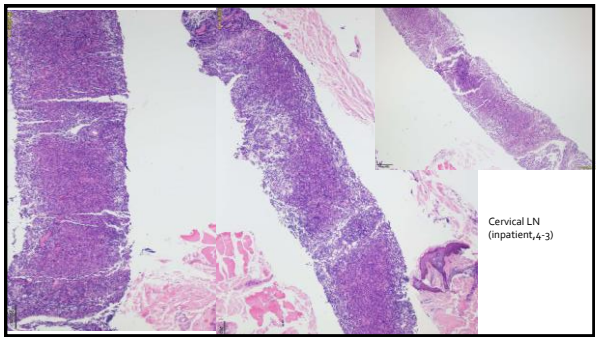
Molecular Analysis:

Interesting Feature(s) of Submitted Case:

LN: Atypical CD8+T cells were EBER positive(consistent with BM biopsy), most T cells and histiocytes were CD4+. Suspected macrophage phagocytosis of red blood cells.
Should skin biopsy be diagnosed as CD30+LPD? (consult case, need to borrow slides and take pictures)
Q: How to treat the patient with CAEBV -T/NK type(2-3) and when is proper?

Proposed Diagnosis:
CAEBV – T/NK cell type, borderline-tumor

Comments:



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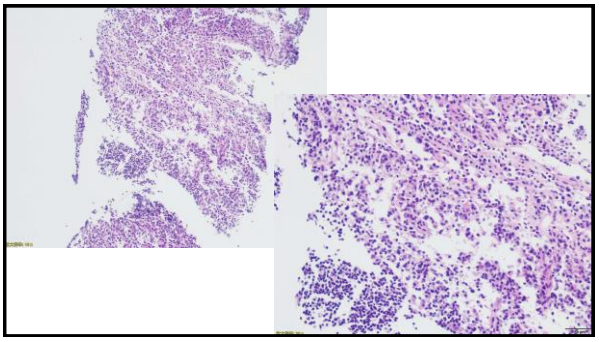

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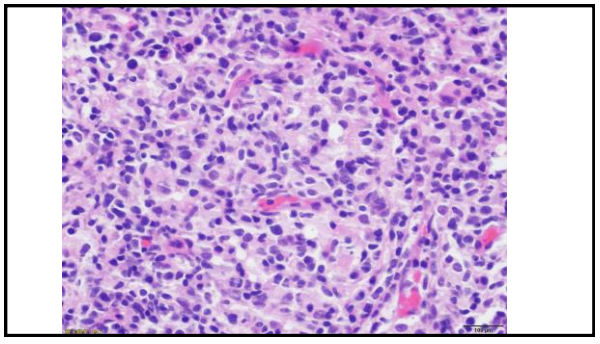

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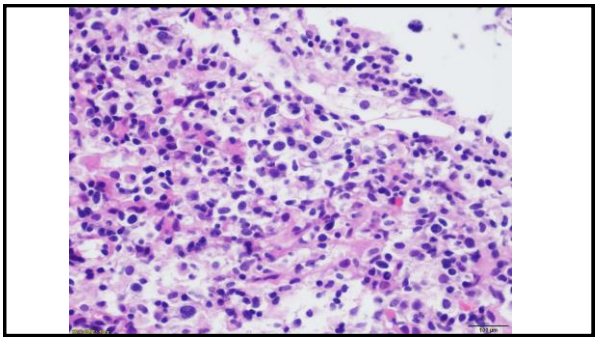

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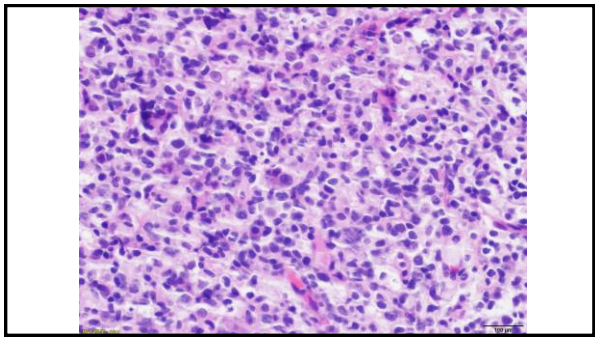

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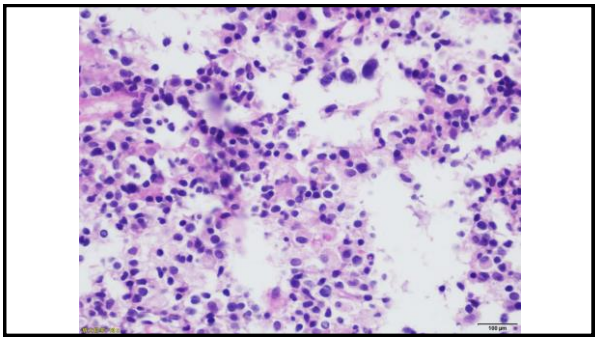

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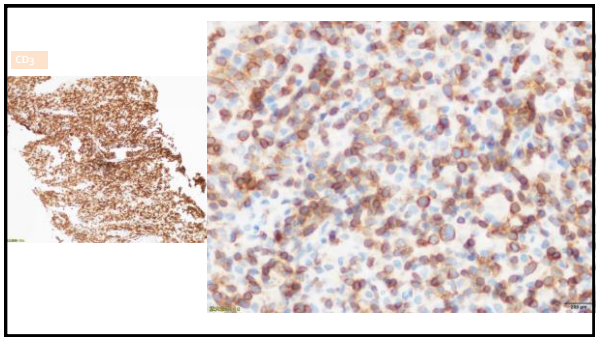

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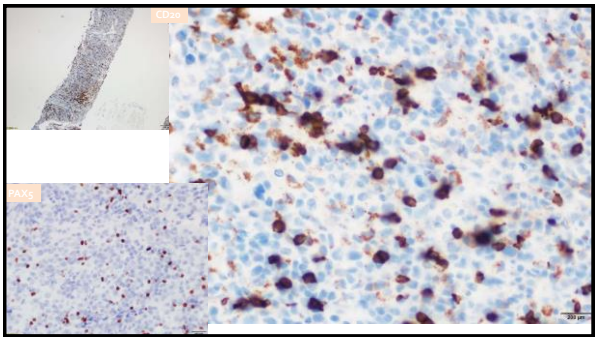

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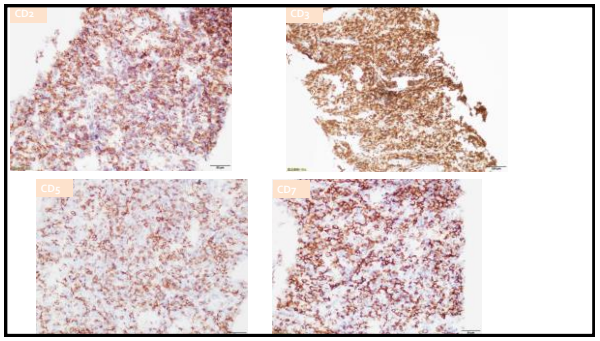

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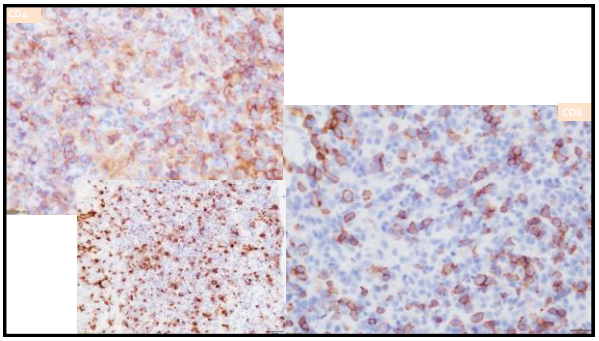

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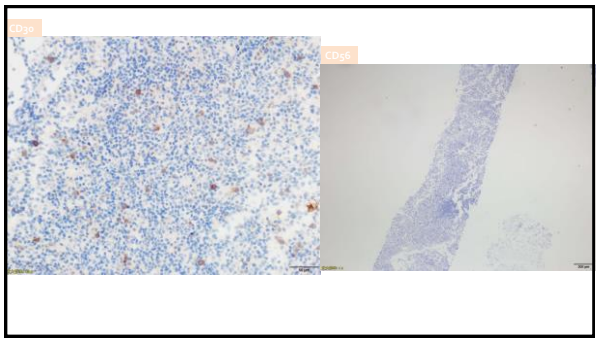

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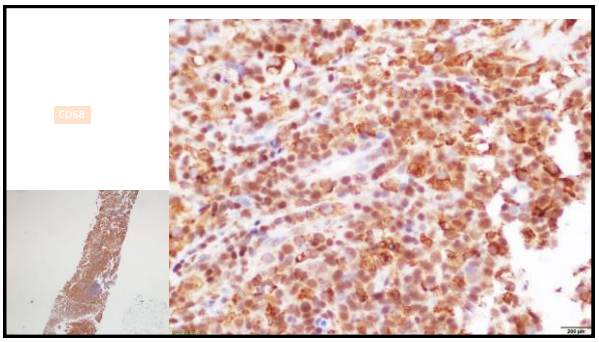

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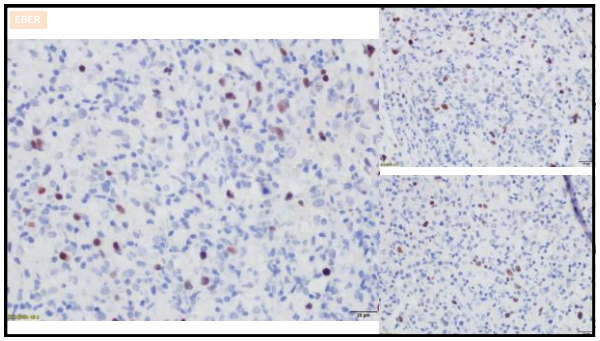

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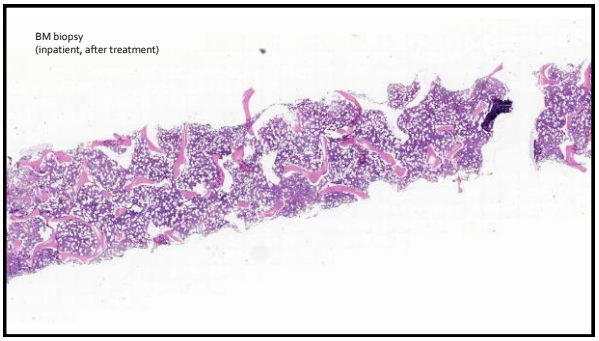

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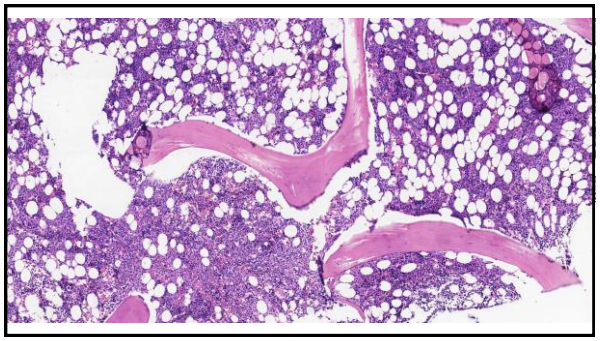

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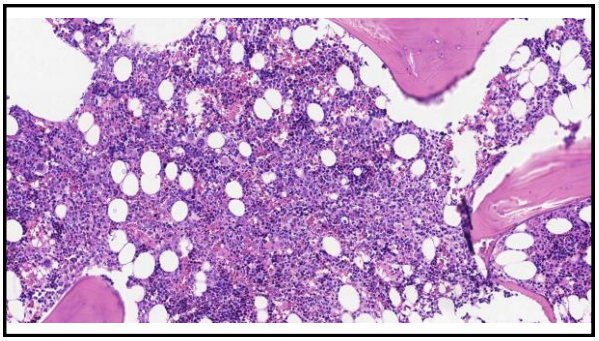

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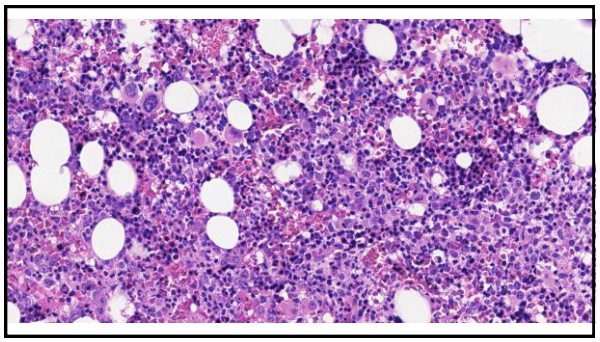

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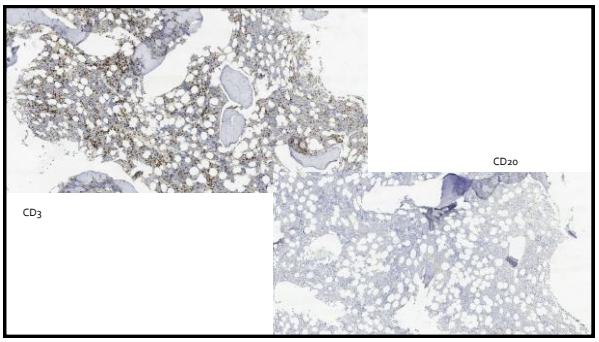

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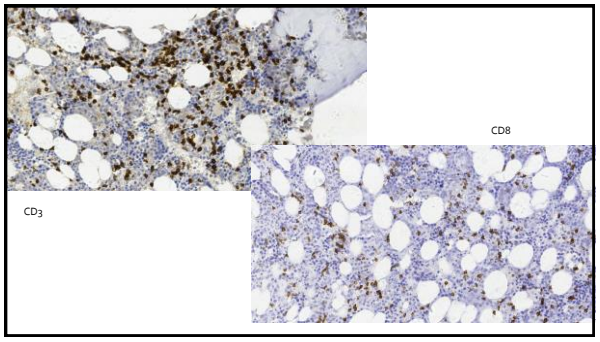

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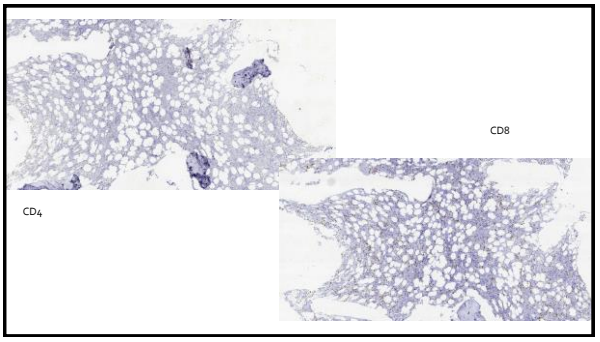

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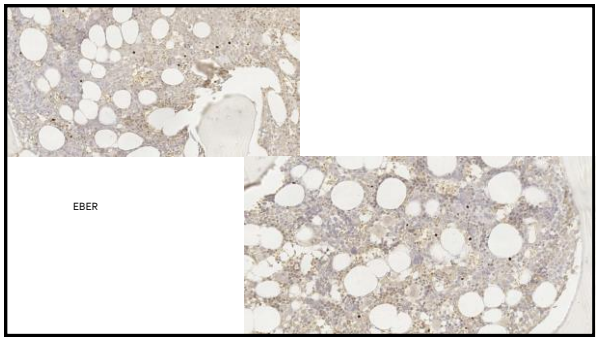

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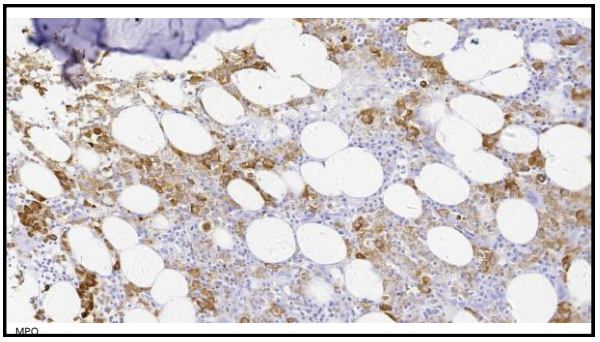


22

Epstein-Barr Virus Positive Lymphoproliferative Disorder and Cytomegalovirus Infection Simultaneously Detected in the Colon

Claudiu V. Cotta and Erica C. Savage
Cleveland Clinic, Cleveland, Ohio, USA

1

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Claudiu V. Cotta, MD., Ph.D. and Erica C. Savage, MD

Affiliation: Cleveland Clinic, Cleveland, Ohio, U.S.A.

E-mail: cottac@ccf.org

Clinical History:
The patient is a 63 y.o. woman initially presenting with diarrhea and hematochezia. Colonoscopy revealed total colonic active colitis and a 6 cm rectal mass. A biopsy showed invasive squamous cell carcinoma. Palliative irradiation was started and the patient was admitted with bacteriemia. A biopsy of the colon was repeated.

Biopsy Fixation Details:
Formalin

Description of Clinical Image if Any:
Image not submitted, but colonoscopy described total active colitis.

Details of Microscopic Findings:
Colonic mucosa with surface ulcerations and granulation tissue. Rare cells with viral inclusions. Numerous plasma cells, in the lamina propria, with mature appearance, occasional immunoblasts, but no normal lymphoid structures.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry: Occasional CD30, MUM1 positive cells, negative for CD3 or CD20. Polytypic plasma cells on the stain for CD138 and in-situ hybridization with probes for kappa or lambda immunoglobulin light chains-encoding mRNA. Cytomegalovirus positive cells. Chromogenic in-situ hybridization with probes for Epstein-Barr virus (EBV)-encoded small RNA (EBER) shows occasional positive cells, not forming sheets. Most plasma cells are negative for EBER.

2

Special Stains:
N/A

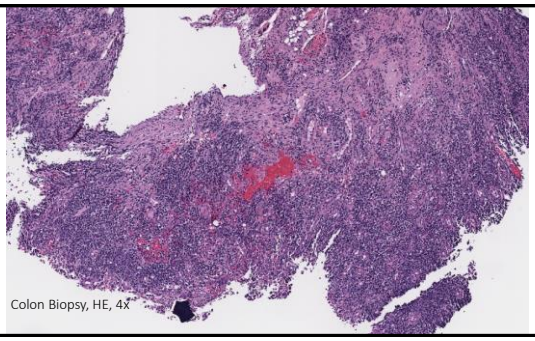
Cytogenetics:
Not available

Molecular Analysis:
Polymerase chain reaction with primers for T-cell receptor (TCR) beta and gamma chain genes and for immunoglobulin heavy and kappa light chain genes (Biomed 2) pending.

Interesting Feature(s) of Submitted Case:
Co-existing CMV and EBV infections in a patient with a non-hematopoietic neoplasm. While the CMV infection is probably limited to epithelial structures, the EBV positive cells are hematolymphoid, associated with an abundant, polytypic plasma cell infiltrate. The possibility that these cells correspond to CD30 positive cells with immunoblastic features is considered likely. Further classification of the EBV positive process is difficult. The differential diagnosis includes mucocutaneous ulcer, EBV positive lymphoma and an acute EBV infection, possibly colitis.

Proposed Diagnosis:
Epstein-Barr virus positive colitis simultaneous with cytomegalovirus infection.

3



Colon Biopsy, HE, 4x

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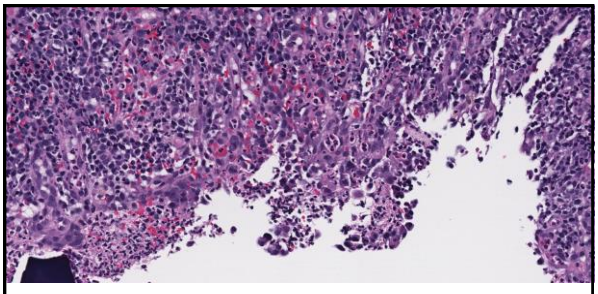

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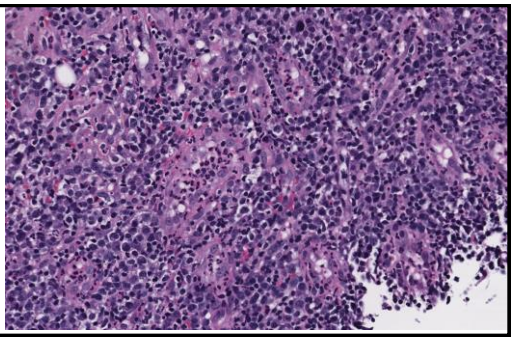

Colon Biopsy
HE, 20x

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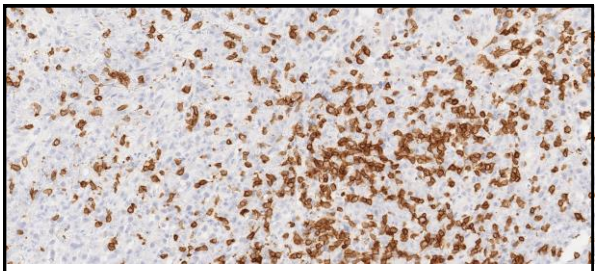

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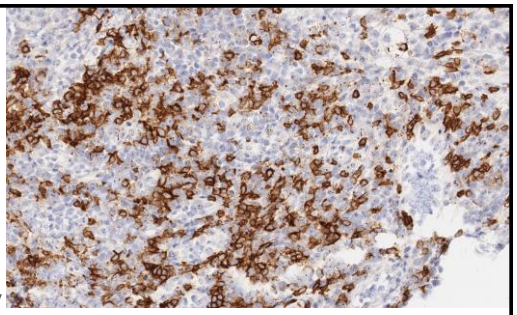

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CD20, 20x

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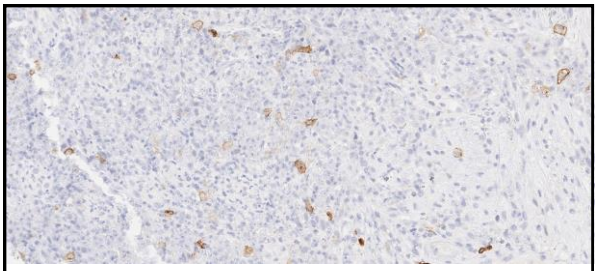

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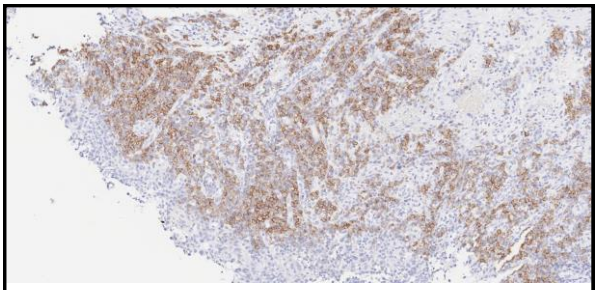

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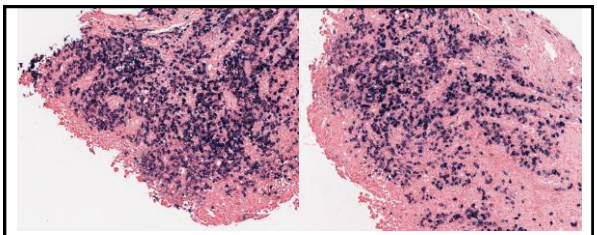
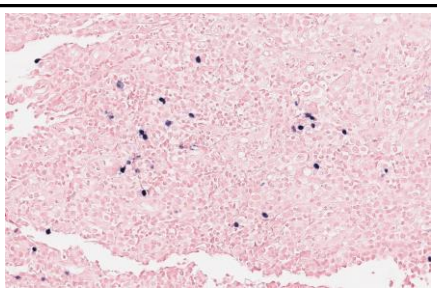


Colon Biopsy, kappa, 10x

Colon Biopsy, lambda, 10x

11



Colon Biopsy, EBER, 20x

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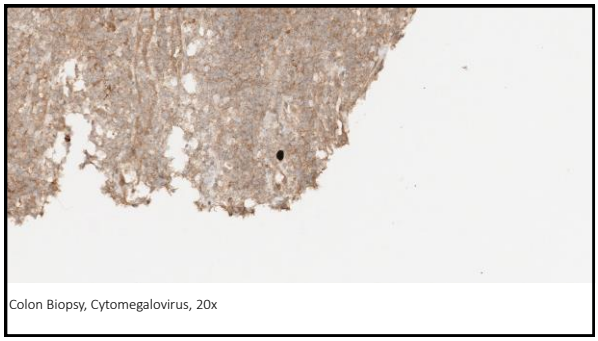

Colon Biopsy, Cytomegalovirus, 20x

13

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Miao Zheng (MD PhD); Yang Yang (MD PhD); Xiangrong Hu (MD); Jianfeng Zhou (MD PhD).

Affiliation: Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology

E-mail: zmzk@sina.com

Clinical History:
A 20 years old male patient.

Chief Complaint: intermittent fever for more than 4 years.

Past history: a history of chronic rhinitis; blood transfusion history. This patient is allergic to tetanus antitoxin. Nothing else special.

Family history: nothing special.

Physical examination: no yellow staining on the skin, no swelling of superficial lymph nodes in the neck and the rest of the body. The throat is not red, the tonsils are not swollen, and no tenderness on the sternum. The liver and spleen can be touched under the ribs.

Current medical history: On January 21, 2014, the patient had swelling and pain in the right nasal face with no obvious cause. He was admitted to the Department of Otorhinolaryngology in Anhui province. Sinus CT showed sphenoid sinus inflammation and bilateral inferior turbinate hypertrophy. After catching a cold in early April 2014, the patient suffered from repeated fever with body temperature up to 40ºC, accompanied by night sweats. In mid-April, the patient had nasal vestibular swelling and pain, sore throat, nasal discharge, headache and he was admitted again to the Department of Otorhinolaryngology in Anhui. Review of sinus CT showed: soft tissue density visible in the sphenoid sinus; no abnormalities in bilateral maxillary sinus, ethmoid sinus and frontal sinus; significant enlargement of double inferior turbinate. Large groups of purulent scabs-like neoplasms of the nasopharynx with uneven surface can be seen under

nasal pharyngoscopy. Pathological examination of neoplasms in the nasopharynx suggested that NK/T-cell lymphoma cannot be ruled out, the surrounding mucosal squamous epithelial showed grade II atypical dysplasia. Pathology Consultation Tips from a hospital in Shanghai showed: combined with immunohistochemistry results, NK/T-cell lymphoma cannot be ruled out. Immunohistochemistry suggested tumor cells: CD20(-), CD3(+), CD4(+), CD43(+), CD56 (+), CD8 (+), GB (+), Ki-67 (+, 30-40%), Perforin (+), TIA-1 (+), EBER (+). The patient was transferred to the hospital in Shanghai and was taken a new pathology examination of the neoplasms under nasopharyngoscopy. The result revealed chronic mucosal inflammation. So the patient was not treated. On August 15, 2014, the pathology examination of the neoplasms under nasopharyngoscopy was carried out in Anhui, and the results still suggest chronic mucosal inflammation.

On February 25,the patient developed fever again, the right submandibular lymph node was swollen, and he was treated again in Anhui. PET/CT showed multiple lymph nodes shadows in right posterior occipital, bilateral sternocleidomastoid and submandibular areas, the FDG metabolism is increased to varying degrees, the bilateral tonsils are enlarged, the FDG metabolism is increased, and the lymphoma is considered; the FDG metabolism of the bilateral nasopharynx is slightly increased; there're nodule-like shadows with increased density in the left upper and the lower right lung, FDG metabolism did not increase; there's sinusitis and splenomegaly. Right submandibular lymph node puncture showed a hyperplasia of the lymphatic network under the microscope, immature cells are easy to see. Peripheral blood flow immunophenotyping suggests increased NK cells with suspected phenotype (clonal hyperplasia). Lymphnode biopsy suggests: reactive hyperplasia with granuloma formation in the right submandibular lymph node, tuberculosis is suggested. The results of the pathological consultation of a hospital in Shanghai suggested that: (right submandibular) lymphoid tissue reactive hyperplasia with granulomatous lesions, specific infection should be excluded with clinical history.

On July 05, the patient developed fever again. He was treated in Anhui. Hemophagocytic syndrome and septic shock occurred. He was then transferred to another hospital, and EBV-DNA was 8.29×10^7 copies/mL at that time. Bone marrow cytology suggested: proliferation of bone marrow nucleated cell is still active, lymphatic ratio is increased, mainly mature lymphocytes, some lymphocytes vary in size, nuclear folding/cytoplasm visible particles and Burr-like protrusions can be seen. Mature lymphocyte tumor is considered. Bone marrow flow cytology suggests: 37.3% of NK/T cells are mature, immunophenotypes are HLA-DR+, $CD2^+$, $CD56^+$, CD3-, CD5-, $CD7^+$, CD4-, CD8- , NK/T cell leukemia is considered. CT indicates splenomegaly.

On July 28, the patient received chemotherapy with SMILE regimen (MTX 2g d1, VP16 100mg d2-4, Pegaspargase, 3750IU, DXM 40mg d2-4), After chemotherapy, CT showed no swelling of the spleen. On August 19, the patient underwent the second chemotherapy, the regimen was the same as before.

On September 24, CT showed splenomegaly, multiple lymph nodes on both sides of the neck. Bone marrow cytology suggests: atypical lymphocytes are visible, about 1% of the cells. Bone marrow flow cytology showed no obvious abnormal cell population, and the proportion of NK lymphocytes increased.

On October 21, bone marrow flow cytology at Anhui found 4.4% abnormal mature NK cells, NK cell lymphoma was considered. Bone marrow biopsy shows: a few mildly irregular tissue-like or lymphocyte-like cells in the medullary cavity. The patient did not continue treatment then.

On September 06, 2016, the patient developed fever again, with intolerance of cold, chills, sore throat, running nose and other discomforts. EBV-DNA test: 6.49×10^5copies/mL. Bone marrow cytology suggests that myeloproliferation is active. Erythrocyte and megakaryocyte cell lines proliferate actively and platelets show aggregated distribution. The bone marrow flow cytology didn't find obvious abnormal cell population. The result of chromosome examination: 46, XY [20].

During October 17 to November 17, 2016, the patient was hospitalised in our hospital due to "intermittent fever for more than 2 years". Blood routine: WBC: 6.11×10^9 / L, NE: 1.57×10^9 / L, HB: 136 g / L, PLT: 133×10^9 / L; EBV DNA in PBMC: $>1.0\times10^7$ copies/mL; Plasma EBV DNA: 6.17×10^5 copy / mL; β2 microglobulin: 3.05↑mg/L. Urine routine: urine protein ±, epithelial cell count 16 / uL. Chest CT: infection in both lungs is possible, it is recommended to review after treatment; the number of bilateral axillary lymph nodes increased; thickening and adhesion of bilateral pleural; a small amount of bilateral pleural and pericardial effusion. Bone marrow cytology: active bone marrow proliferation, the proportion of lymphocytes is obviously high, of which 22% of the lymphocytes are medium sized, the amount of cytoplasm is medium, blue, and the cytoplasm contains purple-red particles. Nuclear is round or depressed, false nucleoli are visible. Conclusion: the proportion of abnormal lymphocytes is higher in the bone marrow. Enzyme-labeled staining of small megakaryocytes indicates megakaryocyte hyperplasia. Bone marrow biopsy suggests slightly reduced bone marrow proliferation in this area. Bone marrow flow cytometry results：about 10.9% NK cells（accounted for total karyocytes, accounted for 73.2% of lymphocytes）expressed CD45bri, CD2bri, CD7, CD94, CD159a, Perforin（94.7% positive）, with partial expression of CD161, CD8, Ki-67（38.6% positive）, without CD3, CD5, CD4, CD45RA, CD45RO, CD57,

CD16, CD11b, TCRαβ, TCRγδ, CD158b, PD-L1, CD158e, PD-1, CD158ah, CD159c expression. It is highly considered that such cells are NK cells with abnormal phenotypes. NK cell lymphoma cannot be excluded. Bone marrow receptor gene rearrangement test: negative. EBV Sorting-PCR suggests: T cells 4.39×10^6copies/2×10^5 cells, B cells 5.68×10^6copies/2×10^5 cells, NK cells 3.25×10^7copies/2×10^5 cells, PBMC 1.73×10^7copies/2×10^5 cells, suggesting that EBV mainly infected NK cells. The positive rate of cytoplasmic perforin in peripheral blood CD56+CD3-NK cells was reduced, and the positive rate of granzyme was generally normal. Next-generation sequencing suggested the presence of a missense mutation of the LYST gene (p.N1526K). PET/CT suggested NK/T-cell lymphoma after treatment: 1. bilateral cervical lymph node enlargement, partial metabolism increased, combined with clinical history, changes after lymphoma treatment is considered; 2. bilateral tonsils are slightly larger, show increased metabolism, clinical history should be considered; 3. small nodules in right lower lung and left upper lung, no increase in metabolism, it is recommended to observe; 4. splenomegaly, no increase in metabolism. After antiviral treatment, review of EBV-DNA in PBMC: $>1.0\times10^7$copies/mL; plasma EBV DNA: 8.80×10^3 copy/mL. LYST gene mutation tests of the peripheral blood of the patient's parents showed: father (+), mother (-). The patient did not have fever anymore.

The patient suffered from repeated intermittent fever after discharge and received treatment with glucocorticoid. In early January 2018, the patient had fever again. On January 18, 2018, he came to a hospital in Anhui. Blood routine: WBC: 1.25×10^9/L, HB: 84g/L, PLT: 22×10^9/L; abnormal blood coagulation. EBV-DNA: 9.08 x 10^5 copies/mL. Ultrasound examination showed: multiple lymph nodes in the neck, axilla and groin, splenomegaly and effusion in the abdominal cavity. Bone marrow cytology showed that granulocyte proliferation is reduced and phagocytosis is visible. Bone marrow flow cytology showed: NK cells account for 39.9% of lymphocytes with abnormal phenotype with expression of CD2, CD56, CD7, CD94. Hemophagocytic syndrome secondary to CAEBV is considered, NK/T cell lymphoma/leukemia should be excluded. After 'HLH-04 regimen' and anti-infection treatment, the patient's situation improved. The patient continue to take dexamethasone tablets, VP-16 and cyclosporine after discharge.

On February 26, 2018, the patient came to our hospital again. Review of the examination: blood routine: WBC: 5.76×10^9 / L, NE: 3.70×10^9 / L, HB: 82g / L, PLT: 164×10^9 / L. ALT: 61 U/L, AST: 22 U/L, creatinine: 55 umol/L, uric acid: 228 umol/L, LDH: 405 U/L. γ-GGT: 223 U/L. EBV-DNA in PBMC: $>1.0\times10^7$ copies/mL, plasma EBV-DNA: 2.68×10^4 copy/mL. IL-6: 6.66 pg/mL. Ferritin: 696.8 ug/L. Bone marrow flow cytometry showed: NK cells with abnormal phenotype, aggressive NK cell leukemia was considered. Sorting-RCR shows: still NK cell infection. The killing ability of NK cells is reduced and the degranulation function is abnormal. The patient received L-

GEMOX regimen chemotherapy on March 06, while continuing to maintain glucocorticoid treatment to control HLH.

The patient's condition deteriorates rapidly, the high fever continues after chemotherapy, and the hemophagocytic index worsens progressively. On March 08, ferritin: >50000ug/L, WBC: 2.25×10^9/L, HB: 71g/L, PLT: 8×10^9/L. On March 07, 10, 14 and 17 the patient was respectively treated with 20 mg of basiliximab to control HLH. The patient's ferritin has once decreased, such as that on March 15: 2667.2ug / L, But soon rebounded. The patient died in March 18, 2018.

Biopsy Fixation Details:
None.

Description of Clinical Image if Any:

1. 2014-02: Sinus CT showed sphenoid sinus inflammation and bilateral inferior turbinate hypertrophy.
2. 2014-04: Review of sinus CT showed: oft tissue density visible in the sphenoid sinus; no abnormalities in bilateral maxillary sinus, ethmoid sinus and frontal sinus; significant enlargement of double inferior turbinate. Large groups of purulent scabs-like neoplasms of the nasopharynx with uneven surface can be seen under nasal pharyngoscopy.
3. 2015-02-25: PET/CT showed multiple lymph nodes shadows in right posterior occipital, bilateral sternocleidomastoid and submandibular areas, the FDG metabolism is increased to varying degrees, the bilateral tonsils are enlarged, the FDG metabolism is increased, and the lymphoma is considered; the FDG metabolism of the bilateral nasopharynx is slightly increased; there're nodule-like shadows with increased density in the left upper and the lower right lung, FDG metabolism did not increase; there's sinusitis and splenomegaly.
4. 2015-07: CT indicated splenomegaly.
5. 2016-10: Chest CT: infection in both lungs is possible, it is recommended to review after treatment; the number of bilateral axillary lymph nodes increased; thickening and adhesion of bilateral pleural; a small amount of bilateral pleural and pericardial effusion.
6. 2016-10-24: PET/CT suggested NK/T-cell lymphoma after treatment: 1. bilateral cervical lymph node enlargement, partial metabolism increased, combined with clinical history, changes after lymphoma treatment is considered; 2. bilateral tonsils are slightly larger, show increased metabolism, clinical history should be considered; 3. small nodules in right lower lung and left upper lung, no increase in metabolism, it is recommended to observe; 4. splenomegaly, no increase in metabolism.

Details of Microscopic Findings:

1. 2015-02: Right submandibular lymph node puncture showed a hyperplasia of the lymphatic network under the microscope, immature cells are easy to see.
2. 2015-07-05: Bone marrow cytology suggested: proliferation of bone marrow nucleated cell is still active, lymphatic ratio is increased, mainly mature lymphocytes, some lymphocytes vary in size, nuclear folding/cytoplasm visible particles and Burr-like protrusions can be seen. Mature lymphocyte tumor is considered.
3. 2015-09-24: Bone marrow cytology suggested atypical lymphocytes are visible, about 1% of the cells.
4. 2015-10-21: Bone marrow biopsy showed a few mildly irregular tissue-like or lymphocyte-like cells in the medullary cavity.
5. 2016-09-06: Bone marrow cytology suggested that myeloproliferation is active. Erythrocyte and megakaryocyte cell lines proliferate actively and platelets show aggregated distribution.
6. 2016-10-19 bone marrow cytology: active bone marrow proliferation, the proportion of lymphocytes is obviously high, of which 22% of the lymphocytes are medium sized, the amount of cytoplasm is medium, blue, and the cytoplasm contains purple-red particles. Nuclear is round or depressed, false nucleoli are visible. Conclusion: the proportion of abnormal lymphocytes is higher in the bone marrow. Bone marrow biopsy suggests slightly reduced bone marrow proliferation in this area.
7. 2018-01 Bone marrow cytology showed that granulocyte proliferation is reduced and phagocytosis is visible.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

1. 2014-04: the results of biopsy of the nasopharynx prompted that NK/T-cell lymphoma cannot be ruled out, and the surrounding mucosal squamous epithelial grade II atypical dysplasia. Pathology consultation from a hospital in Shanghai: combined with immunohistochemistry results, NK / T cell lymphoma cannot be ruled out. Immunohistochemistry results of the tumor cells: CD20(-), CD3(+), CD4(+), CD43(+), CD56 (+), CD8 (+), GB (+), Ki-67 (+, 30-40%), Perforin (+), TIA-1 (+), EBER (+).
2. 2015-07-05: Bone marrow flow cytology suggested: 37.3% of NK/T cells are mature, immunophenotypes are HLA-DR+, CD2+, CD56+, CD3-, CD5-, CD7+, CD4-, CD8- , NK/T cell leukemia is considered.
3. 2015-09-24 Bone marrow flow cytology showed no obvious abnormal cell population, and the proportion of NK lymphocytes increased.
4. 2015-10-21 Bone marrow flow cytology at a hospital in Anhui found 4.4% abnormal mature NK cells, NK cell lymphoma was considered.
5. 2016-09-06 The flow cytology didn't find obvious abnormal cell population.

6. 2016-10-19 Bone marrow flow cytometry results：about 10.9% NK cells （accounted for total karyocytes, accounted for 73.2% of lymphocytes）expressed CD45bri, CD2bri, CD7, CD94, CD159a, Perforin（94.7% positive）, with partial expression of CD161, CD8, Ki-67（38.6% positive）, without CD3, CD5, CD4, CD45RA, CD45RO, CD57, CD16, CD11b, TCRαβ, TCRγδ, CD158b, PD-L1, CD158e, PD-1, CD158ah, CD159c expression. It is highly considered that such cells are NK cells with abnormal phenotypes. NK cell lymphoma cannot be excluded.
7. 2018-01-18 Bone marrow flow cytology showed: NK cells account for 39.9% of lymphocytes with abnormal phenotype with expression of CD2, CD56, CD7, CD94. Hemophagocytic syndrome secondary to CAEBV is considered, NK/T cell lymphoma/leukemia should be excluded.

Special Stains:
None.

Cytogenetics:
1. 2016-09-06 The result of chromosome examination: 46, XY [20].
2. 2016-10-19 Bone marrow receptor gene rearrangement test: negative.

Molecular Analysis:
1. Next-generation sequencing suggested the presence of a missense mutation of the LYST gene (p.N1526K).

Interesting Feature(s) of Submitted Case:
1. EBV Sorting-PCR suggests: T cells 4.39×106copies/2×105 cells, B cells 5.68 × 106 copies/2×105 cells, NK cells 3.25×107copies/2×105 cells, PBMC 1.73×107copies/2×105 cells, suggesting that EBV mainly infected NK cells.
2. Bone marrow flow cytometry results：about 10.9% NK cells（accounted for total karyocytes, accounted for 73.2% of lymphocytes）expressed CD45bri, CD2bri, CD7, CD94, CD159a, Perforin（94.7% positive）, with partial expression of CD161, CD8, Ki-67（38.6% positive）, without CD3, CD5, CD4, CD45RA, CD45RO, CD57, CD16, CD11b, TCRαβ, TCRγδ, CD158b, PD-L1, CD158e, PD-1, CD158ah, CD159c expression. It is highly considered that such cells are NK cells with abnormal phenotypes. NK cell lymphoma cannot be excluded.
3. NK cell killing activity detected by flow cytometry: NK cell activity is significantly reduced.
4. Peripheral blood flow cytometry showed: the positive rate of perforin in CD56+CD3- NK cells is reduced. The positive rate of granzyme is roughly normal.Perforin protein

expression is weakened in both NK cells and CTL cells. The expression of granzyme-B in NK cells and CTL cells is normal.
5. Flow cytometry was used to detect the CD107a degranulation level in NK cells: NK cells account for 42.57% of PBMC, and the proportion exceeded the normal range. At the resting state, the level of degranulation is lowered. The level of degranulation in the activated state is within the normal reference range.
6. Next-generation sequencing of HLH mutations suggests the presence of a missense mutation in exon 13 of LYST gene, c.4578T>A, p.Asn1526Lys (p.N1526K).
7. Family screening was performed. The patient's father was also positive for LYST mutation. And the NK cell killing activity of the mother was significantly decreased, suggesting that the mother also has the possibility of mutation of the HLH gene.

Proposed Diagnosis: 1. Chronic active Epstein-Barr virus infection;
2. Aggressive NK-cell leukemia;
3. Hemophagocytic lymphohistiocytosis, LYST mutation.

Comments:
Aggressive NK-cell leukemia (ANKL) results from the systemic neoplastic proliferation of NK cells. ANKL is a rare malignant lymphoproliferative disorder of mature NK cells closely associated with Epstein-Barr virus (EBV) and more common in East Asia than in other areas. Significant variations exist in the morphology of ANKL tumor cells, from typical large granular lymphocyte morphology to highly atypical features with basophilic cytoplasm containing azurophilic granules.

Patients with ANKL manifest a fulminant clinical course, including high fever, pancytopenia, hepatosplenomegaly, multiple organ failure, cytokine storm and hemophagocytic lymphohistiocytosis, and usually die within days to months, even after receiving prompt therapeutic management. Because of the rarity of the disease, the treatment strategy for ANKL has largely been extrapolated from other lymphoid neoplasms. However, the response to conventional chemotherapy routinely adopted in non-Hodgkin lymphoma and lymphoblastic leukemia is poor. Thus, a better understanding of the pathogenesis of ANKL is critically needed to develop more effective therapies.

As we know, Epstein-Barr virus (EBV) is one of eight human ubiquitous herpes viruses, which often causes symptomatic diseases such as infectious mononucleosis (IM) and the lymphoproliferative disorder (LPD) in immunocompromised individuals. In addition, EBV is found to be linked with some human malignancies, including Burkitt lymphoma (BL), nasopharyngeal carcinoma and gastric carcinoma. The tiny minority of individuals develops chronic EBV infection with persistent IM-like symptoms without apparent immunodeficiency. These patients have high EBV-DNA load in the peripheral

blood and monoclonal T cells or natural killer (NK) cells. Because of life-threatening complications such as malignant lymphomas, hemophagocytic lymphohistiocytosis, and organ failure, they invariably have poor prognosis. In order to be unified, this disease is generally named as “chronic active EBV infection” (CAEBV). The number of reported cases with this entity have significantly increased in the last three decades.

As for treatment, identification of hemophagocytic syndrome and application of ‘HLH-2004 Guideline’ as soon as possible will reduce early mortality. The early application of VP-16 is crucial. Such patients usually require maintenance therapy for HLH before HSCT. The ‘HLH-2004’ is difficult to maintain for more than 2 months, and HLH will recur in these patients. A relapsed/refractory HLH regimen can be considered, such as a DEP regimen.

Once CNS is involved, the condition progresses abnormally quickly, often leading to death in a short period of time. Early lumbar puncture + intrathecal injection (MTX, DXM) is conducive to the prevention of CNS complication. It also has some challenges to do HSCT. It is difficult to find matched donors and detect the specific pathogenic genes of the donor. Due to the time constraints, most of the patients can only receive heterozygous allo-HSCT. The incidence of transplant complications are relatively high and so is the cost.

Aggressive NK-cell Leukemia With LYST Gene Mutation

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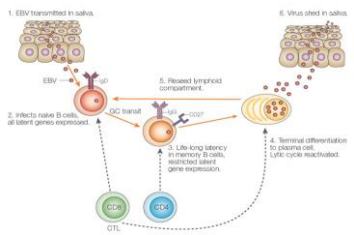
Background - Epstein-Barr virus (EBV)

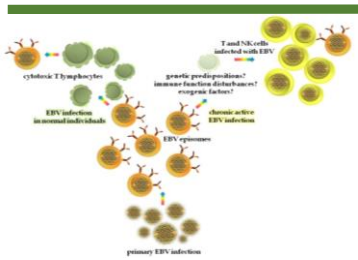


- EBV is a member of the herpes virus family.
- 95% of people have antibodies against the virus.
- Infection is controlled by T cell response.

Nat Rev Microbiol. 2005 Feb;3(2):182-7.

3

Background



- Persistent IM-like symptoms.
- High EBV-DNA load in the peripheral blood.
- Systemic clonal expansion of EBV infected cells.
- Poor prognosis with fatal complications.
- Uneven geographical distribution with most cases in East Asia.

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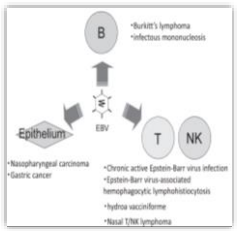
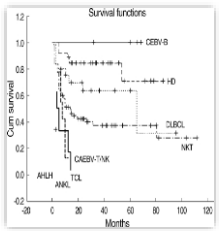
Background - EBV infection and tumors





Rinsho Ketsueki. 2013;54(10):1992-8.
J Korean Med Sci. 2008 Apr;23(2):185-92.

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CONTENTS

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Clinical History

- A male patient, 20 years old;
- Chief Complaint: intermittent fever for more than 4 years.
- Past history: a history of chronic rhinitis; blood transfusion history. This patient is allergic to tetanus antitoxin. Nothing else special.
- Family history: nothing special.
- Physical examination: no yellow staining on the skin, no swelling of superficial lymph nodes in the neck and the rest of the body. The throat is not red, the tonsils are not swollen, and no tenderness on the sternum. The liver and spleen can be touched under the ribs.

7

Pathology

- In April 2014, the results of biopsy of the nasopharynx prompted that NK/T-cell lymphoma cannot be ruled out, and the surrounding mucosal squamous epithelial grade II atypical dysplasia.
- Pathology consultation from a hospital in Shanghai: combined with immunohistochemistry results, NK / T cell lymphoma cannot be ruled out. Immunohistochemistry results of the tumor cells: CD20(-), CD3(+), CD4(+), CD43(+), CD56 (+), CD8 (+), GB (+), Ki-67 (+, 30-40%), Perforin (+), TIA-1 (+), EBER (+).
- A new pathology of neoplasms under nasopharyngoscopy in Shanghai suggested chronic mucosal inflammation.

8

Pathology

- In August 2014, a new biopsy of the nasopharynx in Anhui still suggested chronic mucosal inflammation.
- In February 2015, the right submandibular lymph node puncture showed a hyperplasia of the lymphatic network, and the immature cells were easy to see.
- Lymph node biopsy suggested: (right submandibular) lymph node reactive hyperplasia with granuloma formation, tuberculosis is likely.
- Pathology consultation from a hospital in Shanghai suggested: (right submandibular) lymphoid tissue reactive hyperplasia with granulomatous lesions.

9

Clinical history

- On July 05, 2015, Hemophagocytic lymphohistiocytosis occurred. EBV-DNA was 8.29×107 copies/mL at that time. Bone marrow cytology suggested: proliferation of bone marrow nucleated cell is still active, lymphatic ratio is increased, mainly mature lymphocytes, some lymphocytes vary in size, nuclear folding/cytoplasm visible particles and Burr-like protrusions can be seen. Mature lymphocyte tumor is considered. Bone marrow flow cytology suggests: 37.3% of NK/T cells are mature, immunophenotypes are HLA-DR+, CD2+, CD56+, CD3-, CD5-, CD7+, CD4-, CD8- , NK/T cell leukemia is considered. CT indicates splenomegaly.
- The patient received chemotherapy with SMILE regimen ×2 (MTX 2g d1, VP16 100mg d2-4, Pegaspargase, 3750IU, DXM 40mg d2-4).

10

EBV copies and sorting PCR- our hospital

- EBV-DNA in plasma: 6.17×10^5copy/ml;
- EBV-DNA in PBMC: >1.0×10^7copy/ml.

cell type	copies/number of cells
T cell	$4.39\times10^6/2\times10^5$
B cell	$5.68\times10^6/2\times10^5$
NK cell	**$3.25\times10^7/2\times10^5$**
PBMC	$1.73\times10^7/2\times10^5$

Sorting PCR suggested that EBV mainly infected NK cells.

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NK cell phenotype

- Bone marrow flow cytometry results：about 10.9% NK cells（accounted for total karyocytes, accounted for 73.2% of lymphocytes）expressed CD45bri, CD2bri, CD7, CD94, CD159a, Perforin（94.7% positive）, with partial expression of CD161, CD8, Ki-67（38.6% positive）, without CD3, CD5, CD4, CD45RA, CD45RO, CD57, CD16, CD11b, TCRαβ, TCRγδ, CD158b, PD-L1, CD158e, PD-1, CD158ah, CD159c expression. It is highly considered that such cells are NK cells with abnormal phenotypes. NK-cell lymphoma cannot be excluded.

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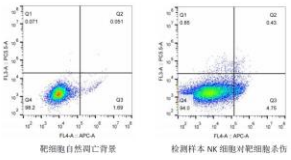
NK cell killing activity

NK 细胞活性检测结果：

名称	结果	正常参考范围
NK 细胞活性检测	3.44%↓↓	≥15.11%

结果附图：



- NK cell killing activity detected by flow cytometry: NK cell activity is significantly reduced.

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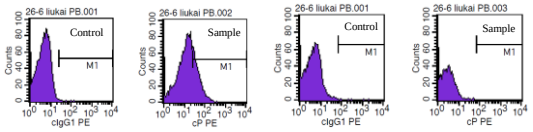
NK cell function

- Peripheral blood flow cytometry showed: the positive rate of perforin in CD56+CD3- NK cells is reduced. The positive rate of granzyme is roughly normal.
- Perforin protein expression is weakened in both NK cells and CTL cells.
- The expression of granzyme-B in NK cells and CTL cells is normal.

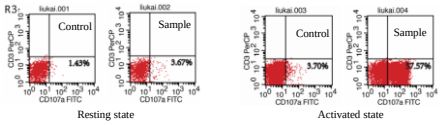


Perforin protein expression is weakened in NK cells. Perforin protein expression is weakened in CTL cells.

14

NK cell function

- Flow cytometry was used to detect the CD107a degranulation level in NK cells: NK cells account for 42.57% of PBMC, and the proportion exceeded the normal range. At the resting state, the level of degranulation is lowered. The level of degranulation in the activated state is within the normal reference range.



Resting state Activated state

15

Next generation sequencing

位点	基因	突变	类型	比例(%)	覆盖度	索引
1：235952111	*LYST*	c.4578T>A p.Asn1526Lys	错义突变	50.72	347	dbSNP:rs117609949

- Next-generation sequencing of HLH mutations suggests the presence of a missense mutation in exon 13 of LYST gene, c.4578T>A, p.Asn1526Lys (p.N1526K).

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Diagnosis

Proposed Diagnosis:

1. Chronic active Epstein-Barr virus infection;
2. Aggressive NK-cell leukemia;
3. Hemophagocytic lymphohistiocytosis, LYST mutation.

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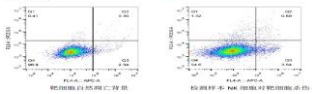
Family screening

Family	LYSTp.Asn1526Lys (c.4578T>A)
Father	(+)
Mather	(−)

NK 细胞活性检测结果：(Mather)

名称	结果（校正后）	正常参考范围
NK 细胞活性检测	1.95%↓↓	≥15.11%

结果附图：



- Family screening was performed. The patient's father was also positive for LYST mutation. And the NK cell killing activity of the mother was significantly decreased, suggesting that the mother also has the possibility of mutation of the HLH gene.

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CONTENTS

01 BACKGROUND

02 CASE REPORT

03 DISCUSSION

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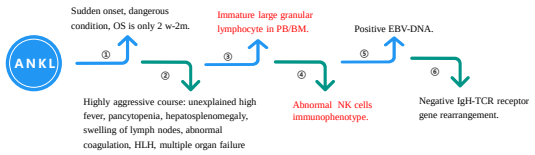
Diagnostic points of ANKL



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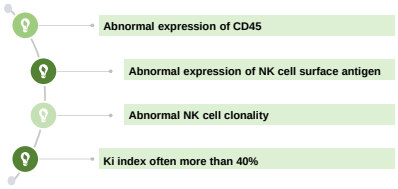
Diagnostic points of FCM in ANKL



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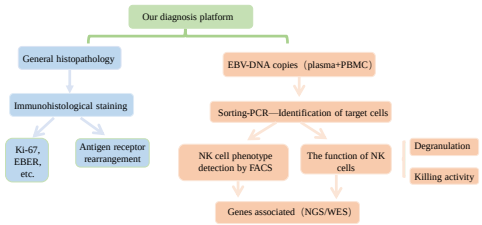
How I diagnose?



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LYST gene mutation

➢The patient has the presence of a missense mutation in exon 13 of LYST gene, c.4578T>A, p.Asn1526Lys (p.N1526K). According to the UniProt database, the protein encoded by LYST gene is involved in the formation of cytotoxic T cells (CTL) and cytotoxic vesicles of NK cells. The LYST gene germline mutation can cause immunodeficiency related to lysosomal membrane defects, which is autosomes inherited. It is so-called Chediak-Higashi syndrome (CHS). This mutation can also lead to the development of hemophagocytic lymphohistiocytosis (HLH).

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How I treat? - Key points

➢Identification of hemophagocytic lymphohistiocytosis and application of 'HLH-2004 Guideline' as soon as possible will reduce early mortality.

➢**The early application of VP-16 is crucial! ! !**

➢Such patients require maintenance therapy for HLH before HSCT.

➢The 'HLH-2004' is difficult to maintain for more than 2 months, and HLH will recur in these patients.

➢A relapsed/refractory HLH regimen can be considered, such as a DEP regimen.

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Prevention of CNS Complication

- Once CNS is involved, the condition progresses abnormally quickly, often leading to death in a short period of time.
- Early lumbar puncture + intrathecal injection (MTX, DXM) is conducive to the prevention of CNS complication.

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HSCT challenge

- Difficult to find matched donors and detect the specific pathogenic genes of the donor.
- Due to the time constraints, most of the patients can only receive heterozygous allo-HSCT.
- The incidence of transplant complications are relatively high and so is the cost.

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THANKS!

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CASE SUBMISSION FORM

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Clinical History:
The patient was a 51 year old healthy man taking zero medications who developed pancytopenia (WBC 0.6 x 10^9/L; Hgb 11.7 g/dL; Plt 77 x 10^9/L), a skin rash, and a transient fever lasting several days. A month later, he again developed fever, accompanied by fatigue, night sweats, and slightly elevated transaminases. A bone marrow biopsy (BMBx #1) was hypercellular with findings suspicious for a T-cell lymphoproliferative process. He received 1 dose of G-CSF and his white blood cell count improved.

After six months a bone marrow biopsy was repeated (BMBx #2) which was normocellular with low-level (5%) involvement by a T-cell lymphoproliferative process.

He was observed for 18 months with repeat complete blood counts, which began to decline at the end of the observation period (WBC 2.3 x 10^9/L; Hgb 14 g/dL; Plt 72 x 10^9/L), and his liver enzymes began to increase (ALT 62 U/L, AST 58 U/L). He developed a febrile illness with no identifiable infectious source except for Epstein-Barr viremia (6,000 copies/mL). A bone marrow biopsy was performed (BMBx #3). The bone marrow showed persistent involvement by a T-cell infiltrate. He was treated with intravenous immunoglobulin and G-CSF.

His neutrophil count continued to decline and approximately 6 months after his last bone marrow biopsy, splenectomy was performed for therapeutic relief of what was thought to be immune-mediated cytopenias. His EBV DNA quantitative test was 14,490 copies/mL.

Three months after splenectomy, he presented with fever of unknown origin. He quickly developed multisystem organ failure including hepatic and renal failure with pleural effusions, and disseminated intravascular coagulation. His EBV DNA quantitative test was 161,200 copies/mL. The

patient was given steroids and transfusion support but died two weeks after admission to the hospital.

Biopsy Fixation Details:
The first bone marrow biopsy was performed elsewhere (unknown).

The second two bone marrow biopsies and spleen were fixed in 10% neutral buffered formalin.

Description of Clinical Image if Any: N/A

Details of Microscopic Findings:

BMBx 1: The bone marrow was hypercellular (95%) with an abnormal interstitial T-cell infiltrate comprising 80% of the bone marrow cellularity.

BMBx 2: The bone marrow was normocellular with trilineage hematopoiesis and low-level involvement by a T-cell process (5% of cellularity)

BMBx 3: The bone marrow was hypercellular (80%) with an atypical ill-defined lymphohistiocytic infiltrate

Spleen: The spleen was enlarged (570 grams) and showed extra-medullary hematopoiesis and an abnormal interstitial T-cell infiltrate. There was hemophagocytosis.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

BMBx1: The interstitial T-cell infiltrate is positive for CD3 and CD5 by immunohistochemistry. Flow cytometry identified slight decrease in CD7 expression. There were scattered EBV positive cells.

BMBx2: Flow cytometry identified a T-cell population representing 82% of lymphocytes, with a subset that lacked CD4 and CD8 expression (18% of lymphocytes), and showed aberrant loss of CD7 and CD26. T-cells appeared to represent 5% of bone marrow cellularity by CD3 immunohistochemistry. Granzyme B was positive in these cells, and scattered EBER-ISH positive cells were present.

BMBx3: Immunostains showed increased numbers of CD3 positive cells with a similar abnormal immunophenotype by flow cytometry as the prior bone marrow biopsy, and representing 27% of the gated lymphocyte population. There was scattered EBER-ISH positivity in the bone marrow.

Spleen: The spleen showed an abnormal CD3 positive infiltrate with coexpression of cytotoxic molecules TIA1, granzymeB and perforin, and EBER-ISH positivity.

Special Stains: None

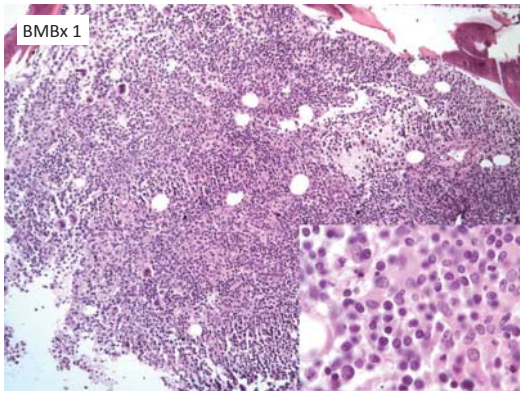
Cytogenetics:
Normal male karyotype for all bone marrow biopsies.

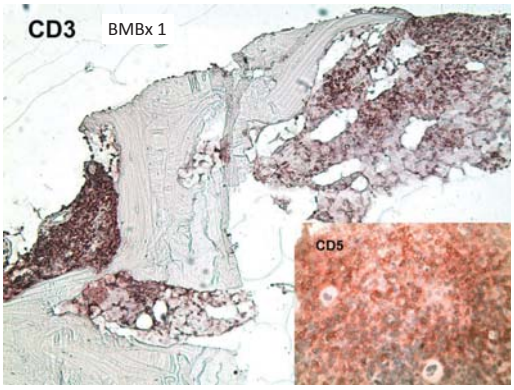
Molecular Analysis: T-cell receptor gene rearrangement studies were attempted on BMBx2 without success. A clonal TCR beta chain gene rearrangement was detected in BMBx3.

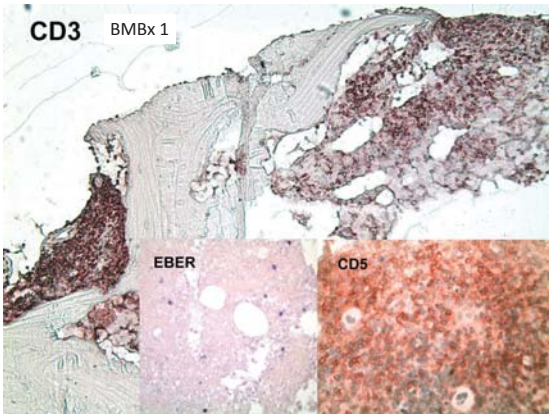
Interesting Feature(s) of Submitted Case: This is an Asian man who developed fever and pancytopenia, and an apparently indolent T-cell lymphoproliferation at the start. He was followed by the infectious disease service for recurrent fevers of unknown origin and the T-cell lymphoproliferation was downplayed as a cause of his cytopenias, which were thought to be cyclical and immune-mediated. It was recognized too late that this was a rare systemic chronic active EBV with ultimate development of multisystem organ failure and hemophagocytic syndrome.

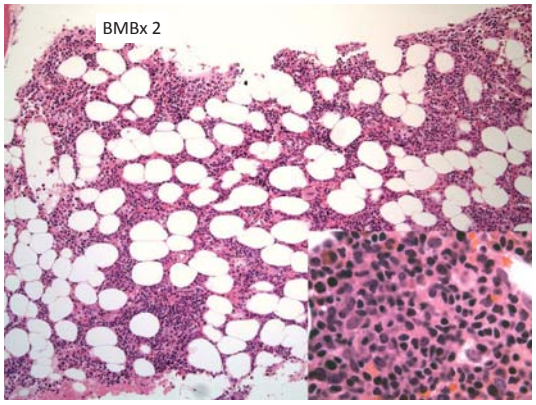
Proposed Diagnosis: Chronic active EBV infection

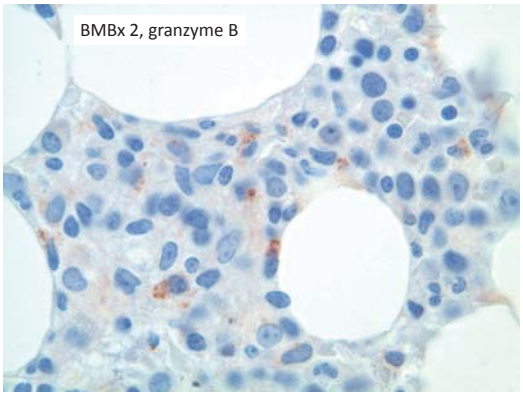
CSHP case submission,
CAEBV, Ondrejka and Hsi

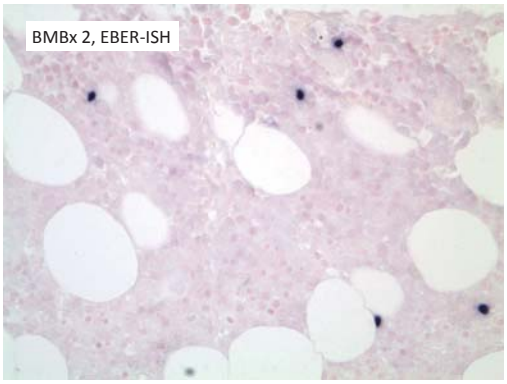


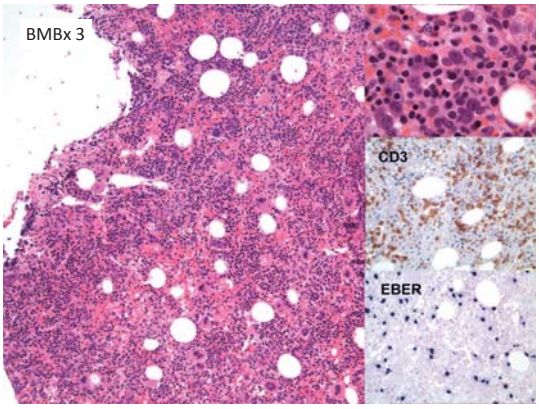


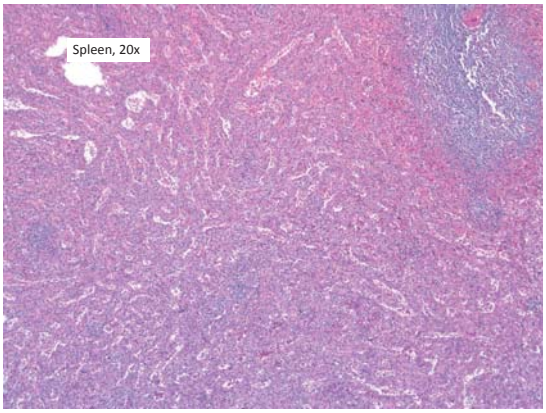


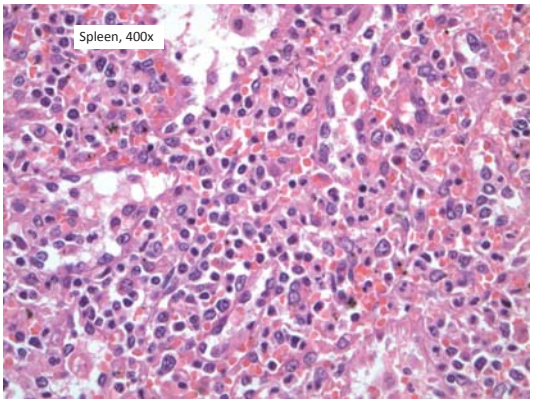


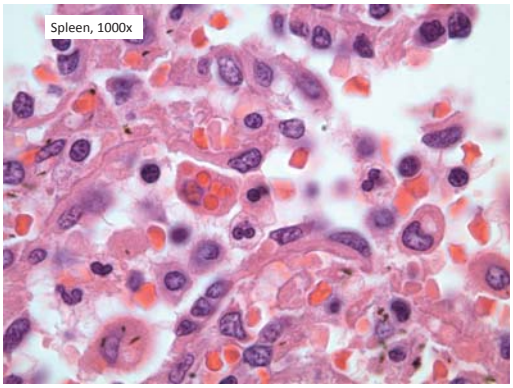


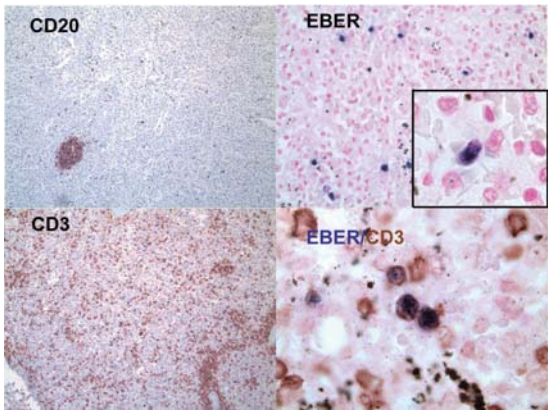

BMBx 2, EBER-ISH

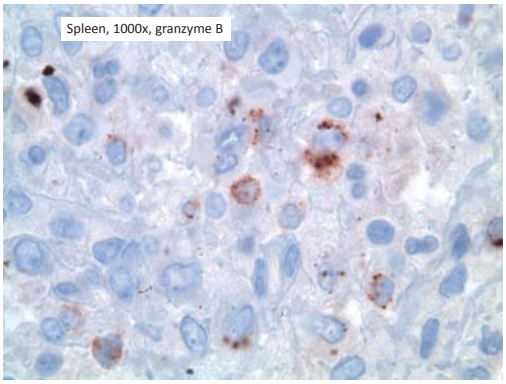

BMBx 3
CD3
EBER

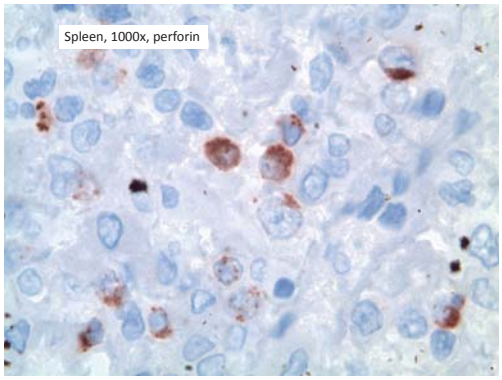

Spleen, 20x


Spleen, 400x


Spleen, 1000x


CD20
EBER
CD3
EBER/CD3


Spleen, 1000x, granzyme B


Spleen, 1000x, perforin

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
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Sarat Kuppachi[2]
Ince Dilek[3]
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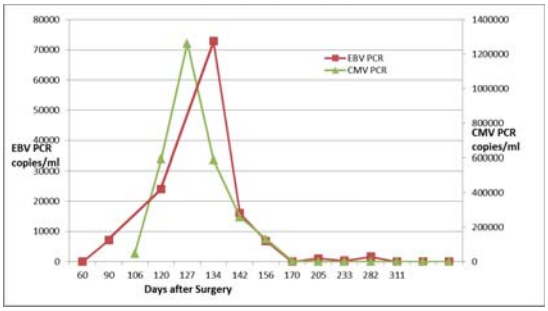
Clinical History:
A 21 year old male received a kidney transplant for advanced chronic kidney disease (CKD) stage 5 from an unrelated non-directed living donor. His renal disease was suspected to be from juvenile nephronophthisis, a part of Sensenbrenner syndrome, as the patient had multiple skeletal abnormalities including plagiocephaly, hemifacial microsomia, torticollis, and scoliosis in addition to CKD. He was CMV and EBV seronegative prior to transplant. The donor was positive for CMV IgG antibodies (Ab), EBV IgG Ab to Viral Capsid Antigen, and EBV Ab to Nuclear Antigen and negative for CMV IgM Ab, EBV Heterophile Ab and EBV IgM Viral Capsid Antigen. These results were indicative of a past donor infection with both CMV and EBV. The recipient was induced with basiliximab and after an uneventful post-operative course was discharged on tacrolimus, mycophenolate mofetil and prednisone. He attained a baseline creatinine ranging from 1.0-1.2 mg/dl (estimated GFR 85.9 – 107.1 ml/m/1.73m^2 – CKD Epidemiology Collaboration). He received valganciclovir 450 mg daily for CMV prophylaxis with assessment for CMV viremia if he became symptomatic. Due to the high risk for a primary EBV infection he was also screened with periodic plasma EBV PCR assays to detect the development of viremia. He tested EBV negative at 2 months, but at 3 months he had 7100 copies/ml of EBV in plasma. Although he was asymptomatic initially, 10 days after the detection of viremia he developed complaints of malaise, cough, sore throat and bleeding gums. He was seen by his local physician and because of noted leukopenia was tested for CMV viremia. He had 46949 copies/ml of CMV in plasma and was admitted to hospital where he complained of a 10 lb weight loss and fevers. On examination he was febrile and tachycardic with bilaterally enlarged tonsils. No other lymph nodes were palpable in the cervical, axillary or inguinal areas. There was no hepatosplenomegaly. He was leukopenic with a white blood cell count (WBC) of 3100/microliter and lymphopenic on differential count. His creatinine was elevated at 1.6 mg/dl, liver functions test were normal and lactate dehydrogenase elevated

to 453 U/L (normal 135-225 U/L). His mycophenolate mofetil was discontinued and he was started on intravenous (IV) ganciclovir for the CMV infection. EBV and CMV quantitative PCR checked six days following the prior CMV level revealed increasing viremia with EBV measuring 24,000 copies/ml and CMV 594,000 copies/ml. Despite initiating IV ganciclovir he continued to have high spiking fevers, a poor appetite, and ongoing weight loss. His CMV viral load increased to 1,263,000 copies/ml. He was tested for CMV resistance and was negative for UL97 and UL54 gene targets. IV Immunoglobulin was added to treat the ongoing CMV infection. His CMV PCR and EBV PCR eventually began decreasing. Yet his symptoms of fevers, night sweats, and poor appetite were still present and he developed dysphagia from his enlarging tonsils. 13 days after admission he underwent tonsillectomy. He was diagnosed with simultaneous EBV and CMV tonsillitis, along with early lesion plasmacytic hyperplasia-type PTLD. Following this his tacrolimus was also discontinued. He underwent a bone marrow biopsy and a PET scan to stage his PTLD. The BM biopsy showed no evidence of lymphoma and the PET scan was unremarkable. He was discharged after a total hospital stay of 21 days. He continued IV ganciclovir until his CMV PCR became undetectable 5 weeks from his initial presentation and then he received valganciclovir orally for 3 months. His CMV IgG, EBV VCA IgG and EBNA antibodies tested positive about one month from his initial presentation. His fevers abated at about 6 weeks and EBV PCR became consistently undetectable at 4 months after his initial viremia (see graph below). He was then started on sirolimus and prednisone for immunosuppression, as there is evidence that adult kidney transplant recipients who receive an mTOR inhibitor have a lower risk of developing CMV infection (1,2). He is now 3.5 years from his transplant; he is asymptomatic with consistently undetectable CMV in the blood and periodic low level EBV viremia (<7000 copies/mL).



References
1. Havenith SH, Yong SL, van Donselaar-van der Pant KA, van Lier RA, ten Berge IJ, Bemelman FJ. Everolimus-treated renal transplant recipients have a more robust CMV-specific CD8+ T-cell response compared with cyclosporine- or mycophenolate-treated patients. Transplantation. 2013;95(1):184-91.
2. Nashan B, Gaston R, Emery V, Saemann MD, Mueller NJ, Couzi L, et al. Review of cytomegalovirus infection findings with mammalian target of rapamycin inhibitor-based immunosuppressive therapy in de novo renal transplant recipients. Transplantation. 2012;93(11):1075-85.

Biopsy Fixation Details:
Formalin fixation

Description of Clinical Image if Any:
none

Details of Microscopic Findings:

Histology of the tonsils showed only a few follicles with germinal centers and marked expansion of the interfollicular area (slide 1, arrow on a rare follicle with germinal center). Portions of the interfollicular area showed sheets of plasma cells (slide 2). Other portions of the interfollicular area showed predominantly small lymphocytes. Scattered large cells with nuclear and cytoplasmic inclusions were seen near small vessels (slides 9 and 10).

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

Flow cytometry was not performed on the tonsil. Flow cytometry on the subsequent bone marrow biopsy showed no immunophenotypic evidence of a non-Hodgkin lymphoma.

Immunohistochemistry was performed on the tonsil. Portions of the interfollicular area showed sheets of plasma cells, as highlighted by CD138 (slides 3 and 4). The plasma cells were polytypic with stains for kappa and lambda (slide 5). In situ hybridization for EBV-encoded RNA (EBER ISH) was positive in the areas with plasma cell expansion (Slide 6). Portions of the interfollicular areas which were not rich in plasma cells showed a mixture of CD20+ B lymphocytes and CD3+ T lymphocytes (slide 8). CMV immunohistochemical stain was positive in the large cells containing inclusions (slide 11).

Special Stains:
EBER ISH. (See Immunohistochemistry section).

Cytogenetics:

none

Molecular Analysis:

CMV resistance testing and PCR for EBV and CMV (see Clinical History)

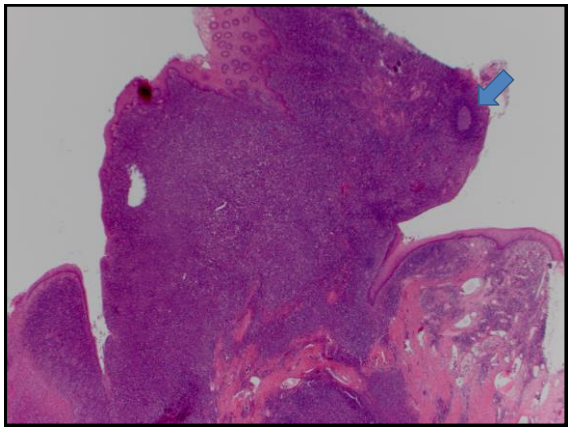
Interesting Feature(s) of Submitted Case:

Our case highlights the complicated, protracted course of simultaneous CMV and EBV infection in the post-transplant setting. It is likely that both infections contributed to the development of this patient's post transplant lymphoproliferative disorder. The presence of these three processes (PTLD – plasmacytic hyperplasia, EBV infection, and CMV infection) together is unusual. The relationship between EBV and PTLD is well recognized; however, the relationship between CMV and PTLD is less clear. It is difficult to determine the inciting infection in our case but with discontinuation of immunosuppression and treatment of CMV disease, his symptoms improved and viremia for both viruses subsequently resolved.

Proposed Diagnosis:

Post-transplant lymphoproliferative disorder, early lesion, plasmacytic hyperplasia type, EBV positive and CMV positive.

Comments:



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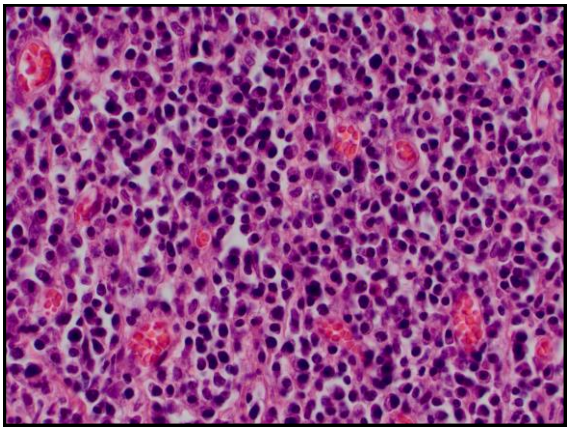

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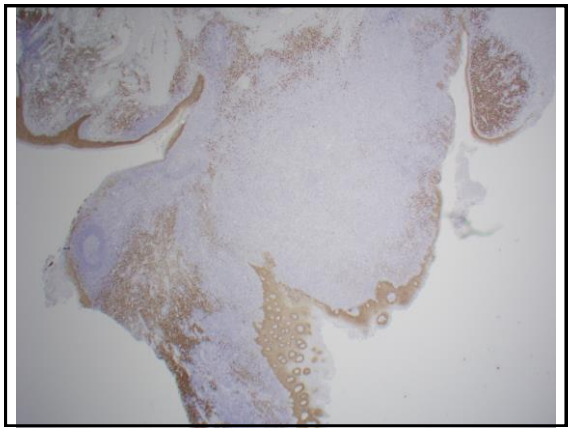

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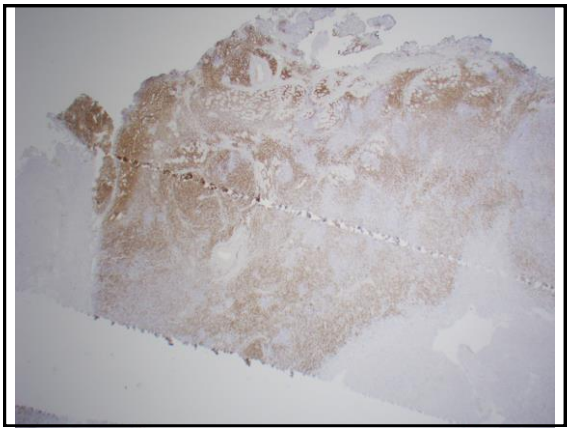

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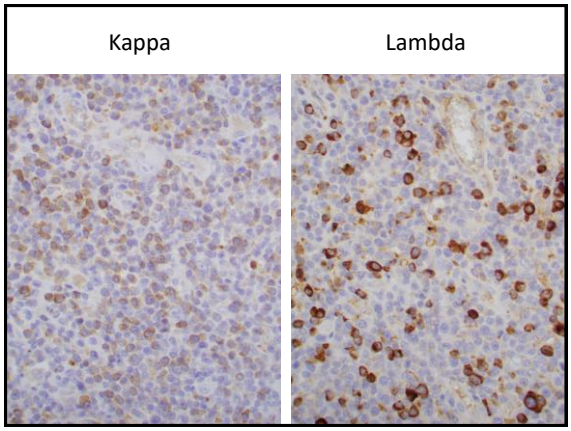

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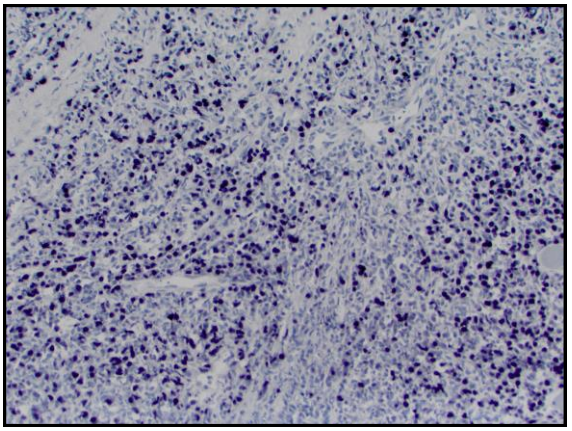

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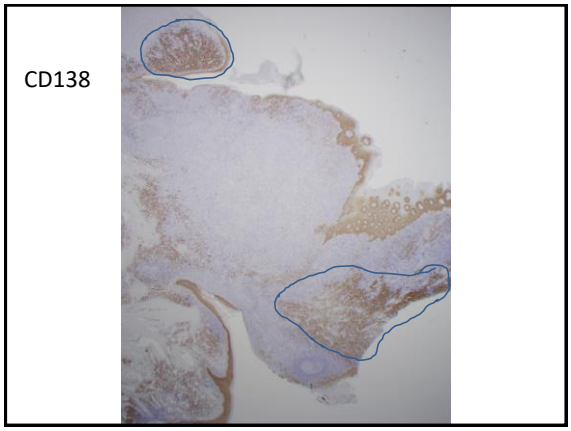

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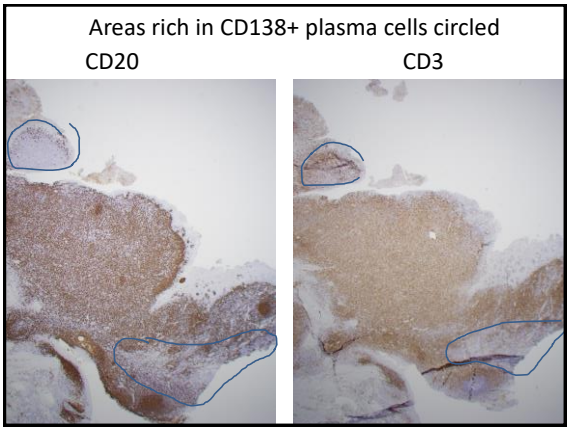

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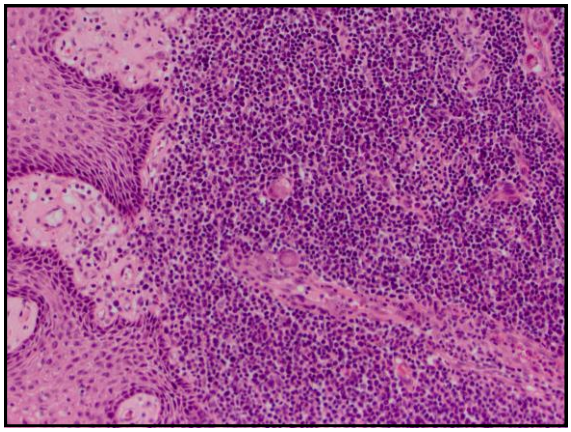

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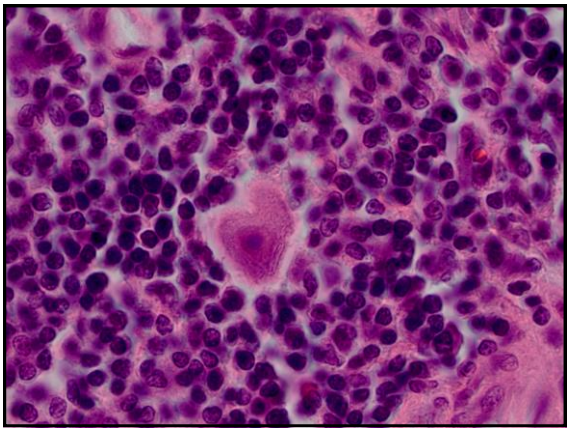

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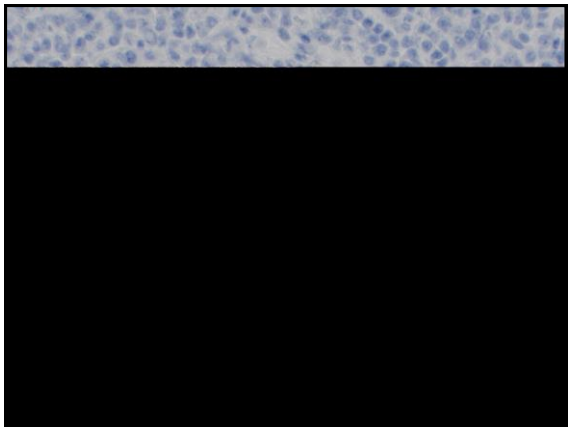

11

Type 2

EBV-related lymphomas (excluding PTLD)

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Yu Li MD PhD

Affiliation:
Chongqing University Affiliated Cancer Hospital & Chongqing Cancer Institute & Chongqing Cancer Hospital

E-mail:
liyu100@163.com

Clinical History:

- 47-year-old female
- Neck masses were found for more than half a year and increased for 2 months. Not accompanied by chills, fever, night sweat, hoarseness and other symptoms, weight loss of 4.5kg.
- Left and right thyroid masses were excised in the outer hospital.
- Complete blood cell counts: RBC 4.87×10^{12}/L, WBC 5.11×10^{9}/L, lymphocyte percentage 28.80%(20-50%), monocyte percentage 6.10%(3-10%), neutrophil percentage 63.30%(40-75%), PLT 215.0×10^{9}/L.
- Blood immune function test: Counts of B cell and NK cell decline
- Human immunodeficiency virus antibody /P24 antigen (HIV ag/ab) negative (-)
- EBV-DNA (postoperative) 3.11E+02 copies/ml (< 5.00e+02)
- EBV EAIgG ↑ 2.26 AU/ml (0-2.00) EBV EAIgA 0.31 AU/ml (0-3.00)
  EBV VCAIgG ↑ >50.00 AU/ml(0-2.00) EBV VCAIgM 0.53 AU/ml (0-3.00)
  EBV VCAIgA 1.58 AU/ml (0-4.00) EBV NAIgG 0.06 AU/ml (0-2.00)

Biopsy Fixation Details: formalin

Description of Clinical Image if Any:
Ultrasound (in the outer hospital): Multiple solid lesions in the thyroid gland, the largest of which was 31.4mm×14.8mm.

Ultrasound examination (postoperative): hypoechoic areas of the middle and lower right neck, right anterior part of neck trachea, right supraclavicular, suprasternal fossa and right subclavian lymph node, with a larger size of about 6×15mm which located in the right neck III area, with reduced cortical echo and disappearance of the portal. CDFI: abundant blood flow signals can be seen inside.

Details of Microscopic Findings: The tissues submitted for examination showed that most thyroid tissues were destroyed and multiple foci of necrosis were seen. Around the necrotic foci, numerous patches of atypical lymphoid cells proliferated and infiltrated. Most of the cells were moderately large, with nucleoli and mitotic figures visible. Lymphoid cells in peripheral tissues are moderately small and of relatively uniform size, showing lymphoepithelial lesions.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

(Immunophenotyping by Immunohistochemistry
IHC：

CK shows LEL
CD21 showed residual atrophic FDC positive and a large number of cells in nodular areas were positive.
CD19, CD20 and PAX5 diffuse positive (+)
CD10(-)，Bcl-6(-)，MUM-1(-)
CD3 and CD5(-)
CyclinD1(-)，CD30 scattered (+)，CD38(-)，C-myc(10%+)，Bcl-2（80%+）P53(wild type)
Ki-67(large cell 50%-60%，perimeter zone10-20%)

In situ hybridization: EBER positive (>200 cell/HPF).

Special Stains: Not done.

Cytogenetics: Not done.

Molecular Analysis: BCR arrangement---IgH(FR2-JH) monoclonal, Ig κ (V κ -J κ) monoclonal

Interesting Feature(s) of Submitted Case: EBV+ low-grade B-cell lymphomas, in particular, EBV+ MALToma, are rare. Histologic features resemble those of typical MALT lymphomas in most cases shows greater prevalence with immune deficiency. Clonal by BCR,EBV Latency I(most LMP1 negative). Clinical behavior similar to other forms of EMZL lymphoma or PTLD Respond to withdraw of immune suppression, Rituximab. This case received 2 cycles of chemotherapy with R-CHOP regimen. Enlarged cervical lymph nodes were reduced and follow-up is continuing at present.

Proposed Diagnosis: EBV+ DLBCL(non-GCB)with EBV+ MALToma in thyroid

Comments:

The 1st Joint Chinese Society for Hematopathology and Society for Hematopathology Workshop/CSHP 2019

EBV+ DLBCL(non-GCB)with EBV+ MALToma in thyroid

Case report

Yu Li, Fang Liu

Department of Pathology, Chongqing University Affiliated Cancer Hospital

Chongqing University Affiliated Cancer Hospital, Chongqing Cancer Institute, Chongqing Cancer Hospital, Chongqing Cancer Center

1

Clinical History

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- Ultrasound (in the outer hospital): Multiple solid lesions in the thyroid gland, the largest of which was 31.4mm*14.8mm.
- Left and right thyroid masses were excised in the outer hospital.
- Complete blood cell counts: RBC 4.87×1012/L, WBC 5.11×109/L, lymphocyte percentage 28.80%(20-50%), monocyte percentage 6.10%(3-10%), neutrophil percentage 63.30%(40-75%), PLT 215.0× 109/L.
- Blood immune function test: Counts of B cell and NK cell decline
- Human immunodeficiency virus antibody /P24 antigen (HIV ag/ab) negative (-)
- EBV-DNA (postoperative) 3.11E+02 copies/ml (< 5.00e+02)
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  EBV VCAIgG ↑ >50.00 AU/ml(0-2.00) EBV VCAIgM 0.53 AU/ml (0-3.00)
  EBV VCAIgA 1.58 AU/ml (0-4.00) EBV NAIgG 0.06 AU/ml (0-2.00)

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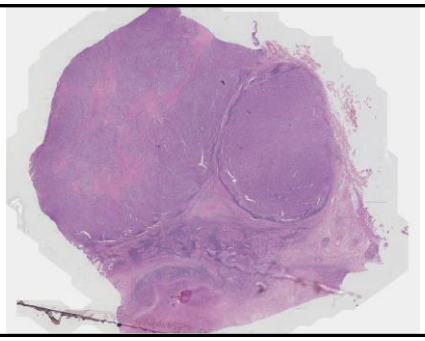

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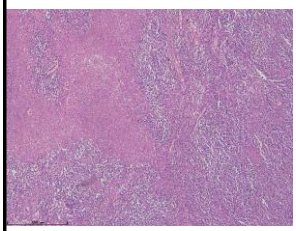
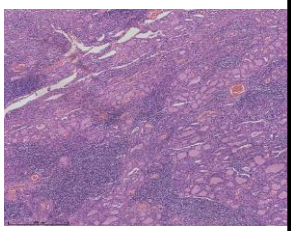
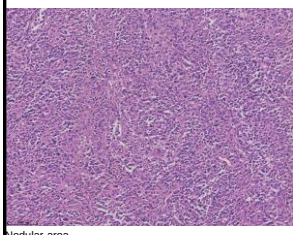


Nodular area

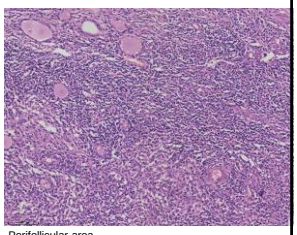


Perifollicular area

4



Nodular area



Perifollicular area

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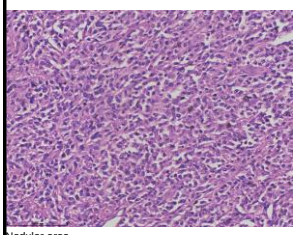

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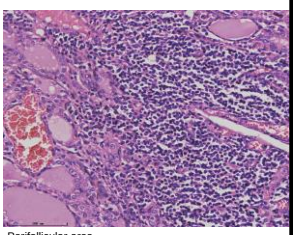

Perifollicular area

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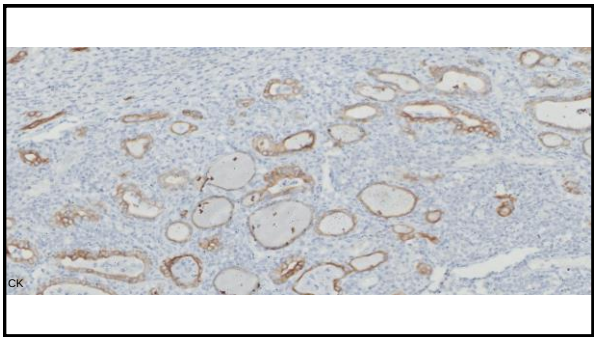
CK

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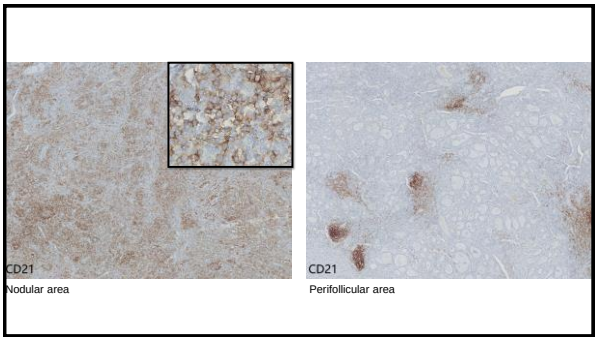
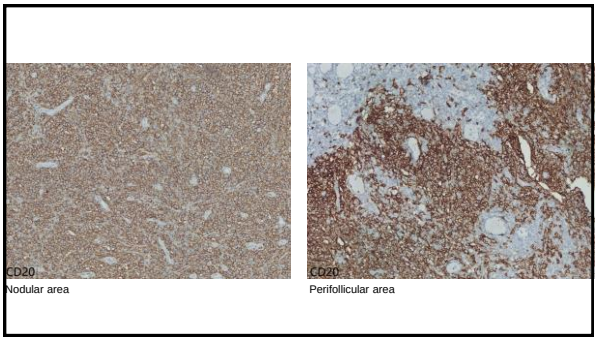
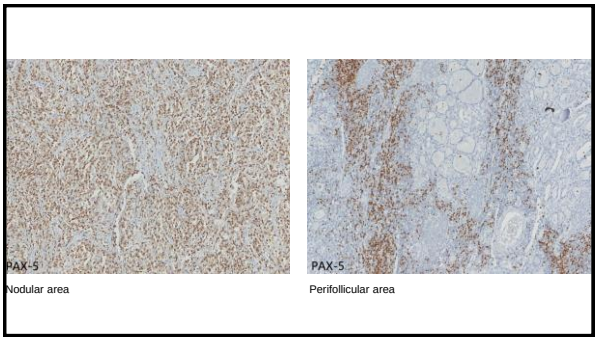

CD21
Nodular area
CD21
Perifollicular area

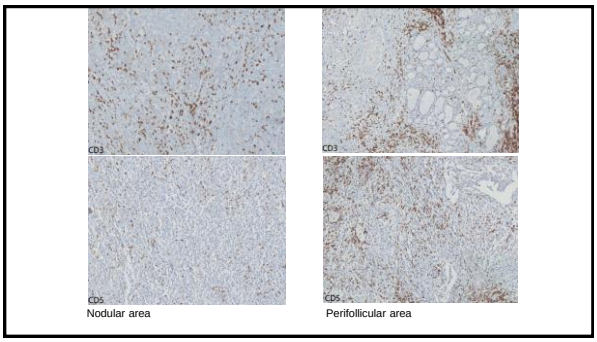
8


CD20
Nodular area
CD20
Perifollicular area

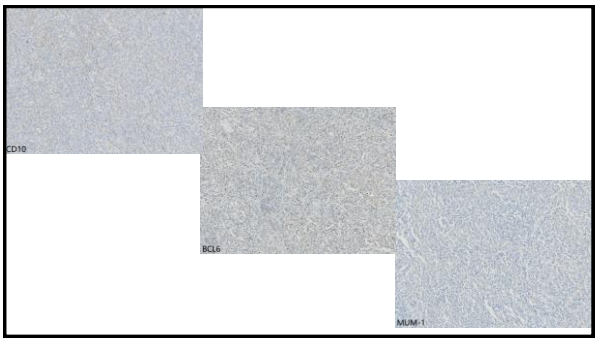
9


PAX-5
Nodular area
PAX-5
Perifollicular area

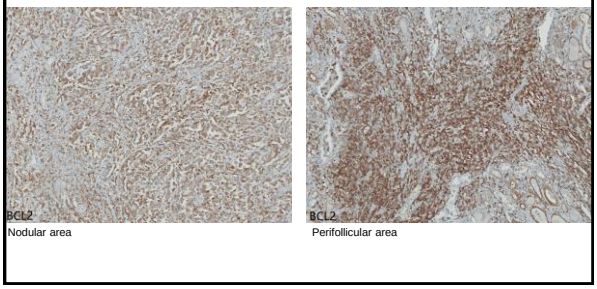
10


CD3
CD3
CD5
CD5
Nodular area
Perifollicular area

11


CD10
BCL6
MUM-1

12



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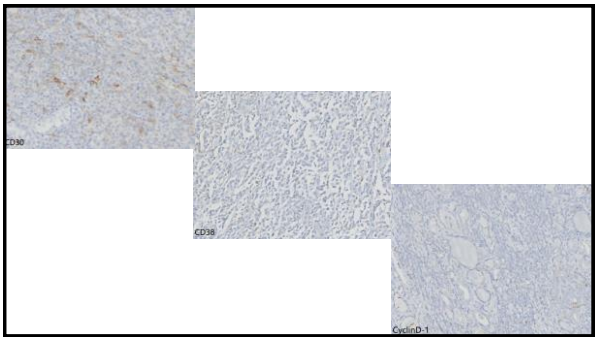

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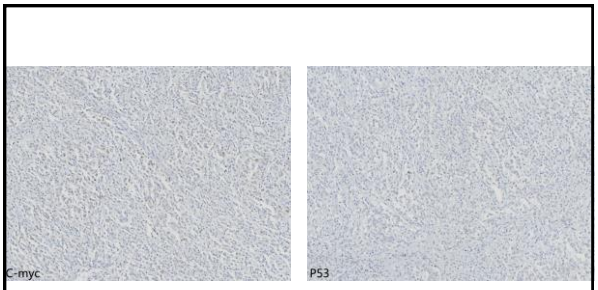

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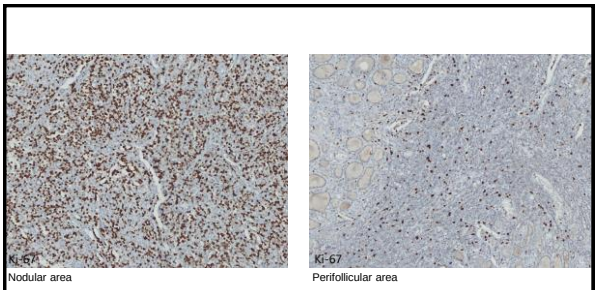

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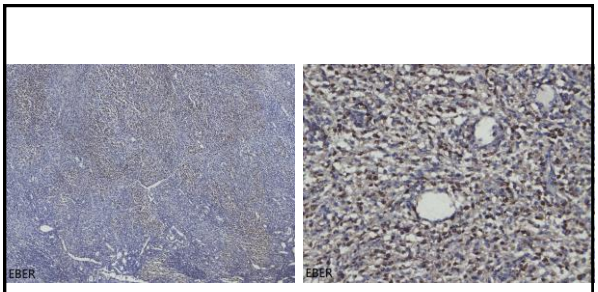


17

- IHC:
  - CK shows LEL
  - CD21 showed residual atrophic FDC positive and a large number of cells in nodular areas were positive.
  - CD19, CD20 and PAX5 diffuse positive (+)
  - CD10(-), Bcl-6(-), MUM-1(-)
  - CD3 and CD5(-)
  - CyclinD1(-), CD30 scattered(+), CD38(-), C-myc(10%+), Bcl-2 (80%+) P53(wild type)
  - Ki-67(large cell 50%-60%, perimeter zone10-20%)
- ISH: EBER + (>200 cell/HPF)
- BCR arrangement: IgH(FR2-JH) monoclonal, Igκ(Vκ-Jκ) monoclonal

18

Diagnosis

EBV+ DLBCL(non-GCB)with EBV+ MALToma in thyroid

19

Interesting Features of Submitted Case

- EBV+ low-grade B-cell lymphomas, in particular, EBV+ MALToma, are rare *(Am J Surg Pathol 42:1306;2018)*
- Histologic features resemble those of typical MALT lymphomas in most cases
- Shows greater prevalence with immune deficiency
- Clonal by BCR,EBV Latency I
- Clinical behavior similar to other forms of EMZL lymphoma or PTLD
- Respond to withdraw of immune suppression, Rituximab

(This case received 2 cycles of chemotherapy with R-CHOP regimen. Enlarged cervical lymph nodes were reduced and follow-up is continuing at present.)

20

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Miaoxia He, MD PhD.

Affiliation:
Department of pathology, Changhai Hospital, Shanghai, The Second Military Medical University, China 200433

E-mail:
hmm26@163.com

Clinical History:
A 19-year-old Chinese female medical student presented with high fever and elevated bilirubin for 3 days accompanied by decreased white blood cell count and platelets. Her temperature ranged from 38.5 degrees Centigrade to 41.1 degrees Centigrade. CBC showed a WBC count of 2.82, hemoglobin 12.3, platelets 90, and C-reactive protein 40.82. Antibiotics were started but it failed to relieve her symptoms.

Three days later, the patient's physical condition deteriorated rapidly. The patient presented with dyspnea and her oxygen saturation was around 85%. The morphology of the bone marrow aspiration showed mild morbidity of the granulocyte and erythrocytic series. Small numbers of abnormal cell are present in the marrow. An increased proportion of neutrophils with nuclear shift to left were observed in the peripheral blood.

EBV IgM antibody was positive and EBV DNA copy number was 2.9 into 10 to the power of 5 per ml.

Then, she had a little bitter enlargement of bilateral cervical and axillary lymph nodes. She also was found splenomegaly by B ultrasound.

Biopsy Fixation Details:
The patient underwent a right cervical lymph node biopsy. The specimen was cut as slides then fixed in 10% neutral buffered formalin about 12 hours.

Description of Clinical Image if Any:
CT revealed inflammation of the right upper lobe and lower lobe and a small amount of pleural effusion on both sides.

Details of Microscopic Findings:
The lymph node reveals a partly effaced nodal architecture by a diffuse atypical lymphoid proliferation. Frequent mitotic figures are seen. There are scattered admixed histiocytes.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
Flow cytometry analysis of Bone marrow showed about 6% of abnormal phenotypic lymphocytes (SSC high, CD3 positive, CD8 positive, CCD3 positive, CD2 positive positive, CD94 positive, HLA-DR positive, TCR alpha beta positive, CD4 negative, CD5 negative, CD7 negative, CD16 negative, CD25 negative, CD30 negative, CD56 negative,

CD57 positive, and TCR, delta negative). The distinctive possibility of the CD8 positive T lymphocyte proliferative disease was considered by the bone marrow analysis.

Immunohistochemistry and in situ hybridization Results of lymph node showed EBER was diffusely positive in the vast majority of the lymphoid cells. The cells have a mature T-cell phenotype and are positive for CD3, CD2, CD8, largely negative for CD5. They have a cytotoxic phenotype, positive for Gran B, TIA1, focally for perforin. KI-67 shows a high proliferative rate. Hemophagocytosis is highlighted with phagocytic vacuoles in histiocytes by the immunostains. P53 is negative. Many of the T-cells are positive for TCR gamma, but Beta F1 is positive in many cells as well. Thus, a derivation from alpha beta vs. gamma delta T-cells is difficult to conclude. CD56 stain is negative and PD-1 is negative.

Special Stains:
EBER is diffusely positive in the vast majority of the lymphoid cells by EBER in situ hybridization.

Cytogenetics:
Chromosomes 46,XX.

Molecular Analysis:
T cell receptor gene rearrangements showed TCR beta, V beta-J beta positive, TCR beta D beta-J beta positive, TCR beta V, 1FTCR10-J, positive in bone marrow liquid by PCR.

No gene mutation of hemophagocytic lymphohistiocytosis was found.

Clonal rearrangement pattern of TRG was detected in lymph node by PCR.

Interesting Feature(s) of Submitted Case:
This is a typical case of Systemic EBV-positive T-cell Lymphoma of childhood. But the patient has a favorable outcome.

Proposed Diagnosis:
Hemophagocytic lymphohistiocytosis with an EBV-positive T-cell proliferation consistent with Systemic EBV-positive T-cell Lymphoma of childhood

Comments:
Based on diffuse EBV-positivity of the atypical T-cell infiltrate, and CD56 is negative. PCR studies identified a clonal T-cell population, further supporting the T-cell, rather than NK-cell, origin of this lesion. These results are consistent with the presence of a significant clonal T-cell population and the pathologic diagnosis of Systemic EBV-positive T-cell Lymphoma of childhood.

19 Y/O. Female

Right cervical lymph node

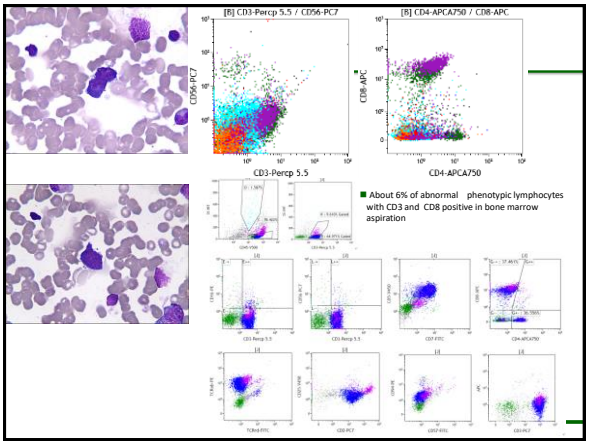
Department of pathology, Changhai hospital
Miaoxia He，MD., Ph.D.

1

Clinical History

- High fever for 3 days
- Bilirubin elevated
- White blood cell count and platelets decreased
- Bone marrow aspiration showed small number of abnormal cells
- Flow cytometry analysis showed about 6% of abnormal phenotypic lymphocytes with CD3 and CD8 positive
- EBV IgM antibody was positive and EBV DNA copy number was 2.9 into 10 to the power of 5 per ml
- B ultrasound: Bilateral cervical and axillary lymph nodes enlarged, Splenomegaly

2



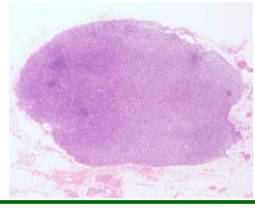
3

Right Cervical LN

- Gross manifestation

  Right cervical LN is 1×0.8×0.8cm in size

  The cut surface is red and soft with capsule
- Morphology



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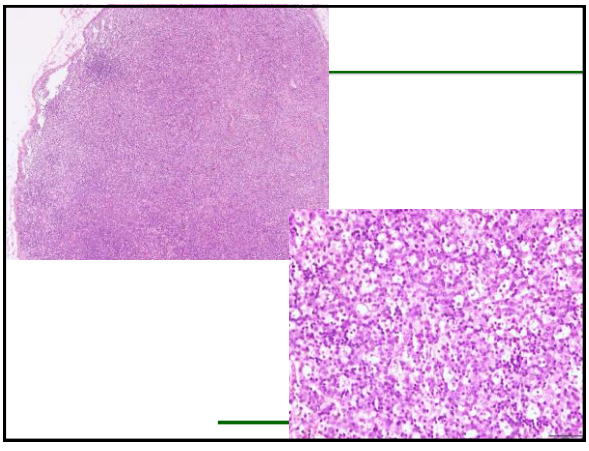

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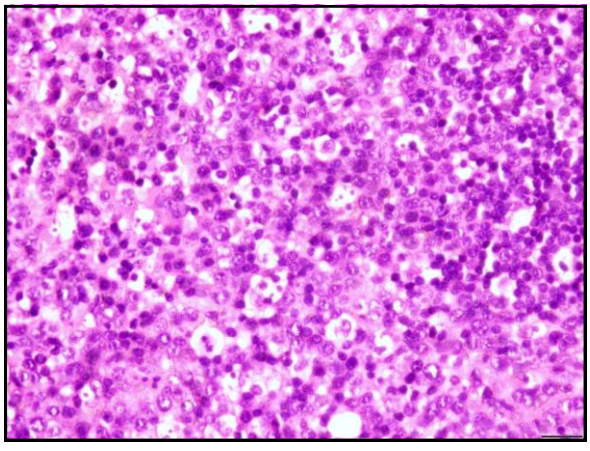

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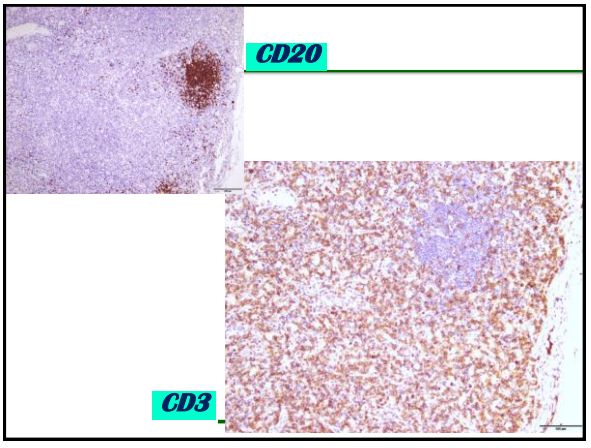
CD20
CD3

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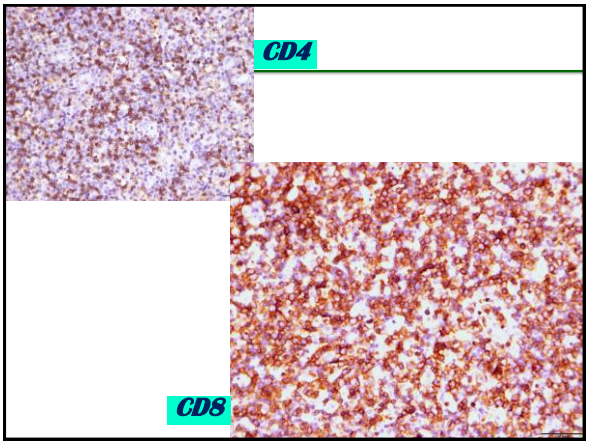
CD4
CD8

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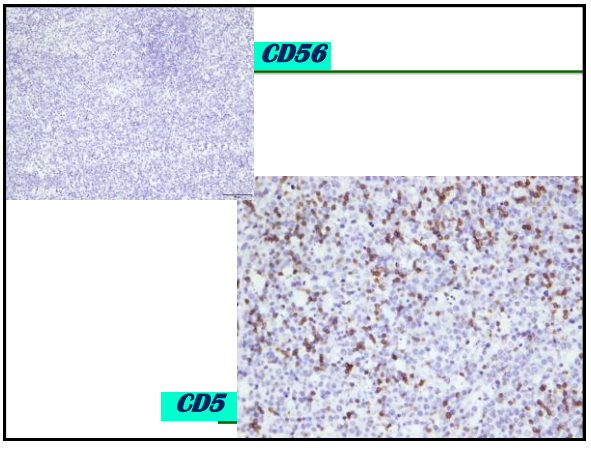
CD56
CD5

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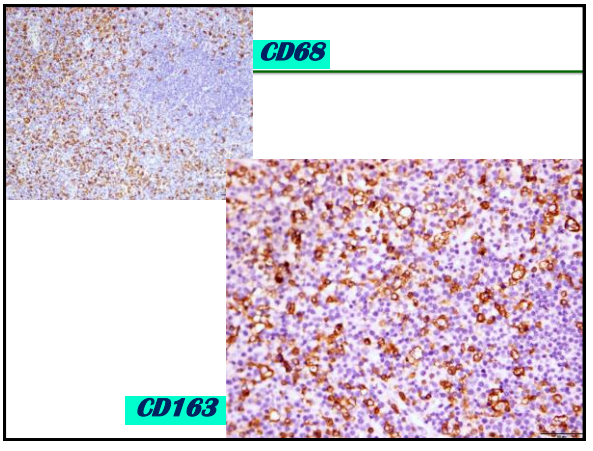
CD68
CD163

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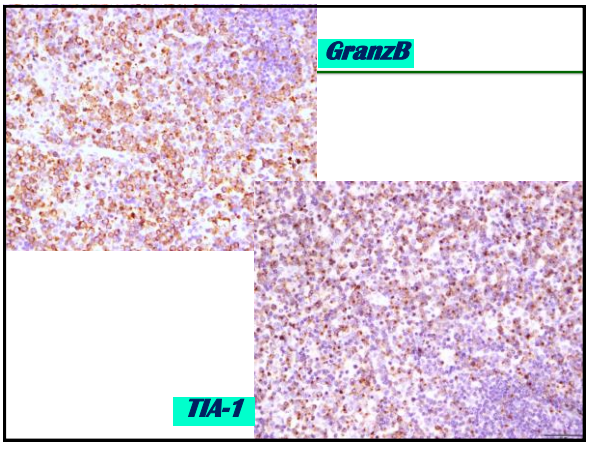
GranzB
TIA-1

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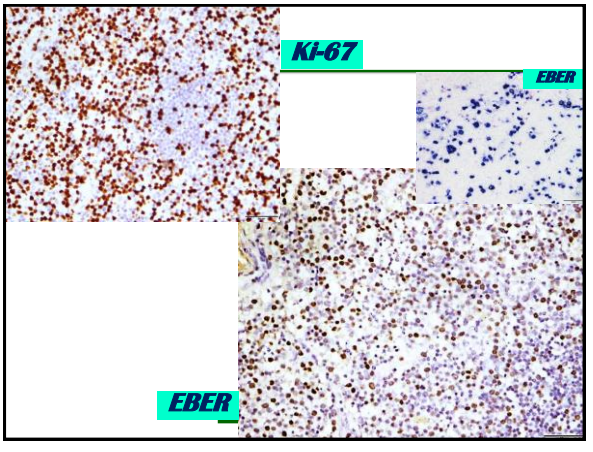
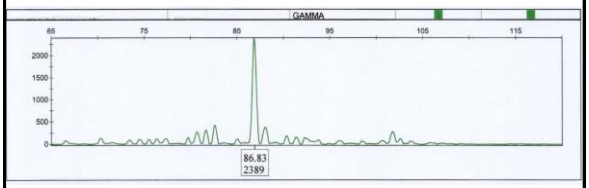

Ki-67
EBER
EBER

12

TCR gene rearrangement

TCRG gene with significant clonal rearrangement



13

Summary of results

- Mature T-cell phenotype: CD3+, CD2+, CD8+, CD5-
- Cytotoxic phenotype: GranzB+, TIA1+
- Ki-67 : High proliferative rate
- TCR Gamma +, BF1 +
- CD56-,PD-1-, P53-
- CD20-, CD79a-,Pax5-
- EBER：Diffuse +
- TCRG gene with significant clonal rearrangement

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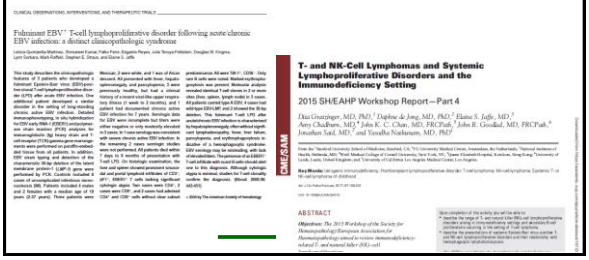
Pathologic Diagnosis

Lymph node, right cervical, excision biopsy :
Hemophagocytic lymphohistiocytosis
with an EBV-positive T-cell proliferation
consistent with ***Systemic EBV-positive T-cell Lymphoma of Childhood***



Fulminant EBV+ T-cell lymphoproliferative disorder following acute/chronic EBV infection: a distinct clinicopathologic syndrome

T- and NK-Cell Lymphomas and Systemic Lymphoproliferative Disorders and the Immunodeficiency Setting

2015 SH/EAHP Workshop Report—Part 4

ONCOGRAM

ABSTRACT

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From www.bloodjournal.org by guest on October 3, 2016. For personal use only.

Review Series

THE UPDATED WHO CLASSIFICATION OF HEMATOLOGICAL MALIGNANCIES

The 2016 revision of the World Health Organization classification of lymphoid neoplasms

Steven H. Swerdlow,[1] Elias Campo,[2] Stefano A. Pileri,[3] Nancy Lee Harris,[4] Harald Stein,[5] Reiner Siebert,[6] Ranjana Advani,[7] Michele Ghielmini,[8] Gilles A. Salles,[9] Andrew D. Zelenetz,[10] and Elaine S. Jaffe[11]

Table 2. Highlights of changes in 2016 WHO classification of lymphoid, histiocytic, and dendritic neoplasms

Entity/category	Change
T-cell large granular lymphocyte leukemia	• New subtypes recognized with clinicopathologic associations. • *STAT3* and *STAT5B* mutations in a subset, latter associated with more clinically aggressive disease.
Systemic EBV+ T-cell lymphoma of childhood	• Name changed from lymphoproliferative disorder to lymphoma due to its fulminant clinical course and desire to clearly distinguish it from chronic active EBV infection.
Hydroa vacciniforme-like lymphoproliferative disorder	• Name changed from lymphoma to lymphoproliferative disorder due to its relationship with chronic active EBV infection and a spectrum in terms of its clinical course.
Enteropathy-associated T-cell lymphoma (EATL)	• Diagnosis only to be used for cases formerly known as type I EATL typically associated with celiac disease.
Monomorphic epitheliotropic intestinal T-cell lymphoma	• Formerly type II EATL segregated from type I EATL and given a new name due to its distinctive nature and lack of association with celiac disease.
Indolent T-cell lymphoproliferative disorder of the GI tract	• New indolent provisional entity with superficial monodonal intestinal T-cell infiltrate, some cases show progression.
Lymphomatoid papulosis	• New subtypes described with similar clinical behavior but atypical histologic/immunophenotypic features.
Primary cutaneous γ δ T-cell lymphoma	• Important to exclude other cutaneous T-cell lymphomas/lymphoproliferative disorders that may also be derived from γ δ T cells such as mycosis fungoides or lymphomatoid papulosis.
Primary cutaneous acral CD8+ T-cell lymphoma	• New indolent provisional entity, originally described as originating in the ear.

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Table 14.01 Classification of EBV-positive T-cell and NK-cell proliferations

Diagnosis	Usual patient age group(s)
EBV-positive haemophagocytic lymphohistiocytosis (benign, may be self-limited)	Paediatric, adolescent
Systemic CAEBV	Paediatric, adolescent
Cutaneous CAEBV, hydroa vacciniforme-like lymphoproliferative disorder	Paediatric, adolescent
Cutaneous CAEBV, severe mosquito bite allergy	Paediatric, adolescent
Systemic EBV-positive T-cell lymphoma	Paediatric, adolescent
Aggressive NK-cell leukaemia	Adult
Extranodal NK/T-cell lymphoma, nasal type	Adult
Nodal peripheral T-cell lymphoma, EBV-positive*	Adult
CAEBV, chronic active EBV infection.	

* Included within the category of peripheral T-cell lymphoma, NOS.

From updated WHO classification

Table 2: HLH-2004 diagnostic criteria [53]

- Genetic defect consistent with HLH **Gene mutation such as PRF1, UNC3D etc**
- **Fulfillment of five of the eight following clinical criteria:**

1. Fever	Temperature > 38.5°C for > 7 days
2. Splenomegaly	Spleen tip palpated > 3 cm below left costal margin
3. Cytopenia	≥ 2 lineages
Hemoglobin	< 90 g/L (neonates < 100 g/L)
Platelets	< 100 ×10⁹/L
Neutrophiles	< 1 ×10⁹/L
4. Hyperferritinemia	> 500 µg/L
5. Hypofibrinogenemia or hypertriglyceridemia	< 1.5 g /L, or > 3 mmol/L
6. Elevated soluble CD25	> 2,400 U/mL
7. Hemophagocytosis	Bone marrow, spleen, liver, lymph node or other tissues
8. Reduced or absent NK cytotoxicity	

Supportive evidence
Elevated transaminases and bilirubin
Elevated lactate dehydrogenase
Elevated d-dimers
Elevated cerebrospinal fluid cells and/or protein
NK: natural killer cells.

Pediatr Blood Cancer 2007;48:124–131

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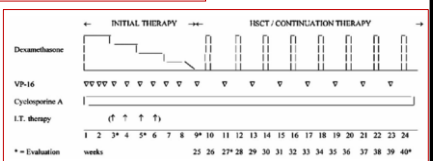
Pediatr Blood Cancer 2007;48:124–131

REVIEW

HLH-2004: Diagnostic and Therapeutic Guidelines for Hemophagocytic Lymphohistiocytosis

Jan-Inge Henter, MD, PhD,[1]* Annacarin Horne, MD,[1] Maurizio Aricó, MD,[2] R. Maarten Egeler, MD, PhD,[3] Alexandra H. Filipovich, MD,[4] Shinsaku Imashuku, MD,[5] Stephan Ladisch, MD,[6] Ann McClain, MD, PhD,[7] David Webb, MD,[8] Jacek Winiarski, MD, PhD,[9] and Gritta Janka, MD, PhD[10] for the Histiocyte Society



中华医学会志 2018 年 1 月 9 日第 98 卷第 2 期 Nat Med J Chin, January 9, 2018, Vol. 98, No. 2 · 91 ·

·标准与规范·

噬血细胞综合征诊治中国专家共识

噬血细胞综合征中国专家联盟 中华医学会儿科学分会血液学组

18

長海醫院 CHANGHAI HOSPITAL

Therapeutic Regimens

- Dex 15mg d1-d3
- VP-16 240mg d1
- CsA 100mg bid

Maintenance treatment

- Anti-virus
- Watch and wait

Follow-up	EBV-DNA
2017/10/2	5.82E+06
2017/10/27	1.32E+05
2017/11/8	2.18E+03
2018/1/3	2.04E+04
2018/2/6	1.33E+04
2018/6/8	1.61E+04
2018/7/20	3.1E+05
2018/8/6	6.56E+05
2018/8/27	1.29E+06
2018/8/31	1.30E+06
2018/9/7	8.10E+04
2018/9/11	9.70E+04
2018/12/12	1.60E+03
2019/03/06	2.30E+02
2019/05/08	negative

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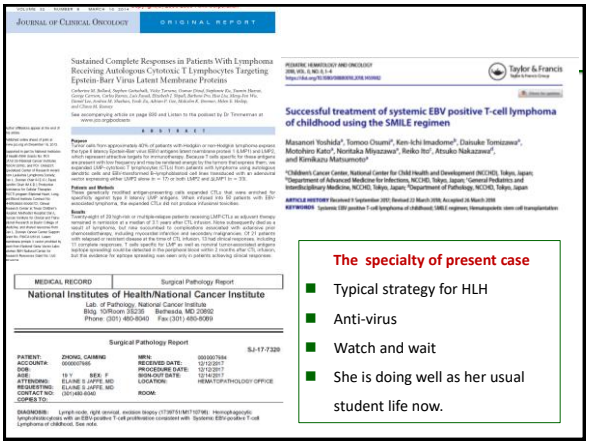
JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Sustained Complete Responses in Patients With Lymphoma Receiving Autologous Cytotoxic T Lymphocytes Targeting Epstein-Barr Virus Latent Membrane Proteins

Successful treatment of systemic EBV positive T-cell lymphoma of childhood using the SMILE regimen

MEDICAL RECORD | Surgical Pathology Report

National Institutes of Health/National Cancer Institute

Lab. of Pathology, National Cancer Institute
Bldg 10/Room 2B236 Bethesda, MD 20892
Phone: (301) 480-8040 Fax (301) 480-8089

Surgical Pathology Report

SJ-17-7320

The specialty of present case

- Typical strategy for HLH
- Anti-virus
- Watch and wait
- She is doing well as her usual student life now.

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長海醫院 CHANGHAI HOSPITAL

Changhai hospital, Navy Military Medical University, Shanghai, China.

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CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):

EBV-positive diffuse large B-cell lymphoma with angioimmunoblastic T-cell lymphoma: a case report.

Yifei Liu MD,Tingting Bian, Jianguo Zhang MD

Affiliation:

Department of the Pathology, Affiliated Hospital of Nantong University

E-mail:

ntdxliuyifei@sina.com

Clinical History:

A 77-year-old male was admitted to our hospital due to continuous fever for more than one month, with a maximum temperature of 38.9 degrees Celsius, cough and white phlegm on November 23, 2018. Chest and abdomen CT showed postoperative changes in the left upper lung, multiple lymph nodes in mediastinum, retroperitoneum, bilateral pelvic wall and inguinal region swellings, abdominal and pelvic effusion. Anti-infective treatment was ineffective, with fever, night sweat and emaciation during the course of the disease. In the past, there was a 40-year history of tuberculosis. Left upper lobectomy was performed seven months ago, and the pathology revealed adenocarcinoma in situ. The patient denied any other previous medical history. Supplementary Examination: PLT 61*109/L, EBV-DNA: 2885 copies/mL (<1000), IgG antibody to EBV capsid antigen: 170 U/ml, IgG antibody to EBV core antigen: 119 U/ml. An evaluation of bone marrow aspirate showed no infiltration of atypical cells. On November 26, 2018, PET/CT showed

that left axillary SUVmax 10.9, intestinal segmental wall thickening and FDG uptake increased significantly, ranging from 5.8*4.5*3.7 cm, SUVmax 20.5.

A left axillary lymph node biopsy demonstrated angioimmunoblastic T-cell lymphoma on November 27. After no treatment, severe abdominal pain broke out on December 12, 2018. Total abdominal CT showed perforation of digestive tract, small intestinal obstruction, abdominal and pelvic effusion, and multiple mild retroperitoneal lymph nodes enlargement. Emergency operation for intestinal perforation was performed. Postoperative pathology showed diffuse large B-cell lymphoma with EBV positive in ileum. R2 chemotherapy regimen was administered at 2018-12-31 after operation: Rituximab 600 mg d0, lenalidomide 25 mg QD 1-10; three courses of R2-miniCHOP regimen chemotherapy were given: Rituximab 600 mg d0, lenalidomide 25 mg QD 1-10, cyclophosphamide 0.6 g d1, doxorubicin liposome 20 mg d1, vinorelbine 30 mg d1, prednisone 60 mg d1-5. EBV-DNA: ＜1000 copies/mL,

IgG antibody to EBV capsid antigen: 129 U/ml, IgG antibody to EBV core antigen: 167 U/ml during follow-up. After four courses of treatment, the patients were generally in good condition, physical recovery and weight gain.

Biopsy Fixation Details:

The specimens were fixed with 10% formalin, routinely treated and embedded in paraffin.

Description of Clinical Image if Any:

No

Details of Microscopic Findings:

Left axillary lymph node biopsy revealed angioimmunoblastic T cell lymphoma. Microscopically, the structure of lymph nodes was destroyed.

In the paracortical area or interfollicular area, various forms of lymphocyte infiltration with small to medium size, transparent cytoplasm, clear membranes and round or irregular nuclei were observed. Eosinophilic granulocytes and plasma cells were mixed, and no medium-large basophilic B-cell mother cells were found. The remaining outer follicles demonstrated prominent proliferation of follicular dendritic cells, which surrounded the high endothelial venules and formed an onion-like FDC network locally.

In left hemicolectomy specimens, the ileal mass is EBV-positive diffuse large B-cell lymphoma. The whole ileal wall was destroyed by diffuse infiltration of atypical lymphoid tissue with obvious coagulation necrosis. Atypical lymphocytes invade nerves and vessels. Atypical cells were medium or large, with round or oval irregular nuclei, rough chromatin, obvious nucleoli, and visible mitosis.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

Immunohistochemical results of needle biopsy of left axillary lymph node: CD3(+), CD5(+), CD4(+), CD8(+),CD7(partial deletion), CD21(FDC net expansion +), Ki67(30%+), PD1(+),CD10(+), Bcl-6(+), CD56(-),CD20(-), CXCL-13 (-), EBER(-). Immunohistochemistry of ileum mass: CD20 (+), CD21 (+), CD10 (+), Bcl-6 (+), MUM1 (+), Ki67 (70%+), EBER (>200/HPF+), PD1 (focus +), CD3 (-), CD8 (-), CD4 (-), CD5 (-), CD7 (-), CXCL13 (-).

Special Stains:
No

Cytogenetics:
No

Molecular Analysis:

TCR gene rearrangement (+) in left axillary lymph nodes.

Interesting Feature(s) of Submitted Case:

Angioimmunoblastic T cell lymphoma and EBV-positive diffuse large B-cell lymphoma occur simultaneously. Lenadomide has a good therapeutic effect.

Proposed Diagnosis:

Angioimmunoblastic T cell lymphoma with EBV-positive diffuse large B-cell lymphoma.

Comments:

Angioimmunoblastic T-cell lymphoma (AITL) is a subtype of peripheral T-cell lymphoma characterized by T-lymphocyte dysplasia with high endothelial vein (HEV) and follicular dendritic cell (FDC), accounting for 1%-2% of non-Hodgkin's lymphoma and 19%-20% of peripheral T-cell lymphoma[1,2]. AITL mainly occurs in the elderly. Its clinical manifestations are superficial lymph node enlargement, fever, rash, hepatosplenomegaly and other systemic symptoms. Some patients show abnormal immune function and polyclonal globulinemia. The prognosis is generally poor and the overall median survival time is less than 3 years. Patients often have severe immune deficiencies, are prone to opportunistic infections, and often die from concurrent infections. Histologically, lymph node structure was destroyed, infiltration of various types of cells, including lymphocytes, granulocytes and plasma cells, and heteromorphic T cells with clear cytoplasm could be seen at the same time. In the vast majority of cases,

significant proliferation of follicular dendritic cells appeared outside the residual follicles, typically enclosing high endothelial venules. There was no abnormal loss of T cell antigen in immunophenotype. CD21 showed the expansion of FDC network, and tumor t-cells presented CXCL13+, PD1+, CD10+, BCL6+ and CD4+. The patient's microscopic morphology and immunophenotype were consistent with that of angioimmunoblastic T-cell lymphoma. Meanwhile, AITL has been proved to be associated with EB virus (EBV) infection, but B cells in AITL showed active viral infection, while tumorous T cells were survived. EB negative was found in a small number of AITL, and the patient had negative immunohistochemistry and no clear medium-large basophilic B-cell under the microscope. The cloned TCR gene rearrangement can be detected in most AILT cases, and molecular genetic analysis has certain practical value for the diagnosis of AILT[3]. Then, the positive TCR rearrangement of this patient further confirmed that the left axillary lymph node was AILT.

Expansion of large B cells with EBV infection in 97% of AITL is considered to be a unique manifestation of potential immune dysfunction in AITL[2]. Although cloned immunoglobulin gene rearrangement can detect up to 20 - 30% of AIL cases [4,5], the amplified B cell components are usually polymorphic and are considered reactive in nature. EBV-related B-cell lymphoma is rare in AITL patients. Only 22 cases of AITL with EBV-related B-cell lymphoma have been reported in the English literature. Among them, 13 cases were intranodal and 9 cases were extranodal. The sites of extranodal involvement included skin, bone marrow, small intestine, soft tissue, lung and nervous system[6-16]. Including this case, DLBCL is the most common subtype of EBV-positive B-cell lymphoma in AITL patients so far.

The patient's left axillary SUVmax 10.9 was shown by PET/CT after consultation. Meanwhile, the intestinal wall of the small intestine was thicker with increased FDG uptake, SUVmax 20.5, and ileal perforation occurred without treatment. The patient was eventually diagnosed as angioimmunoblastic T-cell lymphoma in the left axilla and EBV-positive diffuse large B-cell lymphoma in the ileum. It is worth discussing that the patient had high levels of EBV-DNA in their blood, but EBER in axillary lymph nodes is negative. So can we boldly speculate that ileal EBV+DLBCL is not transformed from AITL? Previous reports mainly focused on the important role of EBV infection in the transformation of AITL into DLBCL after the formation of AITL. In other words, under the action of histocompatibility complex II (MHC II) molecules, EBV-infected B cells can secrete related proteins, such as EBV nuclear antigen-1 (EBNA-1) and latent membrane protein-1 (LMP-1) to T lymphocyte, thereby up-regulating the expression level of CD28 ligand, providing antigen and co-stimulating signal to activate T helper cells and produce CXCL13. CXCL13 also stimulates the activation and proliferation of B lymphocytes, forming a feedback chain[17].The case we studied should be an incongruous lymphoma in different parts of the body under the continuous action of EB virus.

Lenalidomide is an orally active immunomodulatory drug that has direct antineoplastic activity and indirect effects mediated through multiple types of immune cells found in the tumor microenvironment, including B, T, natural killer (NK), and dendritic cells. It stimulates proliferation and activation of NK cells, thereby enhancing NK cell–mediated cytotoxicity and antibody-dependent cellular cytotoxicity[18]. The patient improved the efficacy of the treatment by using lenalidomide in combination.

1

Case Report

Clinical History

- A 77-year-old male was admitted to our hospital due to continuous fever for more than one month, with a maximum temperature of 38.9 degrees Celsius, cough and white phlegm on November 23, 2018.
- Left upper lobectomy was performed seven months ago, and the pathology revealed adenocarcinoma in situ.
- In the past, there was a 40-year history of tuberculosis.

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2

Case Report

Imageological examination

- Chest and abdomen CT: Postoperative changes in the left upper lung, multiple lymph nodes in mediastinum, retroperitoneum, bilateral pelvic wall and inguinal region swellings, abdominal and pelvic effusion.
- PET/CT: Left axillary SUVmax 10.9, intestinal segmental wall thickening and FDG uptake increased significantly, ranging from 5.8*4.5*3.7 cm, SUVmax 20.5.

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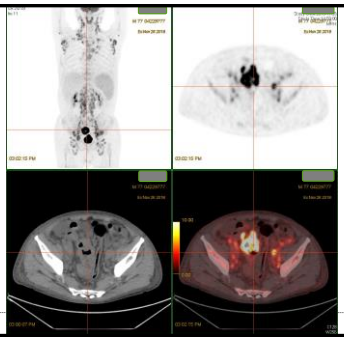
PET/CT



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4

Case Report

EBV virus detection

- EBV-DNA: 2885 copies/mL (<1000)
- IgG antibody to EBV capsid antigen: 170 U/ml
- IgG antibody to EBV core antigen: 119 U/ml.

南通大学附属医院 大医精诚 小宝慈善

5

Case Report

Pathological examination1（2018-11-27）

- A left axillary lymph node biopsy demonstrated angioimmunoblastic T-cell lymphoma.
- Immunohistochemical results of needle biopsy of left axillary lymph node: CD3(+), CD5(+), CD4(+), CD8(+),CD7(partial deletion), CD21(FDC net expansion +), Ki67(30%+), PD1(+),CD10(+), Bcl-6(+)，CD56(-),CD20(-), CXCL-13 (-), EBER(-).
- TCR gene rearrangement (+) in left axillary lymph nodes.

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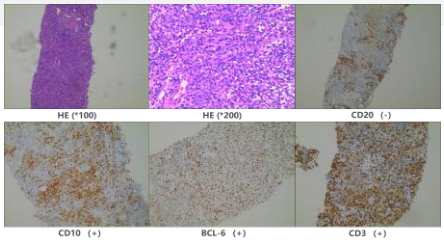
Case Report



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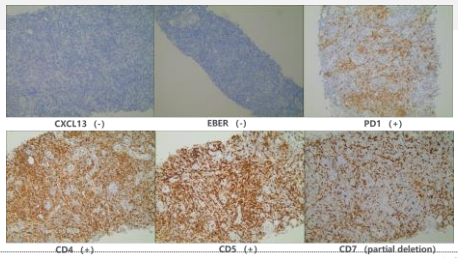
Case Report



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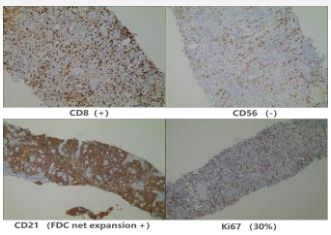
Case Report



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9

Case Report

Disease progression

- After no treatment, severe abdominal pain broke out on December 12, 2018.
- Total abdominal CT showed perforation of digestive tract, small intestinal obstruction, abdominal and pelvic effusion, and multiple mild retroperitoneal lymph nodes enlargement.
- Emergency operation for intestinal perforation was performed.

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Case Report

Pathological examination2（2018-12-12）

- Immunohistochemistry of ileum mass: CD20 (+), CD21 (+), CD10 (+), Bcl-6 (+), MUM1 (+), Ki67 (70%+), EBER (>200/HPF+), PD1 (focus +), CD3 (-), CD8 (-), CD4 (-), CD5 (-), CD7 (-), CXCL13 (-).
- Ileal mass is EBV-positive diffuse large B-cell lymphoma.

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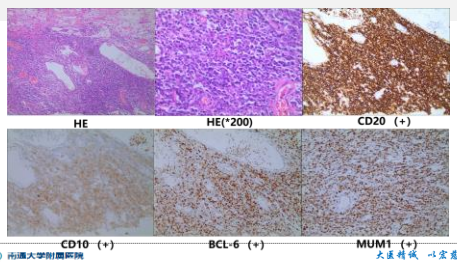
Case Report



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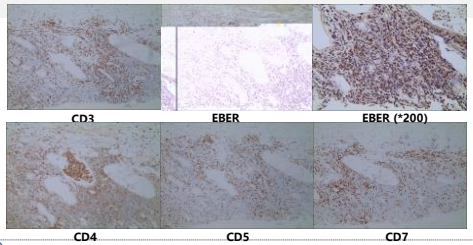
Case Report



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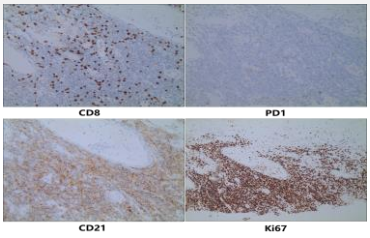
Case Report



14

Case Report

Treatment

- R2 chemotherapy regimen was administered at 2018-12-31 after operation: Rituximab 600 mg d0, lenalidomide 25 mg qd 1-10.
- Three courses of R2-miniCHOP regimen chemotherapy were given: Rituximab 600 mg d0, lenalidomide 25 mg qd 1-10, cyclophosphamide 0.6 g d1, doxorubicin liposome 20 mg d1, vinorelbine 30 mg d1, prednisone 60 mg d1-5.

15

Case Report

Evaluation of curative effect

- The patients were generally in good condition, physical recovery and weight gain.
- EBV-DNA: ＜1000 copies/mL.

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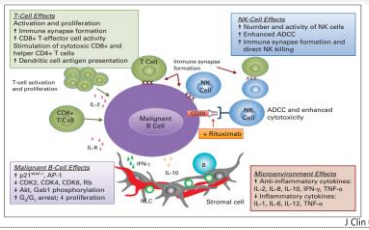
Case Report

Anti-Tumor Mechanism of Lenadomide



J Clin Oncol. 2015; 33(25):2803-11.

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Case Report

Summary

- AITL and DLBCL can exist simultaneously.
- Pathogenesis may be associated with EBV infection.
- The disease progresses rapidly and the prognosis is poor.
- Lenadomide combined therapy can improve the curative effect.

18

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Shanxiang Zhang, MD/PhD

Affiliation:
Indiana University School of Medicine, Indianapolis, Indiana, USA

E-mail:
sz5@iupui.edu

Clinical History:
81 year-old man with history of ear squamous cell carcinoma was found to have enlarged neck lymph nodes during local resection and neck dissection. Subsequent PET/CT revealed generalized lymphadenopathy. Patient was treated with local radiation therapy after resection and four cycles of Rituxan after the current diagnosis of lymphoma. Patient died approximately five months later.

Biopsy Fixation Details:
Resection of the bilateral cervical lymph nodes, which were fixed in neutral buffered formalin overnight.

Description of Clinical Image if Any:
Not applicable.

Details of Microscopic Findings:
The excised lymph nodes showed total nodal architecture effacement by nodular proliferation of predominantly small mature lymphocytes (centrocytes) with morphologically compatible with low-grade follicular lymphoma (FL, accounting for approximately 90% of the tissue sections). However, there were multiple large foci with diffuse proliferation of large atypical cells including frequent very large bizarre cells, morphologically consistent with diffuse large cell lymphoma with anaplastic features (approximately 10%). These large cell areas were clearly demarcated from those of FL.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
Immunohistochemical stains show the large atypical cells were positive for CD45RB (LCA), CD20, PAX-5, CD79a, OCT2, CD10, BCL-6, MUM-1, BCL-2, CD30, p53 and p63. In addition, these large abnormal cells also showed membranous staining for CD3, CD2, CD5, CD7, and CD4 (partial), and CD43. CD15, TCRbeta, TCRgamma, TIA1, granzyme B, perforin, CD56, CD138, myeloperoxidase, EMA, and pan-cytokeratin (AE1/AE3) were negative in these abnormal cells. The FL part was only positive for B-cell markers and BCl-6, BCL-2 with associated CD21-positive follicular dendritic cell meshworks.

EBV study was positive in these large atypical cells but not in FL part by in situ hybridization for EBV-encoded RNAs (EBER).

Flow cytometric analysis showed CD10-positive monoclonal B-cell population with no T-cell lineage markers.

Special Stains:
None

Cytogenetics/FISH:
FISH studies revealed t(14;18) (*IGH*/*BCL2* fusion). There were no rearrangements of *BCL6* and *MYC*. t(8;14) (*MYC*/*IGH* fusion) was negative.

No karyotyping was performed.

Molecular Analysis:
PCR studies using tissue sections enriched for the large cell areas revealed clonal amplification of IGH gene and polyclonal amplification of TCR gamma.

Interesting Feature(s) of Submitted Case:
The current diffuse large B-cell lymphoma (DLBCL), presumably transformed from associated FL, showed multiple unusual features:
1) While FL transforms into DLBCL is relatively common, it is typically not associated with EBV infection.
2) There were multiple very large bizarre tumor cells, suggestive of anaplastic morphology (DLBCL-AF).
3) Most unusually, the large tumor cells showed aberrant expression of multiple T-cell lineage markers including CD2, CD3, CD5, CD7, CD4 (partial) and CD43.

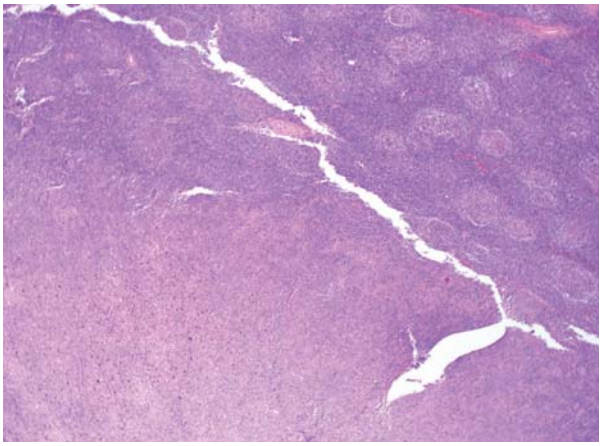
Proposed Diagnosis:
EBV-positive DLBCL with extensive aberrant expression of T-cell markers, 10%
Low-grade FL, 90%.

Comments:
Large B-cell lymphoma with aberrant expression of T-cell markers has been rarely reported with majority of them being EBV-associated lymphoma, such as plasmablasic lymphoma, primary effusion lymphoma, and pyothorax-associated lymphoma. They tended to express one or few T-cell markers except in reported DLBCL-AF. For typical DLBCL, however, most express one or few non-CD3 T-cell markers and are almost all EBV-negative. The current case was almost unique with so many unusual features. It also stresses the importance of performing multiple lineage markers together with molecular studies for both B-cell and T-cell receptor gene rearrangement before we can mark a definitive lineage assignment, which may directly affect patient's management.

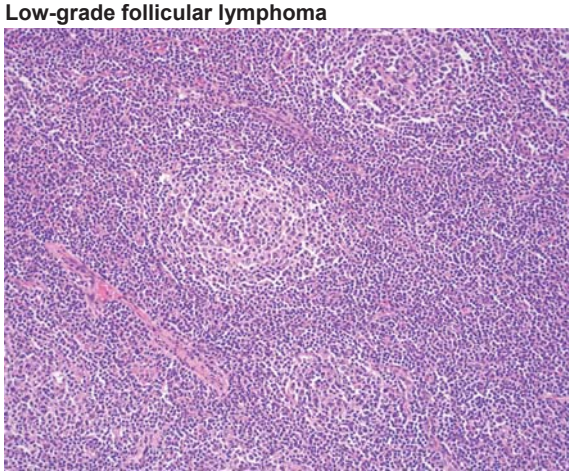
Epstein-Barr virus-positive diffuse large B-cell lymphoma in association with follicular lymphoma with extensive aberrant expression of T-cell markers.

Shanxiang Zhang, MD, PhD
Department of Pathology & Laboratory Medicine
Indiana University School of Medicine
Indiana, USA

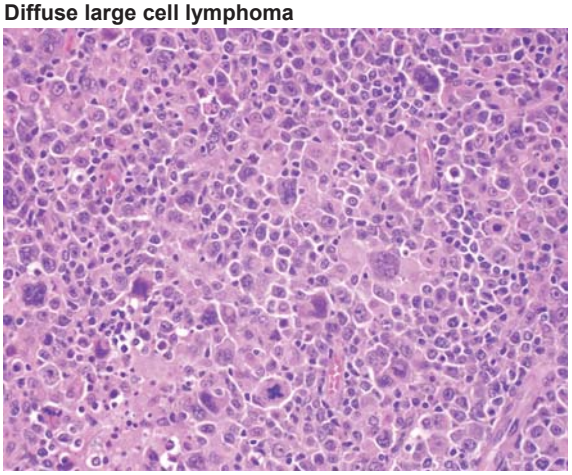
Diffuse large cell lymphoma adjacent to follicular lymphoma



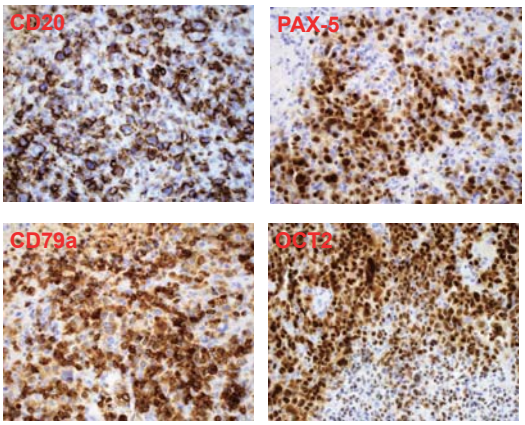
Low-grade follicular lymphoma



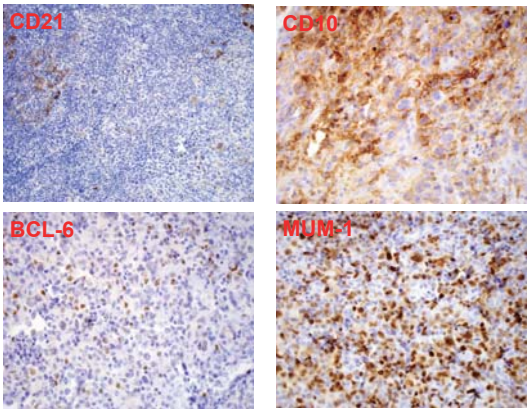
Diffuse large cell lymphoma

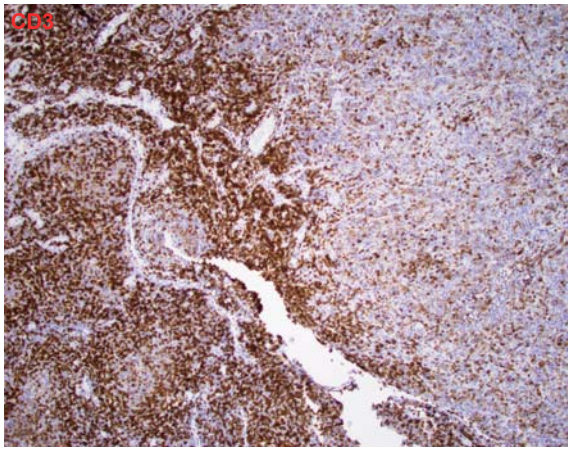


Large atypical cells positive for B-cell markers

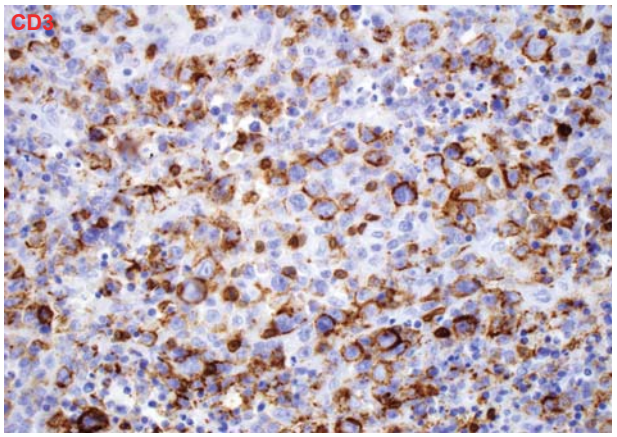


Diffuse large cells

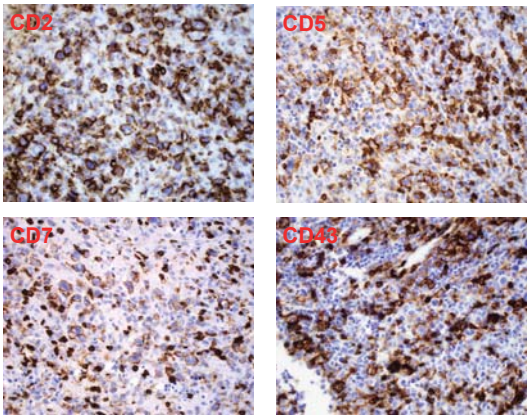




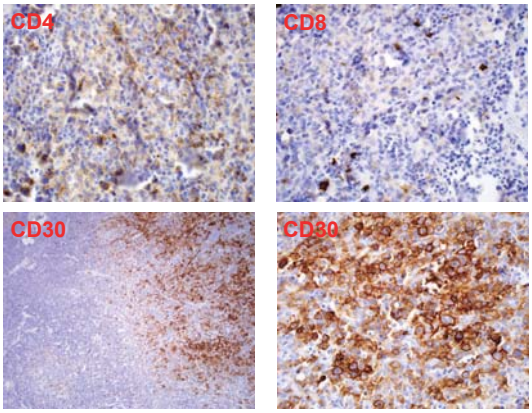
Large atypical cells positive for CD3



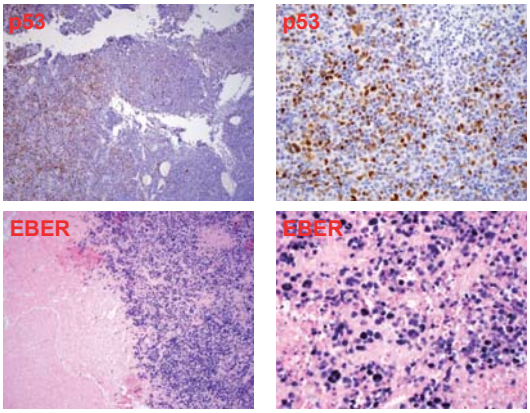
Large atypical cells positive for T-cell markers



Large atypical cells positive for CD30



Large atypical cells positive for p53 and Epstein-Barr virus

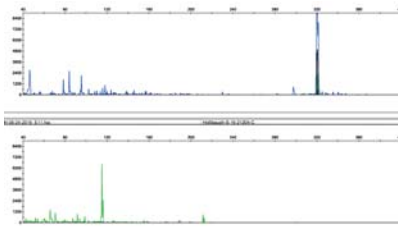


Summary of immunohisotchemical stains of the large atypical cells:

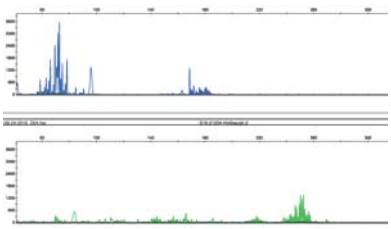
Positive: CD45RB, CD20, PAX-5, OCT2, CD79a, CD10, BCL-6, MUM-1, BCL-2, CD30, CD3, CD2, CD5, CD7, CD4 (partial), CD43, p63, and p53

Negative: CD15, TCRbeta, TCRgamma, TIA1, granzyme B, perforin, CD56, CD138, ALK-1, c-Myc, myeloperoxidase, EMA, and pan-cytokeratin (AE1/AE3)

Epstein-Barr virus: positive by in situ hybridization (EBER).



Clonal amplification of IgH by PCR



Polyclonal amplification of TCR gamma by PCR

The 1st Joint CSHP/SH Workshop and CSHP2019 Case 19-2044

Qilin Ao, Dong Kuang, Yu Hu

Institute of Pathology, Tongji Hospital, Tongji Medical College

Huazhong University of Science and Technology

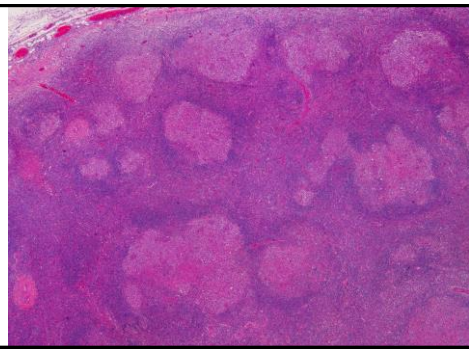
1

Male, 61 years old. The left axillary mass was found for more than 4 months.

Four months ago, the left axillary mass was found, asymptomatic and gradually enlarged, from 0.8cm×0.5cm to about 5 cm×5 cm, soft and unclear boundary.

Laboratory tests showed that positive for hepatitis B, HIV and syphilis.

2



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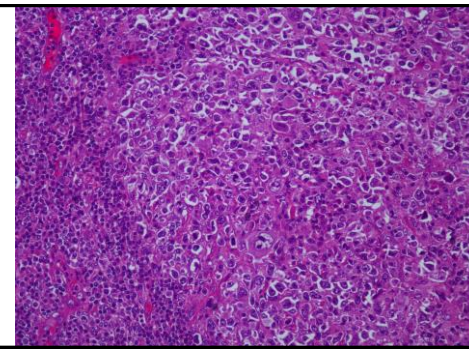

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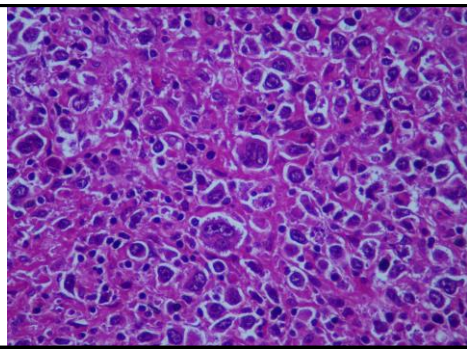

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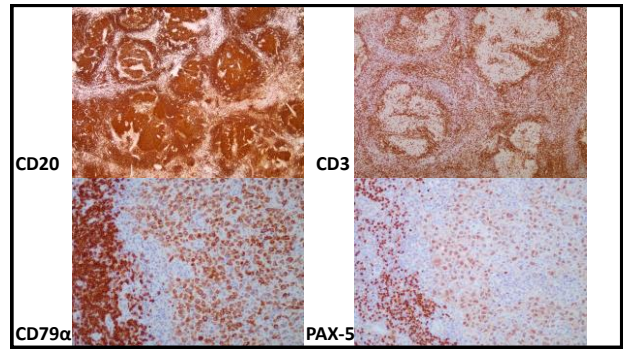

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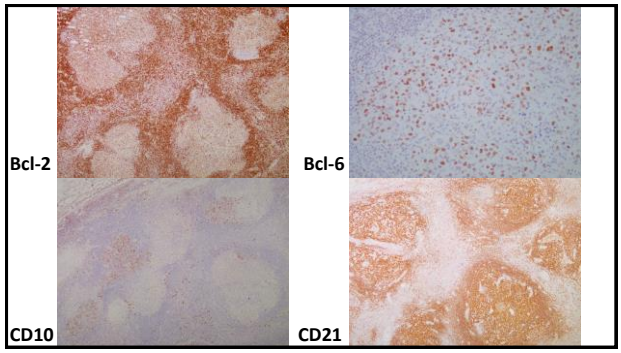

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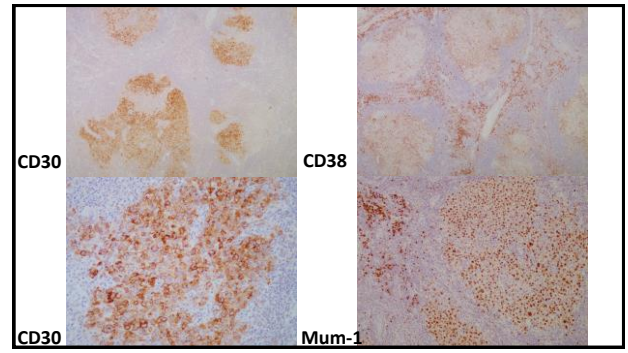

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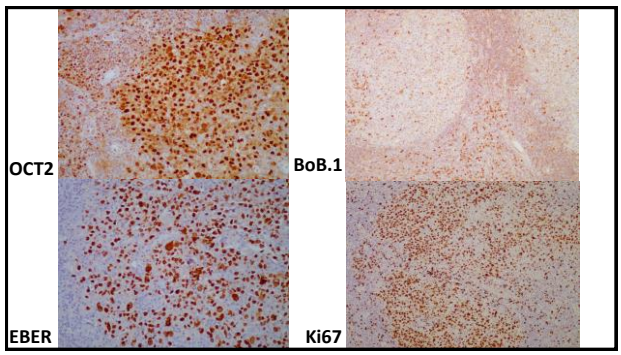


9

华中科技大学同济医学院附属同济医院

Positive:
CD20,CD19,CD79a(weak),PAX5(weak), Bcl-6, Bcl-2(weak),CD30,Mum1,OCT2,Bob.1(scattered), EBER;

Negative:
CD3,CD10,CD21,CD35,CD38,CD138,IgM,HHV8, EBNA2;

Ki-67 LI: 90%.

10

华中科技大学同济医学院附属同济医院

Proposed Diagnosis:

(The left axillary) lymph node EBV positive follicular lymphoma(3B)

11

华中科技大学同济医学院附属同济医院

12

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Shiyong Li, MD, PhD

Affiliation: Emory University School of Medicine

E-mail: sli2@emory.edu

Clinical History: 64-year-old Caucasian female presented to an outside hospital with fatigue and peripheral edema for 3-4 weeks. Complete blood cell count showed pancytopenia. Other laboratory findings included elevated LDH, PT and PTT, and decreased fibrinogen. CMV and HIV was negative. EBV viral load was 1×10^7 IU/ML. Bone marrow biopsy was negative. A diagnosis of HLH was suspected and patient was transferred to Emory University Hospital for further care. Despite aggressive treatment with IVIG, rituximab and high dose steroids, the patient's condition continued to deteriorate. She expired 10 days after transfer and an autopsy limited to chest and abdomen was performed.

Biopsy Fixation Details: Tissues were fixed in buffered formalin.

Description of Clinical Image if Any: N/A

Details of Microscopic Findings: Multiple sections showed intravascular proliferation of large atypical lymphoid cells in lung, heart, kidney, uterus, ovary, fallopian tube, and perilymph node adipose tissue.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry: Immunohistochemical stains show the lymphoma cells positive for CD3 (cytoplasmic and membrane), CD56, and TIA1 and negative for CD4, CD5, CD8, CD20, CD30 and myeloperoxidase. Flow cytometry was not performed.

Special Stains: No performed

Cytogenetics: Not performed

Molecular Analysis: T-cell receptor gamma gene rearrangement was polyclonal.

Interesting Feature(s) of Submitted Case: Intravascular location of the lymphoma cells without mass formation or apparent necrosis.

Proposed Diagnosis: Intravascular NK/T-cell lymphoma

CSHP/SH Workshop 2019

Comments: An extremely rare EBV-positive NK/T-cell lymphoma mainly localized in the vascular space.

EMORY
PATHOLOGY

SH/CSHP case submission

Shiyong Li, MD, PhD

Clinical history

- 64 year old Caucasian female who presented to her primary care physician with fatigue and peripheral edema for 3-4 weeks
- CBC showed pancytopenia
  - WBC 2.0 K/uL
  - HgB 8.0 g/dL
  - Platelet 30 K/uL
- Transferred to an OSH for workup

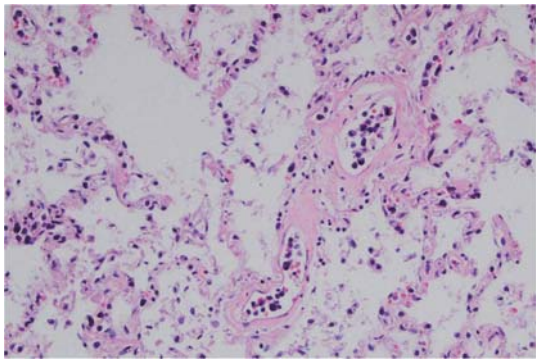
Clinical history

- Findings at the OSH:
  - Physical exam revealed mild peripheral edema. No skin lesions
  - CT showed only mild hepatosplenomegaly
  - Labs:
    - Persistent pancytopenia
    - Bone marrow biopsy negative
    - Elevated LDH, PT and PTT, and decreased fibrinogen
    - CMV, HIV negative
    - EBV viral load 1 x 10^7 IU/mL
  - ?HLH
- Suspected HLH and transferred to Emory for further care

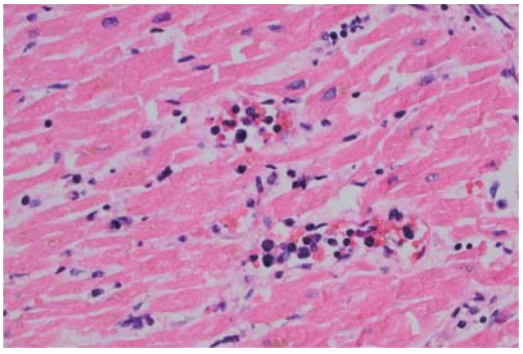
Clinical history

- Repeat bone marrow negative. No definitive hemophagocytic histiocytes
- Meet diagnostic criteria for HLH
  - Fever (highest 39.6°C)
  - Splenomegaly
  - Cytopenia
  - Hypertriglyceremia (563 mg/dL) and hypofibrinogenemia (77 mg/dL)
  - High ferritin level (6000 ng/dL) and
  - Elevated soluble CD25 (13,370 U/mL)
- Conditions continued to deteriorate – respiratory failure requiring intubation and hypotensive requiring vasopressors; DNR per family request
- Expired 10 days after admission, limited autopsy: chest/abdomen

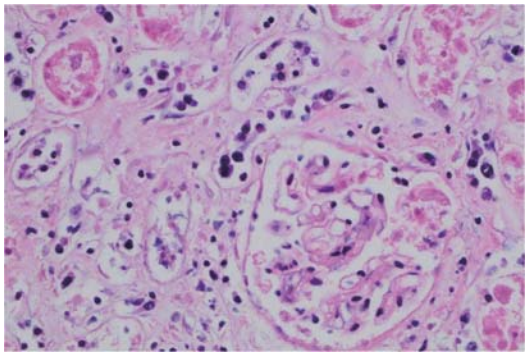
Lung



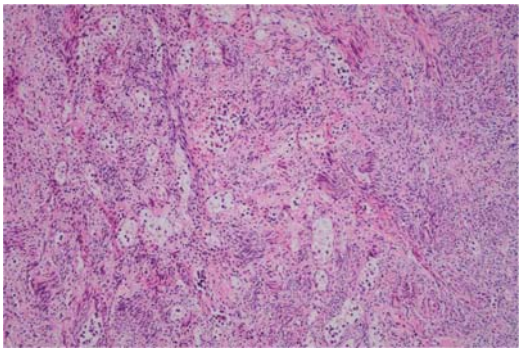
Heart



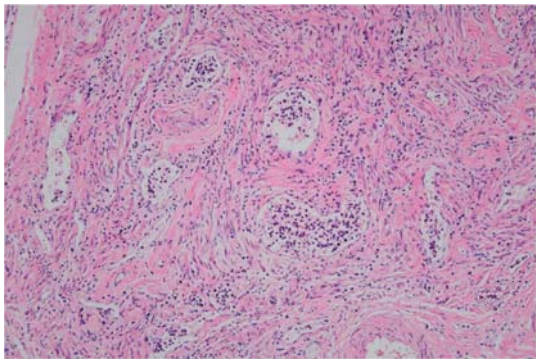
Kidney



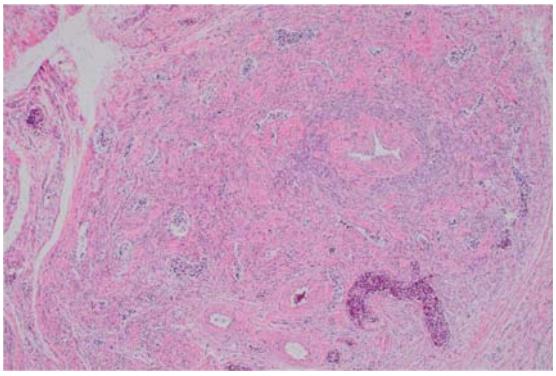
Uterus



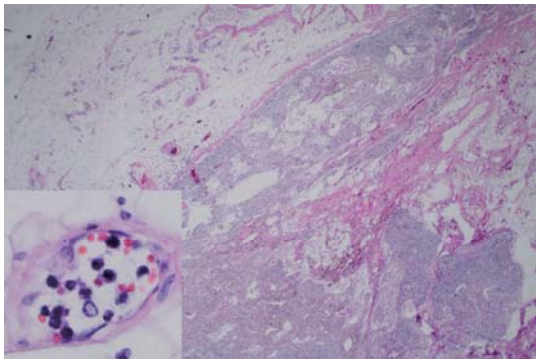
Ovary



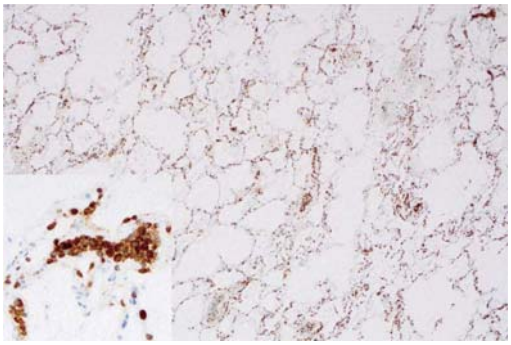
Fallopian tube



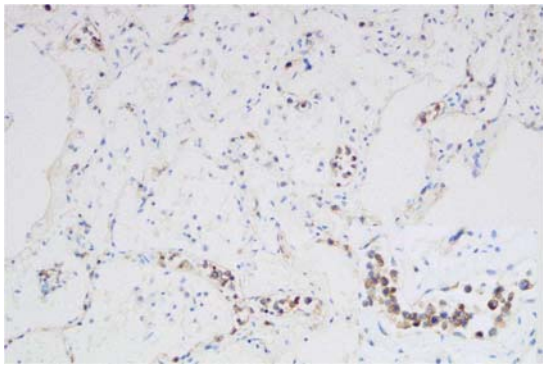
Abdominal lymph node



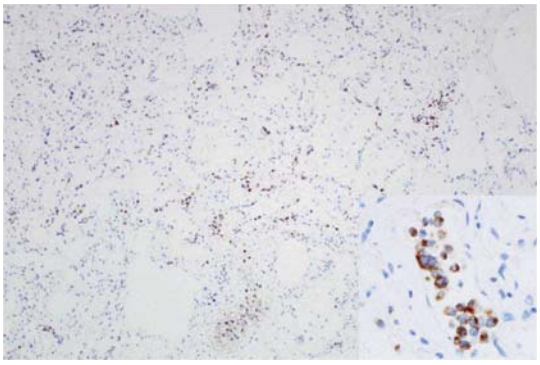
CD3



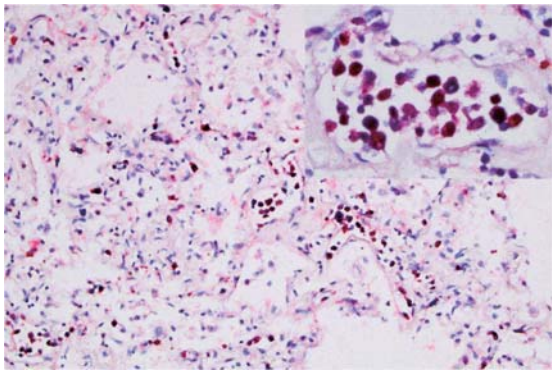
CD56



TIA1



EBVISH



Additional studies

- Immunostains negative for CD20, CD4, CD5, CD8, CD30 and myeloperoxidase
- Molecular study: T-cell receptor gamma gene rearrangement showed a polyclonal pattern.

Final diagnosis

- Intravascular NK/T-cell lymphoma (IVNK/TL)

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Yasodha Natkunam M.D., Ph.D., and Atif Saleem, D.O.

Additional contributors: Rohan Joshi, Li Lei, Lhara Lezema, Shyam S. Raghavan, Nastaran Neishaboori, Mohana Roy, Joe Schroers-Martin, Gregory W. Charville, Christian Kunder, Brent Tan, Beth A. Martin

From the Department of Pathology and Department of Medicine, Division of Hematology, Stanford University School of Medicine, Stanford, CA

Affiliation: Stanford University Medical Center

E-mail: asaleem@stanford.edu

Novel Molecular Insights on Systemic EBV-Positive T-Cell Lymphoma of Childhood

Clinical History: The patient was a 27-year-old male of Vietnamese ancestry with a history of recurrent ear infections as a child, mechanical injury to his foot (7 months prior) requiring amputation with subsequent high fever/superinfection, and repeated bouts of a mononucleosis-like illness. He was referred to out center with one month of evolution characterized by dry cough, fever, fatigue and shortness of breath without response to azithromycin administration. He was found to have acute liver failure and hemophagocytic lymphohistiocytosis (HLH) as defined by the HLH-2004 trial criteria (fever, splenomegaly, peripheral blood cytopenia, hemophagocytosis in the bone marrow, ferritin>500 ng/mL and elevated soluble IL-2 receptor level (9081 U/mL)). He was given etoposide and dexamethasone to initially target the HLH upon diagnosis. Following confirmation of lymphomatous infiltration of the liver and bone marrow, he additionally underwent allopurinol, cyclophosphamide, gemcitabine and filgrastim therapy. After approximately ten days his course was complicated by severe neutropenia, acute kidney injury, encephalopathy and hypervolemia leading to respiratory distress which prompted intubation. Valacyclovir, isavuconazole and atovaquone were added to his regimen. The formation of a hepatohydrothorax necessitated pigtail catherization which ameliorated his condition. Over the next several days he developed vancomycin-resistant enterococcal bacteremia and meningitis culminating in a septic shock recalcitrant to maximum vasopressor support. He was transitioned to comfort care in accordance with his family's wishes and died 26 days after his admission.

Biopsy Fixation Details: The liver biopsy was formalin fixed and paraffin embedded. One core of the bone marrow biopsy was received in Bouin's solution and decalcified whereas the second core was received in formalin and decalcified in EDTA; both were subsequently paraffin embedded. Bone marrow aspirate material was sent for cytogenetics and molecular studies without decalcification.

Description of Clinical Image if Any:

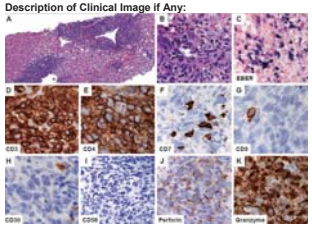


Image 1. Liver biopsy showing systemic EBV-positive T-cell lymphoma of childhood. A dense portal infiltrate mostly sparing the sinusoids and parenchyma is seen (A), and is composed of large, hyperchromatic pleomorphic and dyscohesive cells (B) with in-situ hybridization highlighting expression of EBV (to EBER; C). Immunohistochemistry demonstrates that the tumor cells express CD3 (D), CD4 (E), and lacks CD7 (F), CD8 (G), CD30 (H), and CD56 (I). Cytotoxic granule-associated markers perforin (J) and Granzyme B (K) were expressed. (Original magnifications: panel A x40; panels B-K x400).

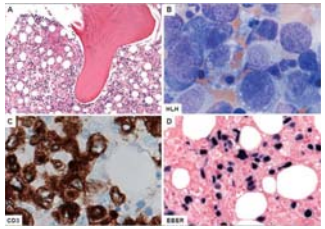


Image 2. Bone marrow biopsy and aspirate showing systemic EBV-positive T-cell lymphoma of childhood. The bone marrow demonstrates a mild histiocytic hyperplasia with erythrophagocytosis and infiltrating large, hyperchromatic and dyscohesive cells (A). The bone marrow aspirate shows hemophagocytic lymphohistiocytosis (HLH) exemplified by hemophagocytosis of a nucleated erythroid precursor (B). By immunohistochemistry, the tumor cells in the bone marrow express CD3 (C) and EBV by EBER in situ hybridization (D). (Original magnifications: panel A x40; panel B x1000; panels C-D x400).

Details of Microscopic Findings: The liver biopsy showed a dense portal infiltrate, mostly sparing the lobular parenchyma, composed of dyscohesive cells that displayed large, pleomorphic nuclei and brisk mitotic activity. A subsequent bone marrow biopsy demonstrated involvement by the same lymphomatous process in addition to extensive HLH. The lymphoma cells expressed strong and diffuse positivity for EBV (by in situ hybridization for EBER) in addition to aberrant cytotoxic markers (described below).

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry: Immunohistochemistry showed up-regulation of PD-L1 (E1L3N clone) by a majority of the tumor cells with expression of CD3, CD4, Granzyme B, perforin, TIA1 and EBV (by in situ hybridization for EBER), and no expression of CD56. Flow cytometry performed on the bone marrow confirmed an abnormal T cell population expressing CD2, CD3, CD4 and partial CD5 without CD7.

Special Stains: Noncontributory (trichrome, reticulin, Prussian blue, Rhodanine and PAS-d stains performed on the liver did not demonstrate increased fibrosis, abnormal hepatic plate thickness, increased stainable iron, increased stainable copper, or globules to support alpha-1 antitrypsin deficiency, respectively).

Cytogenetics: A normal 46,XY male karyotype was present by cytogenetic analysis of the bone marrow aspirate (using the GTW banding method; 20 metaphase cells were analyzed).

Molecular Analysis: A clonal T cell receptor gamma chain gene rearrangement and multiple genetic alterations by targeted next generation sequencing including a pathogenic *TP53* D281 mutation, an intergenic-*CD274* (*PD-L1*) rearrangement, an *ARID1A* P1266T variant, and an *IRF8* T80R variant were detected. Targeted next generation sequencing was performed using the Stanford Actionable Mutation Panel for hematopoietic and lymphoid neoplasms (HEME-STAMP) which is a hybrid capture-based assay spanning 164 cancer-associated genes.

Interesting Feature(s) of Submitted Case:

- A molecular analysis of systemic EBV-positive TCL of childhood by NGS has not been reported previously in the literature to our knowledge. **The cryptic history of a mononucleosis-like illness is interesting in attempting to correlate a potential chronic EBV infection/ineffective host response and subsequent HLH/lymphomagenesis**. **This case recapitulates the ethnogenetic predisposition which is hallmark in certain EBV associated lymphomas.** The monoclonal proliferation of T-cells seen in both EBV-associated HLH and EBV positive T-cell lymphomas have been theorized to exist on a continuum of disordered T-lymphocyte proliferation and hypercytokinemia following infection of T-cells by EBV.
- Recapitulates that there is an underlying ethnogenetic predisposition

- *IRF8* encodes a transcription factor that is expressed primarily in hematopoietic cells and facilitates immune cell differentiation, cell cycle regulation, and apoptosis. The IRF8 T80R variant present in this case results in a substitution of an arginine for the wild-type threonine at codon 80 and has not been studied experimentally. However, a similar variant at this position, T80A, has been found in the germline setting in sporadic cases of autosomal dominant dendritic cell immunodeficiency disorder. T80A has also been found to result in loss-of-function of IRF8 in vitro. *IRF8* loss-of-function has been implicated in the pathogenesis of pediatric-type follicular lymphoma and diffuse large B-cell lymphomas**. In the germline setting, homozygous biallelic mutations in *IRF8* result in NK and dendritic cell immunodeficiency disorder.** Furthermore, IRF8 binds to and enhances *CASP1* gene promoter activity and caspase-1 expression, which is critical for EBV reactivation. **We postulate that a germline (given a variant allele frequency of 49%) *IRF8* mutation present in our patient may have caused a NK cell dysfunction related to EBV reactivation, which, in addition to his genetic predisposition, may have functioned as an essential step to promulgate lymphomagenesis and HLH**.
- The *TP53* D281H mutation present in this case exists in the DNA-binding domain and alterations within it have been shown to be associated with poor prognostic features in NK/T-cell lymphomas.
- The intergenic-*CD274* (*PD-L1*) rearrangement identified is expected to preserve the extracellular and transmembrane domains of *PD-L1* and remove the 3'UTR, which contains a miRNA binding site that is responsible for downregulation of PD-L1 transcripts. Translocations such as these that remove the 3'UTR increase PD-L1 expression, enhance immune evasion, and confer sensitivity to growth inhibition by PD-L1 inhibitors in cell culture and mouse models. **EBV has been shown to up-regulate PD-L1 via LMP1 and EBNA2 (via downregulation of miR-34a) which raises the possibility that a structural intergenic-*CD274* rearrangement coupled with the effects of EBV on PD-L1 synergistically contributes to anti-viral and anti-cancer immunity**. Although this proposed mechanism has promising implications for treatment with immunotherapy, a recent report in the literature suggests that checkpoint inhibitors may perpetuate HLH, particularly in the presence of EBV infection.

Proposed Diagnosis: Systemic EBV-positive T-cell lymphoma of childhood.

Comments: No additional comments (incorporated in interesting features section above).

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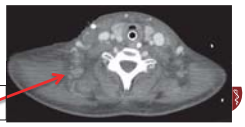
Systemic EBV-Positive T-Cell Lymphoma of Childhood

Yasodha Natkunam and Atif Saleem

Clinical Presentation

- 27-year-old male of Vietnamese ancestry with a history of:
  - Recurrent ear infections as a child
  - Mechanical foot injury (7 months prior) requiring toe amputation/skin graft from left thigh→ complicated by superinfection, resolved after 6-8 weeks abx
  - Repeated bouts of mononucleosis-like illness
    - Most recent episode 1 month prior which progressively evolved to include shortness of breath 2 weeks prior to admission (did not improve following azithromycin)
- Upon admission, the patient was found to have acute liver failure and lymphadenopathy
  - Febrile, splenomegaly, decreased

RBCs and platelets, ferritin 87,079 ng/mL



CT: Level 5 enlarged lymph node 1.7 cm (arrow)

Diagnostic Workup

- Liver biopsy
- HLH evaluation:
  - CBC, coagulation studies, serum ferritin, LFTs, serum triglycerides
  - Blood, bone marrow, urine, (CSF) cultures
  - Viral titers and quantitative PCR testing for suspected viral triggers (EBV, CMV)
  - Bone marrow evaluation
  - Immunologic profile (soluble IL-2 receptor, NK function by flow, Ig levels, perforin/granzyme B, etc…) and genetic testing

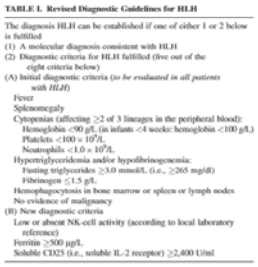


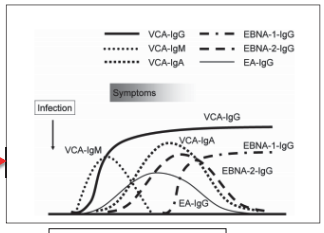
TABLE I. Revised Diagnostic Guidelines for HLH

The diagnosis HLH can be established if one of either 1 or 2 below is fulfilled
(1) A molecular diagnosis consistent with HLH
(2) Diagnostic criteria for HLH fulfilled (five out of the eight criteria below)
(A) Initial diagnostic criteria (*to be evaluated in all patients with HLH*)
Fever
Splenomegaly
Cytopenias (affecting ≥2 of 3 lineages in the peripheral blood):
Hemoglobin <90 g/L (in infants <4 weeks: hemoglobin <100 g/L)
Platelets $<100 \times 10^9$/L
Neutrophils $<1.0 \times 10^9$/L
Hypertriglyceridemia and/or hypofibrinogenemia:
Fasting triglycerides ≥3.0 mmol/L (i.e., ≥265 mg/dl)
Fibrinogen ≤1.5 g/L
Hemophagocytosis in bone marrow or spleen or lymph nodes
No evidence of malignancy
(B) New diagnostic criteria
Low or absent NK-cell activity (according to local laboratory reference)
Ferritin ≥500 µg/L
Soluble CD25 (i.e., soluble IL-2 receptor) ≥2,400 U/ml

Henter JI et al. Pediatr Blood Cancer. 2007 Feb;48(2):124-31.

Laboratory Assays for EBV

- Nucleic acid amplification testing
- Direct examination:
  - Immunohistochemistry
  - In situ hybridization
- Serologic Tests:
  - Heterophile antibodies
  - EBV-specific antibodies

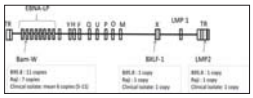


Development of antibodies to EBV antigens following a primary infection in infectious mononucleosis

Manual of Clinical Micro. 12th ed.

EBV Detection/Quantification by Real-Time PCR

- Screening method for HSCT and solid organ transplant patients at risk of developing post-transplant lymphoproliferative disorder (PTLD)
  - Plasma, CSF, bone marrow, BAL, other body fluids, paraffin-embedded tissue
- QIAsymphony instrument Sample* Preparation (automated nucleic acid extraction) and artus* EBV Rotor-Gene kit amplification of the *BKRF1* gene (encodes EBNA-1) *also used for CMV
  - Also EBV DNA detected in plasma of patients with nasopharyngeal carcinoma (primer/probe set that spans the BamH1-W region of the EBV genome, a long internal repeat sequence typically present as 5-11 copies, improving sensitivity)
- Mutations within the targeted portion of the genome may result in under-quantitation of or failure to detect the virus



Sanosyan A, et al. PLoS One. 2017 Aug 29;12(8).

EBV Serology

- Aids in the diagnosis or exclusion of EBV infection
- Trinity Biotech Captia Epstein-Barr Viral Capsid Antigen (VCA) IgG, VCA IgM, Early Antigen Diffuse (EA-D) IgG, and Epstein-Barr Nuclear Antigen (EBNA) IgG ELISAS
  - Ex: VCA IgM sensitivity (acute) = 97.4%, specificity (seronegative) = 96.4%, specificity (seropositive) = 99%

TABLE 3 Typical EBV serological profiles using the most frequently employed antigens and Ig isotypes[a]

Condition	Antigen /Ig isotype					
	VCA IgG	VCA IgM	VCA IgA	EA/D IgG	EA/R IgG	EBNA Ig
Susceptible/no previous exposure	–	–	–	–	–	–
Primary infection	++	+++	+	+	+/–	–
Past infection	+	–	+/–	–	+/–	+
Inconclusive: primary or past infection[b]	+	+	+/–	+/–	+/–	+
Inconclusive: primary or past infection[c]	+	–	+/–	+/–	+/–	–
Chronic active EBV infection	+++	+	++	++	+	+/–
NPC	+++	–	+++	+++	+	+++
X-linked lymphoproliferative syndrome	+/–	–	–	–	–	–

Manual of Clinical Micro. 12th ed.

- VCA: antibodies rise early in acute infection (IgG persists for life, IgM wanes over 3 mos.)
- EA: IgG rises at the onset of illness and wanes over months, but persists in many
- EBNA: IgG appears 6-12 weeks after primary infection, persists for life

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Patient EBV Laboratory Results

	1/9/2019 0531	1/19/2019 0937	1/28/2019 0425
EBV DNA PCR, PLASMA			
SPECIMEN	Plasma *	Plasma *	Plasma *
EBV DNA	Detected * ↑	Detected * ↑	Detected * ↑
Result	824,543 *	26,870 *	10,820 *
Result Log 10	5.92 *	4.43 *	4.03 *

	1/28/2019 1637
EBV DNA PCR QUAL	
Specimen Type	Cerebrospinal ... *
Specimen Source	Cerebrospinal ... *
EBV DNA	Detected *

	1/6/2019 1903
EPSTEIN-BARR VIRUS AB	
VCA IgG Result	Positive * ↑
VCA IgG Level	6.69 *
VCA IgM Result	Negative *
EA IgG Result	Positive * ↑
EA IgG Level	4.48 *
EBNA IgG Result	Negative *
EBV Interpretation	See Comment *

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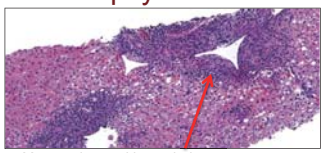
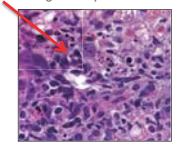
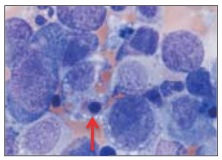
Liver Biopsy and Bone Marrow Evaluation



Liver biopsy: *Pleomorphic, atypical lymphoma cells arranged in a portal distribution*





Bone marrow aspirate: *hemophagocytosis of nucleated cells such as erythroids (arrow)*

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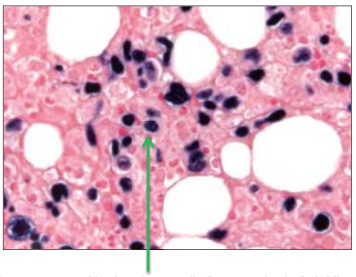

Bone Marrow Biopsy: *EBV positive lymphoma cells (by EBER in situ hybridization)*

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Final Diagnosis

DIAGNOSIS:
PERIPHERAL BLOOD, SMEAR
- **OCCASIONAL CIRCULATING ATYPICAL LYMPHOCYTES CONSISTENT WITH SYSTEMIC EBV-POSITIVE T-CELL LYMPHOMA OF CHILDHOOD**
- **NORMOCHROMIC MICROCYTIC RED BLOOD CELLS**
- **THROMBOCYTOPENIA**

BONE MARROW, ASPIRATE AND BIOPSY
- **INVOLVED BY SYSTEMIC EBV-POSITIVE T-CELL LYMPHOMA OF CHILDHOOD (10-15% OF MARROW CELLULARITY)**
- **HYPOCELLULAR MARROW WITH TRILINEAGE HEMATOPOIESIS**
- **MARKED HEMOPHAGOCYTOSIS OF NUCLEATED CELLS**
- **INCREASED IRON STORES; NO RING SIDEROBLASTS**

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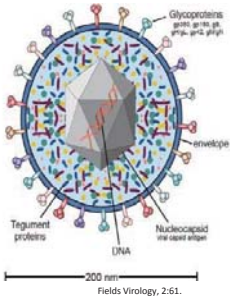
EBV Virology

- **Virology**: Human herpes virus 4, double stranded DNA, EBV-1 and EBV-2
- **Epidemiology**: 90-95% of adults are EBV-seropositive
  - Primary infections: acute infectious mononucleosis, chronic EBV infection
  - Complications: rash, oral hairy leukoplakia, splenic rupture, HLH, lymphomatoid granulomatosis, PTLD
  - Malignancies: Burkitt lymphoma, DLBCL, Hodgkin lymphoma, NPC, ENKTL, malignancies in HIV+



Fields Virology, 2:61.

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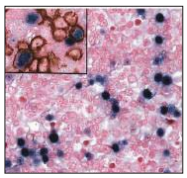
Systemic EBV-Positive T-Cell Lymphoma of Childhood

- Clonal proliferation of EBV-infected T-cells which can occur shortly after primary acute EBV infection or in the setting of chronic active EBV infection
- Children and young adults, Asians/ Native Americans from Central and South America and Mexico, rapid progression with multiorgan failure/ sepsis/ death in days-weeks
  - Etiology unknown but association with EBV and ethnic predisposition suggest a genetic defect in the host immune response to EBV
- Hemophagocytic syndrome is almost always present
- Systemic disease (liver/spleen > lymph nodes, bone marrow) presenting as acute onset of fever/malaise → hepatosplenomegaly, liver failure, lymphadenopathy over weeks
- Pancytopenia, abnormal LFTs, abnormal EBV serology (low or absent VCA IgM)
- Few cases reported to respond to etoposide + dexamethasone regimen followed by HSCT



EBV positive lymphoma cells (by EBER in situ hybridization) in a lymph node which are also positive for CD8 (inset); 2017 WHO Hematopoietic Tumors

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Proposed Mechanisms for EBV-driven HLH and Systemic EBV-Positive T-Cell Lymphoma of Childhood

- Infection of CD8+ T cells by EBV with expression of latency II proteins (LMP-1, LMP-2, EBNA-1) and a failure to produce EBV-specific cytotoxic T-cells suggesting an NK/T-cell dysfunction
  - LMP-1 (part of the TNF superfamily) ultimately activates NF-κB → promotes proliferation, and inhibits TNF-alpha mediated apoptosis through suppression of TNF-alpha/TNFR-1 complex formation, selectively in T-cells
    - Upregulation of TNF-alpha has independently been implicated in HLH
- The monoclonal proliferation of T-cells seen in both EBV-associated HLH and EBV-positive T-cell lymphomas may exist on a continuum of disordered T-lymphocyte proliferation and hypercytokinemia following infection of T-cells by EBV

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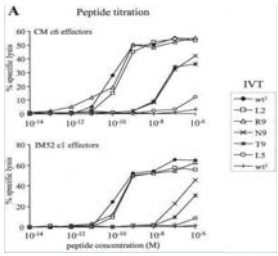
Genetically Determined Susceptibility Alters Response to EBV

TABLE 2. EBV strains of different geographic origins with particular IVT and AVF epitope sequence combinations

Virus type	AVF epitope	IVT epitope	No. of strains from donors who are: Chinese	Caucasian	African
1	wt^a	wt^1	3	28	23
	wt^a	IVT/N9		2	
	wt^b	IVT/L2	15	2	
	AVF/N4	IVT/N9	18		
	AVF/S1F2	IVT/L2	14		
	AVF/S1F2	IVT/N9	1		
	AVF/P1	IVT/L2	4		
	AVF/F1L2	IVT/N9	4		
	AVF/A2	IVT/L5	2		
	AVF/S1L2	IVT/T9	2		
	AVF/S1L2	IVT/N9	1	2	
	AVF/S1L2	IVT/R9		1	
	AVF/S1L2	IVT/L2		9	
	AVF/S1L2	IVT/L2R9		4	
2	wt^2	wt^2	10	5	5

[a] wt^1, Caucasian B95.8 prototype 1 epitope sequence.
[b] wt^2, African Ag876 prototype 2 epitope sequence.



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Midgley RS et al. J Virol. 2003 Nov;77(21):11507-16.

Clinical Follow Up

- Treated with etoposide, gemcitabine, cyclophosphamide, dexamethasone → developed severe neutropenia, acute renal failure, volume overload requiring dialysis and intubation
- Hepatohydrothorax which resolved with pigtail catheter but over the next few days he developed vancomycin resistant enterococcal bacteremia and meningitis which was treated with linezolid and tigecycline but he continued to decline
  - EBV was also detected in his CSF at this time
- Septic shock refractory to maximum vasopressor support and ultimately transitioned to comfort care

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Additional Contributors

- Rohan Joshi, Li Lei, Lhara Lezema, Shyam S. Raghavan, Nastaran Neishaboori, Mohana Roy, Joe Schroers-Martin, Gregory W. Charville, Christian Kunder, Brent Tan, Beth A. Martin
- From the Department of Pathology and Department of Medicine, Division of Hematology, Stanford University School of Medicine, Stanford, CA

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CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Yuhua Huang, MD

Affiliation:
Department of Pathology, Sun Yat-sen University Cancer Center

E-mail:
huangyh@sysucc.org.cn

Clinical History:
- M/44
- Presented with cough, fever and night sweats for 2 months
- Right supraclavicular LN biopsy was performed.

Biopsy Fixation Details:
Formalin-fixed paraffin-embedded tissue sections

Description of Clinical Image if Any:
Multiple masses were revealed in the mediastinum and pulmonary hilum by CT scan; Multiple enlarged LNs were also found in right supraclavicular and axillary regions (2 cm in diameter).

Details of Microscopic Findings:
Large area of caseous necrosis and devoid of granulomas were found in the supraclavicular LN, which showing typical morphology of tuberculous lymphadenitis.Only one small area of the biopsy showed some abnormal large lymphoid cells. Immunohistochemistry showed that these large cells
were positive for CD3, CD56,GranB,TIA1,Ki67（80%+) and EBER.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
CD3+, CD56+,GranB+,TIA1+,Ki67（80%+),c-Myc（80%+),EBER+
CD20-,CD5-,CD4-,CD8-,ALK-

Special Stains:
Acid-fast stain (+)

Cytogenetics:
No

Molecular Analysis:
This case showed germline configuration of TCR.
No Ig gene rearrangement was identified.

Interesting Feature(s) of Submitted Case:
1. Collision lesion consisting of tuberculosis and NK/T-cell Lymphoma is extremely rare, which has not been reported to date.
2. Tuberculosis and NK/T cell lymphoma can share common features, such as coagulation necrosis, a background of inflammatory cells.
3. Our case highlights the importance of thoroughly reviewing all foregoing relevant patient data (most notably pathology samples) in order to rule out the presence of lymphoma that may exist within the shadow of typical morphology of tuberculous lymphadenitis.

Proposed Diagnosis:
(Right supraclavicular LN) Tuberculous Lymphadenitis complicated by NK/T-cell Lymphoma

Comments:
NK/T-cell lymphoma undiagnosed in a patient with tuberculosis: a case report.

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Supraclavicular LN Collision Lesion Consisting of Tuberculosis and NK/T-cell Lymphoma ------Case Presentation

Yuhua Huang

Dept. of Pathology, Sun Yat-sen University Cancer Center

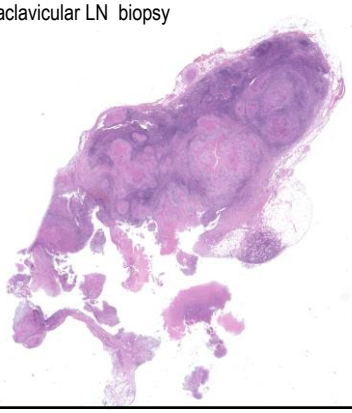
1

Case history

- M/44
- Presented with cough, fever and night sweats for 2 months
- Multiple masses in the mediastinum and pulmonary hilum were revealed by CT scan; Multiple enlarged LNs were also found in right supraclavicular and axillary regions (2 cm in diameter).
- Right supraclavicular LN biopsy was performed.

2

Right supraclavicular LN biopsy



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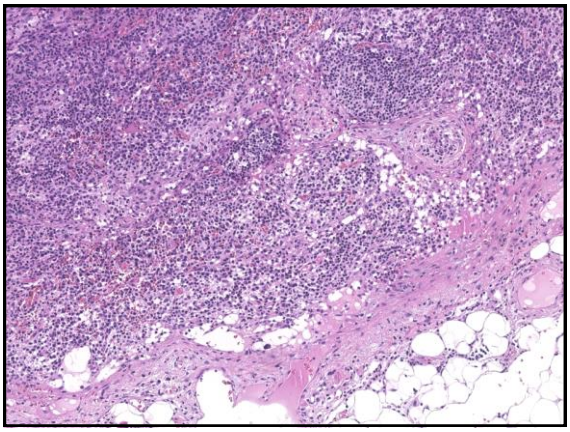

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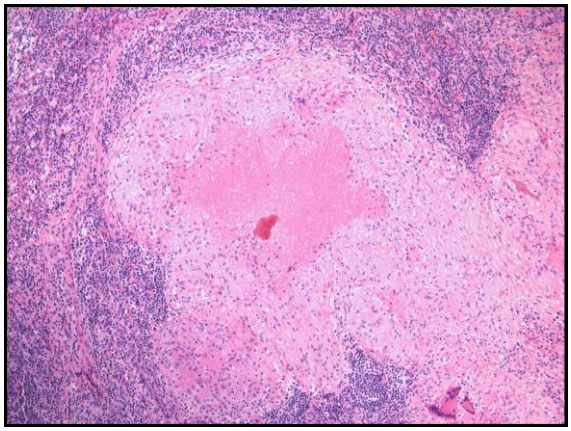

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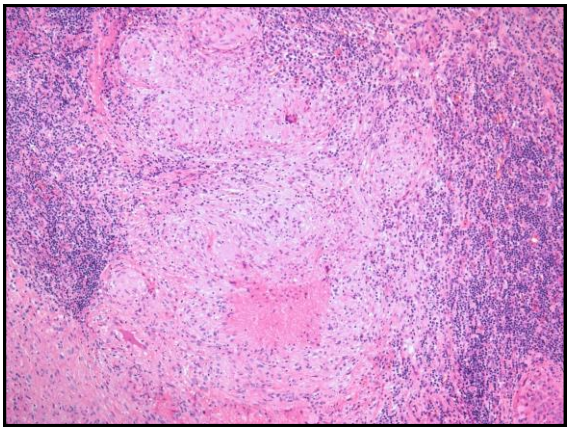

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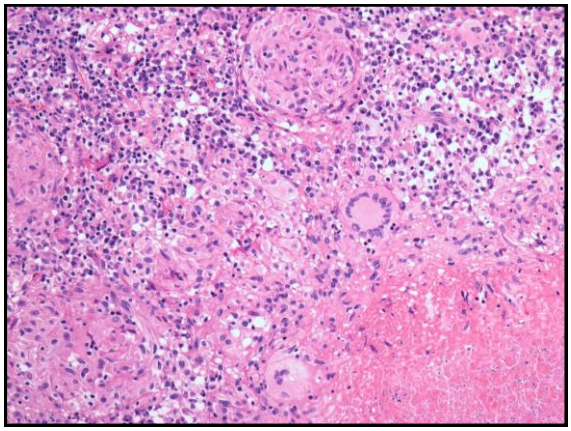

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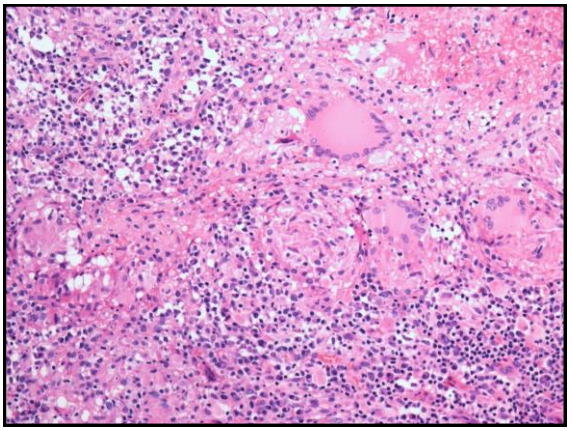

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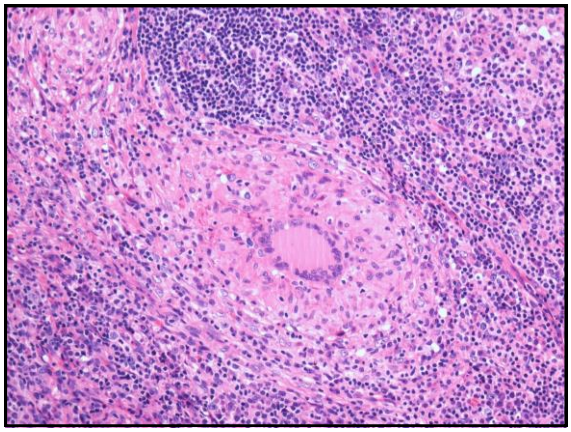

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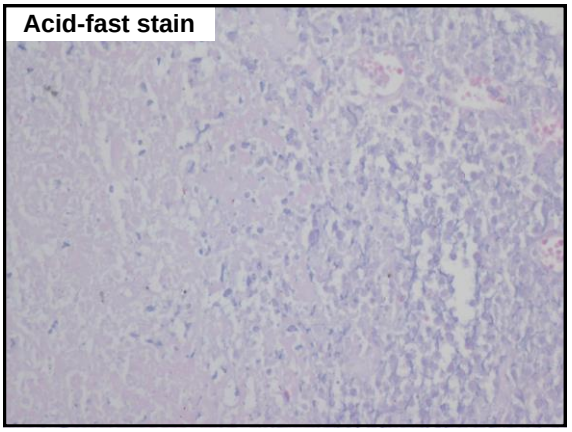

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Molecular Findings

PCR for Mycobacterial tuberculosis using DNA extract from the tissue block was positive.

Prior Diagnosis

(Right supraclavicular LN) Tuberculous Lymphadenitis

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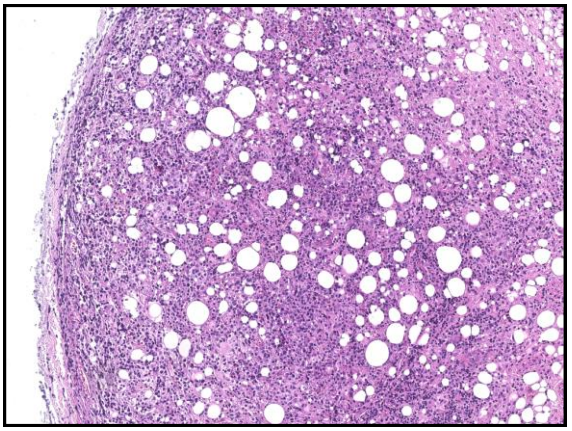

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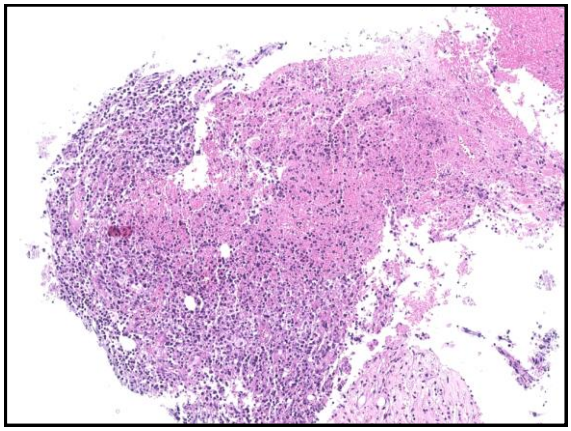

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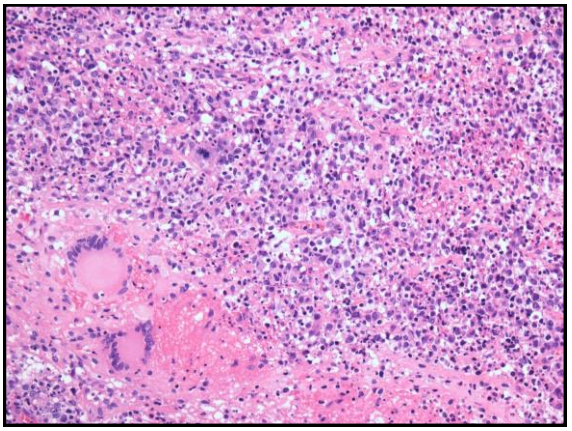

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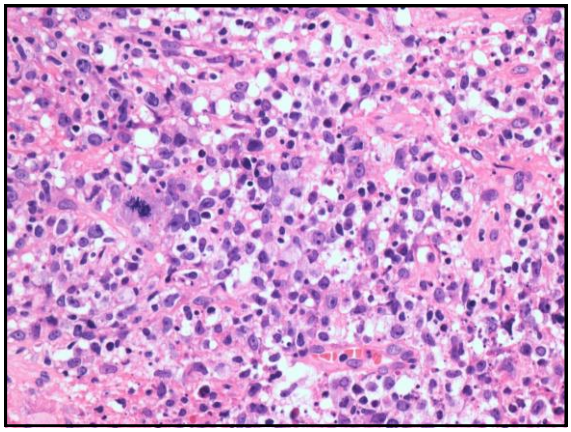

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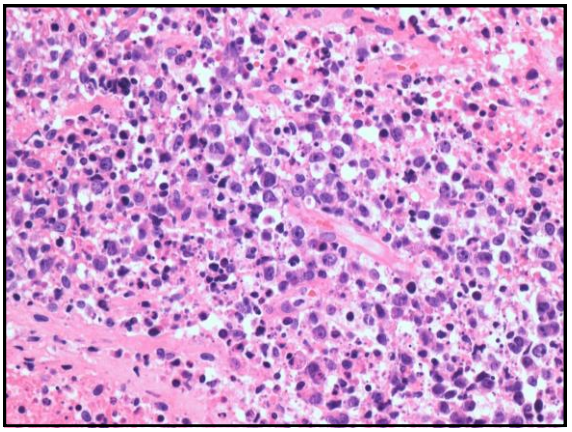

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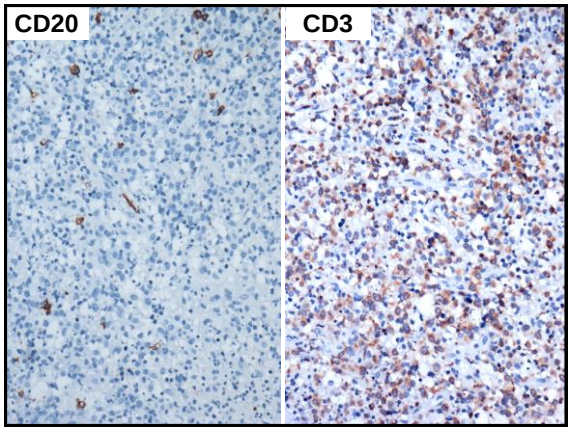

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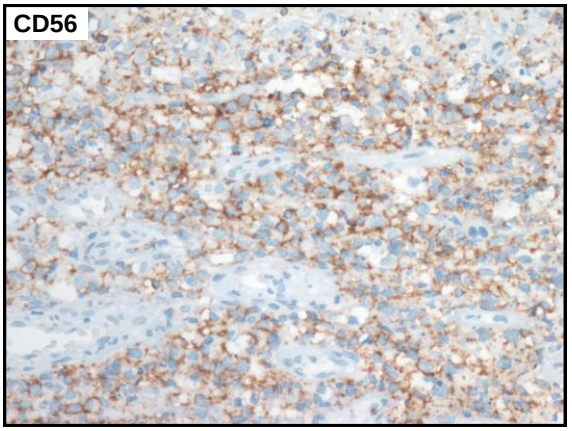

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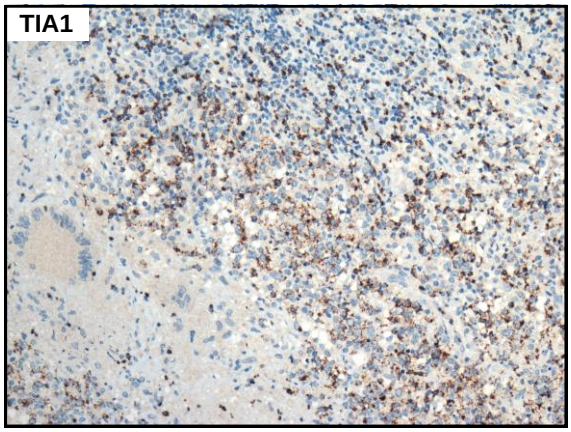

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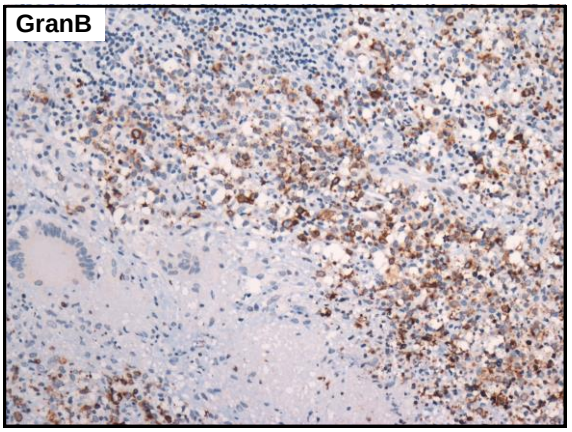

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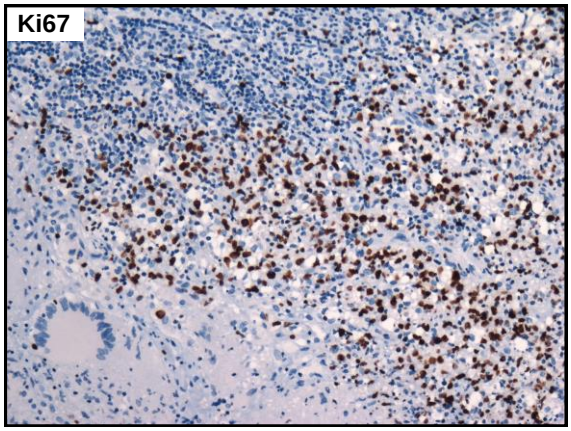

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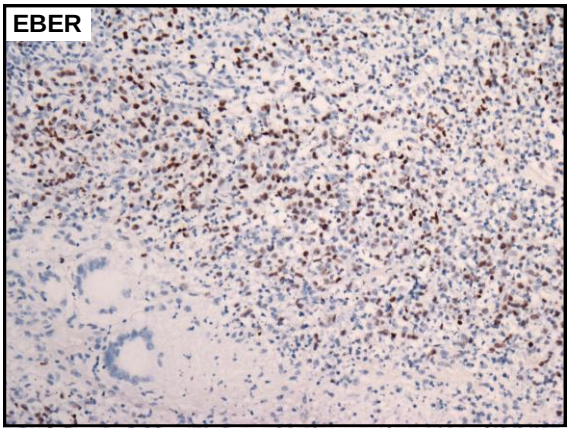

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Final Diagnosis

(Right supraclavicular LN) NK/T-cell Lymphoma

Complicated by Tuberculous Lymphadenitis

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Take-home message

- ✓ Collision lesion consisting of tuberculosis and NK/T cell Lymphoma is extremely rare.
- ✓ Tuberculosis and NK/T cell lymphoma can share common features, such as coagulation necrosis, a background of inflammatory cells.

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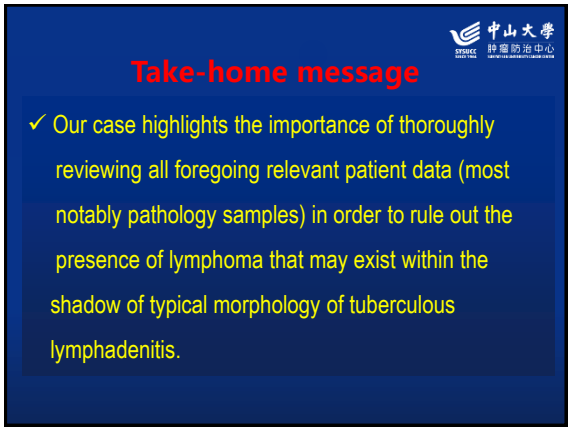
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Take-home message
✓ Our case highlights the importance of thoroughly reviewing all foregoing relevant patient data (most notably pathology samples) in order to rule out the presence of lymphoma that may exist within the shadow of typical morphology of tuberculous lymphadenitis.

25

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Cheng Fei, MD

Affiliation:
The first affiliated hospital of Zhejiang University

E-mail:
18368191527@163.com

Clinical History:
Male, 75 years old.
A mass was found in the right neck for more than 1 month without B symptoms or other discomfort. Examinations showed lymph nodes of bilateral inguinal, abdominal and mediastinal were enlarged.
One right neck lymph node (2×1×1cm) and one right inguinal lymph node (2.5×2×1.5cm) were removed.

Biopsy Fixation Details:
None

Description of Clinical Image if Any:
None

Details of Microscopic Findings:
There are three different zones in one lymph node with clear boundary.
One zone consists of a variable number of large cells. The pattern of nodal involvement is by definition partially diffuse with extranodal extension and sclerosis. Mitoses are numerous.
Another zone shows diffuse infiltrates with medium-sized prominent clear cells with irregular nuclei.
In the third zone, the neoplastic cells are medium-sized lymphocytes, with clear to pale cytoplasm, distinct cell membranes, adjacent to HEVs.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
DLBCL: CD3(-) CD20(+) CD21(-) CD4(-) CD5(-) CD8(-) CD10(+) EBER(+) PAX-5(+) MUM-1(+) Bcl-6(+)
AITL: CD3(+) CD20(+) CD21(-) CD4(+) CD5(+) CD8(+) CD10(+) PD-1(+) EBER(+) PAX-5(-) MUM-1(-) Bcl-6(-)
PTCL: CD3(+) CD20(-) CD21(-) CD4(+) CD5(+) CD8(+) CD10(-) EBER(-) PAX-5(-) MUM-1(-) Bcl-6(-)

Special Stains:

None

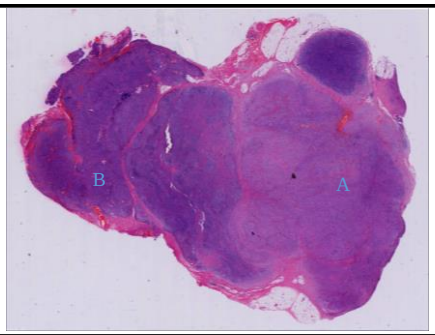
Cytogenetics:
None

Molecular Analysis:
IG and TCR gene rearrangements were detected.

Interesting Feature(s) of Submitted Case:
Three different kinds of lymphoma were found in one lymph node which two of them were associated with EBV.

Proposed Diagnosis:
Composite lymphoma, consists of EBV-positive diffuse large B-cell lymphoma(GCB), peripheral T-cell lymphoma NOS, and AITL with aberrant CD20 expression.

Comments:



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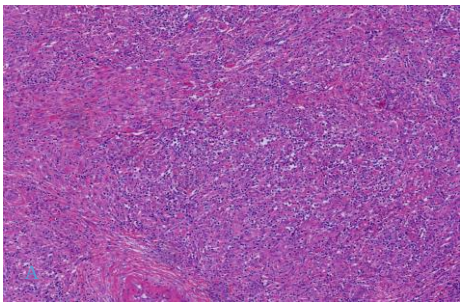

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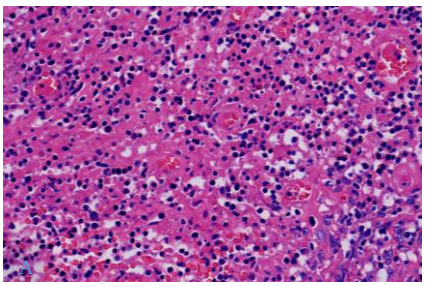

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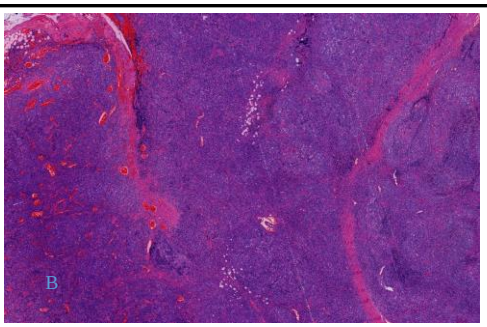

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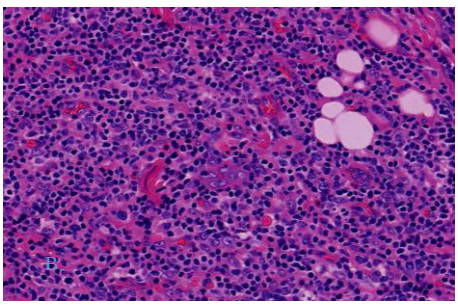

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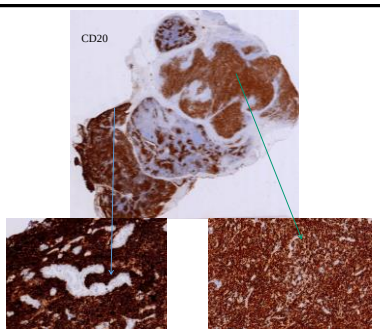

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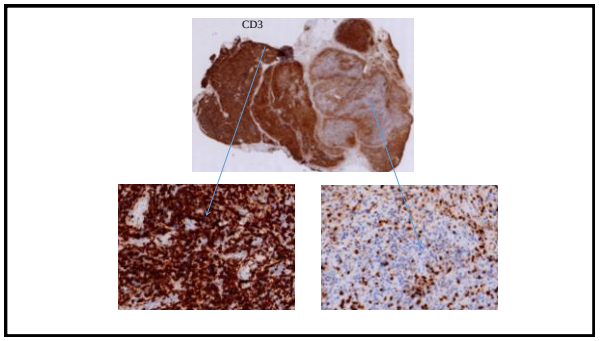

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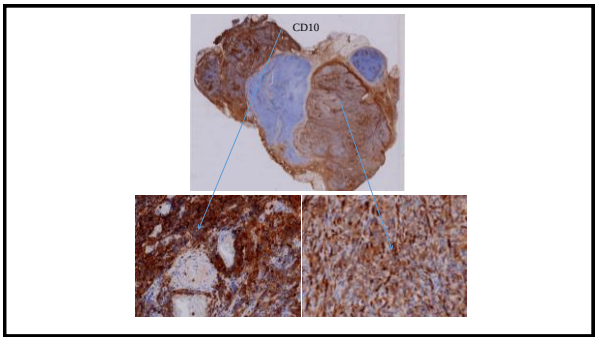

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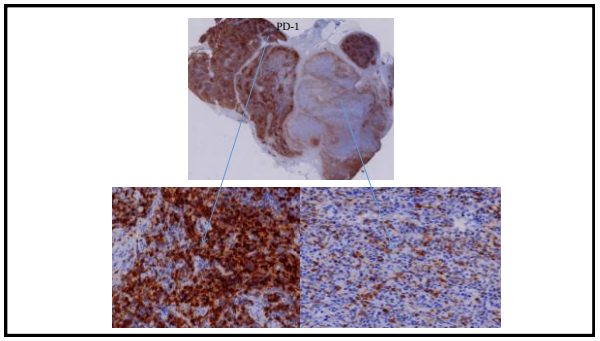

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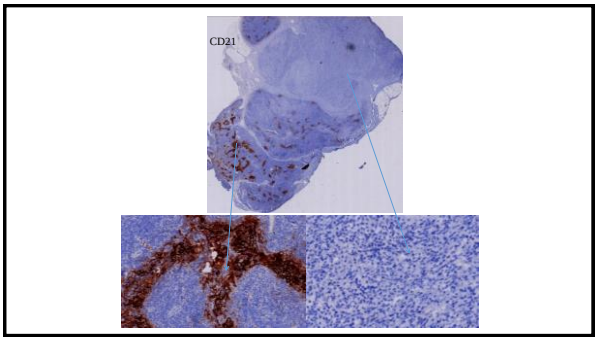

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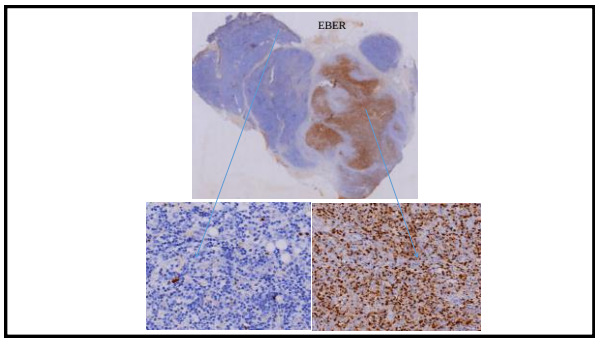

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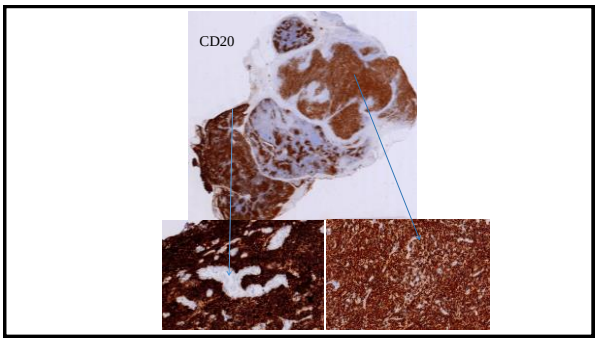

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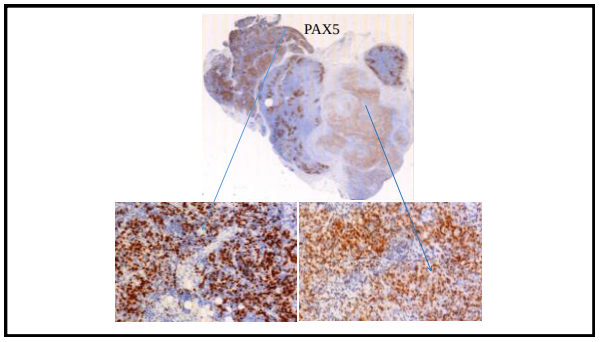

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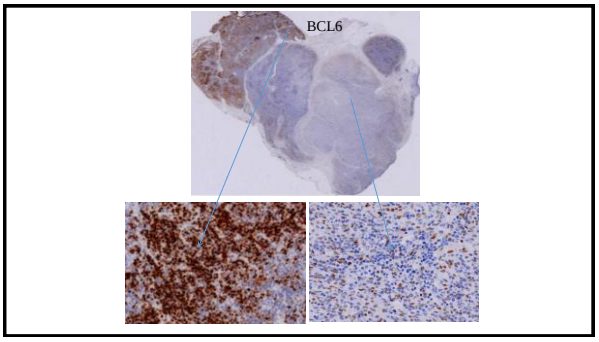

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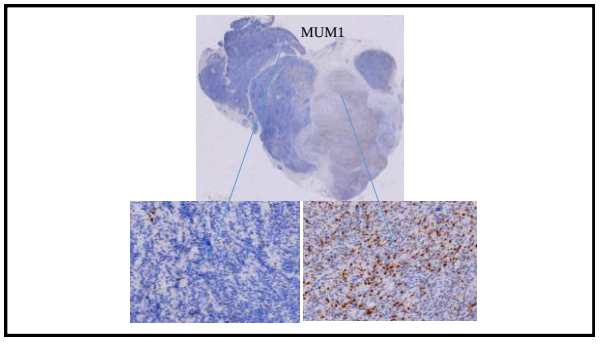

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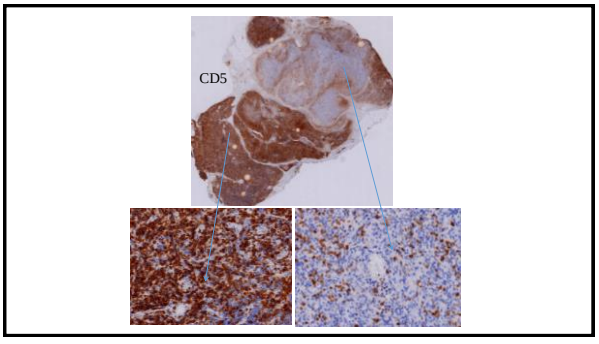

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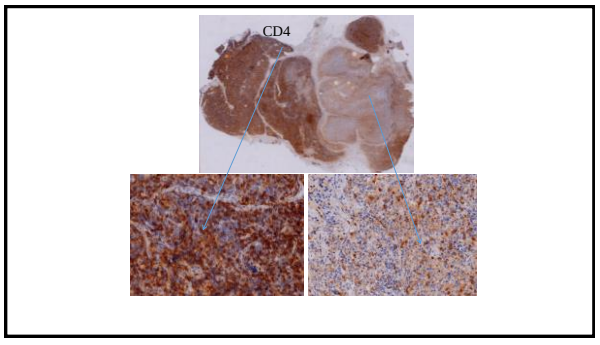

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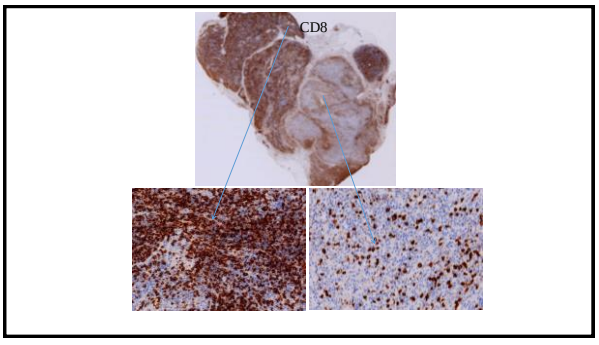

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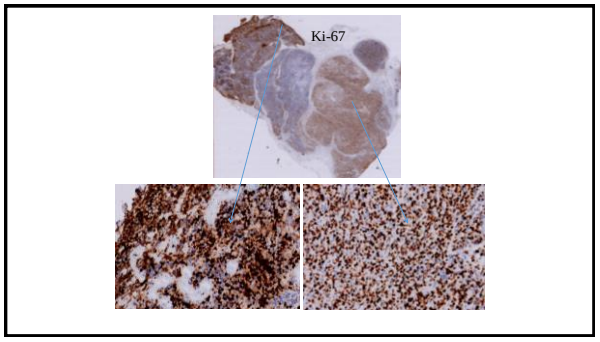

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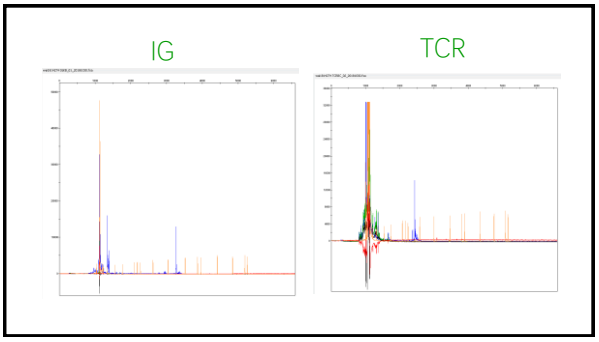

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CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Pei Lin, MD, Zhuang Zuo, MD, PhD, Sergej Konoplev, MD, PhD, M. James You, MD, PhD

Affiliation: The University of Texas-MD Anderson Cancer Center, Houston, TX 77030

E-mail:peilin@mdanderson.org

Clinical History:

A 71 year man noted a "lump" in his neck for several months. Physical exam found a left supraclavicular mass measuring about 3 x 5 cm, bilateral enlarged axillary lymph nodes measuring about 1.5-2 cm and a ~1 cm inguinal lymph node. No hepatosplenomegaly.

HIV, HBV and HCV serological tests were negative. Serum protein electrophoresis and immunofixation electrophoresis were negative for M protein.

An excisional biopsy of the submandibular salivary gland and adjacent lymph node was obtained in 10/2018. The patient was diagnosed with stage IIIA lymphoma with elevated LDH 382 (normal range: 135-225 IU/L), treated with R-CHOP. Restaging by PET/CT at the end of 6th cycle of treatment found stable but low level of disease in the abdomen. A right retrocaval lymph node needle core biopsy was obtained in 4/2019.

Biopsy Fixation Details: Formalin fixed paraffin embedded

Description of Clinical Image if Any:

Before treatment 10/2018: Multi-compartmental hypermetabolic lymphadenopathy above and below the diaphragm:

Left supraclavicular lymph nodes measure 2.4 x 4.1 cm with SUV 33.84.
Abdominal lymph node conglomerate measures 6.7 x 3.7 cm with SUV 32.57
Right inguinal lymph node measures 0.9 cm with SUV 4.89.
Overall, SUV ranges from 46.5 to 15.79 to 4.89 in different compartments.

After 4 cycles of R-CHOP 1/2019: Interim PET/CT show marked improvement

After 6 cycles of R-CHOP 4/2019: Significant reduction of tumor load but persistent low level of lymphadenopathy in retroperitoneal, mesenteric and retrocaval areas, SUV ranging from 2.2-7.8

Details of Microscopic Findings:

Submandibular salivary gland and adjacent lymph node excisional biopsy in 10/2018:

The nodal architecture is effaced by diffuse infiltrate of atypical lymphoid cells composed of large atypical forms admixed with small to intermediate sized cells. The large cells focally form sheets with frequent mitotic figures. Rare small lymphoid aggregates are noted in the salivary gland tissue.

Staging bone marrow was negative for involvement by lymphoma.

Right retrocaval lymph node, needle core biopsy in 4/2019:

Predominantly mature appearing plasma cells.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

A: Excisional biopsy of submandibular salivary gland and adjacent lymph node in 10/2018:

Most infiltrating cells are positive for CD20, PAX-5 and MUM1 (subset), but negative for CD10, CD30 and Cyclin D1. BCL-6 highlights intensely the areas of large cells which are also dimly positive for CD5 but negative for BCL2 and MYC (<30%). EBER highlights rare small cells. Ki-67 is variable, ranging from 50% (mainly BCL6 positive large cells) to 10% in the other areas. Kappa and lambda by IHC and ISH show rare polytypic plasma cells. CD21 and CD23 reveal rare foci of disrupted FDC meshworks, most notable in the areas of large cells. CD3 and CD43 stain the background T-cells.

B: Right retrocaval lymph node, needle core biopsy in 4/2019:

The infiltrating cells are positive for CD138, CD19, CD45, MUM-1 and BCL-2; negative for CD20, Cyclin D1, CD56, CD117, BCL-6 and c-MYC. Rare cells are positive for PAX5. CD3 and CD5 highlight small T-lymphocytes in the background. Focal rare cells are positive for CD21. Ki-67 highlights 5% of cells. Kappa and lambda by ISH show numerous lambda+ cells.

Flow cytometry: not done

Special Stains:

None

Cytogenetics:

Not performed.

Molecular Analysis:

FISH found *BCL-6* rearrangement (30/50 nuclei in the excisional biopsy of cervical lymph node). *MYC* or *IGH@BCL-2* rearrangements are negative.

MYD88 and *CXCR4* mutations are negative in the right retrocaval lymph node needle core biopsy specimen.

Interesting Feature(s) of Submitted Case:

The original diagnosis of diffuse large B cell lymphoma in the cervical lymph node contained only rare EBV+ cells and rare polytypic plasma cells. After completion of 6 cycles of R-CHOP, a restaging biopsy of retrocaval lymph node showed completely different morphology with numerous EBV+ mature plasmacytoid/plasmacytic cells with lambda light chain restriction and a low Ki67 labeling index (5%).

The marked shift in morphology and phenotype suggests chemotherapy induced EBV+ lymphoproliferative disorder (LPD) with plasmacytic differentiation. The differential diagnosis also includes persistence of underlying low grade disease manifesting mainly as a "plasmacytic lesion" after therapy. Despite a plasmacytic morphology, the immunophenotype is more typical of plasmacytoid lymphocytes (CD19+ CD45+ CD138+, CD20-, PAX5-) than plasma cells (CD19 and CD45 usually negative), arguing against a diagnosis of plasmacytoma.

Proposed Diagnosis:

Diffuse large B cell lymphoma arising from a low grade B cell lymphoma followed by chemotherapy induced EBV associated lymphoproliferative disorder with marked plasmacytic differentiation showing Lambda light chain restricted plasma cells.

Comments:

Cancer Therapy-Associated Lymphoproliferative Disorders is a recently described and under-recognized type of immunodeficiency-associated lymphoproliferative disorder (Reference: Pina-Oviedo, S. et al. Am Surg. Pathol. 42:116-129, 2018). In the referenced study, the mean interval from initiation of therapy for initial hematologic malignancy to onset of LPD was 66 months (range, 3 to 299 mo). The lesions involved nodal or extranodal sites, falling into a wide range of morphological spectrum. EBV was a consistent finding, positive in 82% (14/17) of cases.

In this case, the differential diagnosis also includes persistent underlying low grade lymphoma after chemotherapy with selective expansion of EBV+ drug resistant clone. Without biopsy confirmation of the EBV+ "plasmacytic" lesion, the PET/CT findings suggest persistent underlying disease. The biopsy proven EBV+ lesion posed a therapeutic dilemma for the clinicians: whether to continue chemotherapy to eradicate residual disease or to remove further immunosuppression. The patient is currently under observation without further treatment.

EBV+ Cancer Therapy-Associated Lymphoproliferative Disorders Following R-CHOP for Diffuse Large B Cell Lymphoma

- Pei Lin, MD, Zhuang Zuo, MD, PhD, Sergej Konoplev, MD, PhD, M. James You, MD, PhD
- Affiliation: The University of Texas-MD Anderson Cancer Center, Houston, TX 77030
-
- E-mail:peilin@mdanderson.org

1

History

- A 71 year man noted a "lump" in his neck for several months.
- PE found generalized lymphadenopathy, but no hepatosplenomegaly
- Serological tests for HIV, HCV, HBV all negative
- PET/CT revealed multi-compartmental hypermetabolic lymphadenopathy above and below the diaphragm

2

History

- *An excisional biopsy of submandibular salivary gland and adjacent lymph node was obtained in 10/2018*
- A diagnosis of DLBCL was rendered, possibly arising from a low grade B cell lymphoma
- After 6 cycles of R-CHOP, a low level of lymphadenopathy was still detected in the abdomen by PET/CT
- *Restaging needle core biopsy of the right retrocaval lymph node was obtained in 4/2019*

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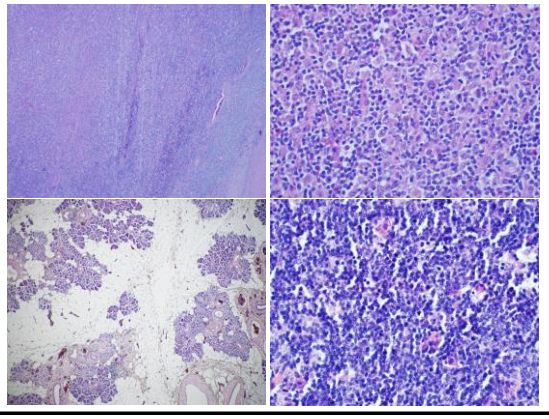
Submandibular salivary gland and adjacent lymph node excisional biopsy in 10/2018



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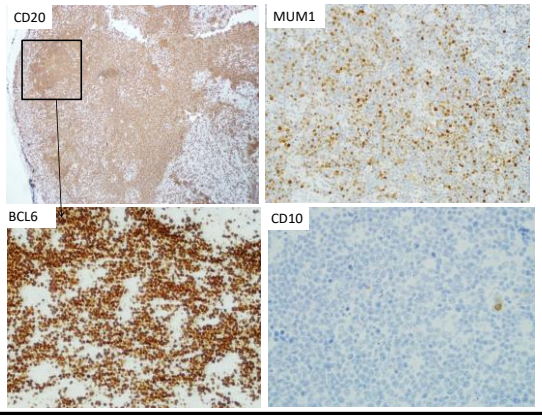

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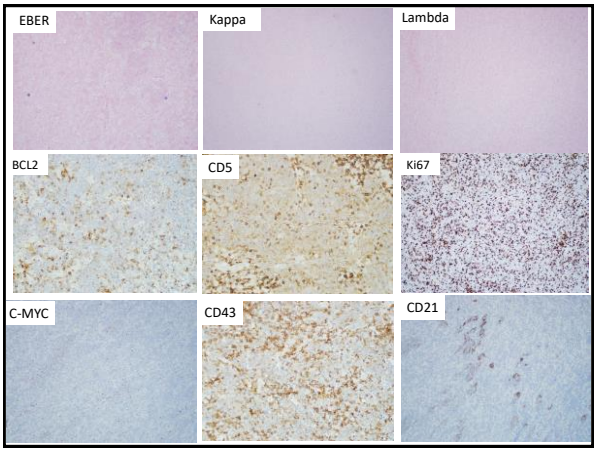

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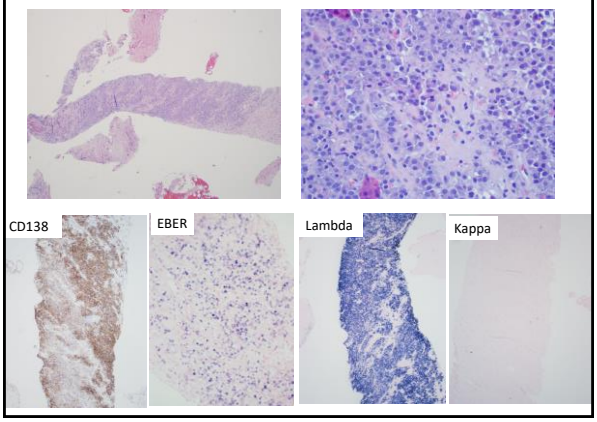
Submandibular salivary gland and adjacent lymph node excisional biopsy in 10/2018

- Most infiltrating cells are positive for CD20, PAX-5 and MUM1 (subset)
- BCL-6 large cells are positive for CD5 but negative for BCL2, CD10, CD30, Cyclin D1 and MYC (<30%)
- EBER highlights rare small cells.
- Ki-67 is variable, ranging from 50% (mainly BCL6 positive large cells) to 10% in the other areas.
- Kappa and lambda by IHC and ISH show rare polytypic plasma cells.
- CD21 and CD23 reveal rare foci of disrupted FDC meshworks
- CD3 and CD43 stain the background T-cells.
- FISH found BCL6 rearrangement but no BCL2 or MYC rearrangement

7

Right retrocaval lymph node, needle core biopsy in 4/2019:



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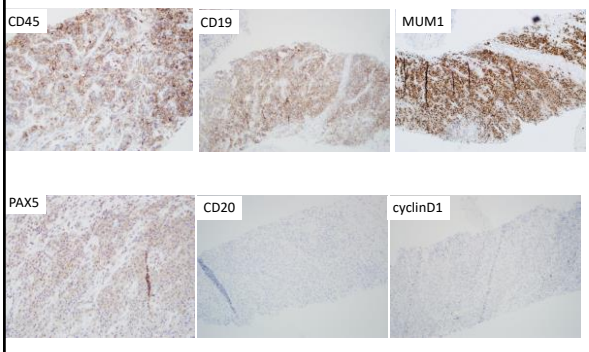
Right retrocaval lymph node, needle core biopsy in 4/2019



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Right retrocaval lymph node, needle core biopsy from 4/2019

- The infiltrating cells are positive for CD138, CD19, CD45, MUM-1 and BCL-2;
- negative for CD20, Cyclin D1, CD56, CD117, BCL-6 and c-MYC.
- Rare cells are positive for PAX5.
- Ki-67 highlights 5% of cells.
- Kappa and lambda by ISH show numerous lambda+ cells
- Molecular study: Negative for MYD88 and CXCR4 mutations

10

Proposed Diagnosis

Diffuse large B cell lymphoma arising from a low grade B cell lymphoma followed by chemotherapy induced EBV associated lymphoproliferative disorder with marked plasmacytic differentiation

11

Interesting Feature(s) of Submitted Case

- The original diagnosis of diffuse large B cell lymphoma in the cervical lymph node contained only rare EBV+ cells and rare polytypic plasma cells. After completion of 6 cycles of R-CHOP, a restaging biopsy of retrocaval lymph node showed completely different morphology with numerous EBV+ cells, marked plasmacytic differentiation with lambda light chain restriction and a low Ki67 labeling index (5%).
- The marked shift in morphology and phenotype suggests chemotherapy induced EBV+ lymphoproliferative disorder (LPD) with plasmacytic differentiation.
- The differential diagnosis also includes persistence of underlying low grade lymphoma manifesting mainly as a "plasmacytic lesion". Despite a plasmacytic morphology, the immunophenotype is more typical of plasmacytoid lymphocytes (CD19+ CD45+ CD138+, CD20-, PAX5-) than plasma cells (CD19 and CD45 usually negative), arguing against a diagnosis of plasmacytoma.

12

Comments

- **Cancer Therapy-Associated Lymphoproliferative Disorders is a recently described and under-recognized type of immunodeficiency-associated LPD (Reference: Pina-Oviedo, S. et al. Am Surg. Pathol. 42:116-129, 2018).**
- **In the referenced study, the mean interval from initiation of therapy for initial hematologic malignancy to onset of LPD was 66 months (range, 3 to 299 mo). The lesions involved nodal or extranodal sites falling into a wide range of morphological spectrum. EBV was a consistent finding, positive in 82% of cases.**

Comments

- **The differential diagnosis also includes persistent underlying low grade lymphoma after chemotherapy with selective expansion of EBV+ drug resistant clone.**
- **Without biopsy confirmation of the EBV+ "plasmacytic" lesion, the PET/CT findings suggest persistent underlying disease.**
- **The biopsy proven EBV+ lesion posed a therapeutic dilemma for the clinicians: whether to continue chemotherapy to eradicate residual disease or to remove further immunosuppression.**
- **The patient is currently under observation without further treatment.**

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Ke Zhenyu, Resident
Guo Shuangping, MD PhD
Wang Zhe, MD PhD

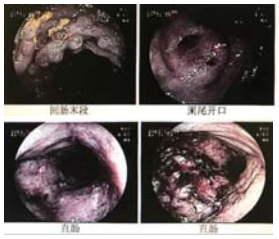
Affiliation:
Department of Pathology, Xijing Hospital and School of Basic Medicine, Air Force Medical University

E-mail:
153417954@qq.com; guoshp@fmmu.edu.cn;

Clinical History:
Male, 58-year-old, hemafecia with change of stool property for more than 6 months.

Biopsy Fixation Details:
(The terminal ileum) one piece of small gray tissue, size: 0.2 × 0.1 × 0.1 cm.
(The appendicitis orifice) two pieces of small gray tissue, size: 0.3 × 0.2 × 0.1 cm.
(Rectum) Six pieces of small gray tissues, size: 0.6 × 0.5 × 0.2 cm.

Description of Clinical Image if any:
A wide mucosal hemispherical bulge can be observed in the terminal ileum and was biopsied under gastrointestinal endoscopes. The surface of mucosa was partially congested, and the biopsy was soft.
A semi-circular bulge of mucosa with smooth surface and was observed at the appendicitis orifice, and lesion was biopsied with firm and brittle texture.
A big cauliflower like tumor with erosion and haemorrhage was visible in the rectum 14-8 cm away from the anus. The tumor was biopsied.



Details of Microscopic Findings:
The architecture of mucosa was destroyed with lose of normal glands. The neoplastic cells diffusely infiltrate the mucosa of the terminal ileum, the appendicitis orifice and rectum with permeation pattern. Reactive follicles with enlarged germinal center and lymphoepithelial lesions were easily observed.The neoplastic cells were small to medium-sized, with slightly irregular nuclei and inconspicuous nucleoli, resembling those of centrocytes, and relatively abundant, pale cytoplasm. Large cells resembling centroblasts or immunoblasts were also present. There were plasmacytic differentiation.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
Positive: CD20、PAX-5、MUM1、EBER
Negative：CD3、CD21、CD23、CD10 、CyclinD1、SOX-11、CD56、TIA-1、Granzyme B、CMV、EBNA2
Kappa/lambda no restriction
Bcl-2 (+10%) 、Bcl-6 (+20%)、C-myc (+20%) 、Ki67 (+, hotspot 30%)

Special Stains:
N

Cytogenetics:
N

Molecular Analysis:
N

Interesting Feature(s) of Submitted Case:
EBV-positive extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue in the gastrointestinal tract.

Proposed Diagnosis:
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue, EBER+.

Comments:
Male, 58 years old, hemafecia with change of stool property for more than 6 months.
Diagnosis：Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue, EBER+.

Case report

Ke Zhenyu/Guo Shuangping

Department of Pathology, Xijing Hospital and School of Basic Medicine, Air Force Medical University

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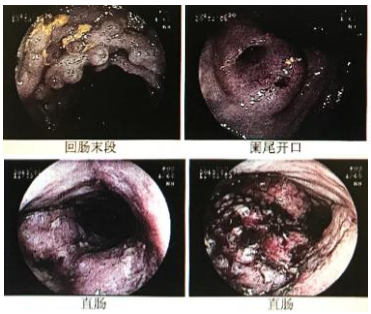
Clinical History

- Male, 58 years old
- hemafecia with change of stool property for more than 6 months

2

Endoscope



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Clinical diagnosis and Generally

- [Clinical diagnosis]:

  Lymphoid follicle protuberance at the terminal ileum?

  Appendectomy mucosal lesions?

  Rectal Cancer ?
- [Generally]:

  (The terminal ileum) one piece of small gray tissue, size: 0.2 × 0.1 × 0.1 cm.

  (The appendicitis orifice) two pieces of small gray tissue, size: 0.3 × 0.2 × 0.1 cm.

  (Rectum) Six pieces of small gray tissues, size: 0.6 × 0.5 × 0.2 cm.

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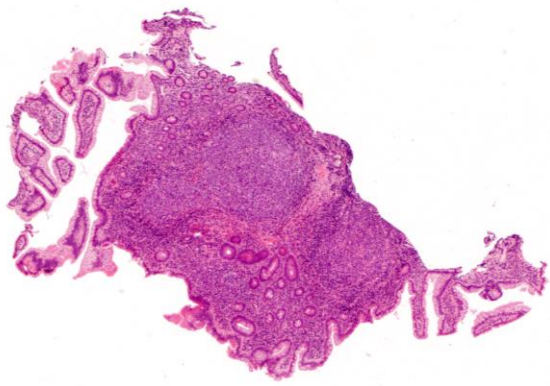
The terminal ileum



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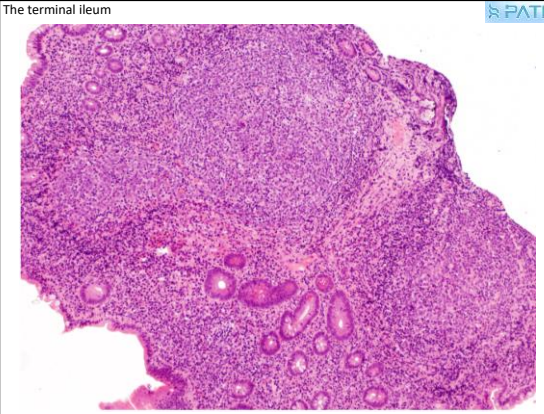
The terminal ileum



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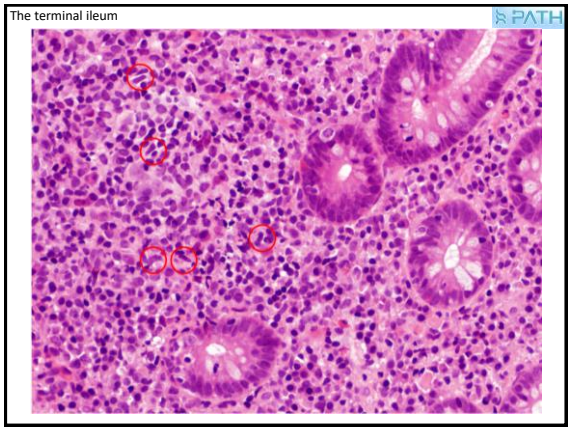
The terminal ileum
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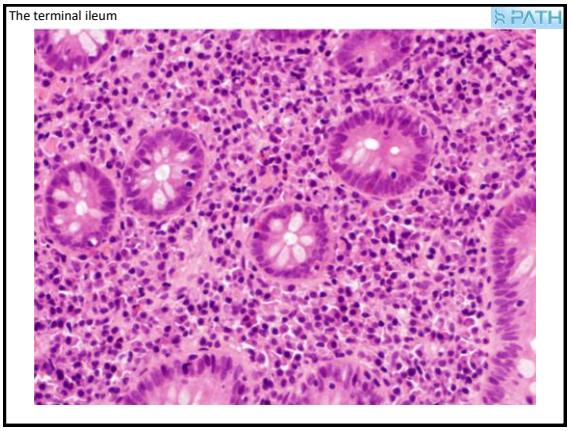
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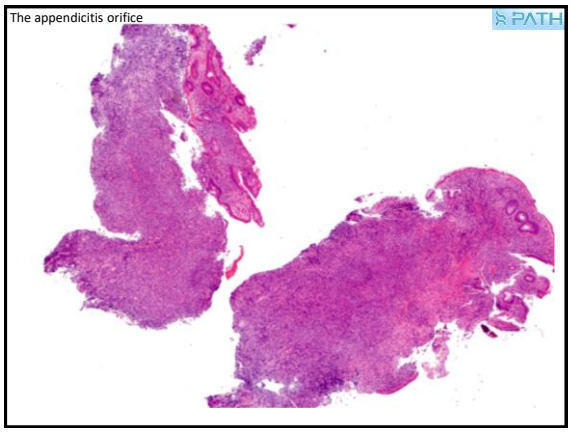
The appendicitis orifice
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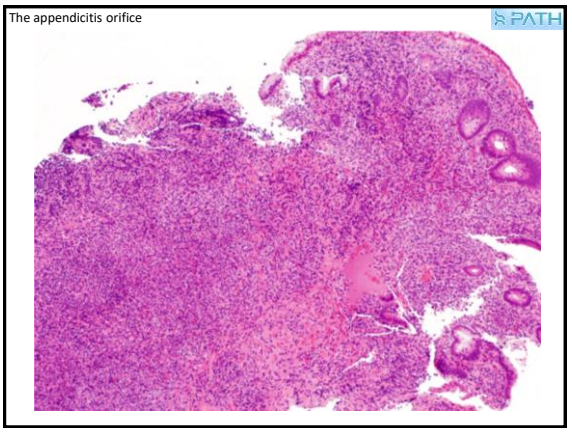
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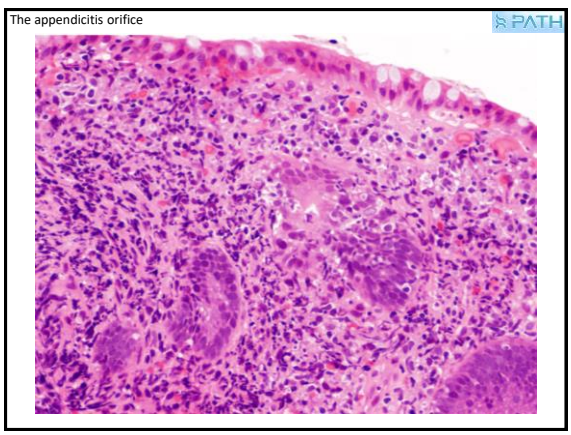
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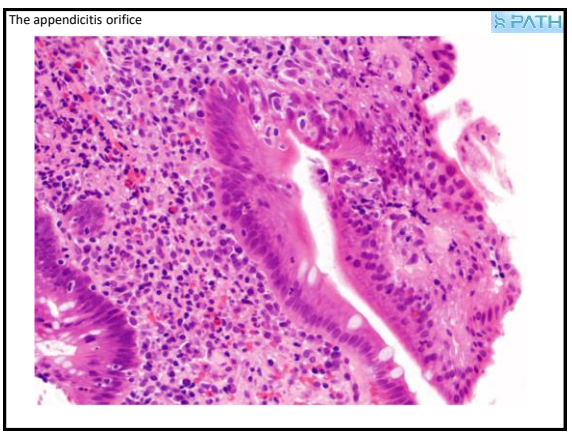
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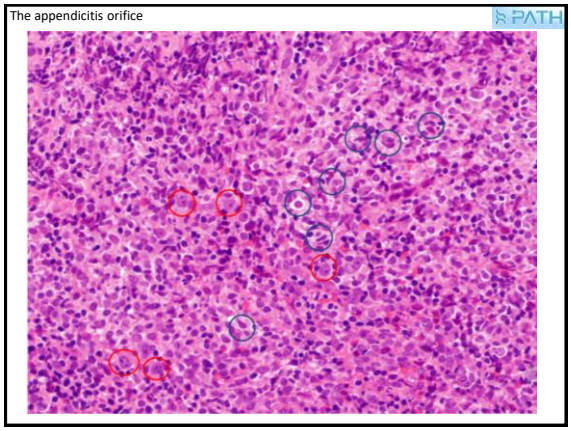

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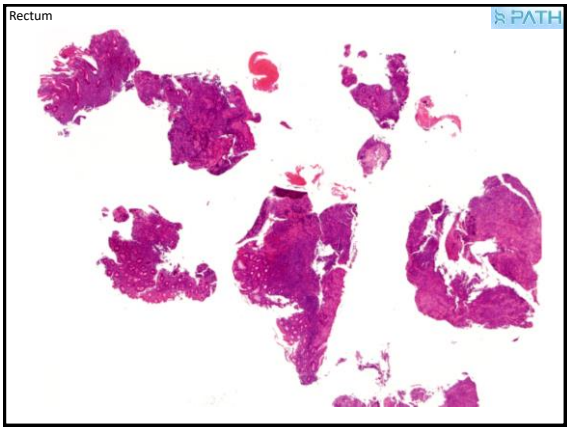

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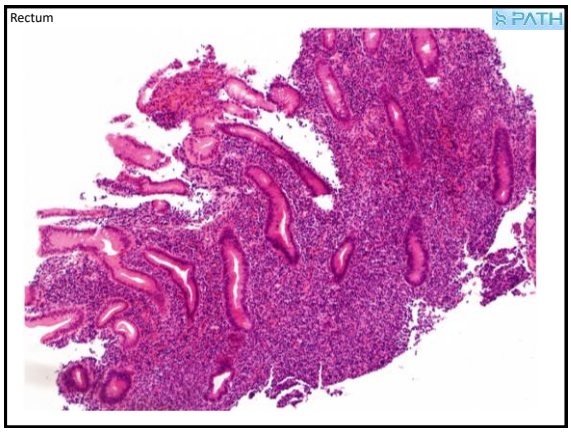

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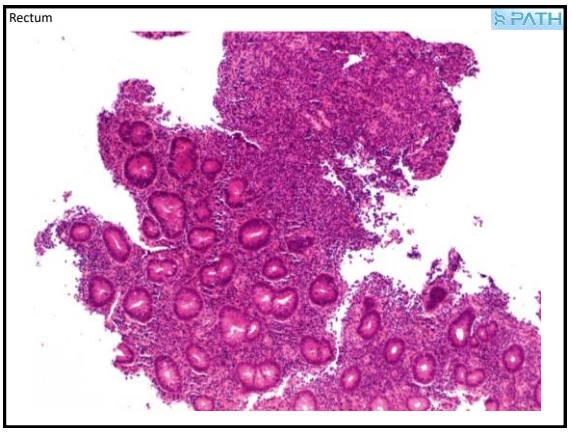

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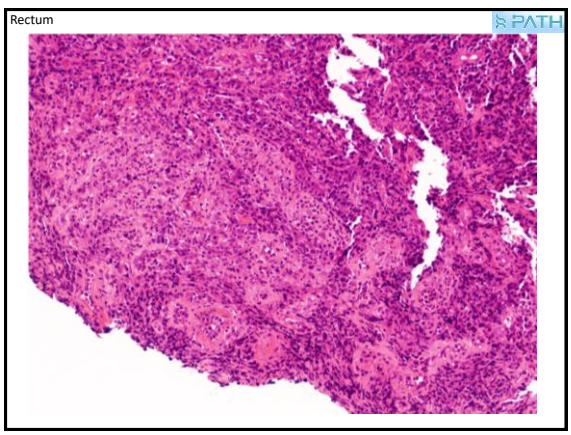

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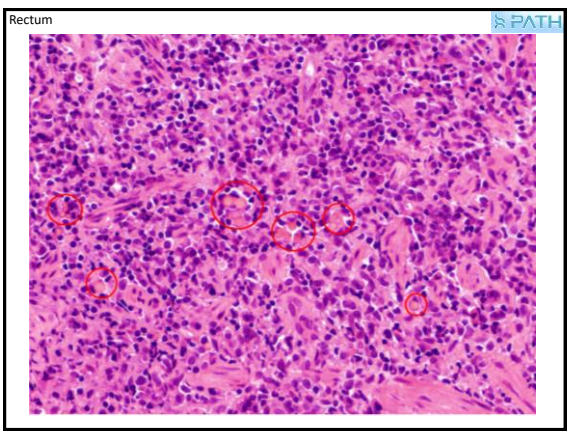

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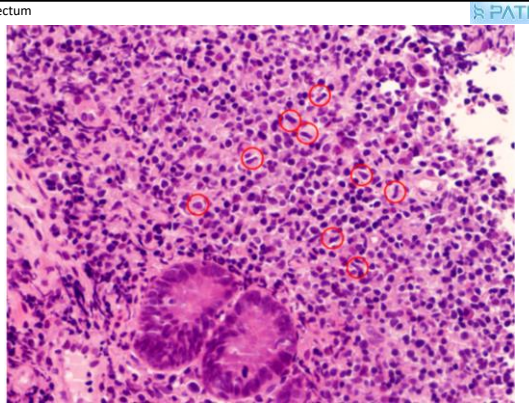
Rectum



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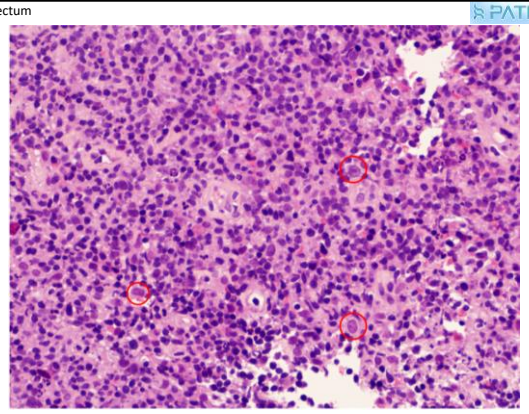
Rectum



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DIFFERENTIAL DIAGNOSIS ?

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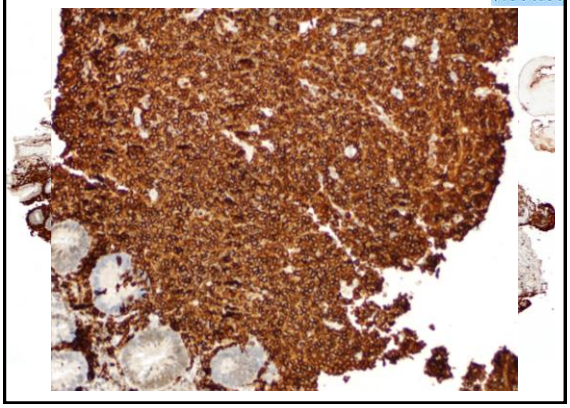
CD20



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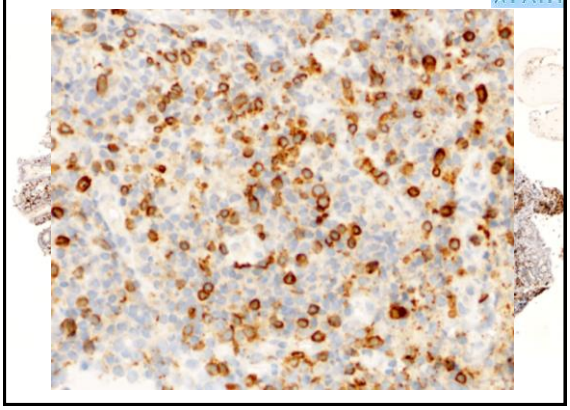
CD3



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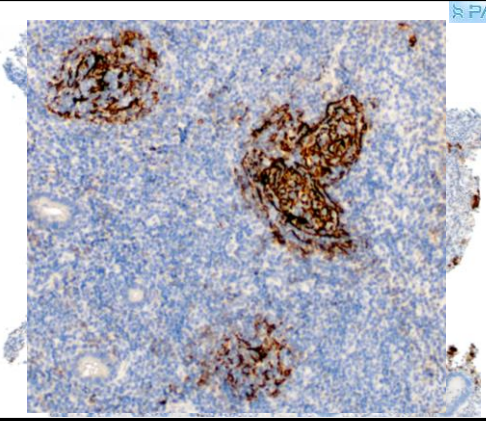
CD21



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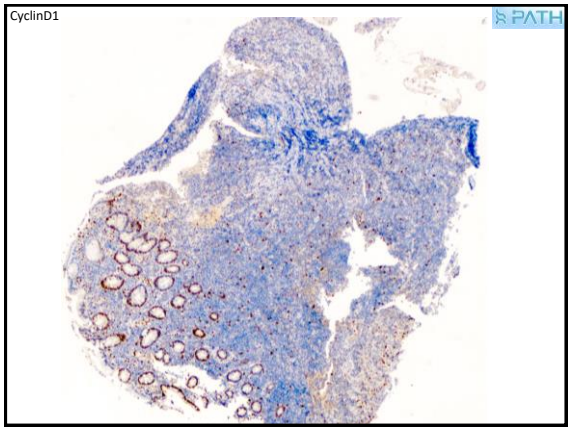

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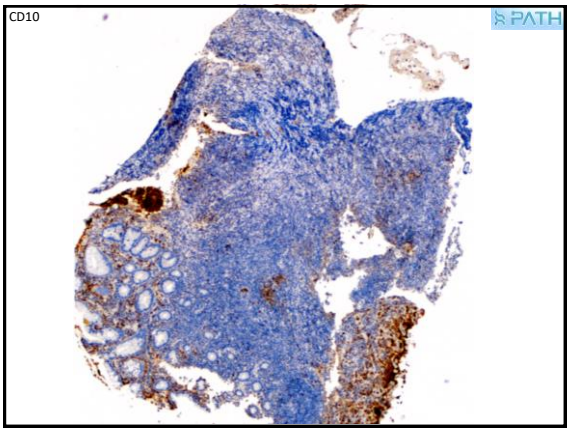

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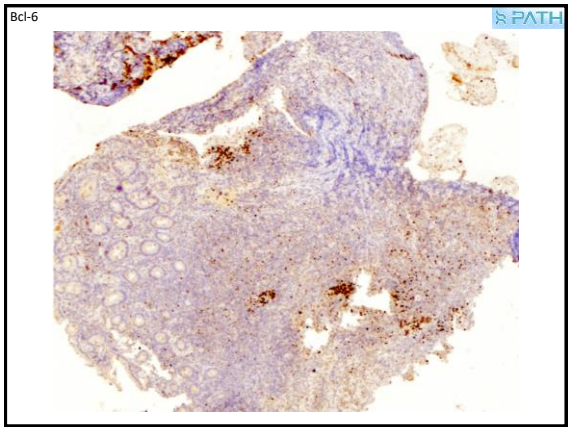

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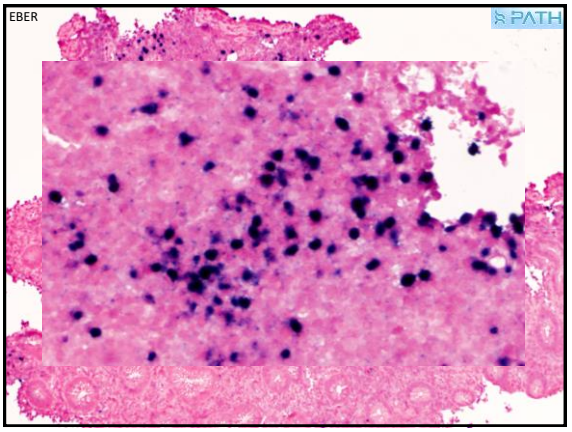

28

PATH

Diagnosis

Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue, EBER +

29

PATH

Literature review

- The marginal zone lymphoma of EBV reported in the literature usually occurs in the skin, lung, tonsil, parotid gland, soft tissue and other parts.
- Currently, there are 1 case of stomach and 1 case of small intestine. However, no reports of rectal EBV+ MALT lymphoma occurred.
- We first reported the EBV+ MALT lymphoma that occurred in the rectum.

30

Literature review

ORIGINAL ARTICLE

Lymphoproliferative disorders in Oxford renal transplant recipients

W D Bates, D W R Gray, M A Dada, R Chetty, K C Gatter, D R Davies, P J Morris

J Clin Pathol 2003;56:439–446

52/M, Kidney transplant

93 months later occur MALT lymphoma in Small bowel, EBV+

American Journal of Transplantation 2005; 5: 2901–2906
Blackwell Munksgaard

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doi: 10.1111/j.1600-6143.2005.01098.x

Effect of Anti-CD 20 Antibody Rituximab in Patients with Post-Transplant Lymphoproliferative Disorder (PTLD)

51/F, Kidney transplant

156.9 months later occur MALT lymphoma in Stomach , EBV+

31

Literature review

- EBV+ B-cell lymphomas are most often high grade.
- EBV+ B-cell lymphomas occur sporadically, but many show greater prevalence in the setting of immune deficiency.
- EBV+ low-grade B-cell lymphomas, in particular, EBV+ extranodal marginal zone lymphoma, are rare and almost exclusively seen in the posttransplant setting.

32

Literature review

Am J Surg Pathol 2018;42:1306–1316)

Expanding the Spectrum of EBV-positive Marginal Zone Lymphomas

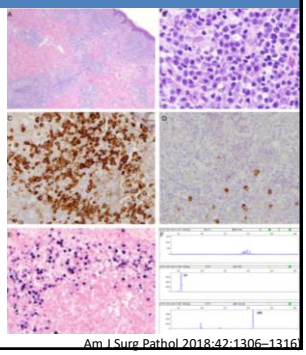
A Lesion Associated With Diverse Immunodeficiency Settings

Three cases (cases 3, 4, and 8) had no underlying risk factors other than age, with presumed immune senescence, presenting with EBV+ MALT lymphoma at ages 69, 70, and 86.

33

Literature review

FIGURE 3. EBV+ MALT lymphoma in a 70-year-old male with no significant past medical history (case 3).
A, The infiltrate involves the dermis and subcutaneous tissue with a perivascular and periadnexal distribution.
B, The infiltrate is composed of monocytoid and plasmacytoid cells.
The plasmacytoid cells are positive for lambda (C) and negative for kappa (D) light chain.
E, EBER shows a similar distribution to lambda.



Am J Surg Pathol 2018;42:1306–1316

34

Conclusion

- EBV infection usually has two states:

  immunodeficiency or non-immune deficiency
- Our case had no history of immunosuppressive therapy or prior malignancy, and EBNA2-.
- Generally, gastric MALT lymphoma is closely related to Hp, but rectal MALT does not have a clear cause.
- Whether EBV infection is a causative factor or a concomitant symptom requires further research.

35

THANK YOU

36

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Shaoying Li, MD, Sa Wang, MD, Sergej Konoplev, MD, and L. Jeffrey Medeiros

Affiliation: MD Anderson Cancer Center

E-mail: sli6@mdanderson.org

Clinical History: The patient is a 40+ year old man with 8-year history of HIV who presented with enlarged lymph nodes not responsive to antibiotics. A right inguinal LN FNA core biopsy was performed.

Biopsy Fixation Details: 10% Formalin

Description of Clinical Image if Any: CT scan showed adenopathy above and below the diaphragm.

Details of Microscopic Findings: The FNA core biopsy showed fragments of lymphoid tissue with no normal nodal architecture. There was diffuse lymphoid infiltration with focal geographic necrosis and angioinvasion. The lymphoma cells were large and pleomorphic, ranging from centroblasts, immunoblasts, plasmablasts, anaplastic large cells to Reed-Sternberg like cells. Apoptotic bodies and mitotic bodies were frequent.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry: The lymphoma cells are:

Positive for: CD20 (small subset), CD22, PAX5 (weak), CD30, CD38 (dim partial), CD43, CD45 (bright), BCL6 (weak), MUM1, and FMC7

Negative for: CD3, CD5, CD10, CD44, CD138, CD200, BCL2, and Surface light chains

Ki67 stain showed a high proliferation rate of >90%.

In situ hybridization study for EBV encoded RNA (EBER) showed strong diffuse stain in all lymphoma cells

Special Stains: None

Cytogenetics: FISH study showed no *MYC* rearrangement.

Molecular Analysis: none

Interesting Feature(s) of Submitted Case:

- A unique case of EBV+ DLBCL in a patient with HIV
  - Lack of expression of several pan-B cell markers (partially or totally) in lymphoma cells: likely related to the EBV infection
  - Strong expression of EBER in all cells: not common in non-immunocompromised patients, even in EBV+ DLBCL
  - Characteristic morphologic features: necrosis, angioinvasion, spectrum of the lymphoma cells including centroblasts, immunoblasts, plasmablasts, anaplastic large cells, and Reed-Sternberg like cells
- The morphologic and immunophenotypic features pose a diagnostic challenge

Proposed Diagnosis:

CD30-positive, EBER-positive Diffuse large B cell lymphoma with necrosis and anaplastic features, non-GCB type

Comments: Although multiple features (listed below) associated with unfavorable prognosis were present, the patient reached complete remission (CR) after 6 cycles of R-EPOCH treatment and has been in CR ever since (6 years by the time of this submission). This might be related to the well-controlled underline disease (HIV).

- Necrosis, anaplastic morphology
- Non-GCB type
- CD30 and EBER coexpression, which was reported to be a worse prognostic factor in DLBCL patients

EBV+ /CD30+ DLBCL in a HIV Patient

Shaoying Li, MD, Sa Wang, MD, Sergej Konoplev, MD, and L. Jeffrey Medeiros

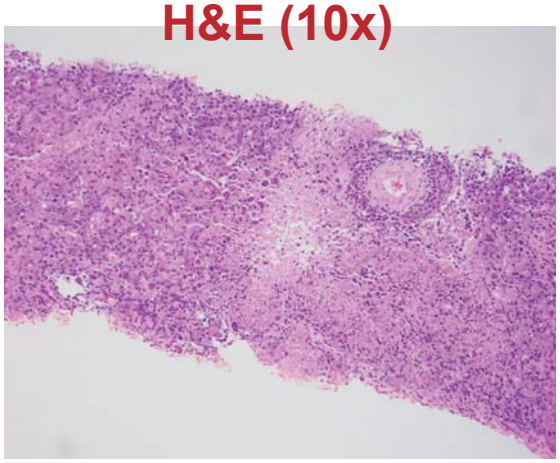
Department of Hematopathology
MD Anderson Cancer Center

History

- A 40+ year old man* with 8 year history of HIV
- CBC: WBC 6.1 (6% Lym, CD4:CD8=1:5.5), Hgb 8.8, MCV 82, Plt 70
- He presented with enlarged lymph nodes, not responsive to antibiotics
- CT scan showed adenopathy above and below the diaphragm
- A right inguinal LN biopsy was performed

*Age<45 yrs, exact number not provided due to HIPPA

H&E (10x)



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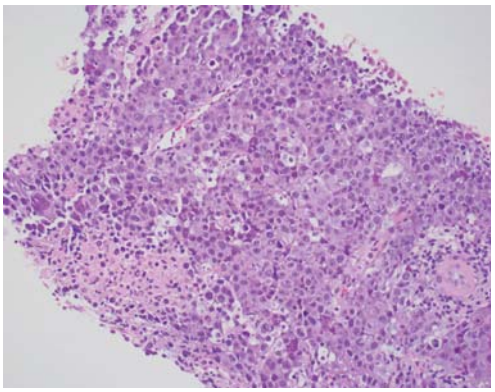

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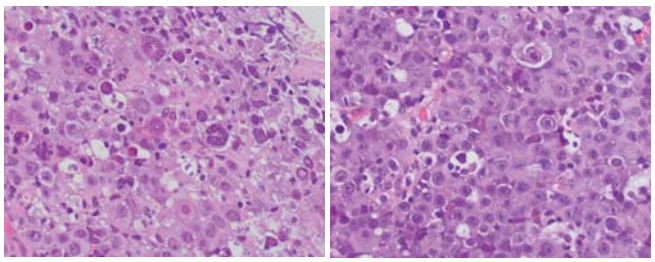
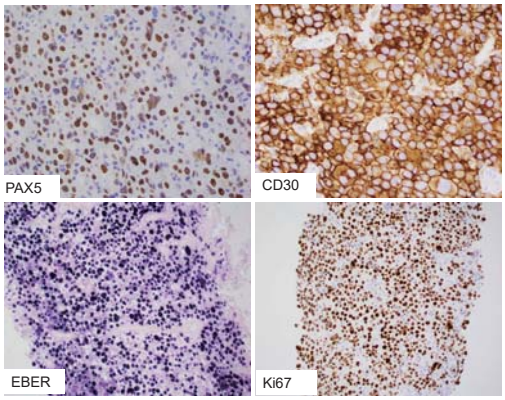


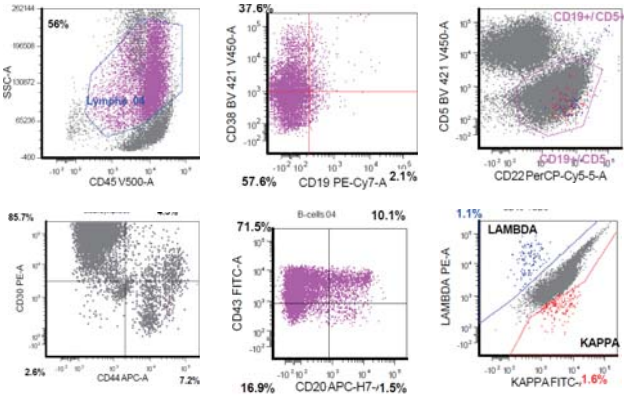
Summary of Morphology

- Diffuse lymphoid infiltration with focal geographic necrosis and angioinvasion
- Lymphoma cells are large and pleomorphic, ranging from centroblasts, immunoblasts, plasmablasts, anaplastic large cells to Reed-Sternberg like cells.
- Frequent apoptotic bodies and mitotic bodies

Immunhistochemistry & In situ Hybridization



Flow Cytometry



Summary of Immunophenotype

- B markers
  - Positive: CD20 (small subset), CD22, PAX5 (weak)
  - Negative: CD19, CD79a
- CD30+ (strong diffuse)
- EBER+ (strong diffuse)
- CD45+ (bright)
- Ki67: >90%
- Others
  - Positive: CD38 (dim partial), CD43, BCL6 (weak), MUM1, FMC7
  - Negative: CD3, CD5, CD10, CD44, CD138, CD200, BCL2, and Surface light chains

FISH

- Negative for MYC rearrangement

Final Diagnosis

- CD30-positive, EBER-positive Diffuse large B cell lymphoma with necrosis and anaplastic features
- Non-GCB type

Follow Up

- Received R-EPOCH x6 (along with HAART for HIV) which finished in 2013
- CR until now (6/2019)
- Current CBC/Differential is normal
  - Current CD4:CD8=1:2.5

Interesting Points of the Case

- A unique case of EBV+ DLBCL in a patient with HIV
  - Lack of expression of several pan-B cell markers (partially or totally) in lymphoma cells: likely related to the EBV infection
  - Strong expression of EBER in all cells: not common in non-immunocompromised patients, even in EBV+ DLBCL
  - Characteristic morphologic features: necrosis, angioinvasion, spectrum of the lymphoma cells including centroblasts, immunoblasts, plasmablasts, anaplastic large cells, and Reed-Sternberg like cells
- The morphologic and immunophenotypic features pose a diagnostic challenge
- Although multiple features associated with unfavorable prognosis were present, the patient had a very good prognosis which might be related to the well controlled underline disease (HIV)
  - Necrosis, anaplastic morphology
  - Non-GCB type
  - CD30 and EBER coexpression, which was reported to be a worse prognostic factor in DLBCL patients

Chronic inflammation-related diffuse large B cell lymphoma: case report

Submitter(s) and Titles (MD or MD PhD):
Qingming Jiang Deputy Chief Physician

Affiliation:
Chongqing University Affiliated Cancer Hospital & Chongqing Cancer Institute & Chongqing Cancer Hospital

E-mail:
jiangqm80@126.com

Clinical History:
A 71-year-old male, presented with the right chest pain for more than 1 month, increased cough, deep inhalation, no obvious fever, night sweats, weight loss and other symptoms.
Past medical history: suffered from tuberculous pleurisy improved after treatment 40 years ago.
Physical examination: A mass with a diameter of about 4cm was palpated in the 5th-6th rib area of the right chest wall, with poor mobility and no tenderness. No enlarged lymph nodes were palpated in bilateral neck, armpit and groin regions.
Complete blood count: RBC 3.59×1012/L, WBC 6.8×109/L, percentage of lymphocytes 12% (20-50%), percentage of neutrophils 80.90% (40-75%), PLT 242×109/L .Blood LDH: 262.50 U / L (109-245).
Blood TB-IgG(-), TB-IgM(-).TB-IGRA:56.60 (<14), lymphocyte immunoassay+IFN-γ 660.30 (≥20), Mycobacterium tuberculosis infection judgment: Positive. Blood immune function test: CD3: 543 × 106/L (955-2860), CD8: 220×106/L (320-1250), CD4: 306×106/L (550-1440). Blood EBV-DNA: 2.04E+03 copies/ml (< 5.00E+02). HIV antibody/P24 antigen: (-).
Blood BUN and CRE (no abnormality is found): CRE 79.10 μmol/L , BUN 7.68 mmol/L. Immunoglobulin test: IgG ↑16.80g/L(7.51-15.60), IgA 2.93g/L(0.82-4.53), IgM 1.45g/L(0.46-3.04), IgE↑1360.00IU/m(l0.00-165.00), κ-LC ↑15.10g/L(6.29-13.50), λ-LC ↑9.06g/L(3.13-7.23).

Serum protein electrophoresis: Albumin(α) 48.30% (55.8-66.1), α1 globulin (α1) ↑ 6.50% (2.9-4.9), α2 globulin (α2) 10.20% (7.1-11.8), β1 globulin (β1) 5.70% (4.7-7.2), γ globulin (γ) 22.70%(11.1-18.8), β2 globulin (β2) ↑ 6.60% (3.2-6.5).
Urinary Bence-Jone protein: Negative
Macroscopic examination: (Right chest wall 5-6 intercostal mass puncture tissue) a pile of grayish white filamentous tissue, 0.5 × 0.5 × 0.1cm3.

Biopsy Fixation Details: neutral formalin

Description of Clinical Image if Any:
Chest contrast-enhanced CT scan: 1. A low-density mass was visible in the right lower thoracic cavity with clear boundary, with a range of about 7.3×12.5cm and thick wall. Multiple strips and nodule calcification foci were visible in the wall. No definite enhancement was found in contrast-enhanced CT.scan 2. Soft tissue nodule shadow could be seen between the 5th and 6th ribs on the right side, the boundary was unclear, the range was about 4.6×3.5cm, the plain scan density was not uniform, patchy low density area could be seen in the plain scan, the enhanced scan had enhancement, and the bone adjacent to the 6th rib was destroyed.
DR: No definite abnormality was found in skull, bilateral femur, tibia and fibula, humerus, ulna and radius and pelvis.
PET-CT: 1. Right chest wall mass (lymphoma imaging features), partially located in parietal pleura; The right 5th/6th anterior rib was involved with bone destruction. 2. Encapsulated pleural effusion with medial subcapsular nodule, increased glucose metabolism, and lymphoma involvement may be considered. 3. Encapsulated pleural effusion in right thoracic cavity. 4. Nodules in upper lobe of both lungs and streak-like high density shadow, combined with medical history, conform to tuberculosis.
Ultrasonography showed that the size and shape of both kidneys were normal, the boundary between the cortex and medulla was clear, and the renal sinus was not separated.

Details of Microscopic Findings: Tumor cells are composed of diffuse larger lymphocytes, mainly composed of central mother/immunoblasts, with pleomorphic features, nuclear round, single or multiple distinct nucleoli, with plasma cell differentiation. Necrosis and blood vessels center growth can be seen. Small lymphocytes and tissue cells are interposed between the tumor cells.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

(Immunophenotyping by Immunohistochemistry)
Positive: CD20 partially(+), CD79α weakly(+), PAX-5(+), CD38 focally(+), CD138 focally(+), MUM-1(+), CD3(+), CD43 weakly(+), CD45(+), Bob.1 partially(+), κ weakly(+), λ focally(+), κ＞λ, CD99(+), Bcl-2 70%(+), C-myc 40%(+), ki-67(+)＞90%.
Negative: CD2, CD5, CD7, CD4, CD8, CD45Ro, CD56, CD30, CD15, CD10, Bcl-6, Oct-2, CD56, EMA, TdT, CD34, CD68(KP1), CD68(PGM1), MPO, CD117, CD123, CD33, CD1a, CD21, CD23, CyclinD1, SOX11, CK-pan.

Flow Cytology of Bone Marrow: 1. No definite abnormality was found in T lymphocyte, B lymphocyte and NK cell; 2. Plasma cells accounted for 0.1% of all nucleated cells. No obvious monoclonal was found. Some cell phenotypes showed reactivity. 3. The proportion of myeloid cells increased, with cells in the mature stage predominated and immature cells decreased.

In situ hybridization: EBER positive.

Special Stains: Not done.

Cytogenetics: FISH for C-myc/IgH fusion gene (-), Bcl-2/IgH fusion gene (-), Bcl-6 break gene (-). .

Molecular Analysis: IgH(FR2-JH) monoclonal, Igκ (Vκ-Jκ) monoclonal, IgL polyclonal. TCRβ, TCRγ and TCRδ polyclonal

Interesting Feature(s) of Submitted Case: The prototype of chronic inflammation-related DLBCL (DLBCL associated with chronic inflammation) is pyothorax-associated lymphoma (PAL). It occurs in the body cavity and narrow space, and is associated with long-term chronic inflammation and EBV infection. The tumor cells are derived from B lymphocytes from the germinal center driven by EBV infection. The median age of onset is around 65-70 years old, male more than female. Patients with a history of chronic pyothorax, often treated with artificial pneumothorax for tuberculosis, accompanying EBV infection, most of the disease sites are located in the pleura and adjacent areas. The appearance of the mass is like a solid tumor, the volume is large, and the soft tissue and ribs of the chest are damaged. In this case, CD20 was partially deleted, with CD38, CD138, MUM-1 partially expression (plasma cell differentiation), and abnormal expression of T cell marker CD3, and EBER was positive. Differential diagnosis includes primary effusion lymphoma, multiple myeloma, extramedullary plasmacytoma, plasminoblastic lymphoma, simple pyothorax, poorly differentiated carcinoma, and malignant mesothelioma.The disease is an invasive lymphoma with a poor prognosis, and median survival time is about 9 months. This case was followed by GDP regimen (gemcitabine + netabaslatine + dexamethasone) chemotherapy for 3 courses, accompanied by esophageal cancer, and carries out radiotherapy and chemotherapy, died after 11 months.

Proposed Diagnosis: Diffuse large B-cell lymphoma associated with chronic inflammation, with plasma cell differentiation and necrosis.

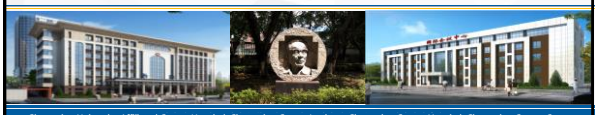
Comments:

The 1st Joint Chinese Society for Hematopathology and Society for Hematopathology Workshop/CSHP 2019

Chronic inflammation-related diffuse large B cell lymphoma: case report

Qingming Jiang Yu Li

Department of Pathology, Chongqing University Affiliated Cancer Hospital



Chongqing University Affiliated Cancer Hospital, Chongqing Cancer Institute, Chongqing Cancer Hospital, Chongqing Cancer Center

1

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Ultrasonography showed that the size and shape of both kidneys were normal, the boundary between the cortex and medulla was clear, and the renal sinus was not separated.

2

Clinical history

Complete blood count: RBC 3.59×1012/L, WBC 6.8×109/L, percentage of lymphocytes 12% (20-50%), percentage of neutrophils 80.90% (40-75%), PLT 242×109/L .

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Mycobacterium tuberculosis infection judgment: Positive.

Blood immune function test: CD3: 543 × 106/L (955-2860), CD8: 220×106/L (320-1250), CD4: 306×106/L (550-1440).

Blood EBV-DNA: 2.04E+03 copies/ml (< 5.00E+02). HIV antibody/P24 antigen: (-).

Blood BUN and CRE (no abnormality is found): CRE 79.10 μmol/L , BUN 7.68 mmol/L.

Immunoglobulin test: IgG ↑16.80g/L(7.51-15.60), IgA 2.93g/L(0.82-4.53), IgM 1.45g/L(0.46-3.04), IgE↑1360.00IU/m(0.00-165.00), κ-LC ↑15.10g/L(6.29-13.50), λ-LC ↑9.06g/L(3.13-7.23).

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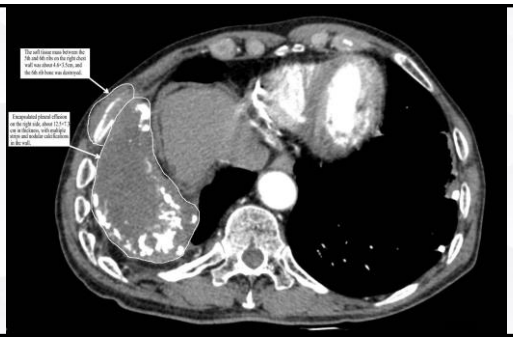
Urinary Bence-Jone protein: Negative

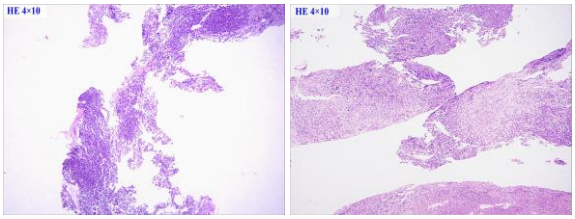
Macroscopic examination: (Right chest wall 5-6 intercostal mass puncture tissue) a pile of grayish white filamentous tissue, 0.5 × 0.5 × 0.1cm3.

3



4

Right chest wall mass biopsy-HE



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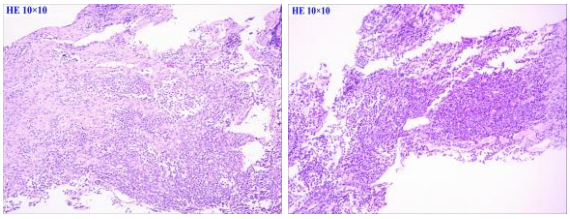
5

Right chest wall mass biopsy-HE



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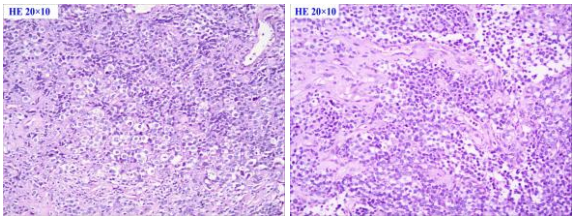
6

Right chest wall mass biopsy-HE



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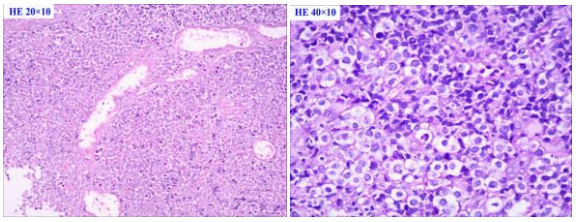
7

Right chest wall mass biopsy-HE



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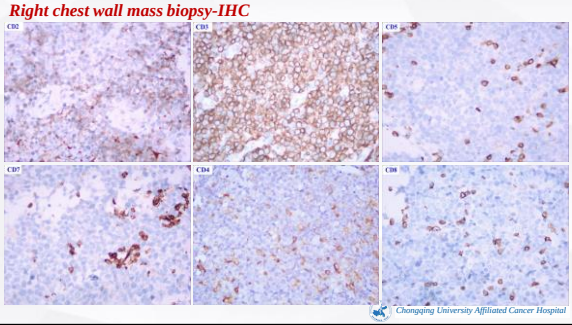
8

Right chest wall mass biopsy-IHC



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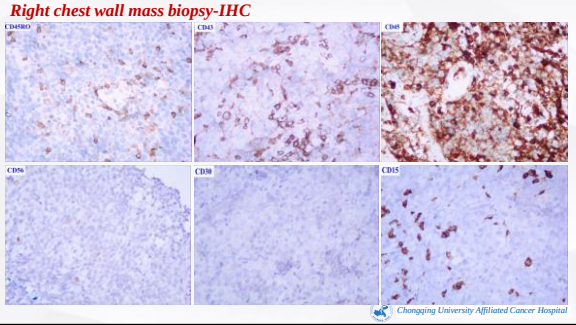
Right chest wall mass biopsy-IHC



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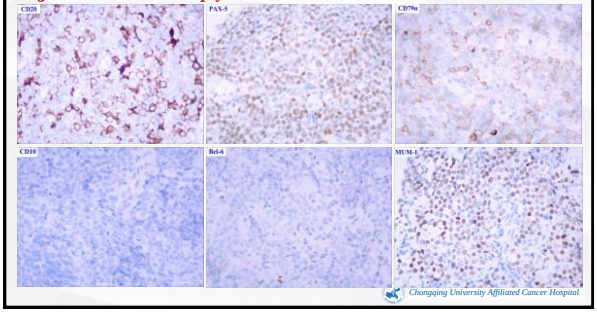
Right chest wall mass biopsy-IHC



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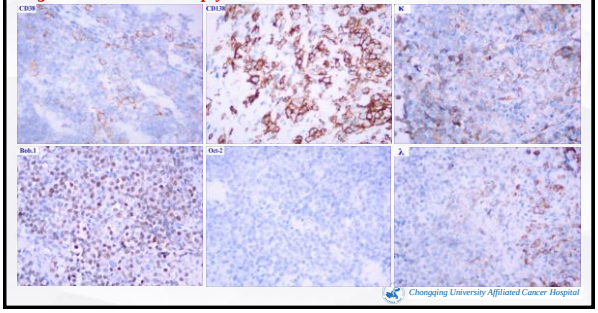
Right chest wall mass biopsy-IHC



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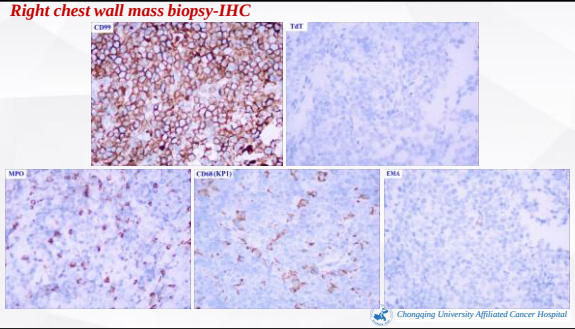
Right chest wall mass biopsy-IHC



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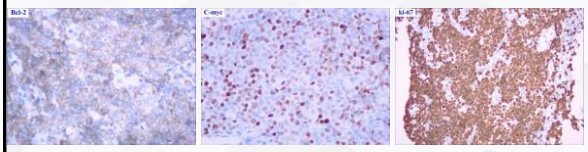
Right chest wall mass biopsy-IHC



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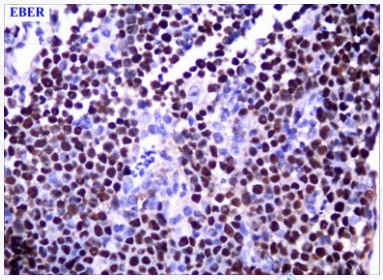
Epstein-Barr virus receptor in situ hybridization



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BCR & TCR

BCR:

IgH (+) <FR2-JH>

Igκ (+) <Vκ-Jκ>

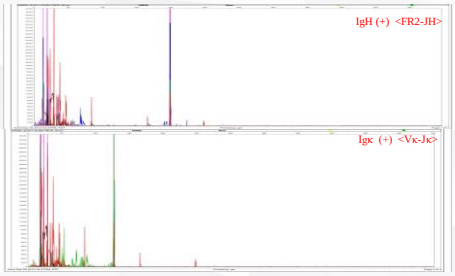
IgL (-)

TCR:

TCRβ (-)

TCRγ (-)

TCRδ (-)



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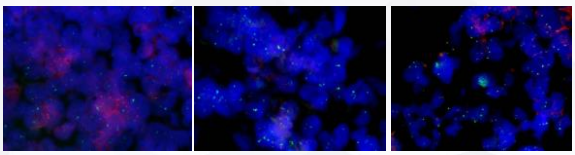
Fluorescence in situ hybridization (C-myc、Bcl-2、Bcl-6)



C-myc/IgH fusion gene : (-) **Bcl-2/IgH fusion gene: (-)** **Bcl-6 break: (-)**

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Summary

<***IHC***> positive: CD20 partially(+), CD79α weakly(+), PAX-5(+), CD38 focally(+), CD138 focally(+), MUM-1(+), CD3(+), CD43 weakly(+), CD45(+), Bob.1 partially(+), κ weakly(+), λ focally(+), κ>λ, CD99(+), Bcl-2 70%(+), C-myc 40%(+), ki-67(+)>90%.

negative: CD2, CD5, CD7, CD4, CD8, CD45Ro, CD56, CD30, CD15, CD10, Bcl-6, Oct-2, CD56, EMA, TdT, CD34, CD68(KP1), CD68(PGM1), MPO, CD117, CD123, CD33, CD1a, CD21, CD23, CyclinD1, SOX11, CK-pan.

<***Epstein-Barr virus receptor in situ hybridization***> : About 80% of cells are positive.

<***Bone marrow cytology and bone marrow biopsy***>: hypoplastic myelogram.

<***Flow Cytology of Bone Marrow***>: 1. No definite abnormality was found in T lymphocyte, B lymphocyte and NK cell; 2. Plasma cells accounted for 0.1% of all nucleated cells. No obvious monoclonal was found. Some cell phenotypes showed reactivity. 3. The proportion of myeloid cells increased, with cells in the mature stage predominated and immature cells decreased.

<***BCR & TCR***> IgH: FR2-JH (+), Igκ: vκ-Jκ(+), IgL(-); TCRβ (-), TCRγ (-), TCRδ (-);

<***Fluorescence in situ hybridization***> C-myc/IgH fusion gene (-), Bcl-2/IgH fusion gene (-), Bcl-6 break gene (-).

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Recommended diagnosis

Chronic inflammation-related diffuse large B cell lymphoma

(Non-GCB), with plasma cell differentiation and necrosis.

[pyothorax-associated lymphoma (PAL)]

*Interesting Features of Submitted Case (*Chronic inflammation-related DLBCL*):*

- The prototype of chronic inflammation-related DLBCL (DLBCL associated with chronic inflammation) is pyothorax-associated lymphoma (PAL).
- It occurs in the body cavity and narrow space, and is associated with long-term chronic inflammation and EBV infection. The tumor cells are derived from B lymphocytes from the germinal center driven by EBV infection.
- The median age of onset is around 65-70 years old, male more than female.
- Patients with a history of chronic pyothorax, often treated with artificial pneumothorax for tuberculosis, accompanying EBV infection, most of the disease sites are located in the pleura and adjacent areas.
- The appearance of the mass is like a solid tumor, the volume is large, and the soft tissue and ribs of the chest are damaged.
- In this case, CD20 was partially deleted, with CD38, CD138, MUM-1 partially expression (plasma cell differentiation), and abnormal expression of T cell marker CD3, and EBER was positive.
- Differential diagnosis includes primary effusion lymphoma, multiple myeloma, extramedullary plasmacytoma, plasminoblastic lymphoma, simple pyothorax, poorly differentiated carcinoma, and malignant mesothelioma.
- The disease is an invasive lymphoma with a poor prognosis, and median survival time is about 9 months.
- This case was followed by GDP regimen (gemcitabine + netabaslatine + dexamethasone) chemotherapy for 3 courses, accompanied by esophageal cancer, and carries out radiotherapy and chemotherapy, died after 11 months.

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Sergej Konoplev, MD, PhD, Beenu Thakral, MD, C. Cameron Yin, MD, PhD, Shaoying Li, MD, Pei Lin, MD,

Affiliation:
The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

E-mail:
skonople@mdanderson.org

Clinical History:

A 49-year-old African America woman presented in April 2019 with cough, fever, severe fatigue, and 5 pound weight loss. CBC demonstrated moderate anemia. Serological tests for HIV, HBV, HCV, and HTLV were negative; CMV test was positive for IgG. Bone marrow biopsy performed at another institution demonstrated hypercellular (80%) bone marrow with 32% neoplastic cells interpreted as blasts, the diagnosis of T lymphoblastic leukemia/lymphoma was established. The patient received two cycles of HCVAD therapy and was transferred to our institution for second opinion regarding her refractory disease. Physical exam revealed a massive hepatosplenomegaly; no lymphadenopathy and no other abnormalities were detected.

Biopsy Fixation Details:
Bone marrow core biopsy: formalin-fixed, formic acid-decalcified, paraffin embedded
Clot specimen: formalin fixed, paraffin embedded

Description of Clinical:

CT chest detected poorly defined nodular and ground glass opacities bilaterally. CT abdomen demonstrated hepatosplenomegaly. The liver was diffusely enlarged (27 cm in length). The spleen was markedly enlarged (22.8 cm in length) and showed geographic areas of low attenuation in the periphery suspicious for splenic infarctions. No enlarged lymph nodes in the abdomen or pelvis were detected.

Details of Microscopic Findings:

Bone marrow aspirate smear shows numerous (50%) medium-size cells with open nuclear chromatin, one or two prominent nucleoli, and moderate amount of

deep basophilic cytoplasm without granules. Bone marrow core biopsy and clot specimen shows hypercellular (60-70%) bone marrow with large clusters of medium-size cells with open nuclear chromatin.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

We performed flow cytometry immunophenotypic studies using bone marrow aspirate material of the specimen. A distinct population of aberrant NK-cells was detected. The neoplastic cells account for 49% of all cells analyzed. The neoplastic cells were positive for CD2, cytoplasmic CD3, CD7 (bright), CD30 (subset dim), CD45 (bright), CD48 (partial), CD56, and CD158b. The neoplastic cells were negative for CD1a, surface CD3, CD4, CD5, CD8, CD10, CD13+CD33, CD19, CD25, CD34, CD117, CD123, CD158a, CD158e, TdT, TCR alpha/beta, TCR gamma/delta, myeloperoxidase, and HLA-DR.

We performed immunohistochemical stains using fixed, paraffin-embedded tissue of clot specimen. The neoplastic cells were positive for CD3, Granzyme B (subset), TIA-1, and p53 (strong) and negative for CD30, TCL-1, ALK-1, and TdT.

We performed *in-situ* hybridization studies for Epstein-Barr virus-encoded small RNA (EBER) using fixed, paraffin embedded tissue of clot specimen. The neoplastic cells were strongly positive for EBER.

Special Stains:

Not performed

Cytogenetics:

We performed Fluorescence *in-situ* hybridization studies using cell culture from bone marrow aspirate material of the specimen and a LSI *KMT2A* (*MLL*) dual color, breakapart rearrangement probe and a LSI *TP53*/CEP 17 dual color probe. 200 interphases were analyzed for each probe; the results were within the normal limits.

Conventional cytogenetic studies are in progress.

Molecular Analysis:

We extracted DNA from bone marrow aspirate material and analyzed it by PCR-based sequencing using a next generation sequencing platform on genomic DNA to screen for mutations in the coding sequences of following genes: *ANKRD26*, *ASXL1*, *ASXL2*, *BCOR*, *BCORL1*, *BRAF*, *BRINP3*, *CALR*, *CBL*, *CBLB*, *CBLC*, *CEBPA*, *CREBBP*, *CRLF2*, *CSF3R*, *CUX1*, *DDX41*, *DNMT3A*, *EED*, *ELANE*, *ETNK1*, *ETV6*, *EZH2*, *FBXW7*, *FLT3*, *GATA1*, *GATA2*, *GFI1*, *GNAS*, *HNRNPK*, *HRAS*, *IDH1*, *IDH2*, *IKZF1*, *IL2RG*, *IL7R*, *JAK1*, *JAK2*, *JAK3*, *KDM6A*, *KIT*,

KMT2A, *KRAS*, *MAP2K1*, *MPL*, *NF1*, *NOTCH1*, *NPM1*, *NRAS*, *PAX5*, *PHF6*, *PIGA*, *PML*, *PRPF40B*, *PTEN*, *PTPN11*, *RAD21*, *RARA*, *RUNX1*, *SETBP1*, *SF1*, *SF3A1*, *SF3B1*, *SH2B3*, *SMC1A*, *SMC3*, *SRSF2*, *STAG1*, *STAG2*, *STAT3*, *STAT5A*, *STAT5B*, *SUZ12*, *TERT*, *TET2*, *TP53*, *U2AF1*, *U2AF2*, *WT1*, *ZRSR2*.
A missense c.587G>C p.R196P *TP53* mutation at exon 6 was detected.

Interesting Features of Submitted Case:

The immature/blast-like appearance of neoplastic cells in combination with cytoplasmic CD3+/surface CD3-/CD4-/CD8- immunophenotype resulted in a wrong diagnosis at an outside institution. This case illustrates that aggressive NK-cell leukemia might show a morphological spectrum and is not restricted to patients of Asian descent.

Proposed Diagnosis:

Aggressive NK-cell leukemia

Aggressive NK-cell leukemia mimicking (and misdiagnosed as) T lymphoblastic leukemia/lymphoma

- Sergej Konoplev, MD, PhD, Beenu Thakral, MD, C. Cameron Yin, MD, PhD, Shaoying Li, MD, Pei Lin, MD,
- Affiliation: The University of Texas-MD Anderson Cancer Center, Houston, TX 77030
- E-mail: skonople@mdanderson.org

1

History

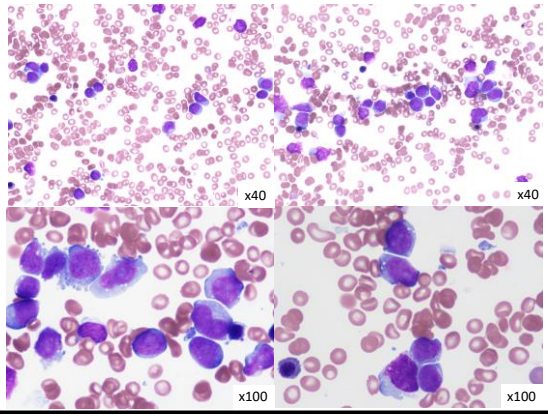
- A 49-year-old African America woman presented in April 2019 with cough, fever, severe fatigue, and 5 pound weight loss.
- Physical exam revealed a massive hepatosplenomegaly; no lymphadenopathy and no other abnormalities were detected.
- CBC demonstrated moderate anemia.
- Serological tests for HIV, HBV, HCV, and HTLV were negative; CMV IgG +.

2

- Bone marrow biopsy performed at another institution demonstrated hypercellular (80%) bone marrow with 32% neoplastic cells interpreted as blasts.
- The diagnosis of T lymphoblastic leukemia/lymphoma was established.
- The patient received two cycles of HCVAD therapy and was transferred to our institution for second opinion regarding her refractory disease.

3

Bone marrow aspirate smear



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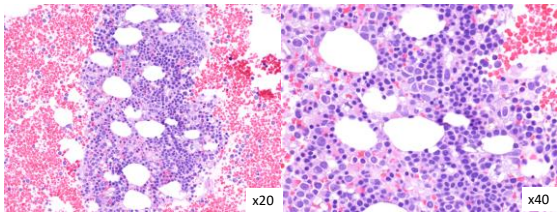
Bone marrow clot



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Immunohistochemical stains

- **Positive:** CD3, Granzyme B (subset), TIA-1, and p53 (strong).
- **Negative:** CD30, TCL-1, ALK-1, and TdT.

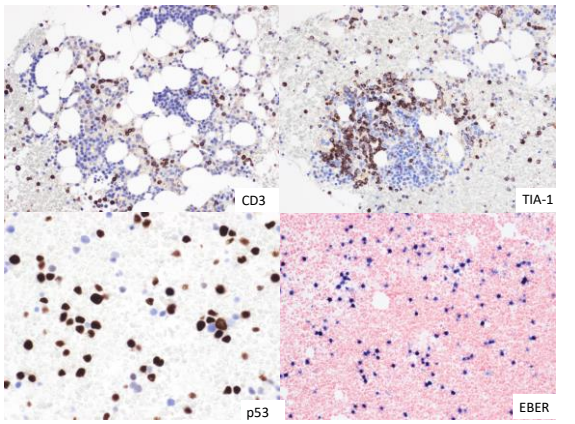
6

Flow cytometry immunophenotypic studies

- **Positive:** CD2, cytoplasmic CD3, CD7 (bright), CD30 (subset dim), CD45 (bright), CD48 (partial), CD56, and CD158b.

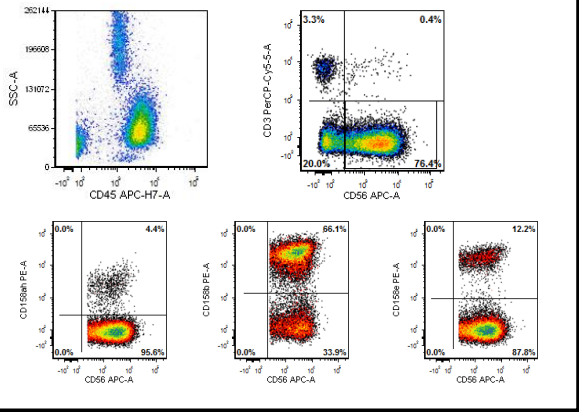
  Negative: The neoplastic cells were negative for CD1a, surface CD3, CD4, CD5, CD8, CD10, CD13+CD33, CD19, CD25, CD34, CD117, CD123, CD158a, CD158e, TdT, TCR alpha/beta, TCR gamma/delta, myeloperoxidase, and HLA-DR.

7



8

Flow cytometry demonstrated KIR restriction



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Fluorescence *in-situ* hybridization studies

- *KMT2A* (*MLL*) dual color, breakapart probe – **normal result**
- *TP53*/CEP 17 dual color probe – **normal result**

10

Next generation sequencing

Missense c.587G>C p.R196P *TP53* mutation at exon 6 was detected.

11

Proposed Diagnosis

Aggressive NK-cell leukemia

12

Interesting Features of Submitted Case

- The immature/blast-like appearance of neoplastic cells in combination with cytoplasmic CD3+/surface CD3-/CD4-/CD8- immunophenotype resulted in misdiagnosis as T-lymphoblastic leukemia/lymphoma
- This case illustrates that aggressive NK-cell leukemia might show a morphological spectrum
- This case also illustrates that aggressive NK-cell leukemia is not restricted to the patients of Asian descent

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Kennosuke Karube, MD PhD

Affiliation:
University of the Ryukyus Graduate School of Medicine

E-mail:
karube@med.u-ryukyu.ac.jp

Clinical History:
A 38-year-old man presented with cervical lymphadenopathy without systemic symptoms. He had no clinical history of an organ transplantation, HIV or HTLV-1 infection, or an immunosuppressive treatment against autoimmune diseases. The cervical lymph node biopsy disclosed features of classic Hodgkin lymphoma (CHL). He underwent combined chemotherapy followed by the achievement of complete remission. After two months, however, he noticed a re-enlargement of the cervical mass, and a thorough workup was performed. Colonoscopy detected multiple ulcers in the cecum and the sigmoid colon. CT and FDG-PET detected enlarged cervical and axillary lymph nodes and multiple liver masses. Biopsies of the cecal and the liver lesions were performed.

Biopsy Fixation Details:
10% buffered formalin

Description of Clinical Image if Any:
Colonoscopy: Multiple ulcers were observed in the cecum and the sigmoid colon.
CT: Enlarged cervical and axillar lymph nodes and multiple liver masses were detected.

Details of Microscopic Findings:
1. Cervical lymph node: Its normal architecture was effaced by the scattered Hodgkin and Reed-Sternberg (HRS) cells and lacunar cells accompanied with numerous histiocytes, diffuse fibrotic changes, and a few lymphocytes.
2. Cecum: "HRS-cell like" atypical large cells were scattered with the infiltration of inflammatory cells, including eosinophils and histiocytes, mainly in the submucosa.

3. Liver: Atypical large lymphoid cells diffusely proliferated with some necrotic foci.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
1. Cervical lymph node: HRS cells were positive for CD30, CD15, PD-L1 and negative for CD20 and BOB.1. HRS cells were also positive for EBV-encoded small RNA in situ hybridization (EBER-ISH).
2. Cecum: HRS-like cells were positive for CD30, CD15 and PAX5, and EBER-ISH, variably positive for CD20, Oct-2 and CD79a, and negative for PD-L1 and BOB.1.
3. Liver: Lymphoma cells were positive for CD30, CD20, BOB.1, Oct-2, and EBER-ISH and negative for CD15 and PD-L1.

Special Stains:
Not done

Cytogenetics:
Not done

Molecular Analysis:
Clonality analysis: PCR for IGH-FR3 using BIOMED-2 primers showed a monoclonal peak for each sample. Three lesions were clonally distinct.

Interesting Feature(s) of Submitted Case:
Cases with the histological transition from CHL to B-cell lymphoma including diffuse large cell lymphoma (DLBCL) or vice versa have been described and mainly showed clonal relevance between the both histological components. This indicates that tumor cells of DLBCL and HRS cells share common precursor cells or transdifferentiate into one another. However, these cases were mostly negative for EBV. Detailed clonal relationship has not been sufficiently analyzed in EBV-positive cases.
Clonality analyses revealed that each lesion had a distinct derivation in the submitted case. There has been only one case report of co-occurrence of EBV-positive CHL and DLBCL, in which clonality analysis was successfully performed. Each component was clonally distinct, like the submitted case. These findings suggest that the histologically and clonally distinct B-cells could simultaneously proliferate in EBV-associated settings in contrast to EBV-negative cases, providing a new insight into the pathogenesis of EBV-associated lymphoproliferative disorders.

Proposed Diagnosis:
Metachronous and synchronous EBV-associated classic Hodgkin lymphoma, EBV-positive mucocutaneous ulcer, and EBV-positive DLBCL, with distinct clonal origins

Comments:

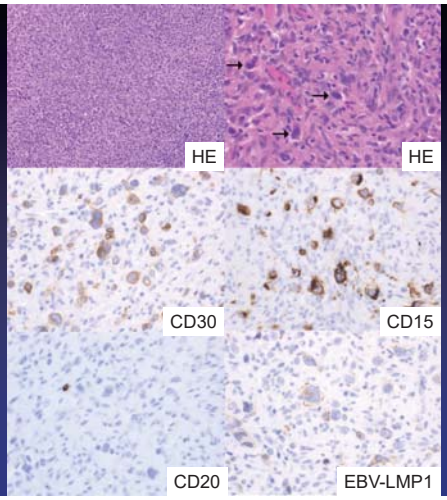
The 1st CSHP/SH Workshop and CSHP 2019 Oct.19-20, 2019

Co-occurrence of EBV+ CHL and DLBCL with distinct clonal origin

Kennosuke Karube
Dept. Pathol. and Cell Biol. University of the Ryukyus
University of the Ryukyus

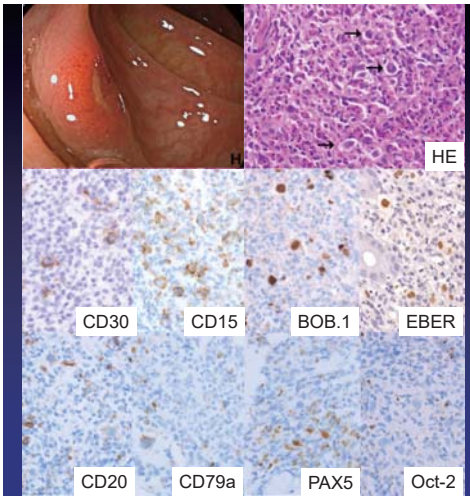
Case report

- 38-year old, 138 cm height male.
- Previously suspected as epiphyseal dysplasia.
- He recognized a cervical lymphadenopathy and the diagnosis of classic Hodgkin lymphoma (CHL) was made.
- After the complete remission of CHL, neck mass re-enlarged combined with multiple cecal ulcers and liver masses.
- There was not any known immunosuppressive state including post-organ transplantation, HIV or HTLV1 infection, or immunosuppressive treatment.



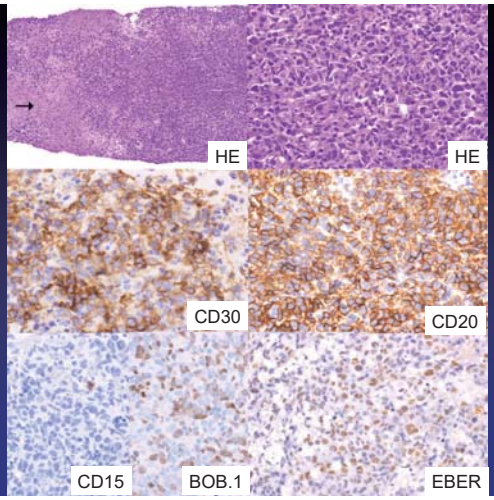
Cervical lymph node

Dx:
Classic Hodgkin lymphoma (CHL), lymphocyte depletion



Cecal ulcer

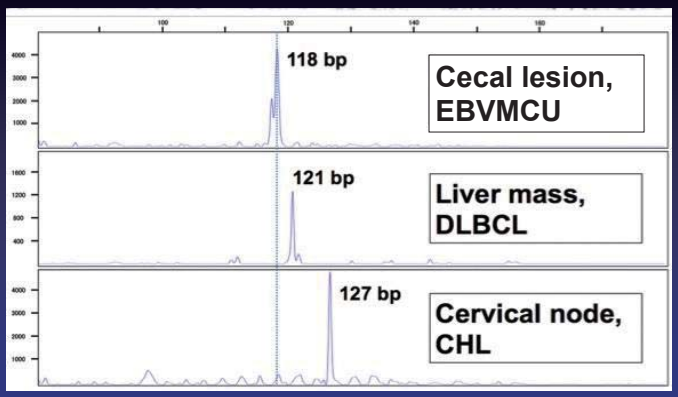
Dx: EBV-positive mucocutaneous ulcer (EBVMCU)



Liver mass

Dx: EBV+DLBCL

PCR for IGH (FR3, BIOMED2 protocol)



Lymphoma cells of three lesions are clonally distinct.

CHL + DLBCL

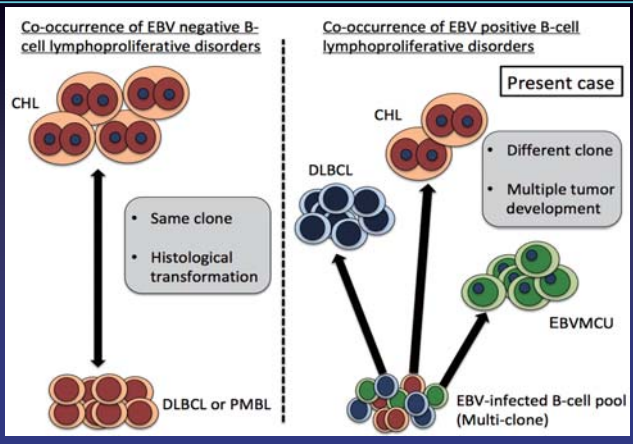
EBV(+)

No.	Patient	Background status	Cooccurrence status	Histology	Clonal association	reference
1	28/F	Post liver transplant	Metachronous	Polymorphic LPD, CHL	Different	Nalesnik et.al, 1993
2	39/M	Chemotherapy?	Metachronous: CHL and others Synchronous: EBVMCU & DLBCL	CHL, EBVMCU, DLBCL	All different	Present case
3	58/M	No	Synchronous	CHL, DLBCL	Not evaluable	Hwang et al., 2011
4	44/M	AIDS	Synchronous (composite)	CHL, DLBCL	Not evaluable	Guaner et al., 1990
5	62/M	MF	Synchronous (composite)	CHL, DLBCL	Not evaluable	Miyagaki et al. , 2009
6	53/F	Immunosuppressive agent (Crohn's disease)	Metachronous	EBVMCU, CHL	Not evaluable	Moran et al., 2015

EBV(-)

No.	Patient	Cooccurrence status	Histology	Site	Clonal association	EBV	reference
1	50/M	metachronous	TCRBL, CHL	Skin, Lymph node	identical	negative	Brauninger et al. 1999
2	46/M	metachronous	MLBL, CHL	Mediastinum, mediastinum	identical	NA	Traverse-Glehen et al. 2005
3	54/F	metachronous	CHL, MLBL	Lymph node, mediastinum	identical	NA	Traverse-Glehen et al. 2005
4	53/F	synchronous	CHL, DLBCL	Stomach	identical	negative	Wang et al. 2013
5	74/F	synchronous (composite)	CHL, DLBCL	Lymph node	identical	negative	Rosenquist et al. 2004
6	56/M	synchronous (composite)	CHL, DLBCL	Small intestine	identical	CHL(+), DLBCL(-)	Huang et al. 2006

Hypothesis of clonal evolution of EBV+/- LPD



Discussion on the immunosuppressive status

- The present case was free from any known immunosuppressive conditions, except for receiving chemotherapy.
- Epiphyseal dysplasia was suspected previously, and he was 138cm tall.
- Schimke immune-osseous dysplasia has been reported as a rare autosomal recessive disease with skeletal dysplasia and immune dysfunction.
- Some congenital genetic abnormality might have affected on this case?

Acknowledgement

Okinawa Prefectural Nambu Medical Center and Children's Medical Center

Department of Pathology
Iwao Nakazato

Department of Hematology
Kazuiku Ohshiro

University of the Ryukyus

Department of Pathol. and Cell Biol.
Mitsuyoshi Takatori
Shugo Sakihama

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Lu Sun. Primary LYG of CNS after treatment of CLPPERS.

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E-mail:
738873919@qq.com

Clinical History:
A 17yo young man complained intermittent headache with fever for 14 months, aggravated 1 month. Subacute onset, gradually worsening, hormone therapy is effective.
In August 2017, the patient started to get sick after staying up late, with headache, and lost 50 pounds in one month. After diagnosed CLPPERS in the provincial hospital, the patient was treated with hormones and was getting well until April 2018. The patient had a transient left limb weakness, pain in temporal region and spread to the top of the head, abnormal cerebrospinal fluid, EBV infection and EBV-associated NK cell hyperplasia. Then there is fever, headache, epileptic seizures, 1-2 minutes relief. BM+FCM found 50.61% were abnormal phenotype NK cells. Hematologist considered the diagnosis of "EBV-associated NK cell LPD, NK cell lymphoma is not excluded." Clinical symptoms got worsening even in the treatment. In October 2018, the patient was admitted to our hospital in an emergency department, and the primary central nervous system lymphoma was suspected.

Biopsy Fixation Details:
FFPE

Description of Clinical Image if Any:
Brain MRI (2017-9-20 local hospital）: The possibility of meningitis.
Brain MRI (2017-9-26 Henan Provincial People's Hospital) : The possibility of ADEM.
Brain MRI (2018-5-30) There were multiple abnormal signals in the brain stem, bilateral cerebellar hemisphere, cerebellar vermis and bilateral cerebral hemisphere white matter areas, and the intracranial pia mater thickening and obvious enhancement, the cerebral surface vascular enhancement shadow increased, bilateral cerebellar tonsil squat.
Brain MRI (2018-8-12) : Consider viral encephalitis, no other than others.

Brain MRI (2018-10-18 our hospital) 1.The possibility of demyelinating lesions, please combine clinically excluded inflammatory lesions; 2. The position of cerebellar tonsils is low, considering secondary changes. 3. Bilateral mastoiditis.
Brain MRI (2018-11-12 our hospital)1. The diffuse abnormal signal in the brain parenchyma and the suspicious enhancement of the pia mater. Compared with the former film of 2018-10-20, the range of brain parenchymal lesions is slightly advanced. Combined with the medical history, it is highly likely to consider the inflammatory lesion caused by low immunity. The possibility of neoplastic lesions (such as diffuse glioma) cannot be completely ruled out, and biopsy is recommended. 2. Cervical spinal cord abnormality signal, MRI examination of cervical spinal cord is recommended. 3. Bilateral mastoiditis.

Details of Microscopic Findings:
(The left caudate nucleus abnormal brain tissue) In a small amount of brain tissue, lymphocytes and histiocytes proliferated, and they were perivascular, some of them were vascular destructive changes, and some areas of tumor cells were diffusely infiltrated, among which there were a few nuclear large-shaped cells. A picture of nuclear fission can be seen. a few reactive astrocytes were also seen.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
astrocytes : GFAP(+)，NeuN(-)，Olig-2(灶状+)，IDH-1(-)，NF(+)，MAP-2(-)，Lmyphocytes:TIA-1(散在+)， CD68(散在+)，CD56(-)，CD3(散在+)，CD20(弥漫+)，CD10(-)，OCT-4(-)，Bcl-2(+)，Ki-67(+40%)，CD30(散在+)，PAX-5(-)，CD23(散在+)，Bcl-6(-)，MUM-1(+)，CyclinD1(-)，CD5(散在+)，C-myc(-)，Granzyme B(个别+)，CD4(散在+)，CD8(-)，Lambda(-)，kappa(+)，ALK(-)。

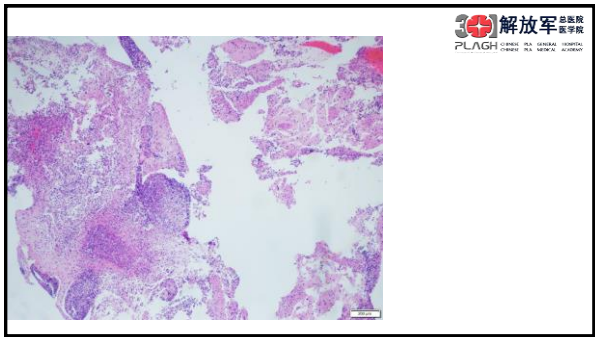
Special Stains:
Luxol fast blue (-)，Reticulin(partial+)，PAS(-)，EBER(＜+50 个/HPF).

Cytogenetics:

Molecular Analysis:

Interesting Feature(s) of Submitted Case:
Rare case, especially with the diagnosis of CLIPPERS.
Is there LYG or other type of lymphoma secondly to CLIPPERS?

Proposed Diagnosis:
Primary Lymphomatoid granuloma (grade 2-3) of CNS



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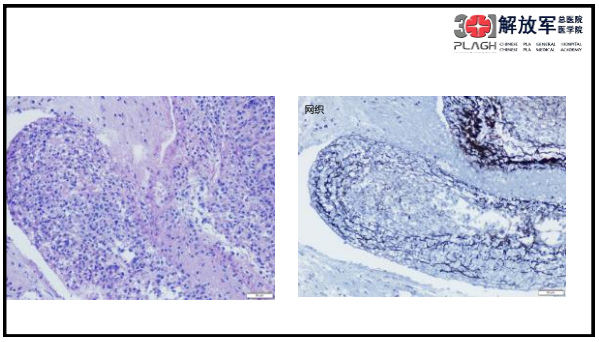

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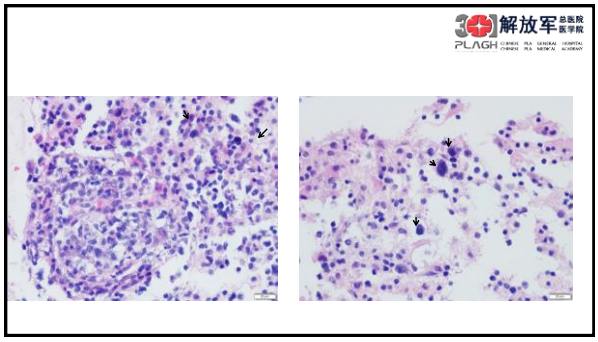

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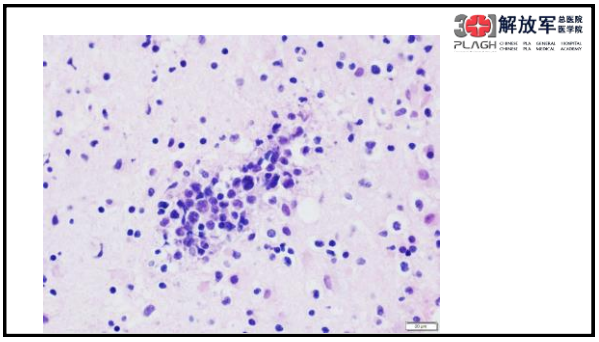

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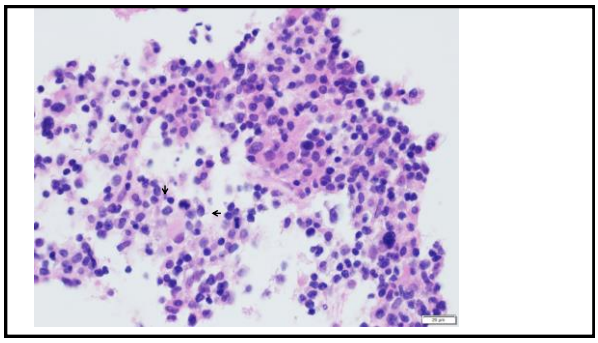

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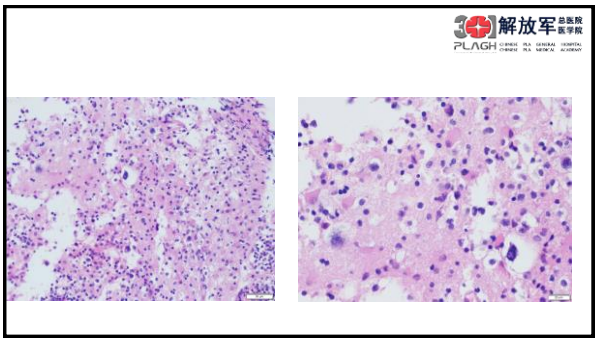

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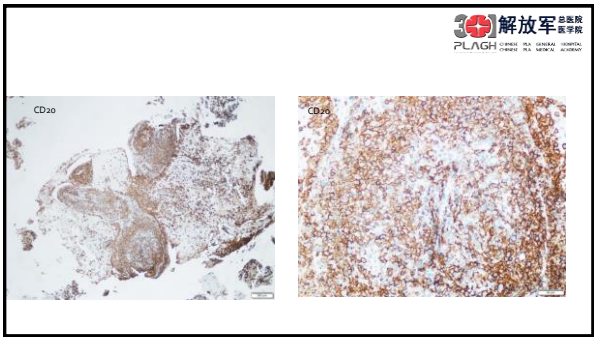

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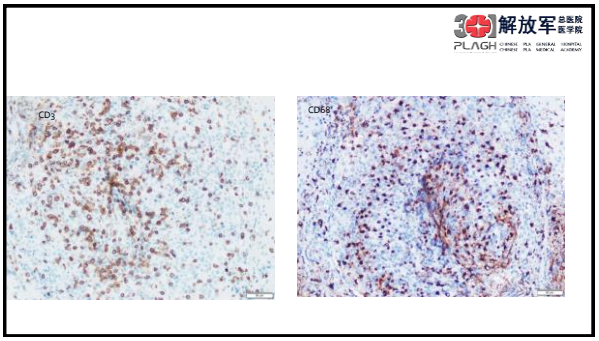

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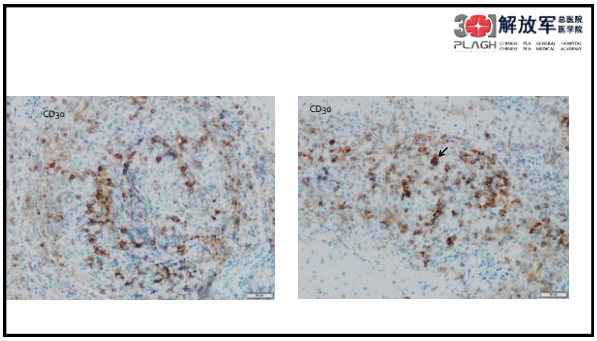

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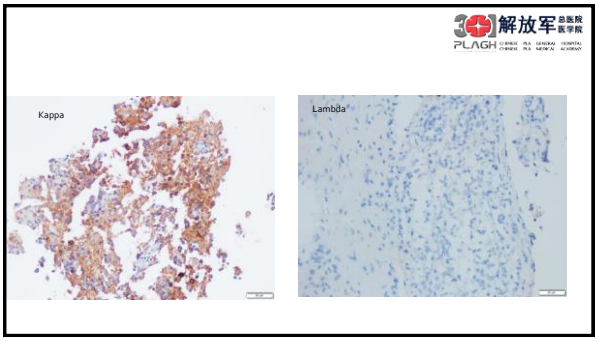

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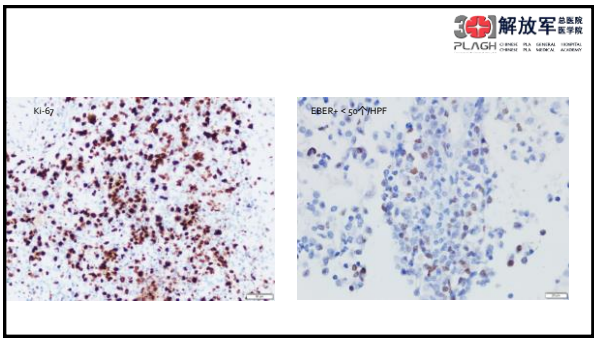


11

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Lu Sun. Primary LYG of CNS after treatment of CLPPERS.

Affiliation:
Department of Pathology, General Hospital of PLA

E-mail:
738873919@qq.com

Clinical History:
24 years old young woman
Chief Complaint: 16 years of muscle and joint pain, intermittent fever, fatigue for half a year, syncope 1 time
Diagnosis:
1. Systemic lupus erythematosus involvement of the nervous system;
2. lymphoma;
3. Hemophagocytic syndrome;

The patient had no obvious cause of generalized muscle pain in February 2003. Clinical diagnosis: systemic lupus erythematosus, juvenile ankylosing spondylitis. Intermittent treatment.
On February 21, 2019, due to fatigue and fasting, the patient developed sweating, shortness of breath, syncope once, no convulsions, and the duration was unknown. After the emergency treatment, she was transferred to the rheumatology department.
anti-nRNP/Sm antibody +, anti-SSA antibody w+, anti-ribosomal P protein antibody +, anti-human globulin direct test 3+
Administration of hormones, hydroxychloroquine sulfate to control systemic lupus erythematosus.
Lumbar puncture in 2019-3-1, 3-8, 3-15, intrathecal injection of 10 mg of methotrexate injection. CSF: anti-nuclear antibody (ANA) 1:5, chloride 117.9 mmol /L, IgG 4.99 mg / dl.

erytheropenia, leukopenia, thrombocytopenia, albumin sustained reduction, bilirubin, LCD increased, splenomegaly, NK cell activity 14.71%, sCD25 > 44000,

Biopsy Fixation Details:
FFPE

Description of Clinical Image if Any:

CSHP/SH Workshop 2019

Details of Microscopic Findings:
(Left clavicular mass puncture) The normal structure of lymph nodes is destroyed, lymphoid tissue hyperplasia, mainly small lymphocytes and histiocytes, with focal necrosis and neutrophil infiltration, including scattered large cells, like HRS cells The number of local atypical cells is large.

Immunohistochemistry and/ or Flow Cytometry:
atypical large cells：CD30 (+), CD15 (-), CD20 (consistent strong +), CD19 (-), PAX-5 (+), PD-L1 (22C3) (+), OCT- 2(+), BOB-1(weak+/-), Ki-67 (hot spot +40%, non-hot spot +10%), CD3 (-, background T cell +), CD8(-), CD4(-), CD68 (-, background tissue cells +), CD1a (-), S-100 (-), CD21 (-), PD-1 (-).

Special Stains:
PAS (-)，EBER (large cell weak +).

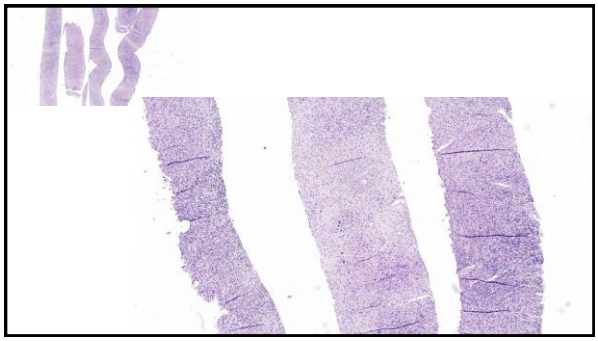
Cytogenetics:

Molecular Analysis:

Interesting Feature(s) of Submitted Case:
B cell lymphoma after treatment of SLE
The differential diagnosis in B cell lymphomas with CD20, CD30, B-cell transcription factors expression.

Proposed Diagnosis:
EBV+LBCL

Comments:



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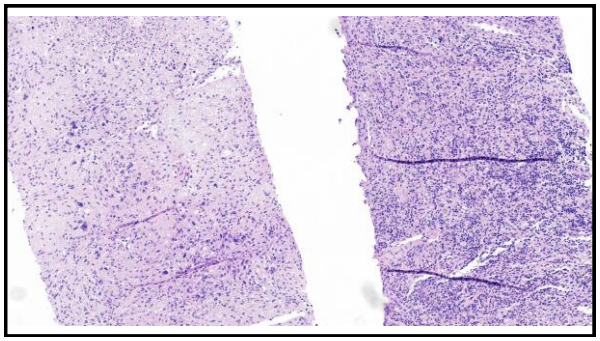

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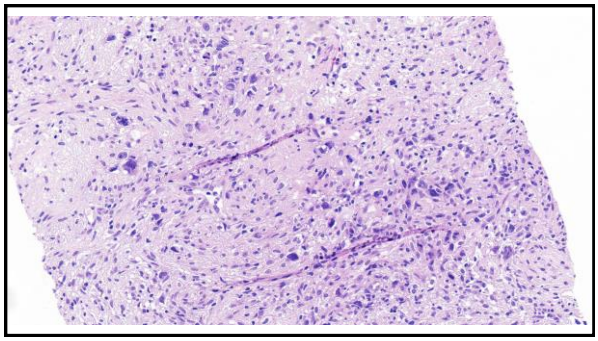

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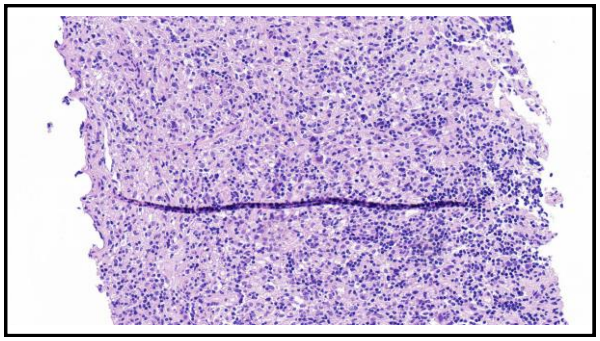

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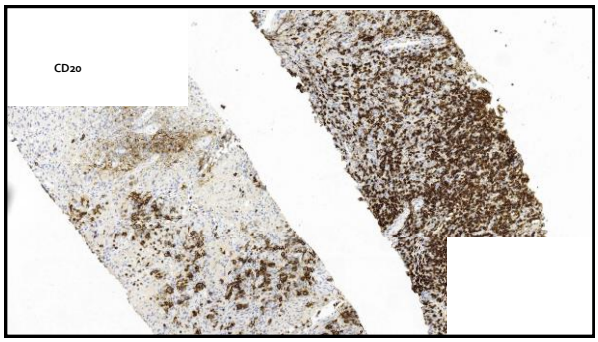

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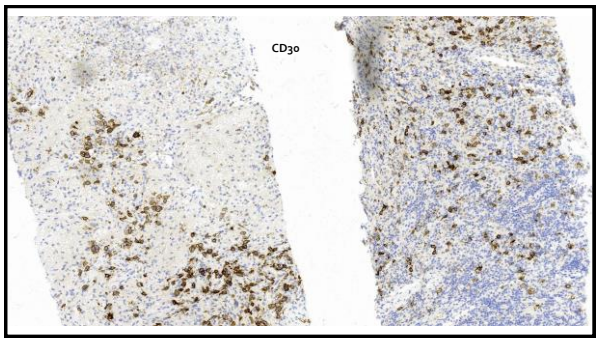

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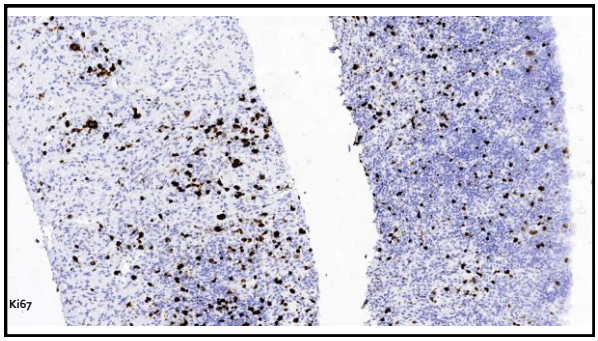

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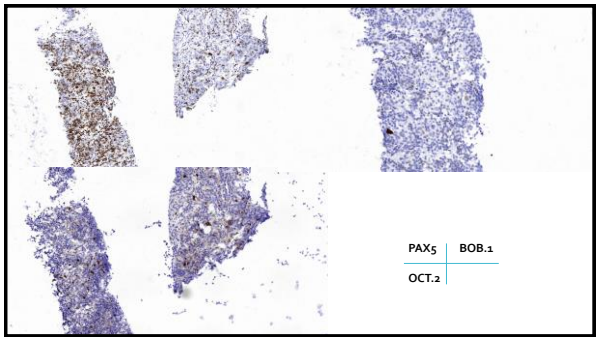

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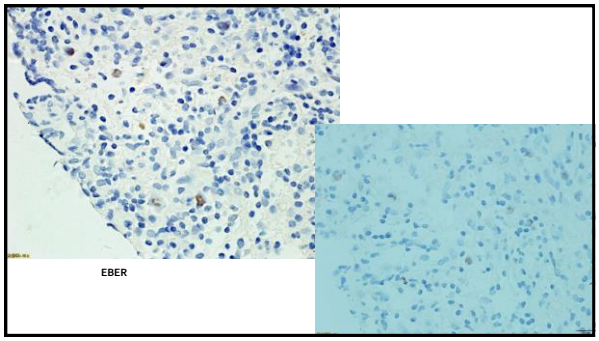


9

CSH/YSH Workshop 2019

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Jagmohan S. Sidhu, MD[1], Steven Gersen, PhD[2], Jonathan Kloss, MD[3]

Affiliation: (1) United Health Services Hospitals, Johnson City, NY, USA. (2) AmeriPath Northeast, Shelton, CT, USA. (3) Broome Oncology, Binghamton, NY, USA

E-mail: jagmohansidhu@aol.com

Clinical History: A 67-year-old male was admitted with severe respiratory difficulty, fever, and night sweats to the intensive care unit. Physical exam revealed bilateral axillary, cervical and inguinal lymphadenopathy. CT scan of chest and abdomen showed bilateral pulmonary infiltrates and mediastinal lymphadenopathy. He received intensive care including mechanical ventilation, antibiotics and heavy dose of corticosteroids. A right groin lymph node excisional biopsy was performed while he was on ventilator. His respiratory symptoms subsided and lymph nodes significantly decreased in size. After the diagnosis of EBV-positive, non-germinal center-like, diffuse large B-cell lymphoma developing in angioimmunoblastic T-cell lymphoma was made, he has been given 4 cycles of dose-adjusted R-EPOCH so far and he is doing well.

Biopsy Fixation Details: 10% Neutral Buffered Formalin

Description of Clinical Image if Any: None

1

Details of Microscopic Findings: The following two separate components, which were imperceptibly merging into each other, were seen:

1. A component of small to medium-sized lymphoid cell infiltrate with clear cytoplasm, high endothelial cell venules, dendritic reticulum cell networks, benign histiocytes, and many scattered and clustered large cells.
2. A component of large sheets of large lymphoid cells with perithelial pattern and areas of necrosis.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

Immunohistochemistry and In situ Hybridization for EBER: Most of the small lymphoid cells with clear cytoplasm had an immunophenotype of T follicular helper cells (positivity for CD4, ICOS, CXCL13 and PD1), also showed CD2/CD5 positivity, and showed CD3/CD7/CD8 negativity. T cells were positive for granzyme A, TIA 1 and perforin. GATA3 was weakly positive and CD31 was strongly positive in T cells. CD31 stained the endothelial cells of increased number of blood vessels. CD23 and CD21 highlighted dendritic reticulum cell networks. CD68 highlighted the increased numbers of benign histiocytes admixed with T-cell infiltrate. Large lymphoid cells were CD20-positive, CD30-positive, MUM-positive, and were negative for BCL2, BCL6, and CD10 with about 95-100% Ki67 labeling index and 100% positivity for MYC protein. Large cells were also positive for EBER and EBV-LMP1.

Flow Cytometric Analysis: About 20% T cells expressing CD2, CD4 and CD5 and not expressing CD3 and CD7

Special Stains: Not performed

Cytogenetics: Not performed

2

Molecular Analysis:

Clonality studies: PCR for T-cell receptor gene rearrangement showed both TCR beta and TCR gamma clonality. B-cell clonality study showed presence of immunoglobulin heavy chain gene rearrangement.

FISH for Aggressive B-cell Lymphoma on the diffuse large B-cell component: No evidence for double or triple hit lymphoma.

Next Generation Sequencing (128 gene Panel): Pathogenic alterations were seen in *DNMT3A* (1 mutation), *IDH2* (one mutation) and *TET2* (two mutations) at 38%, 13%, 18%, and 30% variant allele frequencies. Genomic alterations of uncertain significance were detected in *ABL2* (one mutation) and *RHOA* (one mutation) at 46% and 16% varaint allele frequencies.

Interesting Feature(s) of Submitted Case:

Co-existing EBV-positive diffuse large B-cell lymphoma and angioimmunoblastic T-cell lymphoma (AITL) in the same lymph node at the time of clinical presentation

Occurrence of the most of the most commonly occurring mutations in AITL in our case, but the mutation in *RHOA* gene is different from the commonly described mutation i.e. instead of *RHOA* G17V mutation (which is the mutation described in AITL), our case has *RHOA* G17L mutation. It is possible that *RHOA* G17L mutation is also significant.

Proposed Diagnosis: Development of Non-germinal Center-like Diffuse Large B-cell Lymphoma (AITL-DLBCL, non-GC-like) in Angioimmunoblastic T-cell lymphoma (AITL) in the Same Lymph Node

Comments: None

3

EBV-Positive, Non-germinal Center-Like, Diffuse Large B-Cell Lymphoma Developing in Angioimmunoblastic T-Cell Lymphoma, Presenting in the Same Lymph Node

Jagmohan S. Sidhu, MD[1]
Steven Gersen, PhD[2]
Jonathan Kloss, MD[3]

1. Department of Pathology & Laboratory Medicine, United Health Services Hospitals, Binghamton, NY, USA
2. AmeriPath Northeast, Shelton, CT, USA
3. Broome Oncology, Binghamton, NY, USA

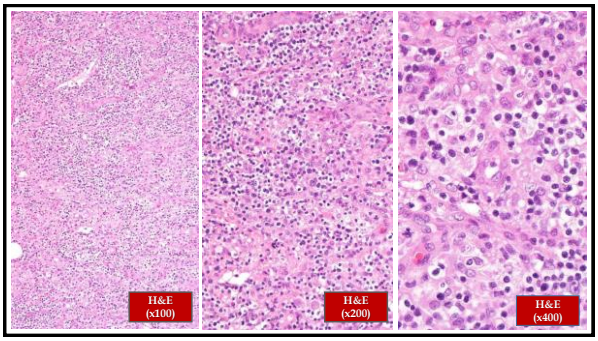
4

Clinical Data

- A 67-year-old male was admitted with severe respiratory difficulty, fever, and night sweats to the intensive care unit.
- Physical exam revealed bilateral axillary, cervical and inguinal lymphadenopathy.
- CT scan of chest and abdomen showed bilateral pulmonary infiltrates and mediastinal lymphadenopathy.
- He received intensive care including mechanical ventilation, antibiotics and heavy dose of corticosteroids.
- A right groin lymph node excisional biopsy was performed while he was on ventilator.
- His respiratory symptoms subsided and lymph nodes significantly decreased in size.

Gross Description

5



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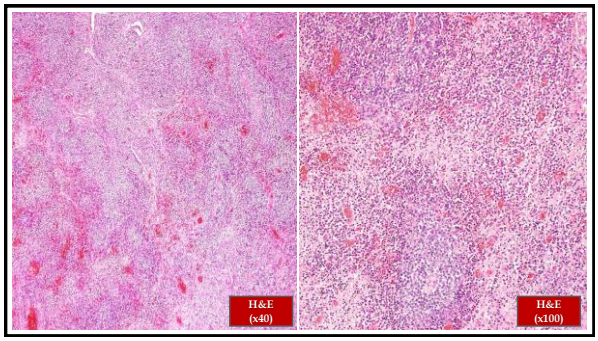
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H&E
(x100)

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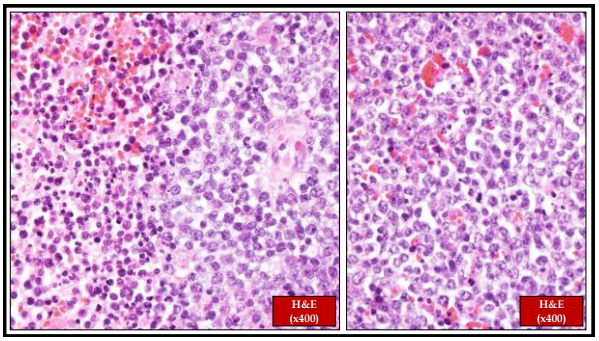
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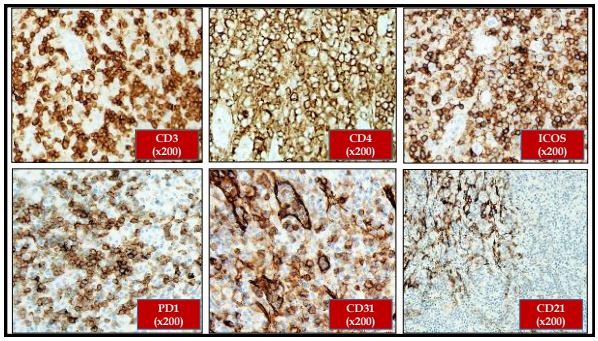
CD3
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CD4
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ICOS
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PD1
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CD31
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CD21
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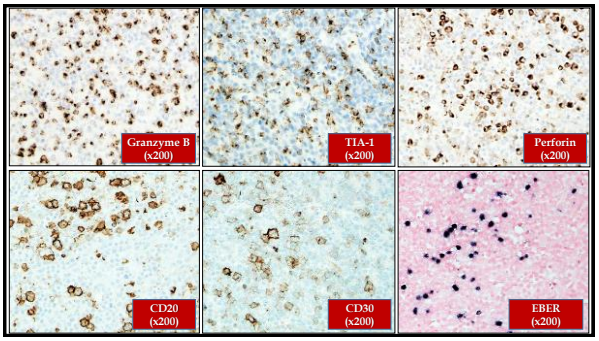
9


Granzyme B
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TIA-1
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Perforin
(x200)
CD20
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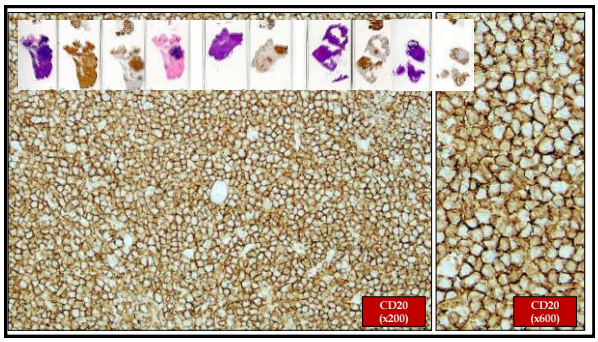
CD30
(x200)
EBER
(x200)

10


CD20
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CD20
(x600)

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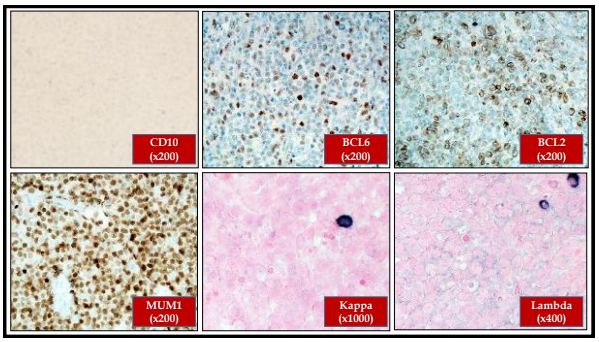
CD10
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BCL6
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BCL2
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MUM1
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Kappa
(x1000)
Lambda
(x400)

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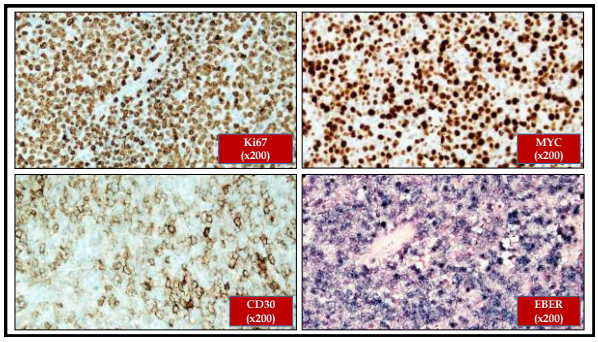

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Flow Cytometric Analysis

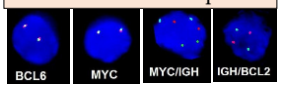
20% T cells expressing CD2, CD4 and CD5 and not expressing CD3 and CD7

Clonality Studies

PCR for T-cell receptor gene rearrangement showed both TCR beta and TCR gamma clonality.

B-cell clonality study showed presence of immunoglobulin heavy chain gene rearrangement.

FISH on DLBCL Component



DNMT3A and *IDH2* mutations are seen in ~30% of AITLs. *TET2* mutations are seen in ~85% of AITLs and in ~12% of DLBCLs.

Next Generation Sequencing (128-Gene Panel)

NGS RESULTS
Pathogenic alterations are DETECTED in the DNMT3A, IDH2, and TET2 genes.
Genomic alterations of uncertain significance are DETECTED in the ABL2 and RHOA genes.

Gene	Exons Tested	Genomic Alteration(s)	Mutation Effect	Allele Frequency	Pathogenic
ABL2	3-5,7-9,11-13	c.1948C>A; p.P650T	MISSENSE	46%	UNCERTAIN
DNMT3A	8-23	c.2645G>A; p.R882H	MISSENSE	38%	YES
IDH2	4	c.515G>A; p.R172K	MISSENSE	13%	YES
RHOA	ALL	c.49_50delGGinsTT; p.G17L	DELETION PLUS INSERTION	16%	UNCERTAIN
TET2	ALL	c.3432delA; p.G1145Vfs*7 c.3997dupA; p.M1333Nfs*6	FRAMESHIFT FRAMESHIFT	18% 30%	YES YES

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Diagnosis

Development of Non-germinal Center-like Diffuse Large B-cell Lymphoma (AITL-DLBCL, non-GC-like) in Angioimmunoblastic T-cell lymphoma (AITL) in the Same Lymph Node

How big a LBC infiltrate has to be in an AITL to call it a DLBCL transformation?

1. Nobody has clarified it in the literature.
2. We know if it is a very large LBC infiltrate with necrosis in an AITL, it is a DLBCL transformation.
3. We also know that if there are only small aggregates of LBCs, it is not a DLBCL transformation.
4. It is not clear whether multiple big aggregates should be called transformation or not.
5. If there are mutations (e.g. *NOTCH1*) in a somewhat large DLBCL component that are entirely different from the mutations in the AITL component, it has to be a DLBCL transformation.

- **Treatment:** He has been given 4 cycles of dose-adjusted R-EPOCH and he is doing well.

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Angioimmunoblastic T-Cell Lymphoma (AITL)

- AITL belongs to the WHO category of **nodal T-cell lymphoma with T follicular helper phenotype.**
- It is an age-related lymphoma, presenting with symptoms of immune system dysregulation.
- There is a massive infiltration of accessory cells in AITL (**Cytokine and Chemokine Storm**: cytokines and chemokines released from TFH-like tumor cells may recruit B cells and FDCs (by CXCL13 and CXCR5 network), mast cells, blood vessels (VEGF by mast cells), and immune cells into the tumor stromal tissues, and activate them to further produce cytokines and chemokines. This positive circuit of cytokines and chemokines may exacerbate the trafficking of these cells into AITL tissues).
- Can transform into DLBCL (usually, but not always, EBV+): 30 cases so far (7 composite lymphomas)

TABLE 1 Recurrent gene mutations in AITL

	Frequencies (%)
RAS superfamily	
RHOA	*RHOA* G17V and rarely K18N G17V is uncommon in non-AITLs. (a hallmark of AITL; diagnostic value) Loss of tumor suppressor function
Epigenetic regulators	
TET2	These modulate methylation status of DNA and histones directly or indirectly affecting gene expression profiles
DNMT3A	
IDH2	
TCR signaling pathway	Extremely rare in non-AITLs (confer the pathologic features of AITL)
PLCγ	
CD28	9-11
FYN	3-4
VAV1	5

Occur at lower VAFs reflecting second hits

Fukumoto K, et al. Cancer Science. 2018;109:490–496.

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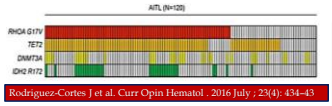
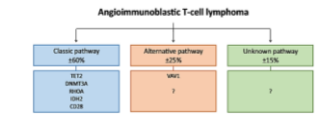
Genomic Landscape of AITL

Co-occurring Mutations in RHOA and Epigenetic Regulators



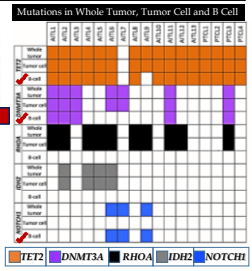
Rodriguez-Cortes J et al. Curr Opin Hematol . 2016 July ; 23(4): 434–43

Three Potentially Distinct Lymphomagenic Pathways



Willemsen et al. Blood Cancer Journal. 2018;8:6
DOI 10.1038/s41408-017-0047-2

Mutations in Whole Tumor, Tumor Cell and B Cell



Fukumoto K, et al. Cancer Science. 2018;109:490–496.

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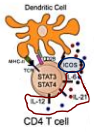
Normal Development and Malignant Transformation of TFH Cells

It remains elusive how TFH cells become malignant. The most common story is:



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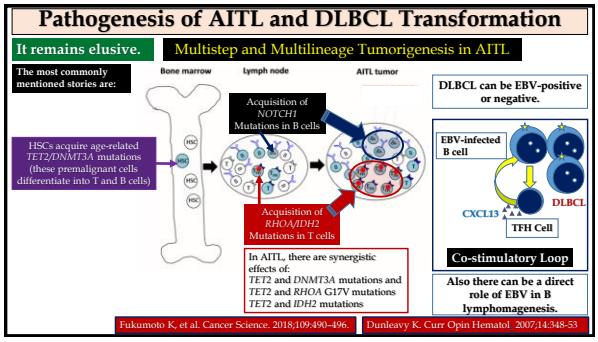

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CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Yunfei Shi MD PhD

Affiliation:
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E-mail:
Shiyunfei2006@sina.com

Clinical History:
Li XX, 52/Male
Enlargement of bilateral cervical and inguinal lymph node was found from October , 2012, fever and night sweating were also found. The right cervical lymph node was thus surgically biopsied for pathology diagnosis.(B1)Right cervical lymph node biopsy: AITL
Stage III B: tumor invasion was found in lymph nodes of bilateral nodes including neck, supraclavicular, axillary mediastinal, abdominal, pelvic and inguinal lymph nodes, spleen was also invaded.
Teatment:
(1) In 2012-10, CHOP×6cycle reach PR after evaluation,
(2) In 2013-4, stem cells were collected following mobilization by CHOP
(3) In May 2013, autologous hematopoietic stem cell transplantation pretreated with BEAM and reach CR, and maintained CR during follow-up.
Recurrence happened in nearly 5 years after autologous transplantation, there were multiple maculopapules all over the body without itching, swelling and pain. In May 2018, the skin lesion was biopsied no significant abnormality was seen (slides not shown).
Symptoms progressed again in 2018-9, including tonsil swelling, pain, dysphagia Multiple cutaneous nodules of extremities，and decreased number of trilineage cells in peripheral blood.
(B2) Skin biopsy of left thigh and (B3) bone marrow biopsy was done in 2018-10.

Biopsy Fixation Details:
All specimens were fixed with 10% formalin.

Description of Clinical Image if Any:
After recurrence, PET/CT showed that the affected site of the disease was: Invasion of multiple lymph nodes around his body; nasopharynx, oropharynx, tonsils, spleen, multiple skin in the whole body; bilateral lungs and bone marrow

Details of Microscopic Findings:
(B1) Right cervical lymph node biopsy
Most of the lymph node structures were destroyed and the paracortical areas were obviously enlarged; pleomorphic tumor cells were diffuse infiltration, small to medium size, clear cytoplasm or light staining, clear nuclear membrane;

follicular dendritic cells were irregularly proliferated, high endothelial vessels were significantly proliferated as dendritic growth. Immunoblasts with large size (large round nuclei and prominent nucleoli) are often seen to be R-S-like.
(B2) Skin biopsy of left thigh
Sheets of atypical lymphocytes can be seen in dermis and subcutaneous tissues. The cell size is large, with prominent nucleus which is large and round, the nucleolus is obvious, and most of them resemble immunoblastic cells, and the mitotic figure can be seen easily. Focal tumor necrosis was seen.
(B3) Bone marrow biopsy
In bone and bone marrow tissues, the ratio of red and yellow pulp is about 1:4. Multiple small foci of dense cells can be seen. At high magnification, those foci were filled with mixed cell components, and most of them are lymphoid cells and histiocyte-like cells. Scattered immunoblastic cells could also be seen.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
Immunohistochemistry
(B1) Results for IHC of tumor cells are CD3, CD4, positive. CD8 was partially expressed. In addition, CD21 markers show irregular proliferation of FDC meshwork outside the germinal center, immunoblasts B cells in background are EBER positive.
(B2) Results for IHC of tumor cells are Bcl-2(+),MUM-1(+),PAX-5(Weak +), CD20(+),CD3(-),CD30(灶+),CD4(-),CD56(-),CD7(-),CD8(-),GramB(-),KI67(90%+),TIA1(-),Bcl-6(-),CD10(-),CXCL13(-)；In situ hybridization: EBER1/2(+)
(B3) Results for IHC of BM proliferating cell foci are CD3(partially+), CD20(scattered +), CD4(mostly +), CD8(partially +), PAX5(scattered +), In-situ hybridization for EBER1/2(-)
Special Stains:

Cytogenetics:

Molecular Analysis:

Interesting Feature(s) of Submitted Case:
Here we had described a secondary cutaneous B -cell neoplastic lesion in a patient with angioimmunoblastic T-cell lymphoma, although this is not so rare, but the patient has received autologous hematopoietic stem cell transplantation and maintained CR during follow-up for nearly 3 years. The initial diagnosis were Bone marrow AITL & secondary skin DLBCL, luckily, after 5 cycles of R-CCOP treatment, the patient reached CR, with negative results in bone marrow biopsy.

So how to diagnose this relapsed lesion? (1) secondary cutaneous Epstein-Barr

virus-associated diffuse large B-cell lymphoma? Or else monomorphic PTLD (B- and T/NK-cell types). And for the BM biopsy, can we call it “BM involved by AITL?” or just reactive lymphocyte infiltration. WE also suppose whether the decreased number of trilineage cells in peripheral blood was caused by EBV infection associated hemophagocytic lymphhistiocytosis in BM or not.

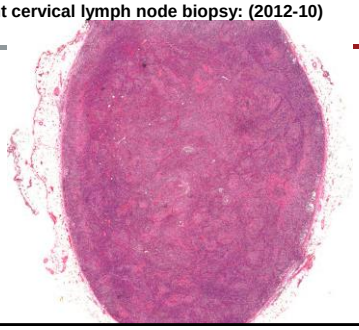
Proposed Diagnosis:
(B1) nodal AITL
(B2) secondary cutaneous Epstein-Barr virus-associated diffuse large B-cell lymphoma.
(B3) BM was not evidently involved by either DLBCL or AITL. (Does it possible that EBV associated hemophagocytic lymphohistiocytosis existed?)
Comments:

Secondary cutaneous Epstein-Barr virus-associated diffuse large B-cell lymphoma.

Yunfei Shi MD PhD, Dept. of Pathology,
Peking University Cancer Hospital

1

B1: Right cervical lymph node biopsy: (2012-10)



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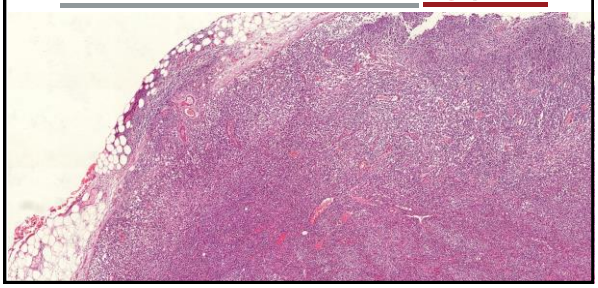
B1: Right cervical lymph node biopsy: (2012-10)



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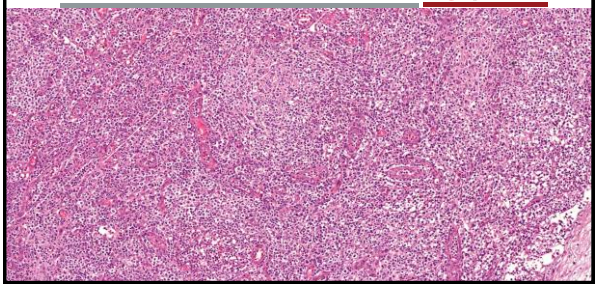
B1: Right cervical lymph node biopsy: (2012-10)



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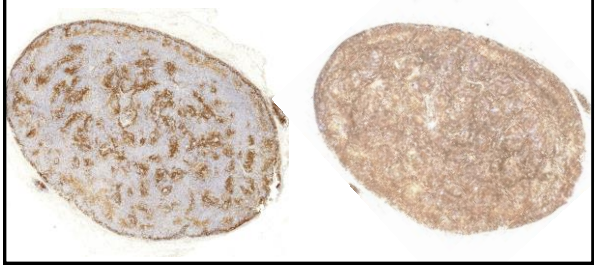
B1: Right cervical lymph node biopsy: (2012-10)

CD20

CD3



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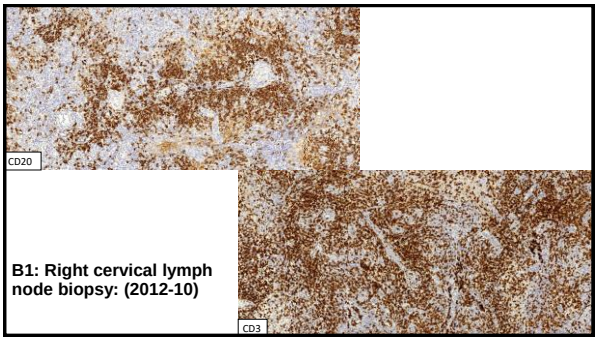

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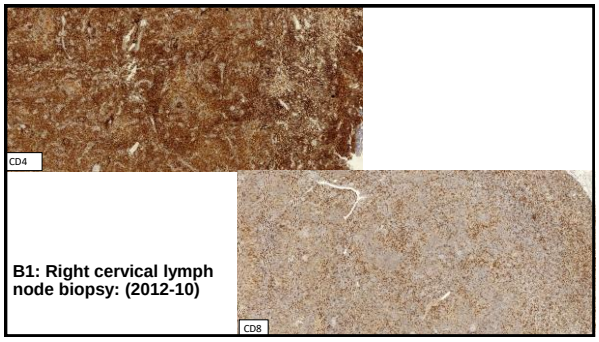
CD4
B1: Right cervical lymph node biopsy: (2012-10)
CD8

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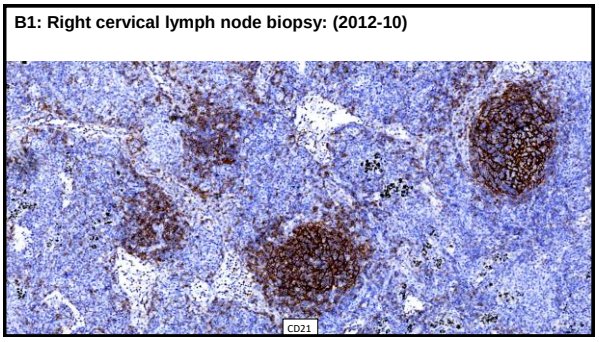
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CD21

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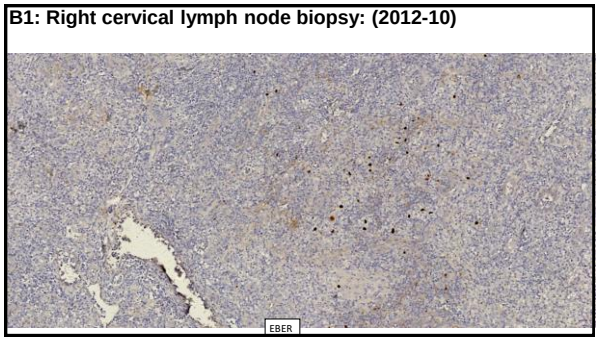
B1: Right cervical lymph node biopsy: (2012-10)
EBER

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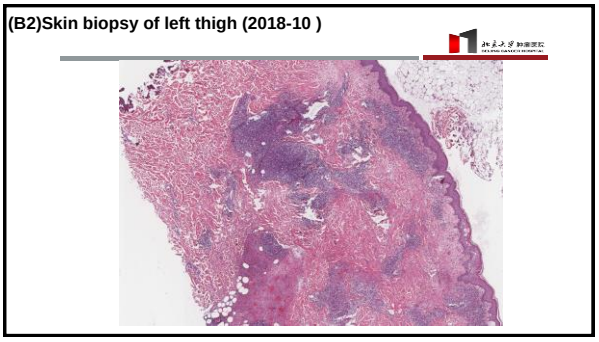
(B2)Skin biopsy of left thigh (2018-10)

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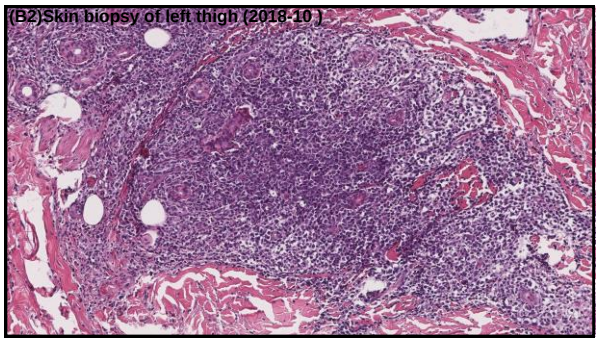
(B2)Skin biopsy of left thigh (2018-10)

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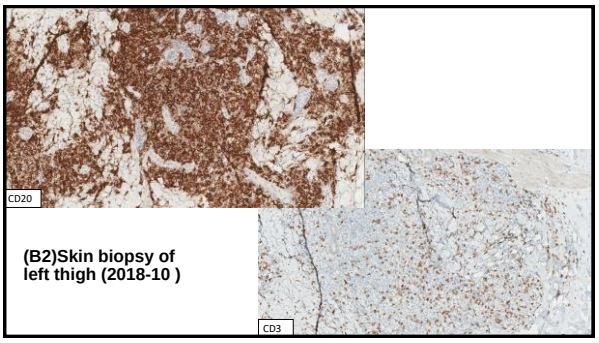
CD20
(B2)Skin biopsy of left thigh (2018-10)
CD3

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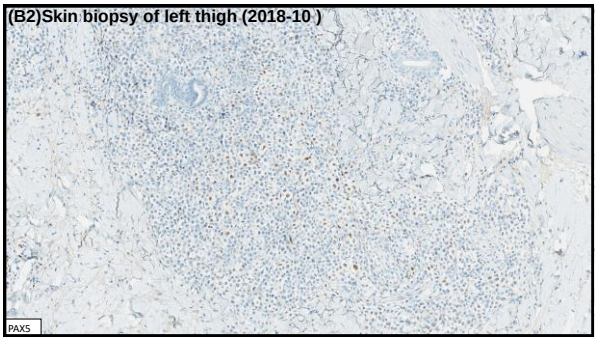

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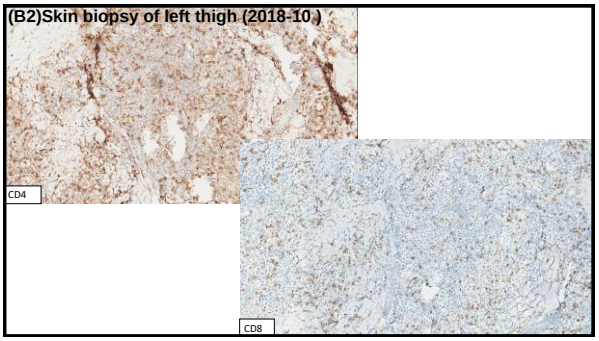

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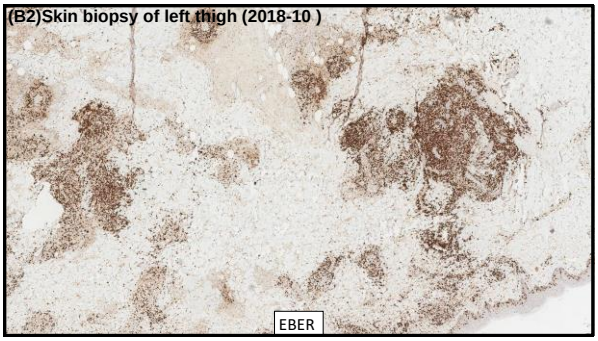

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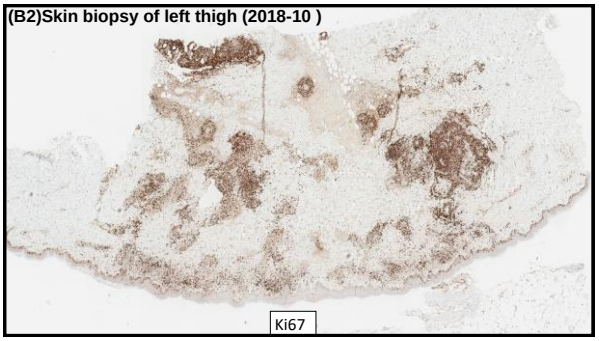

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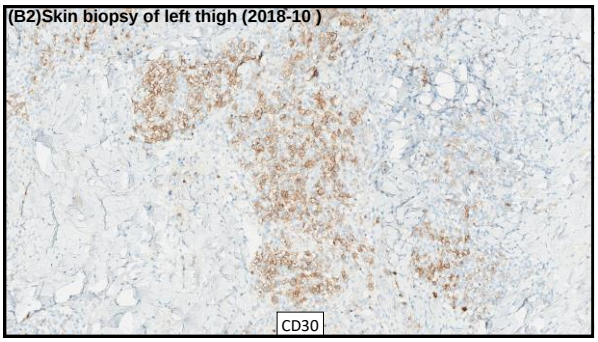

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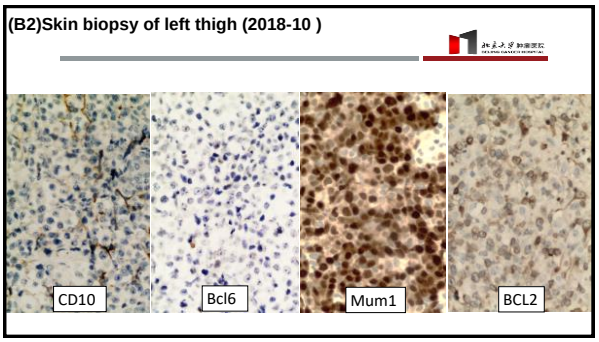

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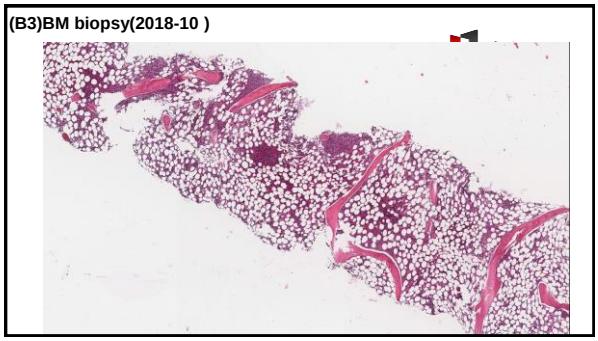

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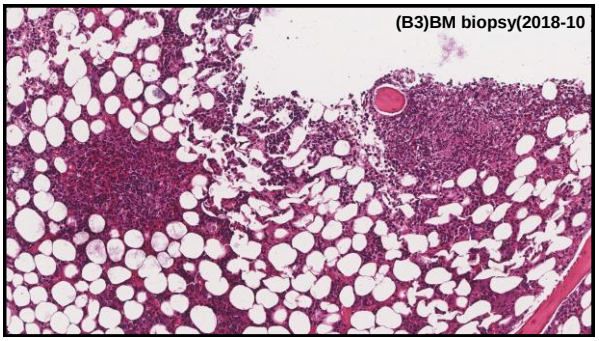

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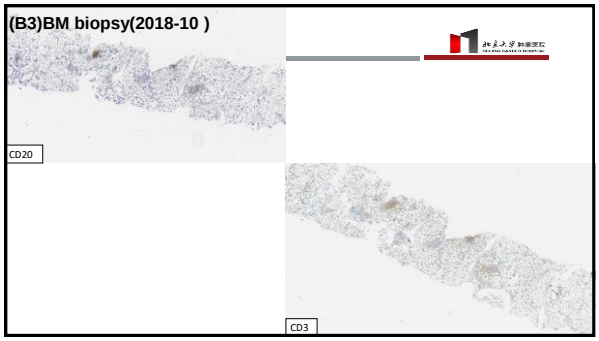

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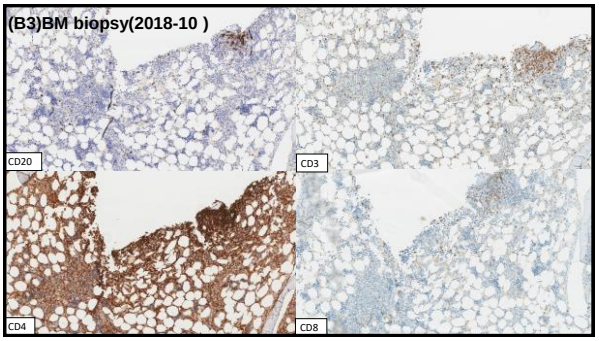

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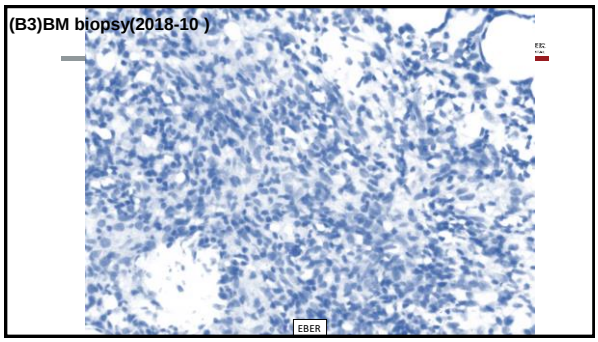

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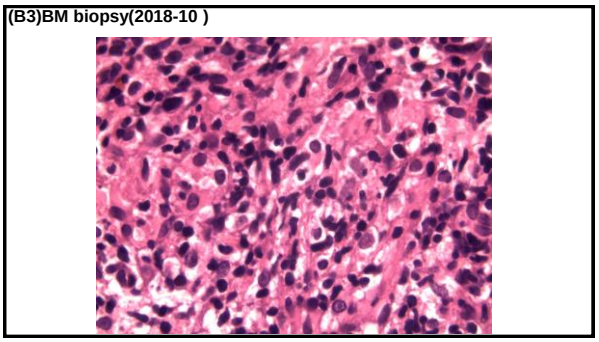

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CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Genevieve M. Crane MD PhD[1], Elizabeth Margolskee MD[1], Susan Mathew PhD[1], Sarah Rutherford MD[2] and Amy Chadburn MD[1]

Affiliation: Departments of [1]Pathology and Laboratory Medicine and [2]Hematology and Oncology, Weill Cornell Medicine/New York Presbyterian Hospital, New York, NY, USA

E-mail: eve945@gmail.com

Clinical History:
A 31-year-old woman with a history of multiple episodes of mononucleosis-like symptoms and cervical lymphadenopathy developed what she felt to be similar symptoms. Such episodes had previously been self-limited and prior documentation of EBV infection had been made. She was treated for possible sinusitis, but symptoms persisted. An ultrasound of the neck demonstrated bilateral lymphadenopathy with the largest node being 5.5 cm in size. Serologic studies showed an elevated EBV viral load. Limited sampling of an accessible node was followed by an excisional biopsy, after which she was treated with steroids. Lymphadenopathy decreased as did her EBV titers. However, her cervical lymphadenopathy remained palpable and a PET/CT scan demonstrated diffuse lymphadenopathy above and below the diaphragm with SUVs ranging from 20 to 38. However, the patient was reluctant to undergo treatment given the partial response to steroids and a continued decline in EBV titers. A repeat PET/CT study three weeks later showed further increase in size of FDG-avid supra- and infra-diaphragmatic lymph nodes with SUVs up to 52. A biopsy of an FDG-avid mesenteric node was then performed.

Pertinent laboratory values:

	December	January	February
LDH (nl 118-320 U/L)	258	184	269
EBV (log)	4.36	3.8	3.11
EBV DNA, quant	23,045	6,088	1,285

EBV serology panel: positive for IgG against VCA and early antigen, negative for IgM and anti-EBNA
HIV negative

Biopsy Fixation Details: Bouin's fixative

Description of Clinical Image if Any: N/A

Details of Microscopic Findings:

Left level 3 lymph node, core needle biopsy (December 2018): Small pieces of tissue with extensive necrosis. A small focal area of viable cells is seen which are intermediate to large in size with large nuclei that show vesicular chromatin and prominent nucleoli. There is a moderate amount of pink cytoplasm.

Cervical lymph nodes, levels 2, 3, and 4, excisional biopsy: Multiple lymph nodes are received. Level 2, 3 lymph nodes demonstrate relatively preserved follicular architecture. The interfollicular areas have increased plasma cells and a heterogeneous population of lymphocytes. The lymph node sinuses are patent. Occasionally large atypical cells can be seen, often at the periphery of germinal centers. Focally, these cells are increased in numbers and form loose clusters. Sheets of cells are not observed. These large atypical lymphoid cells have ample cytoplasm, irregular nuclei and prominent nucleoli. Focal small areas of necrosis are present.

Mesenteric node, excisional biopsy (February 2019): Sections of lymph node demonstrate an effaced architecture with large atypical cells that are highly pleomorphic in appearance. Most have open vesicular nuclei and prominent nucleoli and relatively abundant pink cytoplasm. There are numerous intermixed small lymphocytes and histiocytes. Scattered mitotic figures can be seen along with apoptotic bodies and focal areas of necrosis. The process focally extends into adjacent adipose tissue.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

Left level 3 lymph node, core needle biopsy (December 2018): Immunostains for CD20 highlight increased numbers of large B cells which focally form sheets and clusters. Most of the large B cells are necrotic. CD3 stains scattered small T cells. A subset of the viable atypical B cells are positive for PAX-5, BCL-2, BCL-6 and MUM-1. MYC stains a subset of the viable atypical B cells but is limited for interpretation. Ki-67 stains ~30-40% of the atypical cells, also limited due to viability. The atypical B cells appear negative for: CD5, CD10, CD30 and cyclin D1. In situ hybridization for EBER stains a subset of the viable atypical cells. By flow cytometry, the specimen is predominantly comprised of non-viable debris.

Cervical lymph node, excisional biopsy (December 2018): The large atypical cells are positive for CD20, MUM-1, BCL6, BCL2 and EBER. They are surrounded by numerous CD3 positive T cells. CD138 highlights interspersed plasma cells which are polytpic. They are negative for CD10.

Mesenteric node, excisional biopsy (February 2019): The atypical cells are positive for CD20, BCL-2, BCL-6 and MUM-1. c-Myc is borderline positive, approaching 40% in the atypical cells. CD3 highlights interspersed small to medium-sized T cells. CD30 is positive in rare cells (~1%). EBER highlights scattered cells, predominantly small lymphoid cells. EBV LMP-1, CD10 and p53 are negative.

Flow cytometry: There is a discrete population of kappa-restricted, CD19+, CD20 (bright) B-cells with relatively high forward scatter and side scatter properties and negative for CD5 and CD10. A background population of forward scatter, side scatter smaller lymphocytes is also present which includes a polytypic population of B cells. The large atypical cells overall comprise 21% of total events. Background CD3+ T cells are present without phenotypic abnormality.

Special Stains:
Not applicable.

Cytogenetics:
Cytogenetics and FISH findings (cervical lymph node, excisional biopsy, December 2018): A highly complex female karyotype was observed in 13 of the 20 analyzed metaphase cells. Clonal abnormalities included deletion of 1q (6 cells), one or two copies of a derivative chromosome 3 with a deletion in 3p and a t(3;14) translocation (13 cells), an isochromosome 6p (2 cells), one or two copies of 7q with addition of unknown material (11 cells), deletion of 10q (2 cells), a derivative chromosome 14 either with a t(3;14) translocation (2 cells) or with both t(1;14) and t(3;14) translocations (11 cells), an unbalanced t(1;15) translocation (2 cells), deletion of 16q (4 cells), and addition of unknown material on 21p (6 cells). The remaining 7 cells had a normal female karyotype. Interphase FISH detected BCL6 rearrangement in 3% of the evaluated nuclei (non-dividing cells). There was no evidence of IGH-BCL2 gene rearrangement, but a split IGH signal pattern was found in 3% of the nuclei.

46~48,XX,del(1)(q32q42)[6/13],der(3)del(3)(p23)t(3;14)(q27;q32)[13/13],+der(3)del(3)t(3;14)[2/13],
i(6)(p10)[2/13],add(7)(q22)[11/13],+add(7)(q22)[6/13],del(10)(q24)[2/13],der(14)t(3;14)[2/13],
der(14)t(1;14)(q21;p11.2)t(3;14)[11/13],der(15)t(1;15)(q21;p11.2)[2/13],del(16)(q22)[4/13],
add(21)(p11.2)[6/13][cp13]/46,XX[7]

nuc ish**(BCL6x2)(3'BCL6 sep 5'BCL6x1)[6/200]**,(**IGHx3**,BCL2x2)**[6/200]**

Mesenteric node:
Cytogenetics and FISH Findings (mesenteric lymph node, excisional biopsy, February 2019): A highly complex female karyotype with 79 to 84 chromosomes was observed in all 20 analyzed metaphase cells. It was described as a tetraploid karyotype, with loss of chromosomes (relative to the tetraploidy) specified in the described karyotype (below). Most of the structural abnormalities were observed in the previous cervical lymph node, including deletion of 1q, a t(3;14) translocation, a derivative chromosome 3 with a deletion in 3p and a t(3;14) translocation, an isochromosome 6p, addition of unknown material on 7q, a derivative chromosome 14 with both t(1;14) and t(3;14) translocations, and addition of unknown material on 21p. A deletion in 2q was a newly observed abnormality in this specimen.

Interphase FISH results were consistent with the tetraploid karyotype, showing two copies of rearranged BCL6 gene due to a t(3;14) translocation (51%), four copies each of MYC (49%), IGH (50-52%), and BCL2 (52%), with no evidence of MYC, MYC-IGH or IGH-BCL2 gene rearrangement.

79~84<4n>,XXXX,-1,del(1)(q32q42)x2,-2,del(2)(q13q21)x2,-3,der(3)del(3)(p23)t(3;14)(q27;q32)x2,-5,-6,i(6)(p10),+7,add(7)(q22)x3,-9,-10,-11,-13,-13,-14,der(14)t(1;14)(q21;p11.2)t(3;14)x2,-15,+20,-21,
add(21)(p11.2),-22[cp20]

nuc ish**(BCL6x3)(3'BCL6 sep 5'BCL6x2)**[102/200],**(MYCx4)**[98/200],**(D8Z2,MYC,IGH)x4**[104/200],
(IGH,BCL2)x4[100/200]

Molecular Analysis:
A similar *IGH* monoclonal rearrangement was identified (122-123 bp) in the cervical lymph nodes specimens (core needle and excisional biopsy) and in the subsequent tetraploid mesenteric lymph node. Matching clonal peaks were also identified in IGK consistent with these being clonally-related processes.

Interesting Feature(s) of Submitted Case: EBV lowers the threshold for lymphomagenesis with EBV-driven lymphomas

This case, together with the advantage of supportive data from a series of biopsies, corresponding serological data and molecular and cytogenetic findings, provides evidence to support the "hit-and-run" hypothesis for EBV in lymphomagenesis (see comments below).

The initial presentation of this patient was also interesting given the mononucleosis-like symptoms, which had been severe and recurrent, despite previously documented primary EBV infection. While this might raise the possibility of an underlying immune deficiency, none had been documented, immunoglobulin titers were otherwise essentially within normal limits and HIV testing was negative. Overall, this process appears to be within the spectrum of EBV+ DLBCL, NOS reported in young, immunocompetent patients, which are most often nodal (Nicolae et al. Blood 2015; 126(7): 863–872.) and are now included in the revised 2017 WHO monograph.

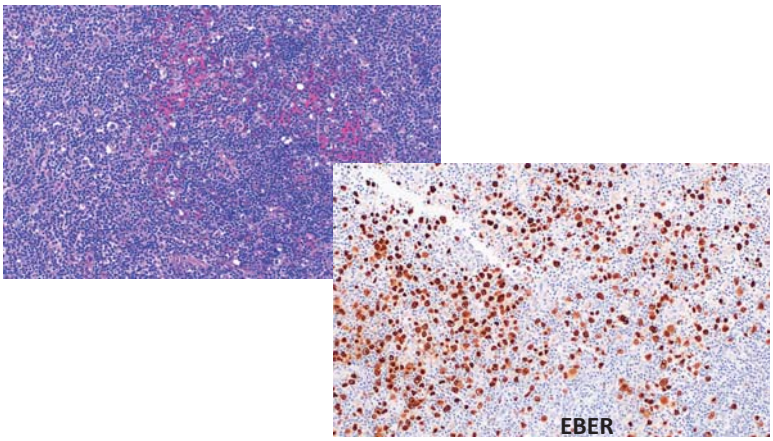
The morphology of the this case was also interesting given the unusual presentation with relatively few atypical cells. Nonetheless, the atypical cells were distinctly EBV+ and the same clone as the subsequently biopsied EBV negative diffuse large B cell lymphoma in the mesentery.

Proposed Diagnosis:
Cervical node: EBV+ diffuse large B cell lymphoma, NOS
Mesenteric node: EBV+ diffuse large B cell lymphoma, NOS with loss of EBV-dependence

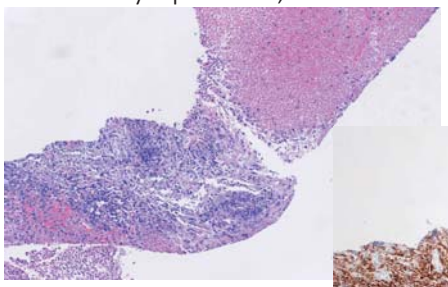
Comments: Because of the patient's prior history of EBV-associated lymphadenopathy and the atypical features on the cervical lymph node biopsies, a greater range of tissue sampling was pursued than typically performed in this setting. As a result, we obtained a unique picture of the evolution of this process between sites and over time with potential proof of the so-called "hit-and-run" hypothesis. That is, EBV may be required for the initiation of lymphomagenesis; however, as a result of additional genetic events, EBV may no longer be required as a driver and be gradually lost in order to evade the immune system (Niller et al. Cancer Lett. 2011; 305(2):200-17). In this case we saw the acquisition of tetraploidy in the tumor cells that no longer required EBV. In addition, a deletion in 2q was a newly observed abnormality in the mesenteric specimen. Detailed molecular studies were not performed but would be of interest to identify acquisition of potential novel driver mutations during the disease progression.

A series of biopsies documenting EBV hit-and-run in this setting are rare, but these processes may show unique features that allow their identification and may be of potential relevance for therapy. For example, previous studies have suggested that viral oncoproteins may result in specific epigenetic signatures that are retained, EBV microRNAs or fragments of the EBV genome (Niller et al. Cancer Lett. 2011; 305(2):200-17 and Mundo et al. Front Microbiol. 2017; 8:229).

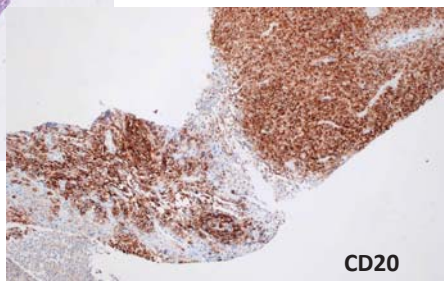
The patient subsequently began therapy with R-CHOP with a plan for 6 cycles. EBV viral load has become undetectable. A follow-up PET/CT scan is planned after completion of the chemotherapy regimen.

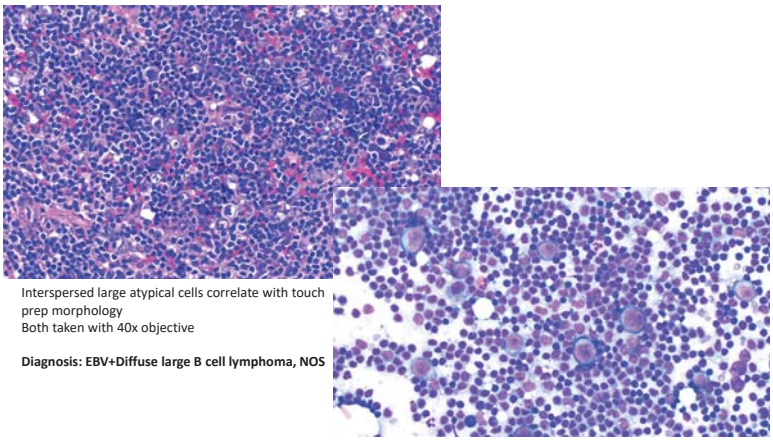


Cervical lymph node, initial core biopsy, December 2018



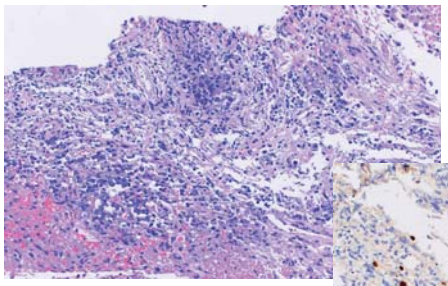
Extensive necrosis which was positive for CD20 along with viable areas
Both taken with 10x objective





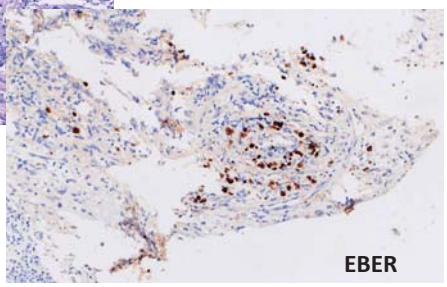
Interspersed large atypical cells correlate with touch prep morphology
Both taken with 40x objective

Diagnosis: EBV+Diffuse large B cell lymphoma, NOS

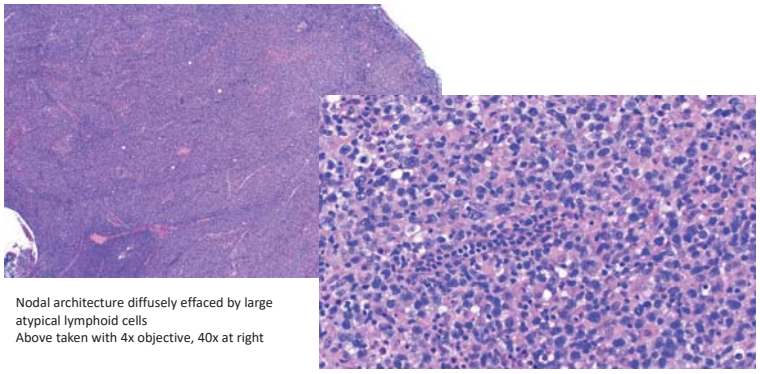


Focal viable area positive for Pax5, Bcl6, Bcl2, MUM-1 and EBER (shown)
Both taken with 20x objective

Diagnosis: Limited biopsy due to extensive necrosis, favor EBV+Diffuse large B cell lymphoma, NOS

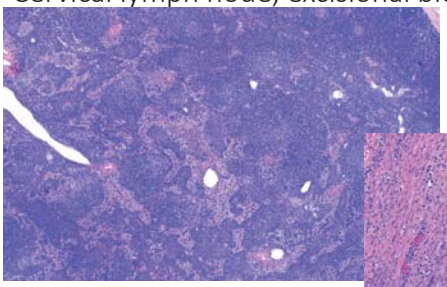


Mesenteric lymph node, excisional biopsy, February 2019



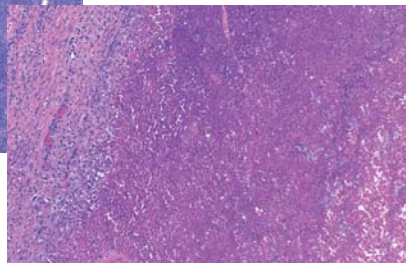
Nodal architecture diffusely effaced by large atypical lymphoid cells
Above taken with 4x objective, 40x at right

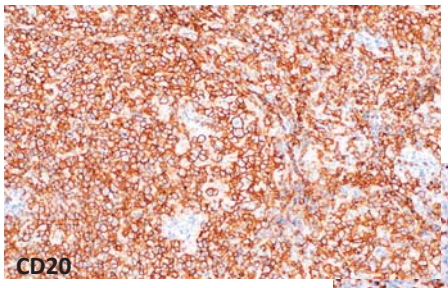
Cervical lymph node, excisional biopsy, December 2018



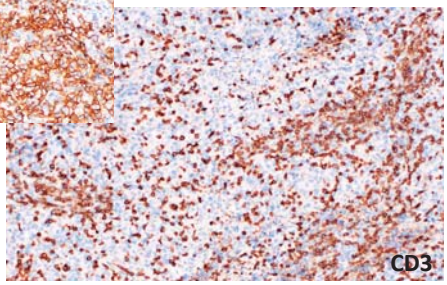
Areas of preserved nodular lymphoid architecture
Taken with 4x objective

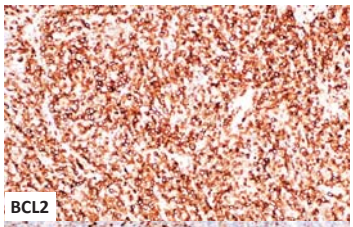
Areas with sheets of necrosis again appreciated
Taken with 10x objective

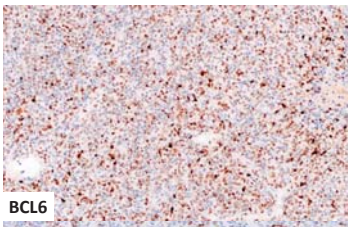


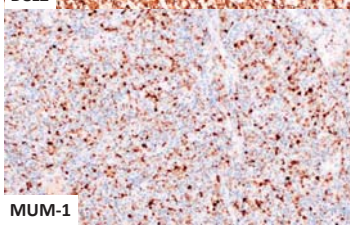


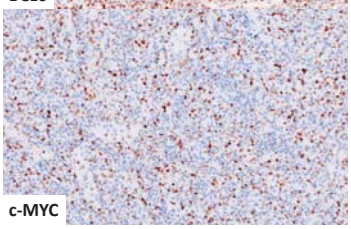
Immunostains taken with 20x objective







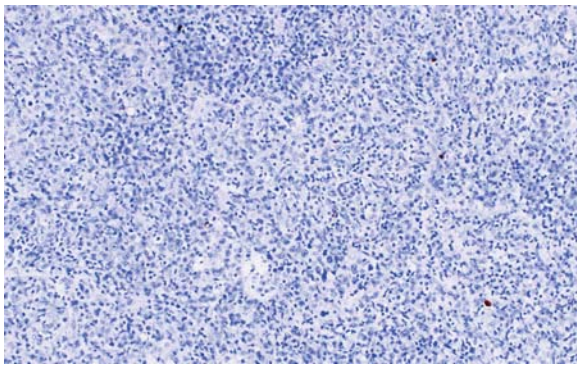




EBER now negative except in isolated small lymphoid cells

Diagnosis: EBV+ DLBCL, NOS with loss of EBV dependence

- **Clonal relation between these processes shown by both cytogenetics and molecular studies**
- **Compatible with "hit-and-run" hypothesis**
- **EBV required for initiation, but not maintenance following accumulation of additional genetic events**
- **(e.g. the lymphoma became tetraploid)**
- **Immune selection may drive loss of EBV**



CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Akira Satou, MD PhD,[1] Tetsuya Tabata, MD PhD,[2] Yasuharu Sato, MD PhD,[3] Tadashi Yoshino, MD PhD,[2] Toyonori Tsuzuki, MD PhD,[1] Shigeo Nakamura, MD PhD,[4]

Affiliation:
[1]Department of Surgical Pathology, Aichi Medical University Hospital, Nagakute, Japan
[2]Department of Pathology, Okayama University Graduate School of Medicine, Okayama, Japan
[3]Division of Pathophysiology, Okayama University Graduate School of Health Sciences, Okayama, Japan
[4]Department of Pathology and Laboratory Medicine, Nagoya University Hospital, Nagoya, Japan

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Clinical History:
A 57-year-old female had been followed up for rheumatoid arthritis (RA) for 25 years. To control the disease, she had taken steroid for 25 years, and methotrexate and etanercept for 5 months. CT revealed enlargement of supraclavicular, axillary, mediastinal, paraaortic, and inguinal lymph nodes, and splenomegaly. Laboratory studies showed elevated level of LDH 734 U/L (normal 120-240 U/L) and sIL-2R 38288 (normal 121-613 U/ml). The patient had an EBV past-infection pattern in a serum antibody test, and was negative for HTLV-1 or HIV serum antibody. A biopsy of bone marrow was performed.

Biopsy Fixation Details:
Tissue sample was fixed in 10% formalin.

Details of Microscopic Findings:
The diagnosis was made by biopsy of bone marrow. Bone marrow exhibited hypercellularity. Intermingled with hematopoietic cells, infiltration of medium-sized atypical lymphocytes was observed.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
Immunohistochemically, atypical lymphocytes were positive for CD3, CD5, CD8, and TIA-1, but negative for CD4, CD56, and granzyme B. The tumor cells were infected with EBV assessed by EBER-in situ hybridization.

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Molecular Analysis:
TCRγ rearrangement was detected by PCR analysis.

Interesting Feature(s) of Submitted Case:
After the diagnosis was made, methotrexate and etanercept were immediately withdrawn. The patient presented with spontaneous regression (CR). The patient has been followed up for 80 months without relapse of lymphoproliferative disorder.

Proposed Diagnosis:
Other iatrogenic immunodeficiency-associated lymphoproliferative disorder (OIIA-LPD), EBV-associated CD8-positive cytotoxic T-cell lymphoproliferative disorder

Comments:
OIIA-LPDs are mainly B-cell LPD or Hodgkin lymphoma types, whereas T-cell LPDs and NK/T-cell LPDs are relatively rare. Methotrexate (MTX) is the most common agent used for OIIA-LPD patients. Previous large studies revealed that T-LPDs or NK/T-LPDs account for only 4-8% of MTX-associated LPDs.

Submitters and Titles:
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Tadashi Yoshino, MD PhD,[2] Toyonori Tsuzuki, MD PhD,[1] Shigeo Nakamura, MD PhD,[4]

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[3]Division of Pathophysiology, Okayama University Graduate School of Health Sciences, Okayama, Japan
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1

Clinical History:
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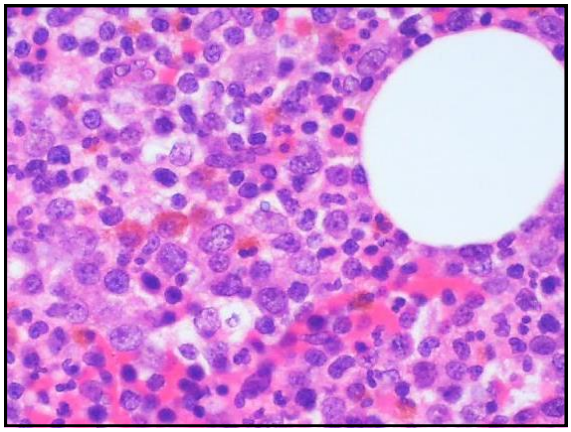
- Lab data:
  WBC 14900, Hb 9.9, LDH 734, sIL-2R 38288, CRP 6.75
- Serum test:
  HIV(-), HBV(-), HCV(-), HTLV-1(-)

2



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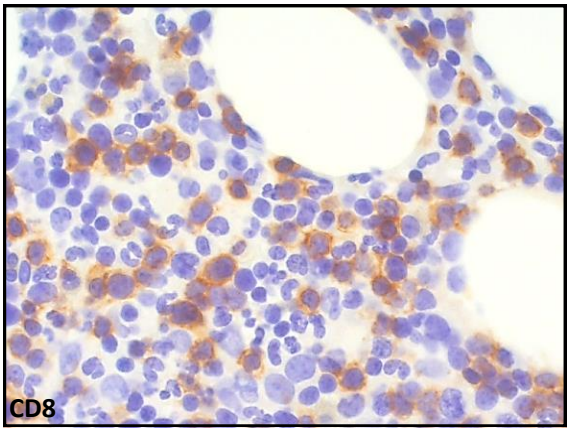

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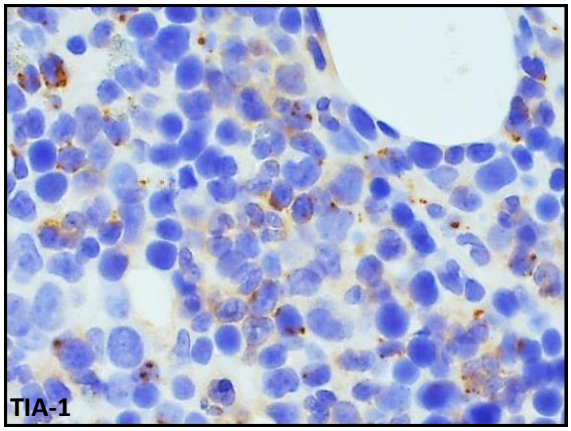

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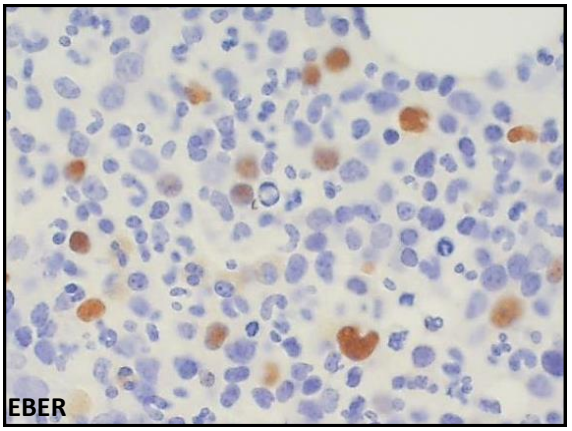

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Results of ancillary tests

- Immunohistochemistry and EBER-ISH

CD3(+), CD5(+), CD4(-), CD8(+), CD56(-), TIA-1(+), granzymeB(-), EBER(+)

- PCR analysis

TCR clonality (-)

7

- Proposed diagnosis

Other iatrogenic immunodeficiency-associated lymphoproliferative disorder, EBV-associated CD8-positive cytotoxic T-cell lymphoproliferative disorder

8

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
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2) Department of Pathology and Laboratory Medicine, Nagoya University Hospital, Nagoya, Japan

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Clinical History:
A 60-year-old male who had no previous history. The presenting symptom was abdominal pain. He received a diagnosis of EBV-positive primary gastric diffuse large B-cell lymphoma (DLBCL) after biopsy of a gastric mass.

Biopsy Fixation Details:
Biopsy samples were fixed in 10% formalin and embedded in paraffin.

Description of Clinical Image if Any:
Endoscopic image reveals an ulcerated mass in the antrum of the stomach.

Details of Microscopic Findings:
Diffuse lymphoid proliferation of medium to large cells are observed.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
CD3(-), CD20(+), CD5(-), CD10(-), BCL-2(+), BCL-6(-), MUM1(+), MYC(-), EBER-ISH(+), neoplastic PD-L1(+, 80%)

Special Stains:

Cytogenetics:

Molecular Analysis:

Interesting Feature(s) of Submitted Case:
In our previous study, PD-L1 expression on tumor cells was detected in only one (0.9%) of 114 patients with primary gastric DLBCL. This rare neoplastic PD-L1-positive (nPD-L1$^+$) gastric DLBCL harbored EBV on tumor cells. Notably, this case showed progressive disease after receiving R-CHP chemotherapy despite in Lugano stage I.

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Proposed Diagnosis:
EBV-positive primary gastric diffuse large B-cell lymphoma

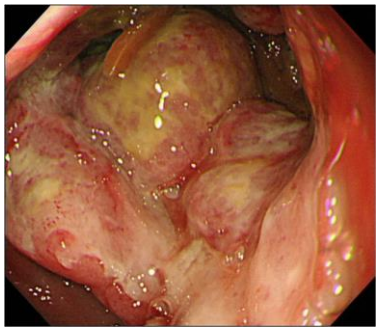
Comments:
Considering the aggressive behavior, patients with nPD-L1+ gastric DLBCL may represent good candidates for anti-PD-1/PD-L1 therapy.

Clinical History

- 60-year-old male had no previous history.
- Patient had abdominal pain, leading to upper endoscopy.

1

Endoscopic image



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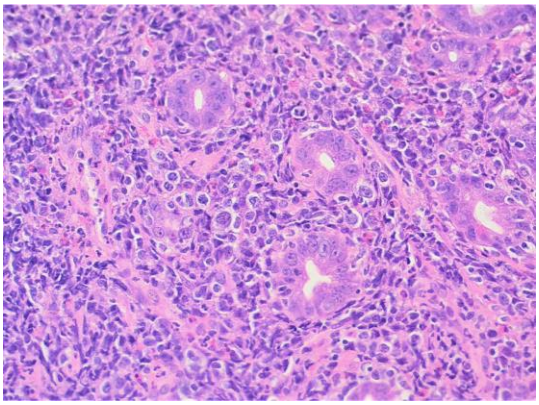

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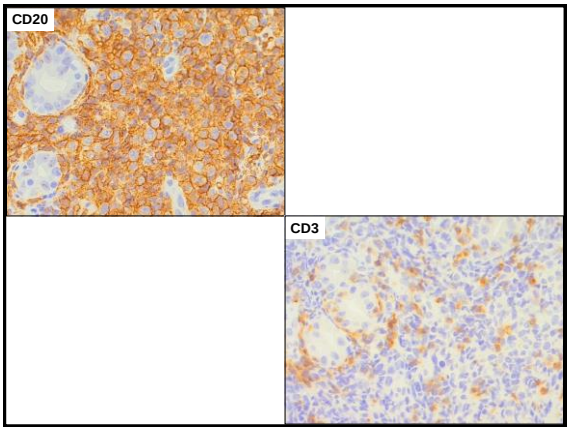

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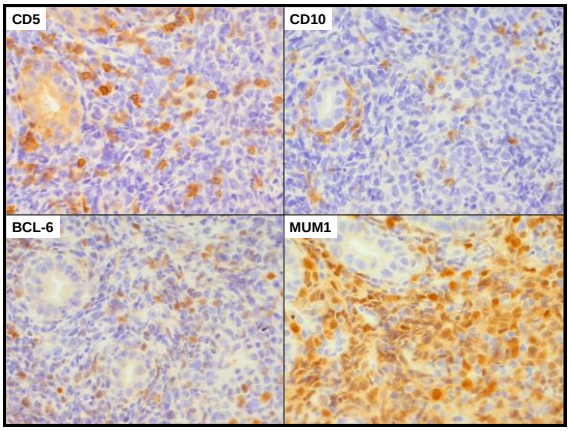

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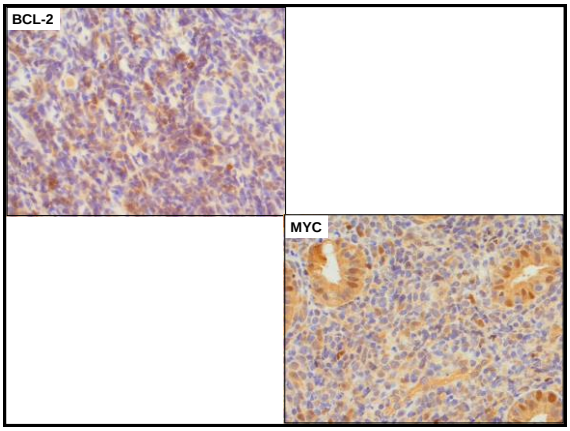

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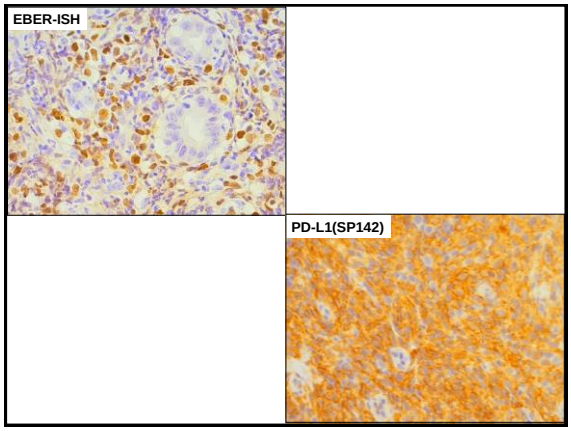

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Diagnosis

EBV-positive primary gastric diffuse large B-cell lymphoma, nPD-L1 positive

8

Treatment and Clinical course

- No evidence of lymphoma was identified outside the stomach. (Lugano stage I)
- Patient received 1 cycle of R-CHP and showed progressive disease.
- Patient underwent autologous peripheral blood stem cell transplantation.
- Patient achieved complete remission and had no relapse at the last follow-up of 10 months after diagnosis.

9

Clinical features of gastric DLBCL

(Ishikawa E et al. Cancer Medicine 2018)

- 240 patients with primary gastric DLBCL diagnosed between 1995 and 2015.
- **25(10%)** cases harbored **EBV** on tumor cells.
- 156 patients received rituximab-containing chemotherapy.

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Clinical features of gastric DLBCL

(Ishikawa E et al. Cancer Medicine 2018)

	Total (n=240) No. %	EBV-positive (n=25) No. %	EBV-negative (n=215) No. %	*P*
Sex (male/female)	136/104 (1.3)	12/13 (0.9)	124/91 (1.4)	0.39
Age (y), median (range)	67 (32-89)	69 (37-85)	67 (32-89)	0.90
Age >60y	170/240 (71)	18/25 (72)	152/215 (71)	1.00
Abdominal pain	39/81 (48)	2/7 (29)	37/74 (50)	0.43
PS 2-4	28/239 (12)	2/25 (8)	26/214 (12)	0.75
Perforation	1/230 (0.4)	0/22 (0)	1/208 (0.5)	1.00
B symptoms present	57/239 (24)	9/25 (36)	48/214 (22)	0.14
Lugano stage II2/IIE/IV	110/239 (46)	13/25 (52)	97/214 (45)	0.76
Ex. involvement >1 site	61/239 (26)	4/25 (16)	57/214 (27)	0.34
Bulky mass (≥10㎝) present	34/240 (14)	3/25 (12)	31/215 (14)	1.00
Helicobacter pylori infection	49/72 (68)	5/7 (71)	44/65 (68)	1.00
IPI High-int, High	69/237 (29)	7/25 (28)	62/212 (29)	1.00
Serum LDH >normal	87/237 (37)	11/25 (44)	76/212 (36)	0.53
sIL-2R ≥1000U/ml	101/211 (48)	9/19 (47)	92/192 (48)	1.00

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Pathological features of gastric DLBCL

(Ishikawa E et al. Cancer Medicine 2018)

	Total (n=240) No. %	EBV-positive (n=25) No. %	EBV-negative (n=215) No. %	*P*
Immunophenotype				
CD5	7/233 (3)	1/23 (4)	6/210 (3)	0.52
CD10	58/234 (25)	7/23 (30)	51/211 (24)	0.61
CD20	231/240 (96)	22/25 (88)	209/215 (97)	0.055
CD30	4/52 (8)	1/13 (8)	3/39 (8)	1.00
BCL-2	95/212 (45)	12/22 (55)	83/190 (44)	0.37
BCL-6	139/228 (61)	12/23 (52)	127/205 (62)	0.38
MUM1	163/228 (72)	17/23 (74)	146/205 (71)	1.00
nPD-L1 (≥5%)	1/128 (0.8)	1/14 (7)	0/114 (0)	0.109
miPD-L1 (≥20%)	60/127 (47)	11/13 (85)	49/114 (43)	0.007
Non-GCB type	145/231 (63)	16/24 (67)	129/207 (62)	0.82

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Clinical course of gastric DLBCL

(Ishikawa E et al. Cancer Medicine 2018)

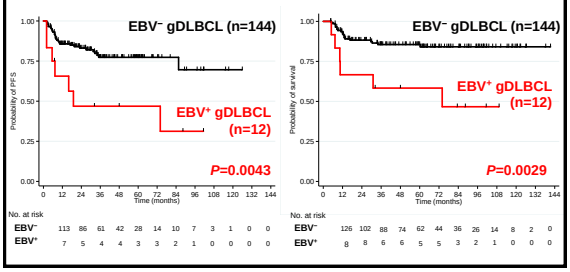
	Total (n=240) No. %	EBV-positive(n=25) No. %	EBV-negative (n=215) No. %	*P*
Treatment				
R-CTx	156/239 (66)	12/25 (48)	144/214 (67)	0.075
	(n=156)	(n=12)	(n=144)	
R-CTx alone	91/156 (58)	8/12 (67)	83/144 (58)	0.76
R-CTx + Radiation	55/156 (35)	3/12 (25)	52/144 (36)	0.54
R-CTx + Surgery	9/156 (6)	0/12 (0)	9/144 (6)	1.00
R-CTx + Surgery + Radiation	1/156 (0.6)	1/12 (8)	0/144 (0)	0.077
No. of cycles, median (range)	5 (1-8)	3.5 (2-8)	5 (1-8)	0.22
Therapeutic response (R-CTx)				
CR	143/156 (87)	9/12 (75)	126/144 (88)	0.21
PR/SD/PD	21/156 (13)	3/12 (25)	18/144 (12)	
follow-up duration (median)	60 mo (4-141)	88 mo (83-109)	59 mo (4-141)	0.17

13

Survival analysis of gastric DLBCL

treated with R-containing chemotherapy

(Ishikawa E et al. Cancer Medicine 2018)



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Interesting Features

- A rare nPD-L1⁺ gastric DLBCL harbored EBV on tumor cells.
- Notable, this case showed aggressive clinical course despite in Lugano stage I.
- Considering the aggressive behavior, patients with nPD-L1⁺ gastric DLBCL may represent good candidates for anti-PD-1/PD-L1 therapy.

15

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Laila Nomani, M.D and Eric D Hsi, MD

Affiliation: Cleveland Clinic

E-mail:Laila.nomani@gmail.com

Clinical History:
A 76 y/o male with a history of ulcerative colitis presented in septic shock in the emergency room. A CT scan revealed a mass in the rectum. Operative findings showed an extensively necrotic tumor 10 cm above anal verge. Initial clinical suspicion was for an adenocarcinoma. The lesion was excised transanally.

Biopsy Fixation Details: Formalin fixed.

Description of Clinical Image if Any: N/A

Details of Microscopic Findings:
The histologic sections of the rectal tumor excision show an ulcerated mucosal mass consisting of a polymorphous infiltrate with underlying soft tissue. There was extensive necrosis. The viable portions at the base of the lesions showed a mixture of small lymphocytes, intermediately sized cells, eosinophils, plasma cells, histiocytes, and scattered large atypical cells with irregular nuclear contours and prominent nucleoli. The lesion appeared circumscribed by small lymphocytes.

Immunophenotyping by Immunohistochemistry:
The large atypical cells were positive for CD30, PAX-5, MUM-1 and BOB-1. Occasional large cells were weakly positive for CD20, while the majority were negative. The large cells were negative for Oct-2, CD15 and CD45. The CD3 stain showed small T-lymphocytes underlying the atypical infiltrate.

Special Stains:
An EBER in situ hybridization stain is strongly positive in the large atypical cells. There are also many positive smaller cells.

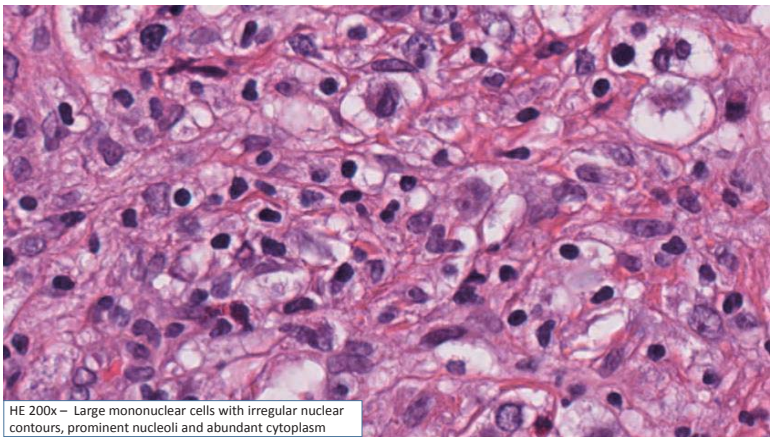
Cytogenetics: N/A

Molecular Analysis: N/A

Interesting Feature(s) of Submitted Case:
This case highlights the classical morphologic features of EBV positive mucocutaneous ulcer. The presenting feature of a large necrotic mass in an elderly patient with ulcerative colitis usually raises the clinical suspicion of an adenocarcinoma. The histologic features clearly point to a lymphoproliferative disorder and the presence of large atypical CD30+ cells with immunophenotypic features of Reed-Sternberg cells, suggests the possibility of classic Hodgkin lymphoma. However, the extranodal site, history of long standing ulcerative colitis, associated immunosuppression and advanced age are clues which raised the possibility of an EBV-driven lymphoproliferative disorder. These lesions are seen in iatrogenically immunosuppressed as well as elderly patients and may have a self-limited course with removal of immunosuppression.

Proposed Diagnosis: EBV-positive mucocutaneous ulcer

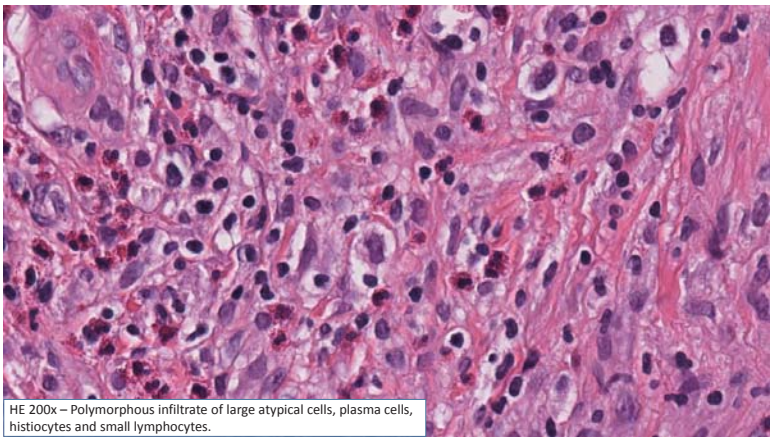
Comments:
The patient's course was complicated by sepsis and abscess around the rectal stump, treated with antibiotics. He also suffered deep venous thrombosis of the upper extremities. He was transferred to a nursing and rehabilitation facility with plans for 4 weekly doses of rituximab therapy. However, poor performance status precluded immediate therapy and was lost to follow-up 3 months after diagnosis.



HE 200x – Large mononuclear cells with irregular nuclear contours, prominent nucleoli and abundant cytoplasm

Case Submission EBV+ Mucocutaneous Ulcer

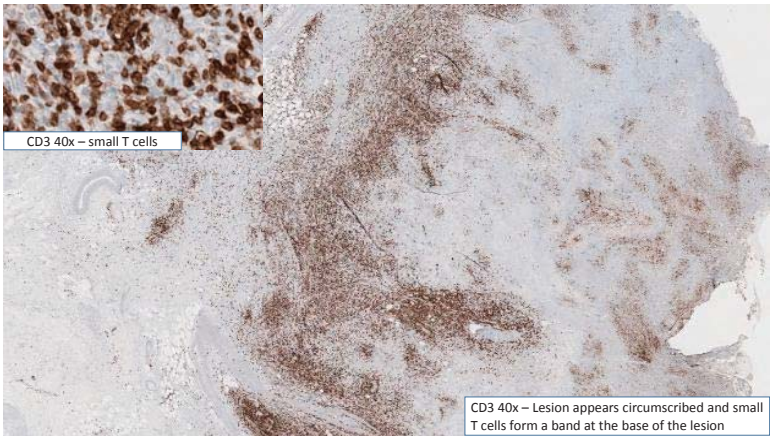
Laila Nomani, MD
Eric D. Hsi, MD
Cleveland Clinic



HE 200x – Polymorphous infiltrate of large atypical cells, plasma cells, histiocytes and small lymphocytes.

Clinical history

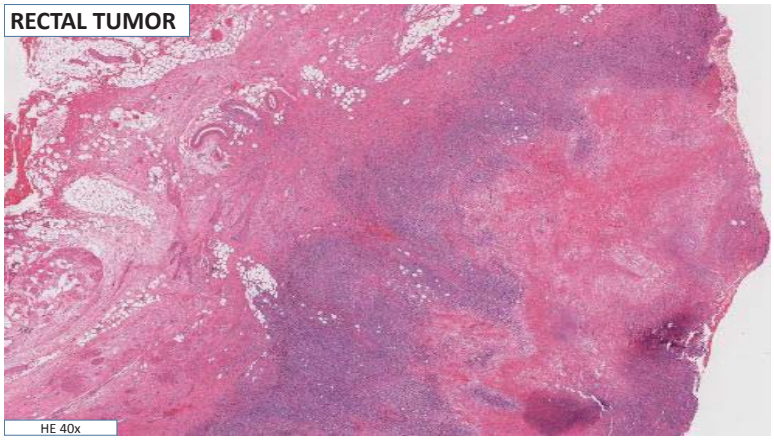
- 76 y/o male with a history of ulcerative colitis and necrotic mass in the rectum.
- A CT scan revealed a mass in the rectum.
- Operative findings showed an extensively necrotic tumor 10 cm above anal verge.
- Initial clinical suspicion was for an adenocarcinoma. The lesion was excised



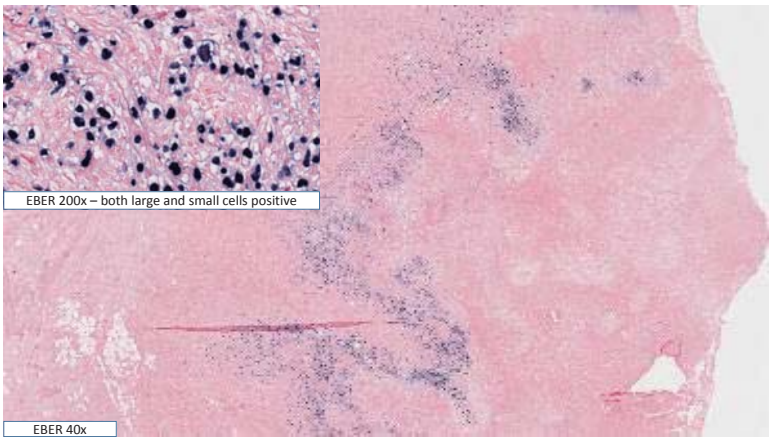
CD3 40x – small T cells

CD3 40x – Lesion appears circumscribed and small T cells form a band at the base of the lesion

RECTAL TUMOR



HE 40x



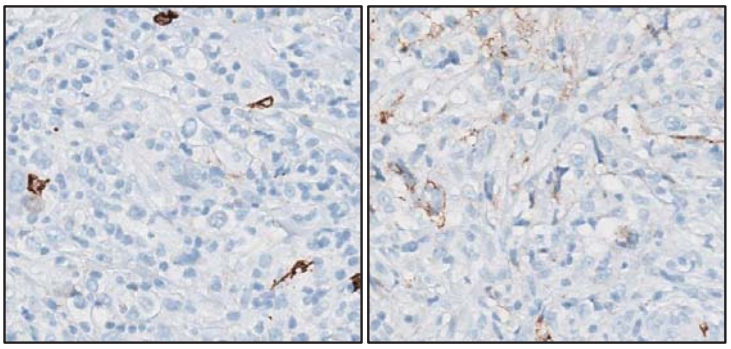
EBER 200x – both large and small cells positive

EBER 40x

CD20 40x – Lesion appears circumscribed

Diagnosis

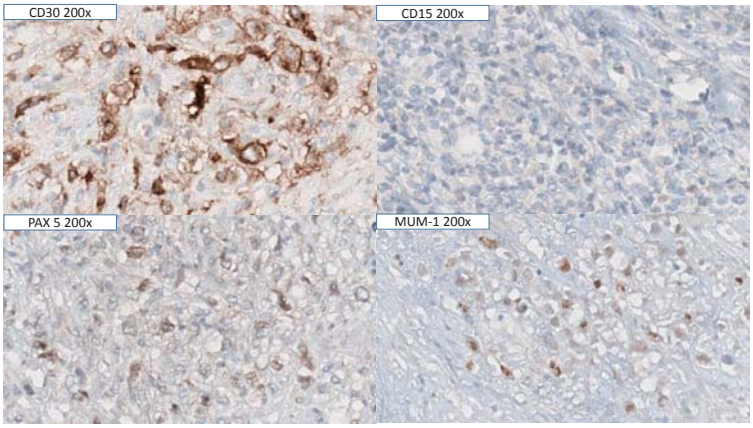
- **EBV+ mucocutaneous ulcer**
- Clinical follow-up:
  - The patient's course was complicated by sepsis and abscess around the rectal stump, treated with antibiotics.
  - He also suffered deep venous thrombosis of the upper extremities.
  - He was transferred to a nursing and rehabilitation facility with plans for 4 weekly doses of rituximab therapy.
  - However, poor performance status precluded immediate therapy and was lost to follow-up 3 months after diagnosis.



CD 20 200x – highlights rare large atypical cells and small scattered B cells

Interesting Features

- This case highlights the classical morphologic features of EBV positive mucocutaneous ulcer.
- The presenting feature of a large necrotic mass in an elderly patient with ulcerative colitis usually raises the clinical suspicion of an adenocarcinoma.
- The histologic features clearly point to a lymphoproliferative disorder and the presence of large atypical CD30+ cells with immunophenotypic features of Reed-Sternberg cells, suggests the possibility of classic Hodgkin lymphoma.
- However, the extranodal site, history of long standing ulcerative colitis, associated immunosuppression and advanced age are clues which raised the possibility of an EBV-driven lymphoproliferative disorder.
- These lesions are seen in iatrogenically immunosuppressed as well as elderly patients and may have a self-limited course with removal of immunosuppression.



CD30 200x

CD15 200x

PAX 5 200x

MUM-1 200x

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):

Hui Liu MD PhD

Affiliation:

The Affiliated Hospital of Xuzhou Medical University

E-mail:

hliu@xzhmu.edu.cnd

Clinical History:

A 54-year-old woman, who presented with bilateral lymph node enlargement in neck, axilla and inguinal for two months, was admitted to the Affiliated Hospital of Xuzhou Medical University in February 2018. The patient showed also multiple lymphadenopathy in abdominal cavity and retroperitoneum on CT scan. No evidence of immunodeficiency or transplant history was found. Serum EBV DNA detection 4.14E+06 copies/ml.

Neck lymph node biopsy was performed, on which a diagnosis of EBV+ LPD was made, based on polymorphic morphological feature and large number EBER positive cells. The patient didn't receive any treatment after diagnosis. No significant relief of symptoms was observed, and serum EBV status of the patients was followed up: 1733 copies/ml on May 23, 2018; 32222 copies/ml, on May 29; and 136657 copies/ml, on May 31; but lower than 1000 copies/ml on June 25.

Six months after, in July 2018, another biopsy of neck lymph node was performed, which presented with typical angioimmunoblastic T-cell lymphoma (AILT) features morphologically and immunohistochemically, with scattered EBER positive cells.

Biopsy Fixation Details:

The tissue was fixed in 10% neutral formalin for 6~24h before FFPE preparation.

Description of Clinical Image if Any:

N/A

Details of Microscopic Findings:

Biopsy 1st：The architecture of the lymph node was effaced diffusely. Small to medium-sized lymphocytes and plasmacytic cells diffusely distributed. No necrosis displayed.

Biopsy 2nd: the architecture of the lymph node was totally effaced, and displaced by the expanded paracortex. The neoplastic cells were small to medium-sized, some large, with clear cytoplasm and atypical nuclear, closely adjacent to HEVs. Inflammatory cells scattered in the background.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

Biopsy 1st：

IHC: CD20focal+, CD19 diffuse+, CD21、CD23 irregular FDC meshwork+ Kappa、Lambda focal+, CD2+,CD3+,CD5+，CD7decrease CD4+,CD8+,TIA-1focal+,CD30 scattered+, CD10 focal+,Bcl-6-,PD-1 focal+ Ki-67 80%

ISH: EBER diffuse+

Biopsy 2nd:

IHC: CD20 focal+, CD2+,CD3+,CD5+,CD7 focal+, CD4>CD8, CD10+,BCL-6+,PD-1+, CD30+, Ki-67 60%

ISH: EBER focal+

Special Stains:

N/A

Cytogenetics:

N/A

Molecular Analysis:

N/A

Interesting Feature(s) of Submitted Case:

As the patient developed into AITL in only half a year, the two biopsies should be closely related. So is it appropriate to classify the initial one as EBV+LPD? When developed into AITL, the morphology of the neoplasm was totally changed, and EBER positive cells decreased dramatically, which is coordinated with serum EBV-DNA test. So, what roles could EBV play in this process?

Proposed Diagnosis:

EBV+ LPD progress into AITL

EBV+ LPD progress into AITL

Reporter
Hui Liu MD, PhD

Affiliation
The Affiliated Hospital of Xuzhou Medical University

1

Clinical History:

A 54-year-old woman, who presented with bilateral lymph node enlargement in neck, axilla and inguinal for two months, was admitted to the Affiliated Hospital of Xuzhou Medical University in February 2018. The patient showed also multiple lymphadenopathy in abdominal cavity and retroperitoneum on CT scan. No evidence of immunodeficiency or transplant history was found. Serum EBV DNA detection 4.14E+06 copies/ml.

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DETAILS OF MICROSCOPIC FINDINGS:

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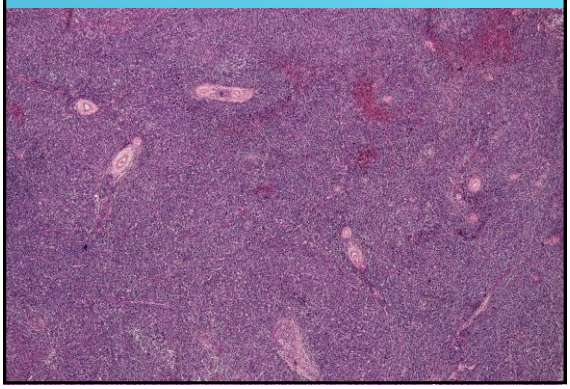
Biopsy 1st: The architecture of the lymph node was effaced diffusely. Small to medium-sized lymphocytes and plasmacytic cells diffusely distributed. No necrosis displayed.



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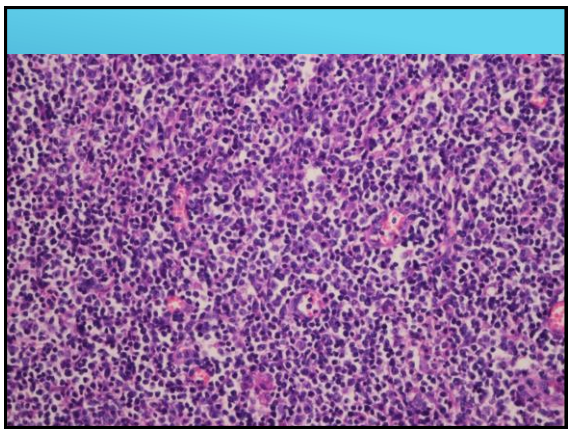

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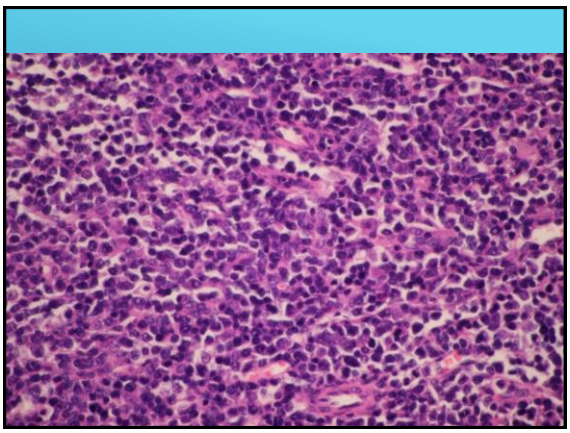

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IMMUNOPHENOTYPING BY IMMUNOHISTOCHEMISTRY:

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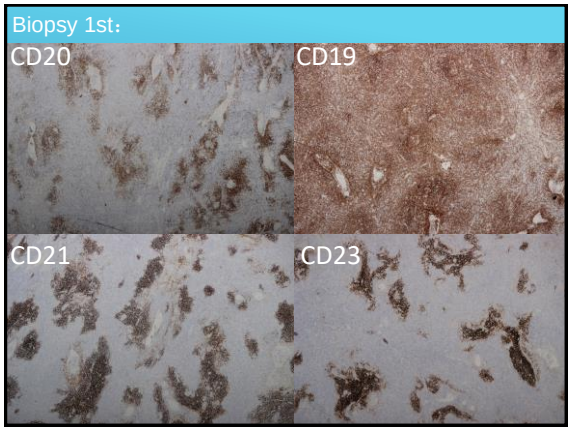

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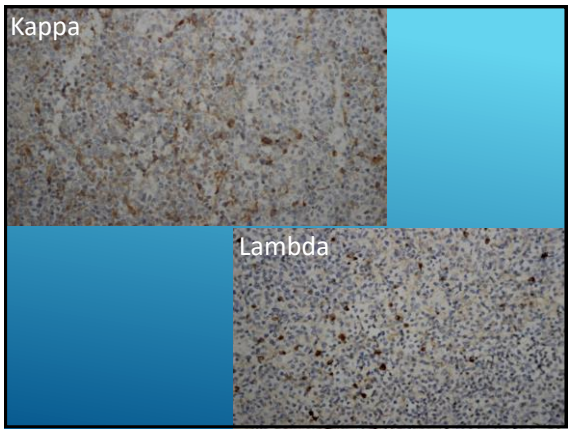

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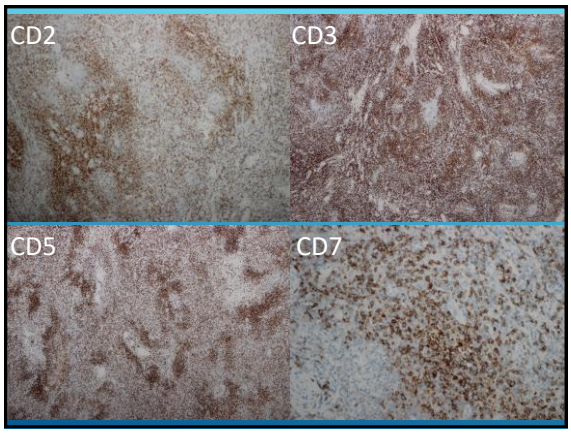

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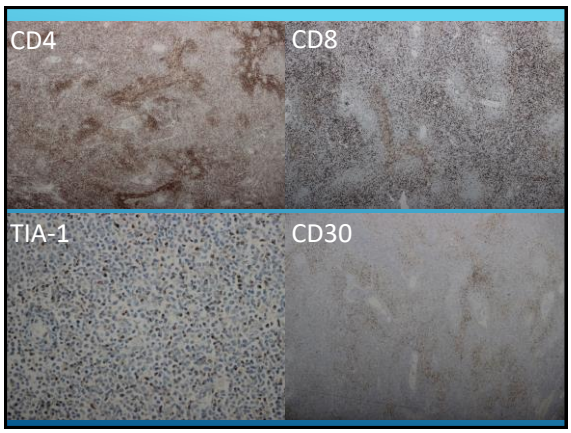

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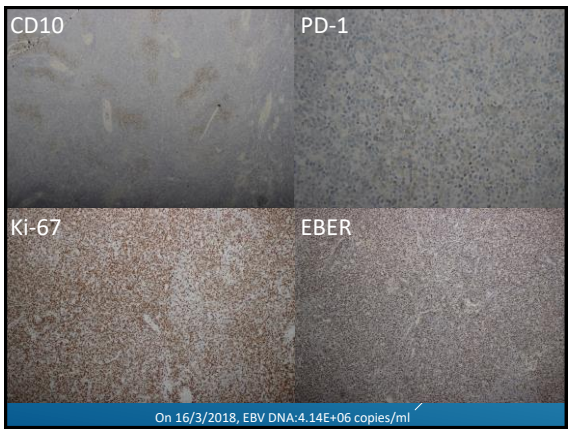

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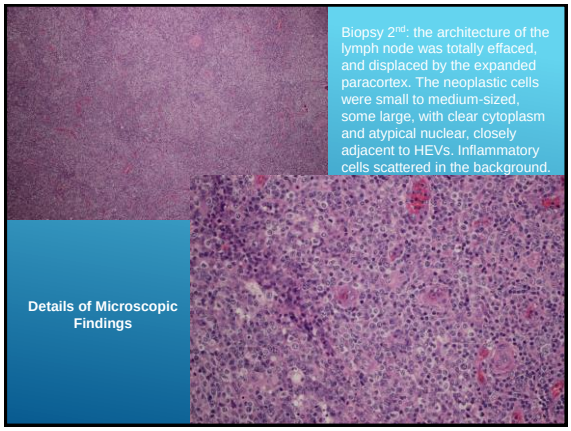

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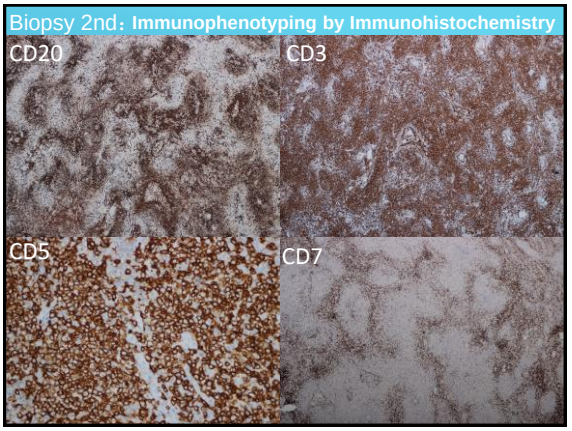

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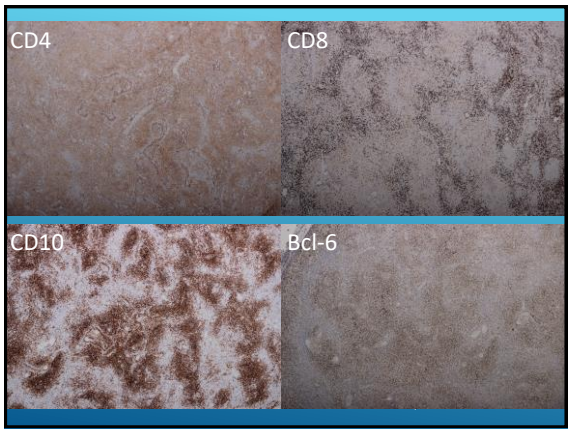

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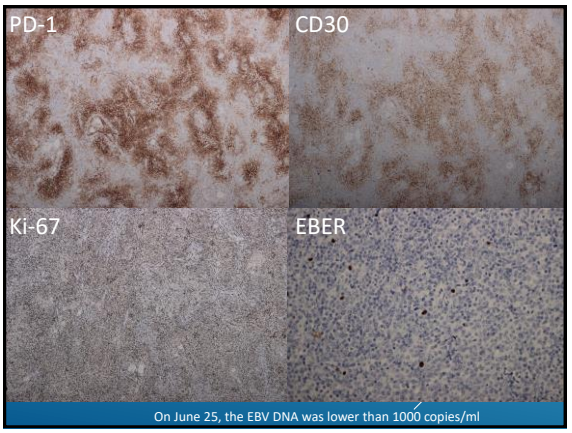


16

PROPOSED DIAGNOSIS:

EBV+ LPD progress into AITL

17

DISCUSSION:

As the patient developed into AITL in only half a year, the two biopsies should be closely related. So is it appropriate to classify the initial one as EBV+LPD? When developed into AITL, the morphology of the neoplasm was totally changed, and EBER positive cells decreased dramatically, which is coordinated with serum EBV-DNA test. So, what roles could EBV play in this process?

18

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Haiyan Lu, MD; Ph.D, Sindhu, Shetty, MD; Sarah Ondrejka, DO.

Affiliation: Cleveland Clinic Foundation

E-mail: luh@ccf.org

Clinical History:

The patient is a 66-year-old male with past medical history of a left adrenal cyst on observation. He presents with increased left-sided abdominal pressure. Computed tomography scan shows a retroperitoneal mass measuring 12.9 x 14.2 x 15.8 cm. During exploratory laparectomy surgery the mass is determined to be unresectable, at least 30 cm in greatest dimension, and involving pancreas, stomach, colonic mesentery and possible left renal hilus. This is an incisional biopsy of the retroperitoneal tumor.

Biopsy Fixation Details: 10% formalin

Description of Clinical Image if Any:

Not applicable

Details of Microscopic Findings:

Histologic sections show a diffuse abnormal lymphoid infiltrate composed of monotonous somewhat epithelioid cells with round nuclei, fine chromatin, prominent nucleoli and occasional multinucleated forms. The cells have a moderate amount of cytoplasm.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

Immunostains show that these cells are positive for CD20, PAX-5, CD79A, CD43, MUM1, MYC, BCL2, and aberrantly positive for CD3 and CD7

(subset). The cells are negative for AE1/3, S-100, CD45, CD30, BCL6, TDT, cyclinD1, CD2, CD4, CD8, CD5, CD21, CD10, ALK-1, TIA, CD56, granzyme B, TCL1A, PD1, betaF1, and TCR-delta. The Ki-67 proliferative index is 80%. EBER in situ hybridization is strongly positive in all of the neoplastic cells.

Special Stains: Not applicable

Cytogenetics: Not applicable

Molecular Analysis: IGH/IGK and T-cell receptor (TCRB and TCRG) gene rearrangements are pending at the time of this writing.

Interesting Feature(s) of Submitted Case:

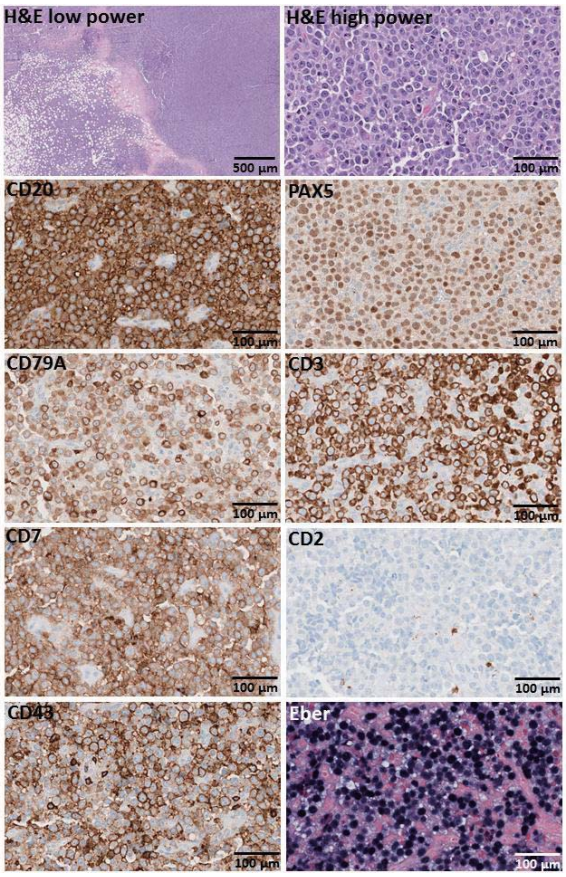
The patient presents with an aggressive, diffusely EBV positive B-cell neoplasm without evidence of immunosuppression other than immune senescence. The pathologic findings are diagnostic of EBV positive diffuse large B cell lymphoma, not otherwise specified, according to the WHO 2016 classification. T-cell antigen expression in EBV-positive large B-cell lymphomas is highly unusual and caused some initial confusion in this case, since the differential diagnosis of a CD3+/EBV+ tumor includes extranodal NK/T-cell lymphoma of nasal type. Plasmablastic lymphoma was excluded on the basis of strong and diffuse CD20 expression and lack of plasmablastic cytologic features. The combined expression of CD20, PAX5, and CD79a allowed classification as a B-cell process.

Proposed Diagnosis:

EBV positive diffuse large B cell lymphoma, not otherwise specified.

Comments:

T-cell antigen expression in EBV-positive large B-cell lymphomas is highly unusual. CD3, CD7, and CD43 were checked to ensure that the positive and negative control tissue stained appropriately. This may be analogous to the paradox of T-cell marker expression that can occasionally occur in EBV-driven plasmablastic lymphomas or rarely in the case of Reed-Sternberg cells in classic Hodgkin lymphoma.



CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Timothy Clifford Carll, MD
Benjamin Kaumeyer, MD
Shiraz Fidai, MD
Shu-Yuan Xiao, MD
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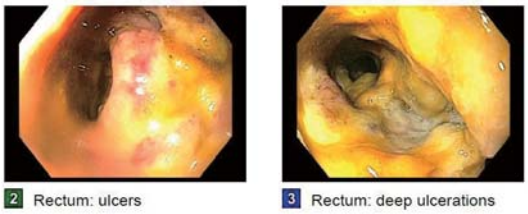
Clinical History:
A 62-year-old man with ulcerative colitis is status-post total colectomy with end-ileostomy & Hartmann's pouch 20 years ago. He is on no systemic therapy for his ulcerative colitis but uses mesalamine and budesonide enemas. He recently developed increased ostomy output and began to pass blood and mucus per rectum. He underwent flexible sigmoidoscopy which revealed erythema, friable mucosa, and inflammatory polyps. Biopsies were taken from multiple sites and submitted to pathology.

Biopsy Fixation Details:
Specimens were fixed in 10% normal buffered formalin.

Description of Clinical Image if Any:



2 Rectum: ulcers 3 Rectum: deep ulcerations

Flexible sigmoidoscopy of Hartmann's pouch reveals diffuse edema, erythema, friable mucosa and inflammatory pseudopolyps.

Details of Microscopic Findings:
The specimen contains multiple fragments of submucosal tissue completely effaced by atypical lymphoid infiltrate. No epithelium is present. The infiltrate is composed chiefly of medium to large pleomorphic cells with variable morphology; ranging from plasmablastic to occasionally Hodgkin Reed-Sternberg-like. There are increased background mitoses, apoptosis, and numerous histiocytes.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
Immunohistochemical staining is performed on paraffinized tissue sections. It highlights the aberrant cells with CD20, MUM1 (30%), and CD30 (subset). EBER ISH is positive in these cells. Ki-67 (MIB-1) proliferation index is 30-40%. CD5 and CD10 are negative. Staining for CD3, CD4, CD5, and CD8 also highlight normal CD4+ preponderant background T-cells.

Special Stains:
Not applicable.

Cytogenetics:
Not applicable.

Molecular Analysis:
Not applicable.

Interesting Feature(s) of Submitted Case:

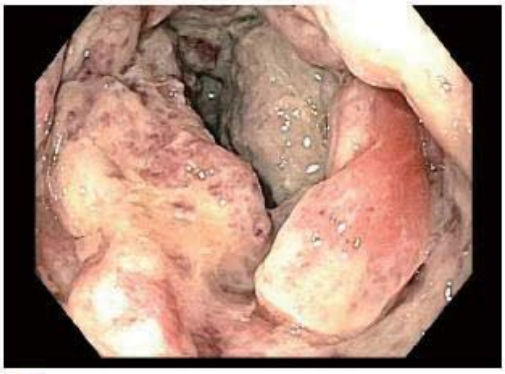
This case features an EBV+ lymphoid proliferation in an ulcerative colitis patient most consistent with EBV+ mucocutaneous ulcer. EBV+ mucocutaneous ulcer is described as usually solitary GI tract lesions in patients with systemically impaired immune surveillance. This patient's Hartmann pouch is involved in a multifocal fashion. The patient is not on systemic immunosuppression for his ulcerative colitis, but only receives local/topical treatment.

This lymphoid proliferation exhibits distinctly variable and heterogeneous morphology including Hodgkin Reed-Sternberg-like cells, with a relatively consistent immunophenotype that is CD20+, CD30+, and EBER ISH+.

Proposed Diagnosis:
EBV+ mucocutaneous ulcer (EBV-MCU).

Comments:

Sigmoidoscopy images



2 Hartmann Pouch

Atypical lymphoproliferation in a Hartmann's pouch

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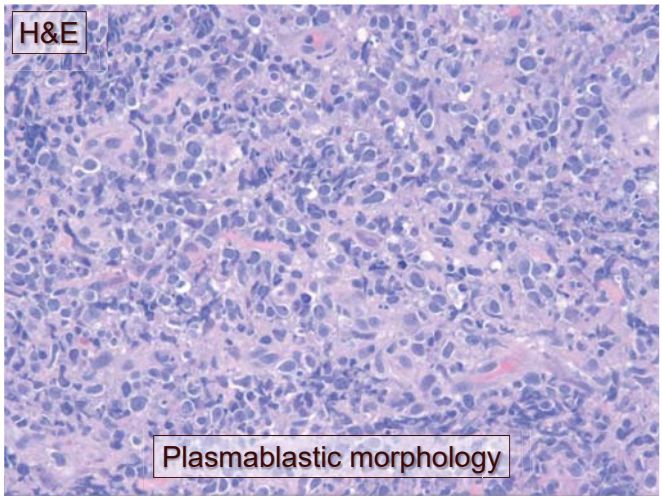

Clinical history

- The patient is a 62-year-old man with a longstanding history of ulcerative colitis.
- He underwent total colectomy with ileostomy and Hartmann's pouch 20 years ago.
- He is on no systemic anti-inflammatory agents (e.g. oral 5-ASA, steroids, TNFα inhibitors).
- Only topical therapy with mesalamine (5-ASA) and budesonide enemas.

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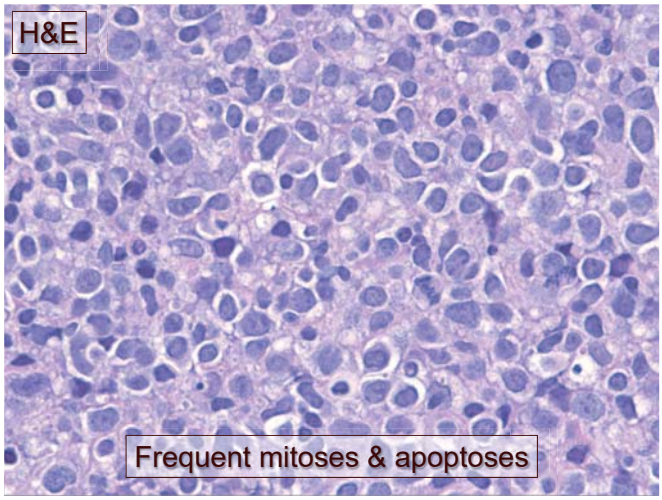

Clinical history

- Prior to current encounter, he began passing increased blood and mucous per rectum.
- Flexible sigmoidoscopy of rectum and Hartmann's pouch demonstrated edema, erythema, friable mucosa and inflammatory pseudopolyps.
- Multiple biopsies of the rectum and Hartmann's pouch were obtained and submitted to pathology in 10% NBF.

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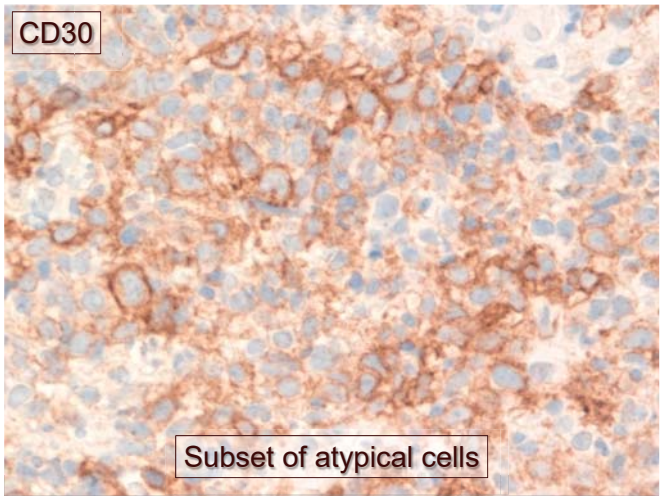
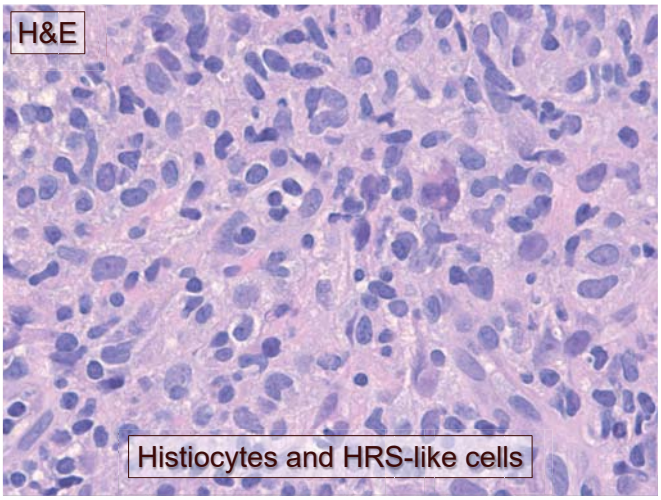
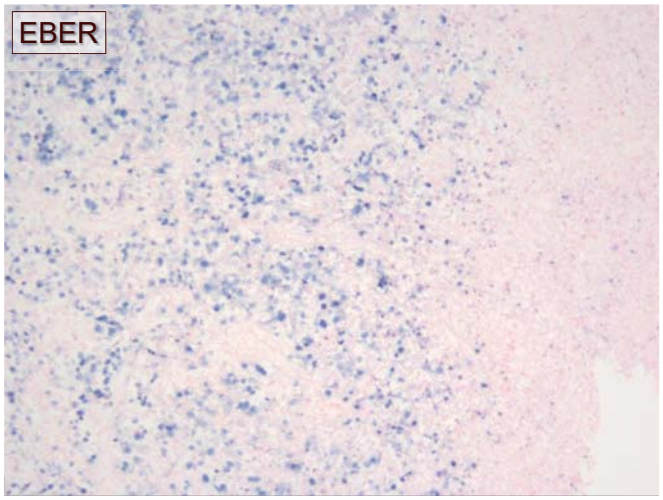
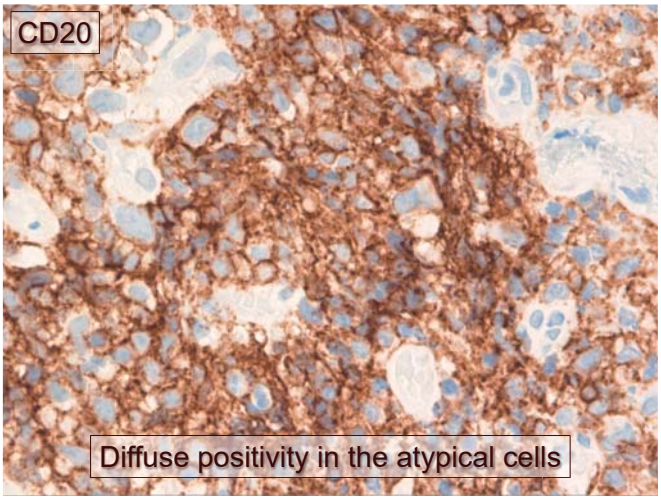
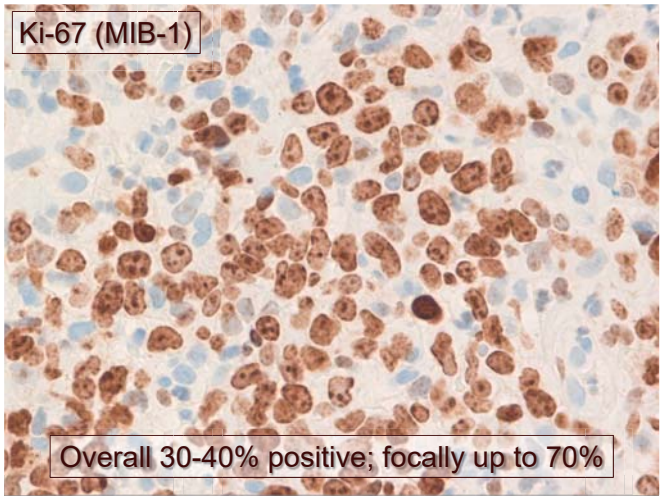
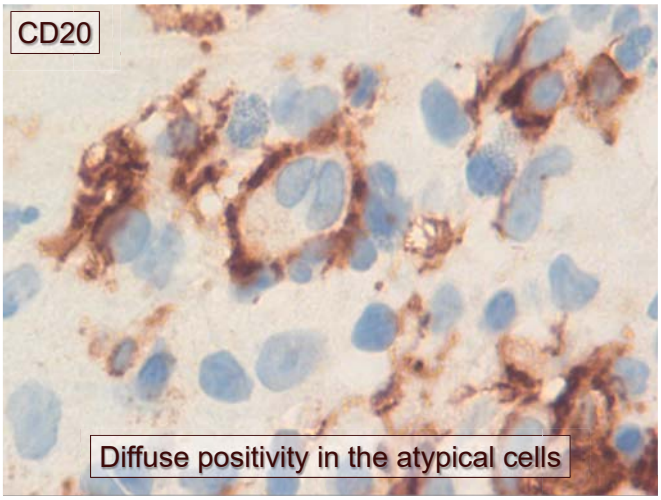

CD30
Subset of atypical cells

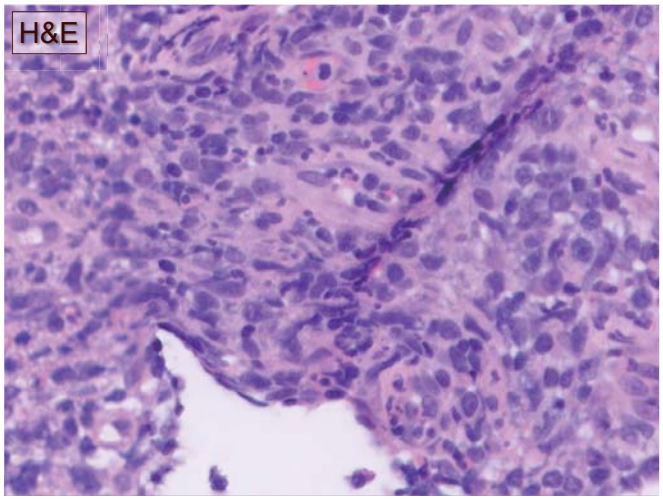

H&E
Histiocytes and HRS-like cells


EBER


CD20
Diffuse positivity in the atypical cells


Ki-67 (MIB-1)
Overall 30-40% positive; focally up to 70%


CD20
Diffuse positivity in the atypical cells



Final diagnosis

- EBV+ lymphoid proliferation most consistent with **EBV+ mucocutaneous ulcer**.
- Conservative management (immunosuppression vs observation) was recommended.

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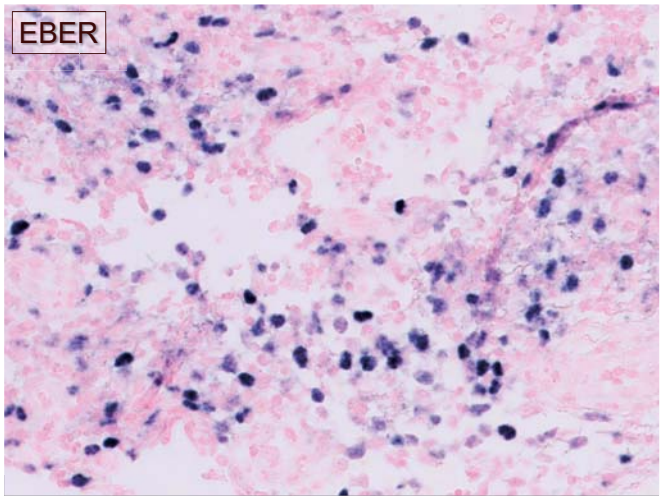

Follow-up

- Patient had been discharged with an oral prednisone regimen for presumed recurrence of ulcerative colitis.
- The patient returned a month later with continued blood per rectum, increased ostomy output, fevers, leukocytosis, and severe dehydration.
- He was treated with IV fluids and antibiotics for a suspected infectious colitis (CMV colitis or *C. difficile*)
  - Stool PCR positive for *C. difficile* toxin A/B
- Repeat sigmoidoscopy demonstrated deep ulcerations of the rectum with friable mucosa.

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Case resolution

- Fevers, leukocytosis resolved with IV fluids and antibiotics.
- Clinical team did not suspect a systemic lymphoproliferative disorder given absence of lymphadenopathy on CT imaging.
- Mesalamine and budesonide enemas were discontinued. The patient is scheduled to follow up for **completion proctectomy**.

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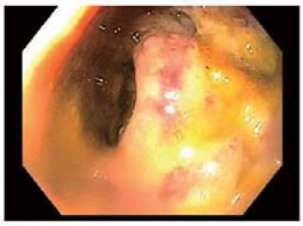
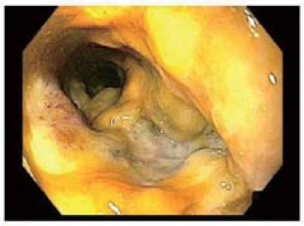
Sigmoidoscopy images



2 Rectum: ulcers



3 Rectum: deep ulcerations

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EBV-Positive Mucocutaneous Ulcer in Inflammatory Bowel Disease

- EBV-positive mucocutaneous ulcer (EBVMCU) is a new clinicopathological entity (*WHO 2016 provisional entity*) occurring in patients with iatrogenic or age related immunosuppression[1].
- Mucosal or cutaneous ulcers with an outgrowth of EBV positive polymorphic infiltrate of lymphoid cells often with Hodgkin like features[1].
  - Considered clinically indolent; many cases will resolve spontaneously[1,5].

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[1] Swerdlow SH, et al. *WHO Classification of Tumours of Hematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: International Agency for Research on Cancer; 2017.
[2] Juan A, et al. Epstein–Barr virus-positive mucocutaneous ulcer in Crohn's disease. A condition to consider in immunosuppressed IBD patients. *Am J Surg Pathol* 2019;43:201-210.
[3] Moran N, et al. Epstein Barr virus-positive mucocutaneous ulcer of the colon associated Hodgkin lymphoma in Crohn's disease. *World J Gastroenterol*. 2015; 21: 8072-8076.
[4] Matnani R, Peker D. Azathioprine induced Epstein Barr virus-positive muco-cutaneous ulcer arising in perianal fistula and abscess associated with Crohn'sdisease. *Journal of Crohn's and Colitis* 2014;8:1747–1748.
[5] Dojcinov S, et al. EBV positive mucocutaneous ulcer – a study of 26 cases associated with various sources of immunosuppression. *Am J Surg Pathol*. 2010;34:405-417.

EBV-Positive Mucocutaneous Ulcer in Inflammatory Bowel Disease

- Cases are reported of EBV-positive mucocutaneous ulcers arising in the gastrointestinal tract of IBD patients[2-6].
  - Almost exclusively associated with systemic administration of immunosuppressive drugs to treat IBD (methotrexate, infliximab, and azathioprine[2]).
  - One known case of EBV MCU arising in gastrointestinal tract of a patient without systemic immunosuppression (patient on oral mesalazine)[6].

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[2] Juan A, et al. Epstein–Barr virus-positive mucocutaneous ulcer in Crohn's disease. A condition to consider in immunosuppressed IBD patients. *Am J Surg Pathol* 2019;43:201-210.
[3] Moran N, et al. Epstein Barr virus-positive mucocutaneous ulcer of the colon associated Hodgkin lymphoma in Crohn's disease. *World J Gastroenterol*. 2015; 21: 8072-8076.
[4] Matnani R, Peker D. Azathioprine induced Epstein Barr virus-positive muco-cutaneous ulcer arising in perianal fistula and abscess associated with Crohn'sdisease. *Journal of Crohn's and Colitis* 2014;8:1747–1748.
[5] Dojcinov S, et al. EBV positive mucocutaneous ulcer – a study of 26 cases associated with various sources of immunosuppression. *Am J Surg Pathol*. 2010;34:405-417.
[6] Prieto-Torres L, Erana I, et al. The spectrum of EBV-Positive Mucocutaneous Ulcer: A Study of 9 Cases. Am J Surg Pathol. 2019; 43:201-210.

Interesting features

- Heterogeneous cytomorphology including plasmablastic and Hodgkin-like morphology.
- Patient under no systemic immunosuppression nor oral 5-ASA agents--entirely topical therapies.

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Type 3

HIV- and HHV8-related lymphomas

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Fang Yu (MD., PhD) and Wei Wang (MD., PhD)

Affiliation: Fang Yu (The First Affiliated Hospital of Zhejiang University) and Wei Wang (The University of Texas MD Anderson Cancer Center)

E-mail: 3311002@zju.edu.cn and wwang13@mdanderson.org

Clinical History: A 61-year-old man presented with several enlarged lymph nodes in the left axilla, the largest 9 cm. PET–CT scan demonstrated that lymphadenopathy was localized to the left axilla, and no other lymphadenopathy was identified. The patient had no clinical symptoms and denied any unintentional weight loss, night sweats or fever. He reported an enlarged lymph node in the same area 2 years ago, which resolved without intervention. He had no history of immunodeficiency and serology studies for HIV, HBV and HCV were all negative.

Biopsy Fixation Details: Formalin fixed paraffin embedded

Description of Clinical Image if Any: N/A

Details of Microscopic Findings: The lymphoma cells predominantly surrounded germinal centers, occupying and disrupting mantle zones with extension into interfollicular areas. In very focal areas, lymphoma cells involved germinal centers. Focal sinusoidal infiltration was also present. At high power, the lymphoma cells were large with a moderate amount of eosinophilic cytoplasm, round to slightly irregular nuclei and central prominent nucleoli consistent with plasmablasts and/or immunoblasts. In other areas that were not involved by lymphoma, there were some morphological features consistent with Castleman disease, including lymphocyte depleted germinal centers surrounded by concentric rings of small lymphocytes and sclerotic blood vessels penetrating radially into germinal centers. Vascular proliferation and plasma cell aggregates were also present in interfollicular areas.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry: By immunohistochemistry, neoplastic cells were positive for CD3 (weak), CD20, CD43, MUM1/IRF4, BCL2 (not shown) and HHV-8. EBER was also positive in the lymphoma cells, as shown by in-situ hybridization. Almost all lymphoma cells were positive for Ki-67 (not shown). CD21 highlighted disrupted follicular dendritic cell networks and a subset of lymphoma cells was also weakly positive. CD10 and BCL6 (not shown) were negative in lymphoma cells, but highlighted reactive germinal centers. The neoplastic cells were negative for CD138, IgM, kappa and lambda light chains. The background plasma cells were small in size and polytypic by kappa and lambda immunostains.

Special Stains: N/A

Cytogenetics: N/A

Molecular Analysis: Molecular studies for IGH, TRB and TRG showed no evidence of monoclonal IGH or T cell receptor gene rearrangements.

Interesting Feature(s) of Submitted Case: Lymphoproliferative disorders (LPD) characterized by concurrent HHV8 and EBV infection are rare and two entities are included in the WHO classification: primary effusion lymphoma (PEL) and germinotropic LPD. These two entities have very different clinicopathological presentations and prognosis. The case we described here showed concurrent HHV8 and EBV positivity but its clinicopathological features are not consistent with either PEL or germinotropic LPD. This indicates that HHV8+/ EBV+ LPDs are a spectrum and consist of cases beyond PEL and germinotropic LPD.

Proposed Diagnosis: HHV8+, EBV+ large B-cell lymphoma

Comments: The differential diagnosis for this case includes: 1), solid variant PEL, 2), germinotropic LPD and 3), HHV8-associated multicentric Castleman disease (MCD) (**Table 1**). The distinct clinicopathological features did not fit readily with any of these three categories.

PEL occurs in HIV-positive patients and predominantly involves body cavities with occasional cases presenting as solid tumors. Rare cases have been described in HIV-negative patients with similar clinicopathological findings. Morphologically, there is a plasmablast/immunoblast proliferation with a diffuse pattern in body cavity or extracavitary sites. Immunophenotypically, lymphoma cells are typically positive for CD138 and negative for pan-B cell markers and immunoglobulins, although variants of PEL with unusual immonophe-notypical features (CD138 and IgM+) have been described. The prognosis of PEL patients is dismal. In comparison, the patient described in this case had no clinical symptoms and HIV status was negative. He had no history of immunosuppression. Morphologically, the lymphoma cells had a plasmablastic/im-munoblastic appearance, but with a distinct growth pattern; lymphoma cells were located around germinal centers, disrupting mantle zones and invading into interfollicular areas. Background changes consistent with Castleman disease were present, including hyalinized blood vessels penetrating atrophic germinal centers, polytypic plasma cells and vascular proliferation in the stroma. Immunophenotypically, this lymphoma was positive for CD20 and negative for CD138, opposite to that described in primary effusion lymphoma.

Another differential diagnosis is germinotropic LPD, a rare entity described in HIV-negative and immunocompetent patients. Clinically, these patients present with localized lymphadenopathy with a good prognosis. The atypical cells have a germinotropic growth pattern and are confined predominantly to germinal centers. The cells are positive for cytoplasmic immunoglobulin heavy chain with monotypic light chain. B cell markers including CD20 are negative, as is CD138. Molecular studies show that the B cells are polyclonal. The patient in this case had a similar clinical presentation to patients with germinotropic LPD, but with different pathological features. In contrast to the germinotropic pattern with cells confined to germinal centers, the lymphoma cells in the current case grew predominantly around germinal centers and only a focal germinotropism was identified. The interfollicular growth and sinusoidal invasion identified

in this case are also not characteristic for germinotropic LPD. Moreover, the presence of morphological features of Castleman disease in the background has not been described in germinotropic LPD. Other features that are not consistent with germinotropic LPD include the positive B cell marker CD20 and negative light chain expression.

There are some morphological features of Castleman disease present in the background, which raises the differential diagnosis of large B cell lymphoma arising in HHV8-positive MCD. EBV, however, is often negative in those lymphomas, although rare cases positive for EBV infection have been described previously. In addition, MCD-associated large B cell lymphoma often occurs in immunosuppressed individuals, especially HIV-positive patients, and presented with systemic disease with aggressive clinical behavior. These tumors express exclusively IgM and lambda light chain. All these features are not present in the case we present here.

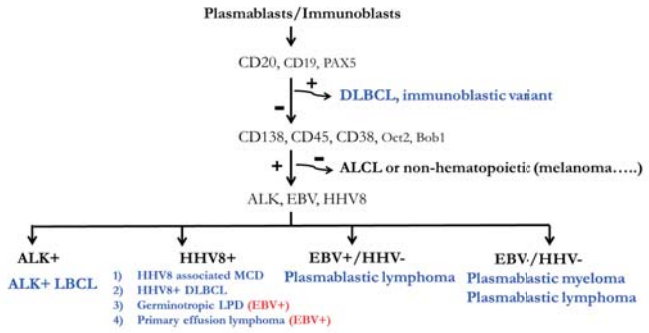
The characteristic cytomorphology of this case is plasmablasts/immunoblasts. In addition to the HHV8-positive entities described in Table 1, there are other neoplasms sharing similar plasmablastic/immunoblastic morphology. These neoplasms include: 1) DLBCL, immunoblastic variant; 2) plasmablastic lymphoma; 3) plasmablastic myeloma, and 4) ALK-positive large B-cell lymphoma. Immunophenotypic analysis is valuable in discriminating between these entities (**Fig 1**). Positive pan-B cell markers, such as CD19, CD20 and PAX5 will justify the diagnosis of DLBCL, immunoblastic variant. If pan-B cell markers are negative, the next step would be to evaluate plasma cell-related markers and other non-lineage associated hematopoietic markers. The absence of these antigens will raise a possibility of T cell lymphoma especially ALCL or non-hematopoietic disease such as melanoma. The positive (even partial) expression will trigger the workup for ALK-1, HHV8 and EBV, and based on their positivity, cases can be further subclassified into different entities as shown in Fig 1. In addition to their immunophenotypic differences, the clinical and laboratory features can also offer clues in differential diagnosis. ALK-positive large B-cell lymphoma occurs more often in younger patients with no history of immunosuppression and tends to involve lymph nodes, especially in the cervical areas. In case of negative EBV, the differential diagnosis between plasmablastic lymphoma and plasmablastic myeloma can be challenging, but the absence of immunosuppression, the presence of bone involvement and the presence of paraprotein favor myeloma over lymphoma.

Table 1: The differential diagnosis of HHV8 positive lymphoproliferative disorders

	HHV8+MCD	HHV8+DLBCL	GLD	PEL	Current Case
Immunosuppression	+	+	-	+	-
Presentation	Generalized	Generalized	Localized	Generalized	Localized
Prognosis	Poor	Poor	Good	Poor	N/A
Morphology	PB/IB in mantle zones	PB/IB, diffuse	PB/IB within germinal centers	PB/IB in fluid or extracavitary sites	PB/IB in mantle zones and interfollicular areas
Castleman features	+	+ or -	-	-	+
HIV status	+/-	+/-	-	+/-	-
HHV8	+	+	+	+	+
EBV	-	-	+	+	+
CD138	+/-	+	-/+	+	-
CD20	-/+	-	-	-	+
Ig	IgM, λ	monotypic	monotypic	-	-
Molecular (IgH)	Polyclonal	Clonal	Polyclonal	Clonal	Polyclonal

Note: **MCD**, multicentric Castleman disease; **DLBCL**, diffuse large B-cell lymphoma; **GLD**, germinotropic lymphoproliferative disorder; **PEL**, primary effusion lymphoma; **N/A**, not available; **PB/IB**: plasmablastic/immunoblastic.

Fig 1: The algorithm for differential diagnosis of lymphomas with plasmablastic/immunoblastic morphology



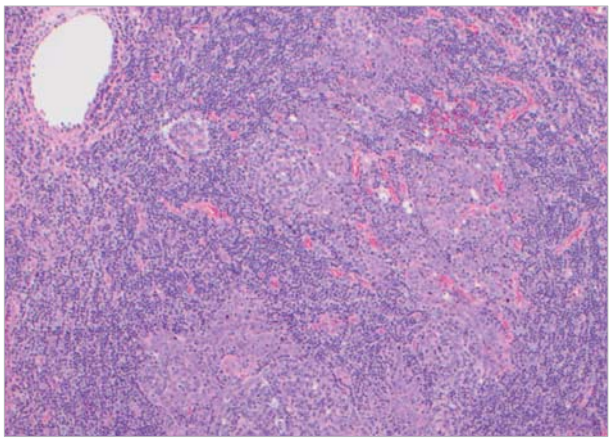
THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

HHV8+, EBV+ Large B-cell Lymphoma

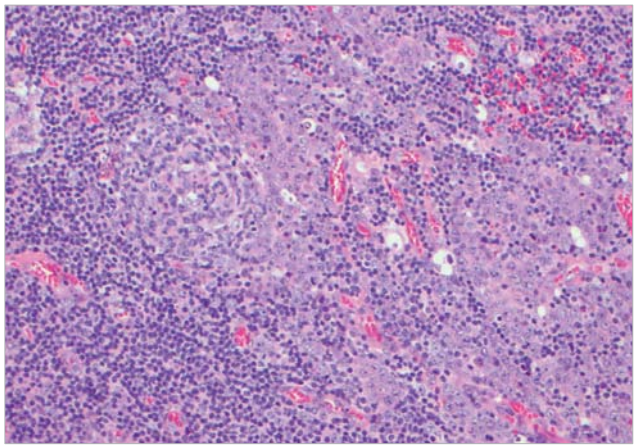
Fang Yu and Wei Wang
Dept. of Hematopathology
The University of Texas MD Anderson Cancer Center

Clinical History

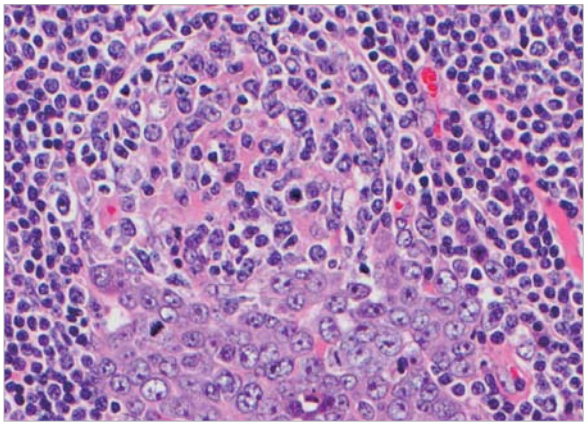
1) A 61-year-old man presented with several enlarged lymph nodes in the left axilla, the largest 9 cm.
2) PET–CT scan demonstrated that lymphadenopathy was localized to the left axilla, and no other lymphadenopathy was identified.
3) The patient had no clinical symptoms and denied any unintentional weight loss, night sweats or fever. He reported an enlarged lymph node in the same area 2 years ago, which resolved without intervention. He had no history of immunodeficiency and serology studies for HIV, HBV and HCV were all negative.
4) An excisional biopsy was performed



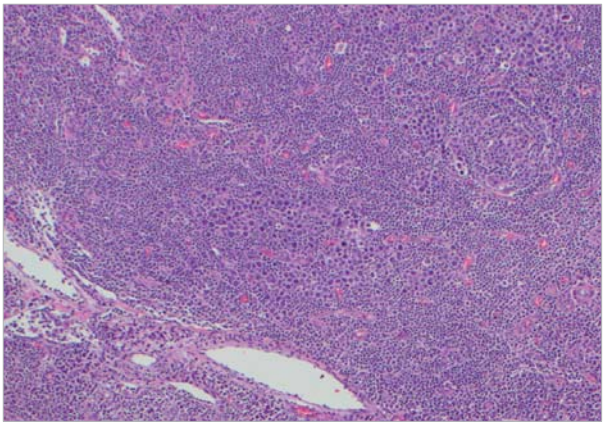
Lymphoma cells form clusters and sheets, surrounding germinal centers with extension into interfollicular areas.



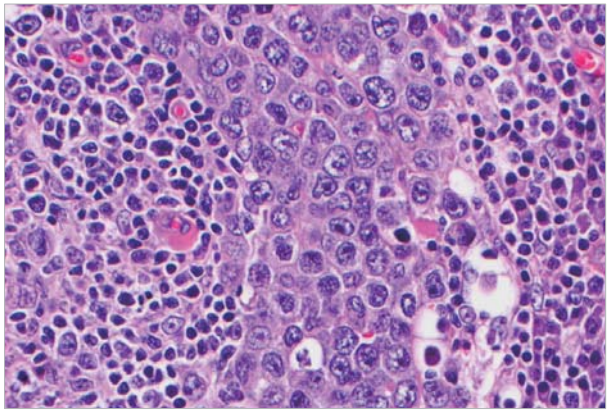
Lymphoma cells surround germinal centers, occupy and disrupt mantle zones.



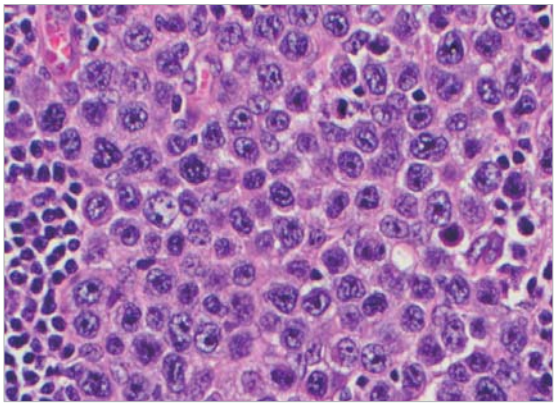
Lymphoma cells occupy and disrupt mantle zones.



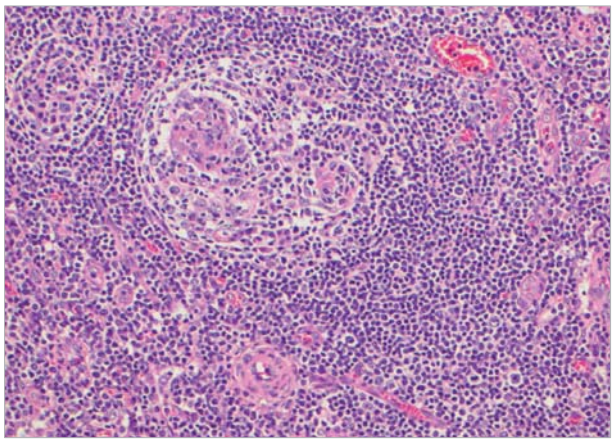
Focal sinusoidal infiltrate is suggested



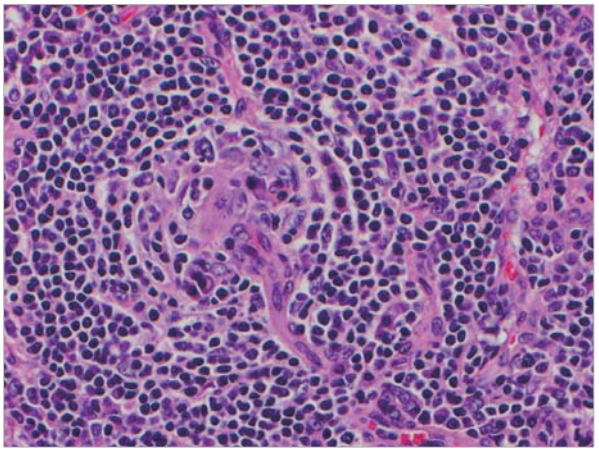
From left to right: residual germinal center, large lymphoma cells, and reactive small plasma cells



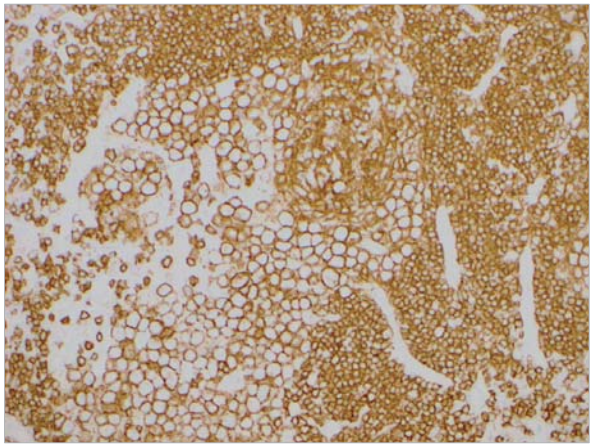
The plasmablastic/immunoblastic morphology of lymphoma cells



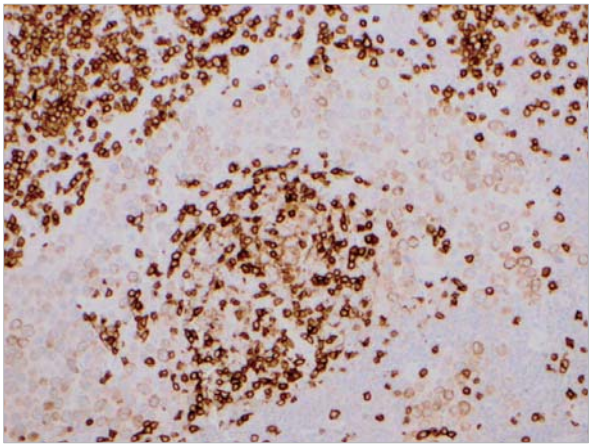
In other areas that are not involved by lymphoma, there are some morphological features consistent with Castleman disease



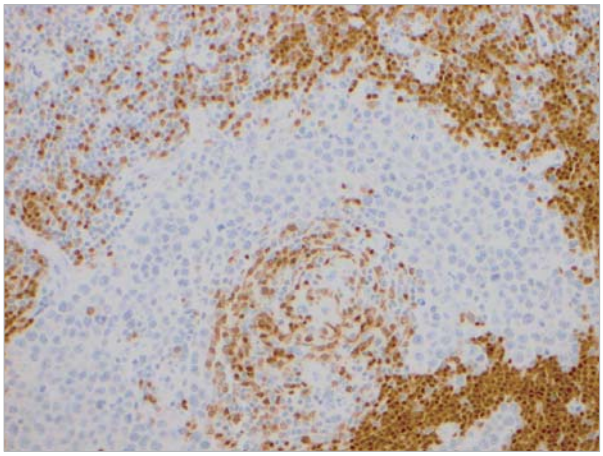
In other areas that are not involved by lymphoma, there are some morphological features consistent with Castleman disease



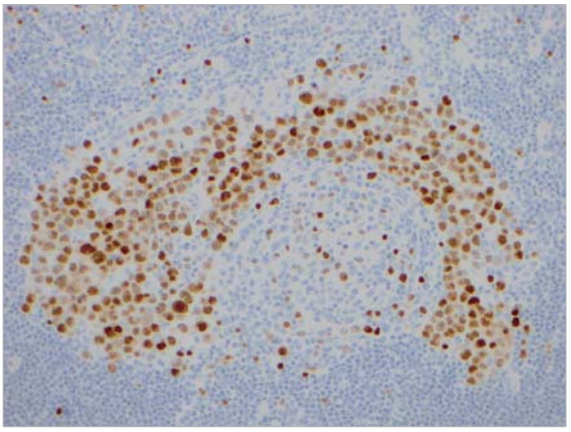
Lymphoma cells are CD20 positive



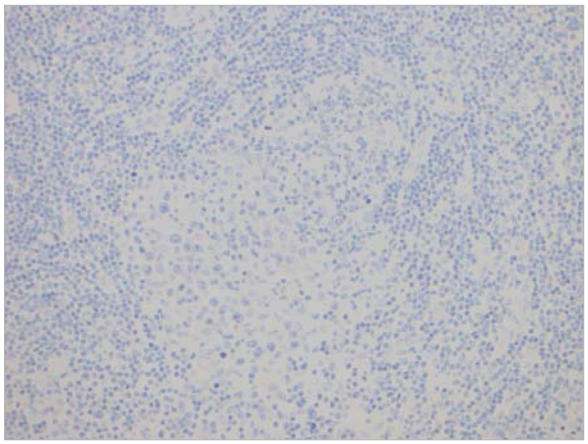
Lymphoma cells are CD3 weakly positive



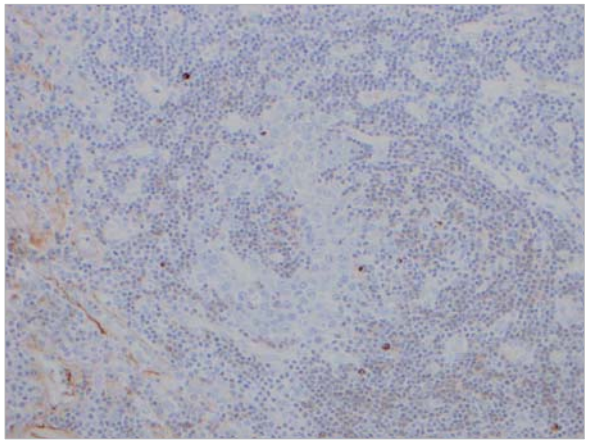
Lymphoma cells are PAX5 negative



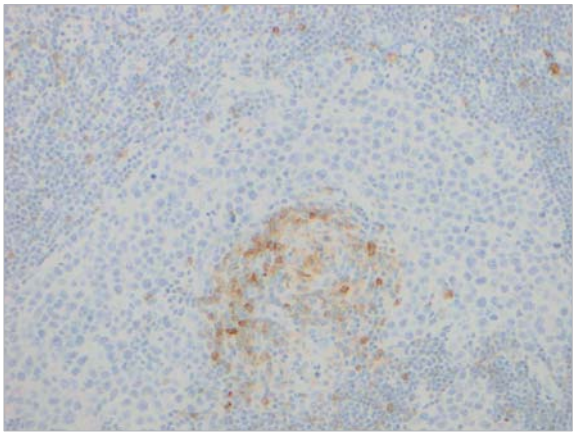
Lymphoma cells are MUM1 positive



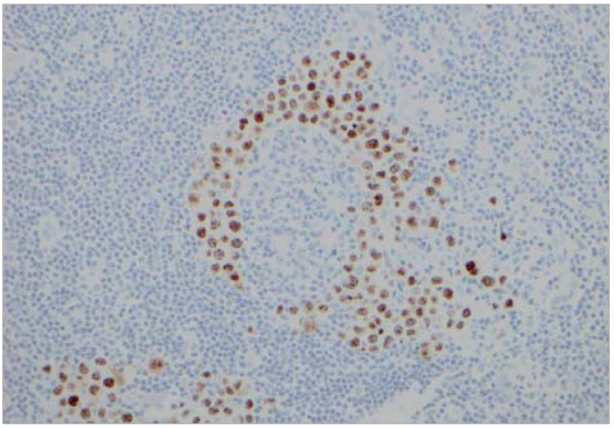
Lymphoma cells are CD138 negative



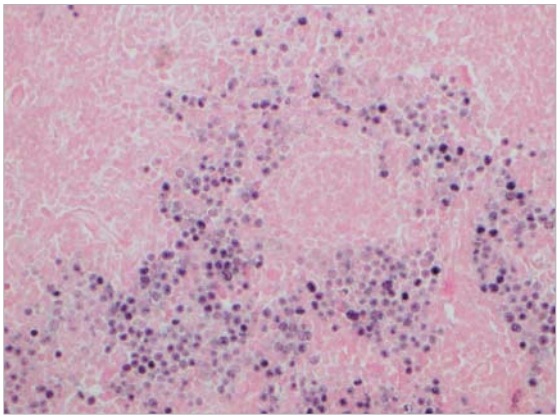
Lymphoma cells are IgM negative



CD10 immunostain highlights residual germinal center cells whereas lymphoma is negative



Lymphoma cells are HHV8 positive



Lymphoma cells are EBV positive

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Jun Wang, MD, Jeremy Deisch, MD

Affiliation:
Loma Linda University Medical Center

E-mail:
jwang@llu.edu

Clinical History:
A 35 year-old Hispanic male presenting with right side blurry vision was found to have ocular syphilis, who was given penicillin therapy. Subsequent work-up revealed HIV/AIDS with an HIV load of 136,000 copies/mL and CD4 T cell count of 97 cells/ml (normal range: 500-1500/ml). He was then put on HAART/cART for HIV/AIDS. He complained complete right vision loss 3 months later, which was found due to right-side retinal detachment/acute retinal necrosis by ophthalmology service. A right retinal biopsy procedure was performed for pathologic evaluation.

Staging work-up: Physical exam revealed no palpable lymphadenopathy or organomegaly. Total body PET scan showed no lymphadenopathy, masses, or organomegaly except increased right ocular uptake activity. Bilateral bone marrow biopsies were negative for involvement by lymphoma or other malignancies morphologically and immunophenotypically (immunohistochemistry and flow cytometry).

Biopsy Fixation Details:
The right retinal biopsy measured approximately 0.2 x 0.1 x 0.1 cm. It was fixed in 10% neutral buffered formalin, and then embedded in paraffin, sectioned at 3 to 4-mm thickness, and stained with standard hematoxylin-eosin or hematoxylin counterstain for immunohistochemistry.

Description of Clinical Image if Any:
Head MRI showed retinal thickening/detachment of the right eye with no other lesions noted.

Details of Microscopic Findings:
Histologic section of the right retinal biopsy showed large polymorphic mononuclear infiltrate with irregular nuclei with hyperchromatic chromatin, prominent nucleoli, and small to moderate amount of amphophilic cytoplasm. Mitotic figures and apoptotic bodies were also seen.

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Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
These pleomorphic mononuclear cells were positive for CD20, PAX5, BCL2, and MUM1, with a high Ki-67 nuclear proliferative index (~90%), but negative for CD3, CD5, C10, BCL6 by immunohistochemistry. Immunohistochemical stains were also negative for HSV I & II, CMV, and syphilis.

Flow cytometry of cells from CSF showed no monotypic B-cells.

Special Stains:
Steiner was negative for spirochetes, PAS and GMS stains were negative for fungi, and Gram stain was negative for bacteria.
CSF culture were negative for microorganisms.

Cytogenetics:
Many tumor cell nuclei were positive for EBV demonstrated by EBER in situ hybridization
No other cytogenetics were performed due to scarcity of the biopsy specimen.

Molecular Analysis:
No IGH/IGK gene rearrangement studies were performed due to scarcity of the biopsy specimen except a negative CMV in the CSF by PCR analysis.

Interesting Feature(s) of Submitted Case:
This case is a special type of HIV-associated EBV+ lymphoma with an extremely unusual clinical presentation as single side visual blurring/loss and had been initially considered due to ocular syphilis. Further work-up demonstrated HIV/AIDS in this patient. The patient eventually lost his total vision of the right eye despite receiving HAART/cART. A lymphoma involving only a single side retina is extremely unusual and diagnostically challenging in such a tiny biopsy as from retina and/or vitrectomy.

Proposed Diagnosis:
EBV+ Primary CNS **High-grade B-cell lymphoma**, most consistent with diffuse large B-cell lymphoma, non-germinal center subtype, involving the right retina
Compatible with **Lymphoma associated with HIV infection**

Comments:
Unusual presentation of sexually transmitted disease such as ocular syphilis in this case should prompt work up for HIV/AIDS, as well as HIV-associated lymphoma. Judicious use of appropriate physical exam and subspecialty (e.g., ophthalmology) exam, as well as radiological imaging studies and biopsy of any abnormalities for a timely pathological evaluation are critical to avoid missing the diagnosis of this aggressive lymphoma and its severe consequence.

EBV+ primary CNS DLBCL involving the right retina of a 35 year-old male with HIV/AIDS

Jun Wang, MD and Jeremy Deisch, MD
Department of Pathology and Laboratory Medicine
Loma Linda University Medical Center
Loma Linda, CA 92354

1

Case Presentation

- A 35 year-old male presenting with right side blurry vision in December 2016 was found to have ocular syphilis, s/p penicillin therapy
- Subsequent work-up revealed HIV/AIDS with an HIV load of 136,000 copies/mL and CD4 T cell count of 97 cells/μl (normal range: 500-1500/μl)
- He was then put on HAART/cART for HIV/AIDS
- He complained complete right vision loss in later February 2017, which was found due to right-side retinal detachment/acute retinal necrosis by ophthalmology service
- MRI shows retinal thickening/detachment of the right eye (arrow) with no other lesions noted
- A right vitrectomy/retinal biopsy procedure was performed

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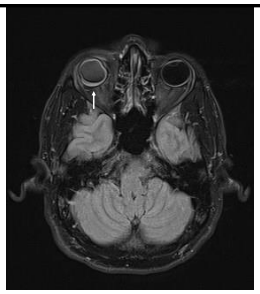

MRI shows retinal thickening/detachment of the right eye (arrow) with no other lesions noted

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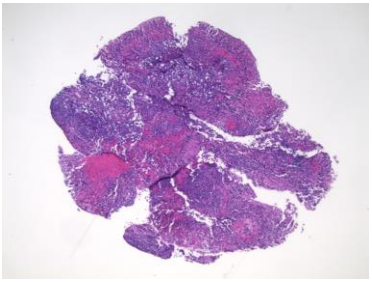

The right retinal biopsy shows a heavy diffuse mononuclear infiltrate (H&E, 20x)

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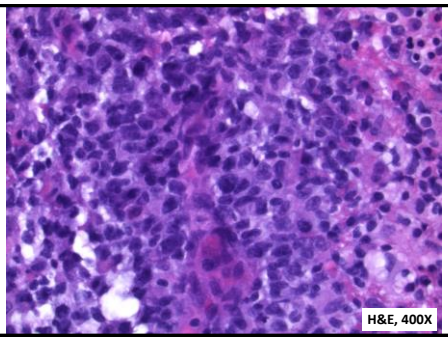

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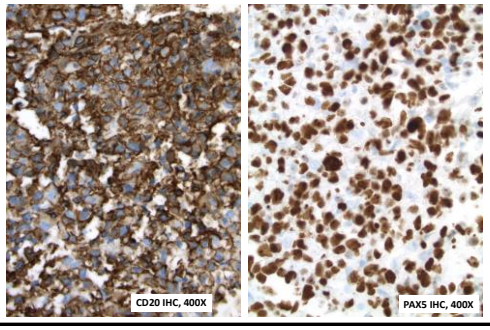

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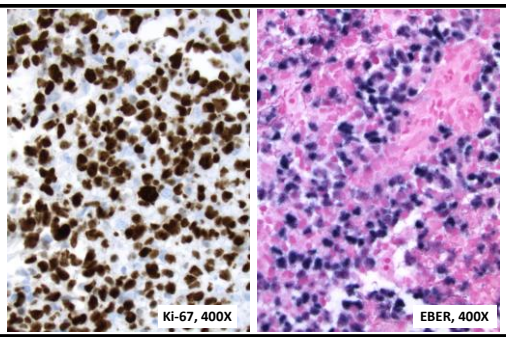

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Additional Immunohistochemical and Histochemial Studies

- CD3-/CD5- by IHC
- CD10-/Bcl-6- by IHC
- Bcl-2+/MUM1 by IHC
- HSV 1&2 and CMV negative by IHC
- Syphilis negative by IHC
- PAS/GMS/Steiner negative for fungi or spirochetes
- Gram stain negative for bacteria

8

Staging Work-up

- Physical exam: No palpable lymphadenopathy or organomegaly
- CSF culture negative; CMV negative by PCR
- CSF flow cytometry negative for lymphoma
- Total body PET scan: No lymphadenopathy, mass(s) or organomegaly except **increased right ocular uptake activity**
- Bilateral bone marrow biopsies: Negative for lymphoma by morphology, immunohistochemistry, and flow cytometry

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Proposed Diagnosis

- EBV+ Primary CNS **High-grade B-cell lymphoma**, most consistent with diffuse large B-cell lymphoma, non-germinal center subtype
- Compatible with **Lymphoma associated with HIV infection**

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Interesting Feature(s) of Submitted Case

- This case is a special type of HIV-associated EBV+ lymphoma with an extremely unusual clinical presentation as single side visual blurring/loss and had been initially considered due to ocular syphilis
- Further work-up demonstrated HIV/AIDS in this patient
- The patient eventually lost his total vision of the right eye despite receiving HAART/cART
- A lymphoma involving only a single-sided retina is extremely unusual and diagnostically challenging in such a tiny biopsy as from retina and/or vitrectomy

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Conclusion

- Unusual presentation of sexually transmitted disease such as ocular syphilis in this case should prompt work up for HIV/AIDS, as well as HIV-associated lymphoma
- Judicious use of appropriate physical exam and subspecialty (e.g., ophthalmology) exam, as well as radiological imaging studies and biopsy of any abnormalities for a timely pathological evaluation are critical to avoid missing the diagnosis of this aggressive lymphoma and its severe consequence

12

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):

Kirill Lyapichev, MD and Sanam Loghavi, MD

Affiliation:

Department of Hematopathology
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Houston, TX, USA

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SLoghavi@mdanderson.org

Clinical History:

A 73 year-old woman presented with shortness of breath and was found to have bilateral pleural effusions. She was being treated with rituximab for marginal zone B-cell lymphoma involving the bone marrow diagnosed 3 months prior to presentation. Subsequent thoracentesis was performed and the pleural fluid was submitted for cytological evaluation and flow cytometric immunophenotyping.

Biopsy Fixation Details:

N/A

Description of Clinical Image if Any:

N/A

Details of Microscopic Findings:

Cytological evaluation the pleural fluid revealed an atypical lymphoid population comprised of intermediate and large sized cells with eccentrically placed nuclei, multiple prominent nucleoli and scant to moderate amounts of basophilic cytoplasm. Initial evaluation of the cytology material was concerning for large-cell transformation of the patient's previously diagnosed marginal zone B cell lymphoma.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

The flow cytometric studies performed on the pleural fluid showed a large population of aberrant cells, positive for CD30 and CD38 expression with partial dim expression CD138 and CD7. These cells were negative for B-cell lineage markers including CD19, CD20, CD22, CD79a as well as T-cell lineage markers including CD2, CD3, CD4, CD5, and CD8. There was no expression of CD16, CD25, CD26, CD52, CD56, CD57, CD94, TCR-AB, TCR-GD, CD56 or CD117.

Due to the morphologic features and ambiguous immunophenotype additional immunohistochemical studies were performed on the cell block material. The neoplastic cells were positive for CD30 and a subset were positive for CD138. There was no expression of kappa or lambda light chains. The neoplastic cells were uniformly and diffusely positive for HHV8 and negative for EBV by in situ hybridization.

Special Stains:

N/A

Cytogenetics:

Fluorescence in situ hybridization (FISH) was negative for *BCL2* rearrangement

Molecular Analysis:
N/A

Interesting Feature(s) of Submitted Case:

The previous history of low grade B cell lymphoma was a confounding factor and raised the possibility of large cell transformation of the patient's previously diagnosed marginal zone lymphoma. Given the unusual immunophenotype a diagnosis of B cell lymphoma seemed less likely and other possibilities were considered. Detection of HHV8 antigen and the clinical presentation of bilateral pleural effusion were helpful in establishing the correct diagnosis of primary effusion lymphoma.

Proposed Diagnosis:

Primary effusion lymphoma (HHV8 positive)

Comments:

A diagnosis of primary effusion lymphoma should be considered in a patient presenting with effusions in which the neoplastic cells lack expression of lineage defining markers. Assessment for HHV8 should be performed as these findings will help establish a correct diagnosis. Availability of fresh material for detailed

immunophenotyping by flow cytometry was also very helpful in the work up of this complicated case.

Primary effusion lymphoma

Kirill Lyapichev, MD and Sanam Loghavi, MD
Department of Hematopathology
MD Anderson Cancer Center
Houston, TX

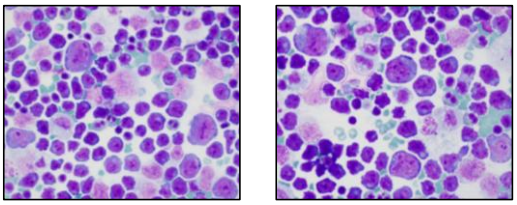
1

Clinical history

- A 73 year-old woman presented with shortness of breath and was found to have bilateral pleural effusions.
- She was being treated with rituximab for marginal zone B-cell lymphoma involving the bone marrow diagnosed 3 months prior to presentation.
- Thoracentesis revealed an atypical lymphoid population comprised of intermediate and large sized cells with eccentrically placed nuclei, multiple prominent nucleoli and scant to moderate amounts of basophilic cytoplasm.
  - Initial evaluation of the cytology material was concerning for large-cell transformation of the patient's previously diagnosed marginal zone B cell lymphoma
- A representative portion of the fine needle aspirate sample was sent for flow cytometric immunophenotyping.

2

Cytology (diff quik, 400X)



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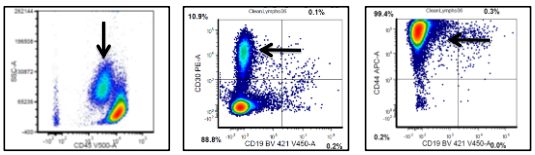
Flow cytometry

These CD19-negative B-cells showed expression of CD45 (dim), CD30 (partial), and CD44. (**black arrows**)



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- Given the unusual immunophenotype a diagnosis of B cell lymphoma seemed less likely at this point and other possibilities were considered.
- No other lineage defining markers were expressed.
- The presence of CD30 expression and the plasmablastic morphologic features together with the clinical presentation with effusions raised the possibility of primary effusion lymphoma
- IHC for anti-HHV8 was performed on the cell block sample

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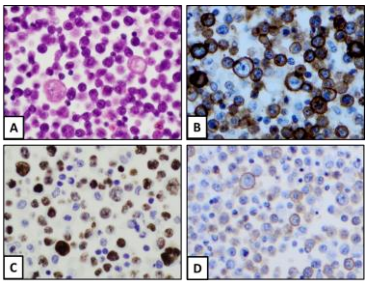
Cytology (cell block 400X)



A. Hematoxylin and eosin stain of the cell block reveals large, atypical lymphoid cells; Small and large atypical lymphoid cells are highlighted by CD30 (B), HHV8 (LANA-1) (C), and CD138 (focally) (D).

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Diagnosis

Primary effusion lymphoma (HHV8 positive)

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Primary effusion lymphoma (PEL)

- Primary effusion lymphoma (PEL) is a large B-cell neoplasm usually presenting as serous effusions without detectable tumor masses.
- It is universally associated with the human herpesvirus 8 (HHV8).
- It usually occurs in the setting of immunodeficiency.
- Some patients with PEL secondarily develop solid tumors in adjacent structures such as the pleura.

WHO 2017

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Flow cytometry and immunohistochemistry

- **POSITIVE**: CD45, HLA-DR, CD30, CD38, VS38c, CD138, EMA, HHV8 (LANA-8)
- **NEGATIVE**: pan- B-cell markers (CD19, CD20, and CD79a), surface and cytoplasmic Ig, and BCL6.
- PEL usually negative for T/NK-cell antigens, although aberrant expression of T-cell markers may occur.
- Usually positive for EBV by in situ hybridization for EBV-encoded small RNA (EBER), but negative for EBV by LMP1.
- EBV-negative PEL is common in elderly HIV-negative patients from HHV8-endemic regions (Mediterranean).

Dupin N, FisherC, Kellam P, et. al. Distribution of human herpesvirus-8 latently infected cells in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma. Proc Natl Acad Sci USA. 96:4546-51.

9

Differential Diagnosis

Most common cavities involved by PEL: pleural, pericardial, and peritoneal.

Must to remember that PEL can involve the unusual cavities, such as an artificial cavity related to the capsule of a breast implant.

The breast implant-associated anaplastic large cell lymphoma (BI-ALCL) is positive for CD30 and negative for common B cell marker (CD19, CD20). To distinguish these two neoplasms T cell markers and HHV-8 immunohistochemical study should be performed where BI-ALCL will have aberrant T cell immunophenotype and negative for HHV-8 by immunohistochemistry.

Said JW, Tasaka T, Takeuchi S, et al. Primary effusion lymphoma in women: report of two cases of Kaposi's sarcoma herpes virus-associated effusion-based lymphoma in human immunodeficiency virus-negative women. Blood. 88:3124-8.

10

Primary effusion lymphoma (PEL)

- The prognosis is very unfavorable
- Median survival is < 6 months.
- Rare cases have responded to chemotherapy and/or immune modulation

Ghosh SK, Wood C, Boise LH, et al. Potentiation of TRAIL-induced apoptosis in primary effusion lymphoma through azidothymidine-mediated inhibition of NF-kappa B. Blood. 101 :2321-7.

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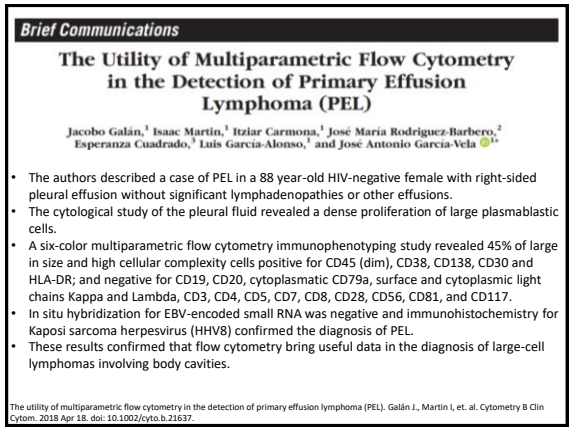

Brief Communications

The Utility of Multiparametric Flow Cytometry in the Detection of Primary Effusion Lymphoma (PEL)

Jacobo Galán,[1] Isaac Martín,[1] Itziar Carmona,[1] José María Rodríguez-Barbero,[2] Esperanza Cuadrado,[3] Luis García-Alonso,[1] and José Antonio García-Vela[1]*

- The authors described a case of PEL in a 88 year-old HIV-negative female with right-sided pleural effusion without significant lymphadenopathies or other effusions.
- The cytological study of the pleural fluid revealed a dense proliferation of large plasmablastic cells.
- A six-color multiparametric flow cytometry immunophenotyping study revealed 45% of large in size and high cellular complexity cells positive for CD45 (dim), CD38, CD138, CD30 and HLA-DR; and negative for CD19, CD20, cytoplasmatic CD79a, surface and cytoplasmic light chains Kappa and Lambda, CD3, CD4, CD5, CD7, CD8, CD28, CD56, CD81, and CD117.
- In situ hybridization for EBV-encoded small RNA was negative and immunohistochemistry for Kaposi sarcoma herpesvirus (HHV8) confirmed the diagnosis of PEL.
- These results confirmed that flow cytometry bring useful data in the diagnosis of large-cell lymphomas involving body cavities.

The utility of multiparametric flow cytometry in the detection of primary effusion lymphoma (PEL). Galán J., Martín I, et. al. Cytometry B Clin Cytom. 2018 Apr 18. doi: 10.1002/cyto.b.21637.

12

Summary

- PEL is associated with a proliferation of large B-cells which are positive for HHV8, CD45 (dim), CD30, CD38, and CD138 and negative for B cell markers (CD19, CD20, and CD79a).
- Although PEL is very rare lymphoma, it is important to rule it out in the patients with pleural, pericardial, peritoneal, and artificial (breast implant capsule) cavities' effusions by sending sample for cytological and flow cytometrical examination.
- Due to lack of mass lesion, cytology and flow cytometry is essential for PEL diagnosis.
- Be cautious when diagnosing PEL, other HHV8 positive lymphomas should be ruled out.

References

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- Ghosh SK, Wood C, Boise LH, et al. Potentiation of TRAIL-induced apoptosis in primary effusion lymphoma through azidothymidine-mediated inhibition of NF-kappa B. Blood. 101 :2321-7.
- Said JW, Tasaka T, Takeuchi S, et al. Primary effusion lymphoma in women: report of two cases of Kaposi's sarcoma herpes virus-associated effusion-based lymphoma in human immunodeficiency virus-negative women. Blood. 88:3124-8.
- Dupin N, FisherC, Kellam P, et. al. Distribution of human herpesvirus-8 latently infected cells in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma. Proc Natl Acad Sci USA. 96:4546-51.

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Josean Ramos, MD; Fernando A. Ocampo, MD, Nicholas Ward, MD

Affiliation:
Rush University Medical Center, Chicago, IL

E-mail:
Nicholas_d_ward@rush.edu

Clinical History:
This is a 29-year-old African American male, with past medical history of sickle cell trait who presented on Dec 2018 with symptoms of weakness, fatigue and fever for 3 weeks. Physical examination was remarkable for left axillary lymphadenopathy and labs showed anemia (Hgb 6.5 g/dL), leukocytosis (11.78 K/Mcl) and thrombocytopenia (Plt 49 K/Mcl). Given these findings a bone marrow biopsy was performed, which showed evidence of focal active hemophagocytosis, and axillary lymph node excision which revealed nodular lymphocyte predominant Hodgkin lymphoma with concurrent Kaposi sarcoma. Further lab studies for HIV were consistently negative. The patient underwent 2 cycles of R-CHOP and presented again with fevers and advancement of lymphadenopathy. The patient underwent repeat lymph node biopsy from the occipital region which revealed an EBV+ and HHV-8+ B-lineage lymphoma with plasmablastic morphology, most consistent with primary effusion lymphoma, extracavitary (solid variant). The patient was then subsequently treated with DA-EPOCH with consideration for possible autologous stem cell transplantation.
Given the unique pathologic findings the patient was referred to immunology which found no history of recurrent infections with a recommendation for an immunodeficiency profile, but given the ongoing chemotherapy testing is currently deferred.

Biopsy Fixation Details:
All specimens were fixed on 10% buffered formalin.

Description of Clinical Image if Any:
N/A

Details of Microscopic Findings:
SPECIMEN #1 (Lymph node, left axillary): The lymph node is effaced by a vaguely nodular infiltrate of scattered large, frankly malignant lymphoid cells in a background of predominately small mature appearing T-cells. The abnormal large lymphoid cells contain large and irregular nuclei with vesicular chromatin, one or more variably prominent nucleoli, and scant to moderate amounts of

cytoplasm, and are morphologically consistent with "popcorn" cells. The pattern of involvement is predominately T-cell rich nodular with some areas revealing a diffuse T-cell rich B-cell lymphoma-like pattern. In addition, a distinct moderately cellular spindle cell proliferation organized into interlacing fascicles with scattered slit-like vascular spaces is seen. The nuclei are enlarged and moderately pleomorphic, with vesicular chromatin.

SPECIMEN #2 (Lymph node, occipital): There is lymph node architecture effacement by a diffuse population of large cells neoplastic cells that have plasmablastic morphology, showing round to oval-shaped nuclei, speckled chromatin, and moderate amphophilic cytoplasm with occasional paranuclear hofs, also evident on the touch preps.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
SPECIMEN #1 (Lymph node, left axillary): Malignant large cells express CD20, PAX-5, CD79a, EMA and MUM-1 and negative for CD30, CD15 and CD10. CD21 highlights no follicular dendritic meshworks in these areas. CD57-positive small lymphocytes are markedly increased.CD3 highlights numerous T-cells. Immunohistochemical stains on this spindle cell proliferation reveal SMA(weak+), CD31 and HHV8 positivity.

Flow cytometry (performed at outside institution): Elevated CD4 to CD8 T-cell ratio. No phenotypic evidence of non-Hodgkin lymphoma.

SPECIMEN #2 (Lymph node, occipital): Neoplastic cells are positive for MUM-1, EBER, c-myc (about 50% positive), and they show lambda restriction by ISH. About 20% are moderately to strongly positive for CD30. Kappa ISH highlights background polytypic plasma cells. CD3 highlights many background T-cells. Ki-67 shows a proliferation index of about 90% in the neoplastic cells. CD20, CD10, and Bcl-6 are negative, Bcl-2 stain is weak to negative in the neoplastic population.

Flow cytometry study performed on the lymph node tissue showed about 11% of the total gated cells to be bright CD38 (+), dim CD45 intermediate side scatter cells that are CD138 (-), CD19 (-), CD56 (-), and CD117 (-) abnormal lymphoid cells that show surface and cytoplasmic lambda light chain restriction. Normal B cells are absent.

Special Stains:
N/A

Cytogenetics:
SPECIMEN #2 (Lymph node, occipital): No evidence of MYC rearrangement by FISH analysis.

Molecular Analysis:

N/A

Interesting Feature(s) of Submitted Case:
This case shows a synchronous diagnosis of three malignancies: Nodular lymphocyte-predominant Hodgkin lymphoma with Kaposi sarcoma in the same lymph node, and primary effusion lymphoma in a separate lymph node. Two of these malignancies are found most commonly on HIV-positive patients, and in this case the patient tested negative for HIV on multiple occasions. The rare occurrences of these tumors added to the unusual locations and with no apparent immunodeficiency, make this a very interesting case.

Proposed Diagnosis:
Nodular lymphocyte-predominant Hodgkin lymphoma with concurrent Kaposi sarcoma. Primary effusion lymphoma, extracavitary (solid variant).

Comments:
N/A

CSHP/SH Workshop 2019

Josean Ramos
Fernando A. Ocampo
Nicholas D. Ward

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Specimen #1

Diagnosis: Nodular lymphocyte-predominant Hodgkin lymphoma and Kaposi sarcoma

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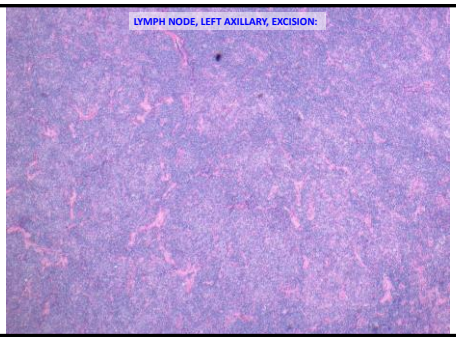

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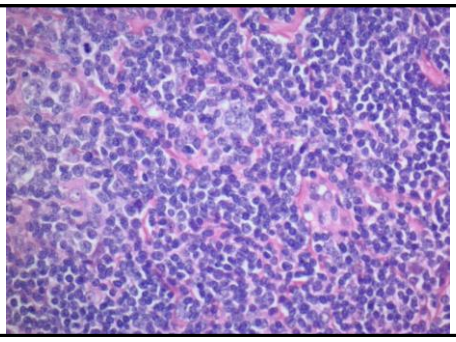

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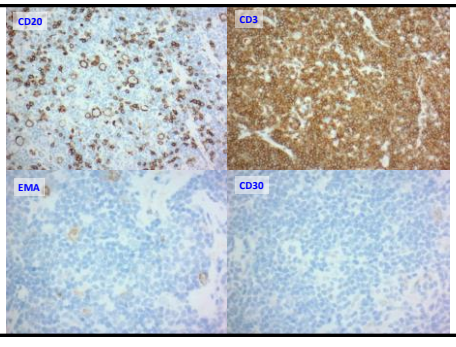

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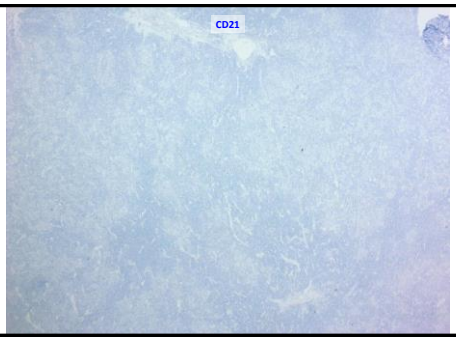

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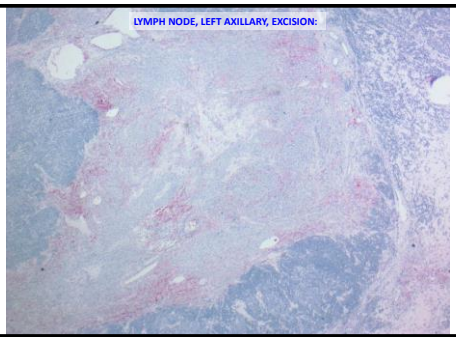

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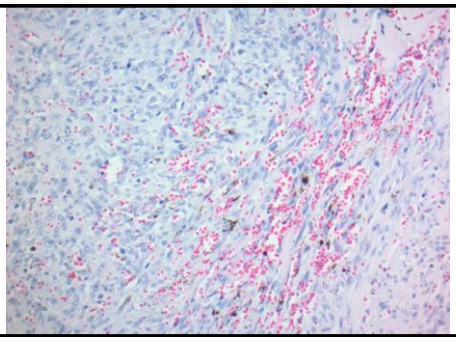

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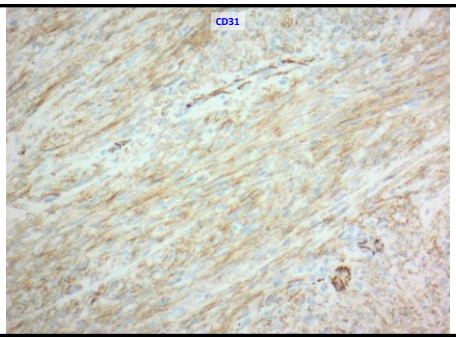

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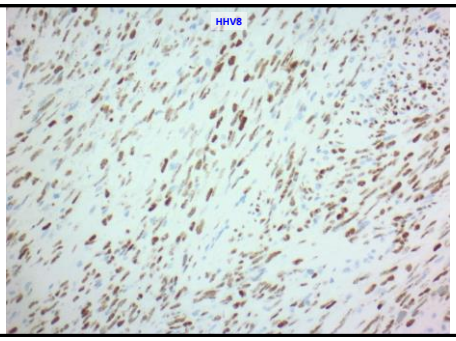

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Specimen #2

Diagnosis: EBV(+) and HHV-8(+) B cell lineage lymphoma with plasmablastic morphology, most consistent with primary effusion lymphoma

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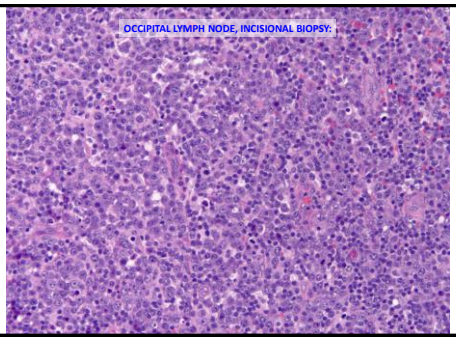

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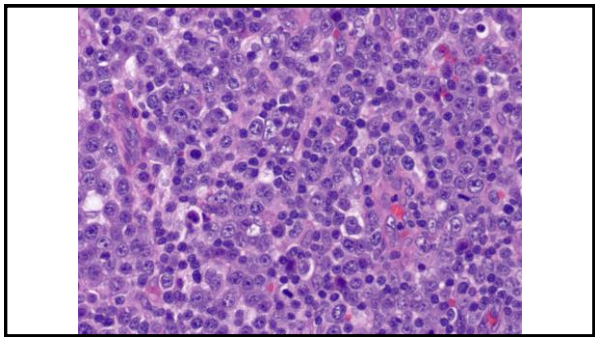

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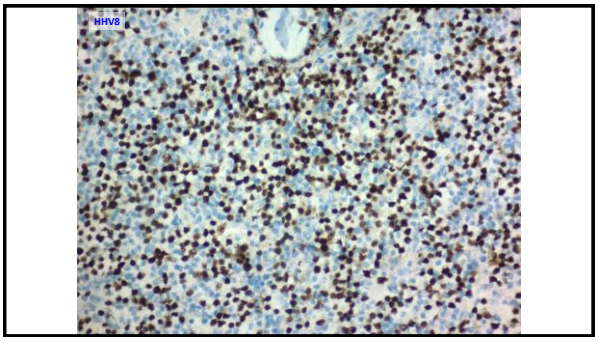

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CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Amandeep Kaur MD, MBBS[1]; Dr. Wei Liu MD, PhD[3], Megan McNerney, MD PhD[2], Girish Venkataraman MD, MBBS[2]

Affiliation:
1- University of Chicago at NorthShore, 2- University of Chicago 3- UnityPoint Health- Methodist Hospital

E-mail:
amanprince88@gmail.com

Clinical History:
A 62-year-old gentleman presented with facial swelling and pressure for a few months. He is reportedly HIV positive and has been on antiviral medication. Reportedly, CT scan showed cervical, axillary and mediastinal lymphadenopathy, largest lymph node measuring 2.4 x 2.7 cm. A neck lymph node was biopsied with a concern for a reactive process versus lymphoma.

Biopsy Fixation Details:
10% neutral buffered formalin

Description of Clinical Image if Any:
-

Details of Microscopic Findings:
Sections demonstrated distortion of the lymph node architecture by multiple foci of subcapsular and sinusoidal involvement by large pleomorphic anaplastic lymphoid cells with abundant basophilic cytoplasm. In addition, there was evidence of variable follicular regression and reactive follicular hyperplasia in the adjoining lymphoid tissue.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
Immunohistochemical stains including CD20, CD3, CD15, S100, CD2, CD30, CD56, TIA 1, BCL-6, CD138, CD21, ALK1, Pax-5, CD45, EMA, CD79a, CD10, CD5, CD7, CD43, CD4, CD8), Oct-2, IgG, IgM, kappa, lambda, CD30, Mum1 and HHV8/KSHV/LANA) were examined. The large neoplastic cells were positive for CD3, Mum1, IgM, EBER and HHV8 and negative for all B-cell markers including CD20, Pax-5, CD79a, Oct-2), plasmacytic markers (CD138, kappa, lambda) and all other markers listed above. Although there was no definite lineage-defining marker, the positivity for both HHV8 and EBV as well as expression of Mum1 and IgM in the setting of HIV supported the above rendered diagnosis. Although the predominant sinusoidal involvement was also unusual

but has been described in rare instances. The lack of both light chain expression in the neoplastic cells was notable and is likely related to down regulation of light chain production by the EBV coinfection.

Special Stains:
-

Cytogenetics:
-

Molecular Analysis:
For B- and T-cell clonality in process.

Interesting Feature(s) of Submitted Case:
- Nodal involvement of Primary effusion lymphoma (PEL) is rare with approximately 50 reported cases in literature.
- They usually have poor prognosis but this patient had an indolent course with excellent response to therapy.
- Sinusoidal involvement as seen in this case is rare in this entity.
- Strong aberrant CD3 expression as seen in this case is also rare in this entity.

Proposed Diagnosis:
Primary effusion lymphoma, EBV+/HHV8+, solid extracavitary variant, with predominant intravascular/sinusoidal involvement.

Comments:
Although there is no definite lineage-defining marker, the positivity for both HHV8 and EBV as well as expression of Mum1 and IgM in the setting of HIV support the diagnosis. Although the predominant sinusoidal involvement is also unusual but has been described in rare instances. The lack of both light chain expression in the neoplastic cells is notable and is likely related to down regulation of light chain production by the EBV coinfection. These tumors frequently express aberrant CD3 (nearly 29% of cases) based on a large recently published series of similar cases.

Case presentation

Dr. Amandeep Kaur MD, MBBS
Dr. Wei Liu MD, PhD
Dr. Megan McNerney MD, PhD
Dr. Girish Venkataraman MD, MBBS

1

Case history

- A 62-year-old gentleman presented with facial swelling and pressure for past few months
- He is reportedly HIV positive for past 20 years and has been on antiviral medications.
- CD4 and HIV viral load: unknown at the time of presentation
- Patient does not have any history of recent opportunistic infections

2

Case history

- CT scan showed cervical, axillary, mediastinum and pelvic lymphadenopathy, largest node measuring 2.4 x 2.7 cm
- A neck lymph node was biopsied with a concern for a reactive process versus lymphoma

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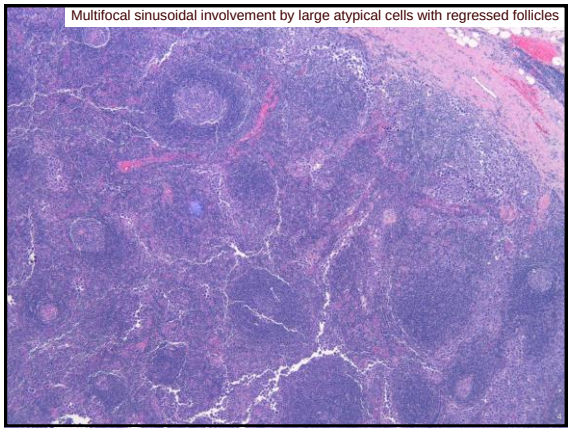

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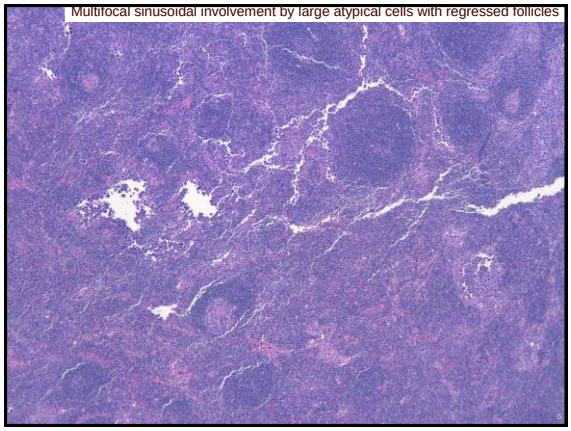

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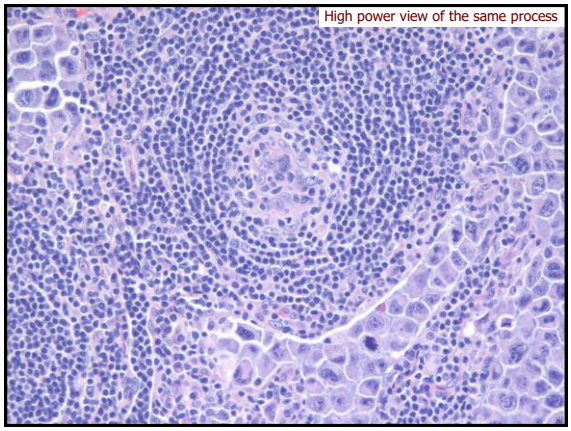

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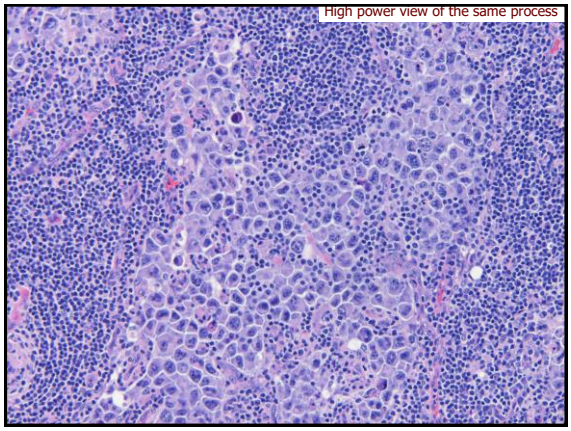

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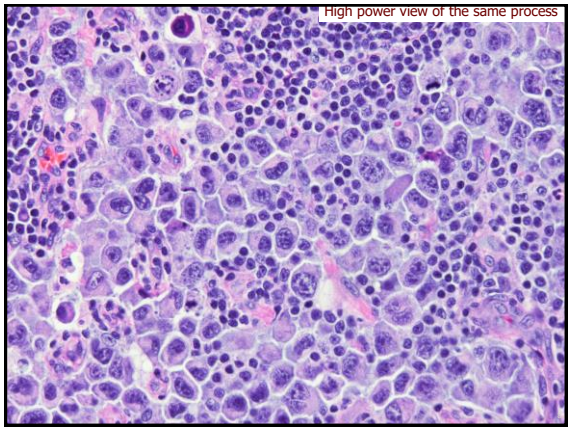

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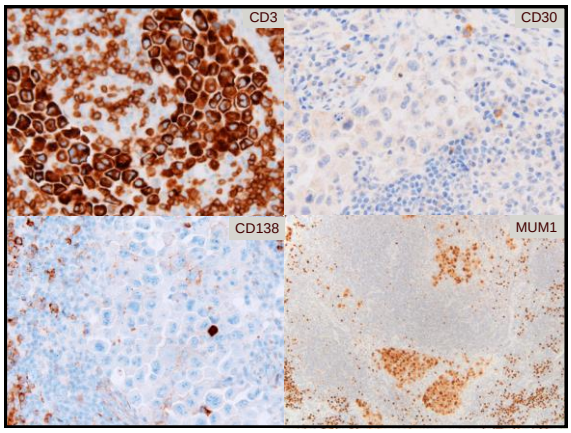

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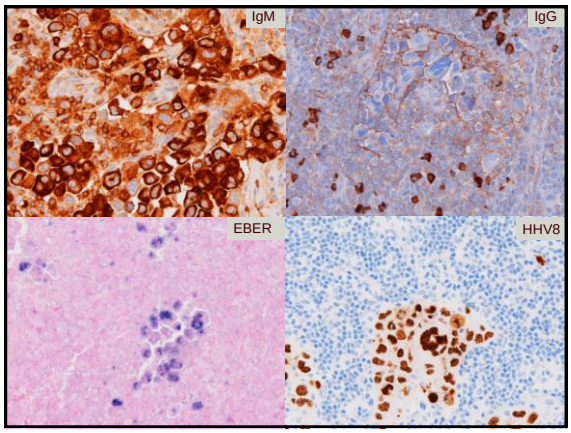

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Negative stains

- CD43
- CD45
- CD20
- PAX5
- CD2
- CD5
- CD7
- Kappa
- CD4
- CD8
- CD15
- ALK
- CD30
- CD79a
- CD138
- IgG
- Lambda

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Final Diagnosis

Submitters' diagnosis:

Primary effusion lymphoma, EBV+/HHV8+, solid extracavitary variant, with predominant intravascular/sinusoidal involvement

WHO (2016): Primary effusion lymphoma

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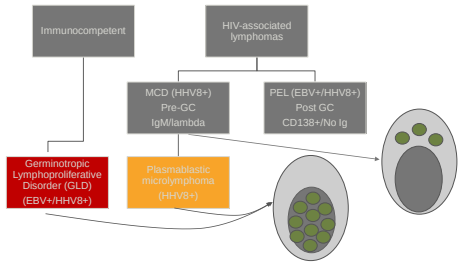
HHV8-addicted lymphoproliferations



KSHV- and EBV-associated germinotropic lymphoproliferative disorder. Ming-Qing Du, and Peter G. Isaacson coworkers Blood 2002 100:3415-3418;

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Characteristics of classic and extracavitary PEL

Characteristics	Classic group (n=34)	Extracavitary group (n=17)
Sex male (%)	92	91
CD4 cell count (median)	204	185
Undetectable plasma HIV-RNA (%)	49	47
cART at diagnosis (%)	68.6	70.6
Kaposi sarcoma (%)	49	56
Castleman disease (%)	35.3	32.3
Treatment with standard chemo (%)	88.2	82.3
Treatment with low dose/no chemo (%)	11.7	17.6

Guillet S, Gérard L, Meignin V, Agbalika F, Cuccini W, Denis B, Katlama C, Galicier L, Oksenhendler E. Classic and extracavitary primary effusion lymphoma in 51 HIV-infected patients from a single institution. Am J Hematol. 2016 Feb;91(2):233-7. doi: 10.1002/ajh.24251. PubMed PMID: 26799611.

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Difference between PEL and extra-cavitary PEL

PEL:

- Serous effusions only
- Lack pan-B cell markers
- Surface and cytoplasmic immunoglobulin is absent
- Lack T/NK cell antigens

Extra-cavitary PEL:

- Solid tumor masses
- Can express B-cell markers
- Can express immunoglobulins more frequently
- Aberrant expression of T-cell antigens can be seen

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Extra-cavitary/solid variant of PEL in a Lymph node

	Age (yrs)	Sex	Site	Morphology	HHV-8	EBER	CD3	CD138	MUM1	Follow-up (mo)
Case 1	42	M	LN	sinusoidal	+	+	-	+ (subset)	+	13 No dis
Case 2	42	M	LN	sinusoidal	+	-	-	+	ND	25 No dis
Our case	62	M	LN	sinusoidal	+	+	+	-	+	37 No dis

Kim Y, Leventaki V, Bhaijee F, Jackson CC, Medeiros LJ, Vega F. Extracavitary/solid variant of primary effusion lymphoma. Ann Diagn Pathol. 2012 Dec;16(6):441-6. doi: 10.1016/j.anndiagpath.2012.03.004. Epub 2012 Apr 11. PubMed PMID: 22497739.

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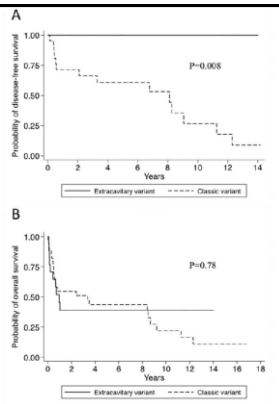
Prognosis



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Unique features of our case

- Nodal involvement is rare in PELs.
- Usually have poor prognosis (not in this case).
- Sinusoidal involvement is rare.
- Strong CD3 expression is rare and may be misleading.

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Follow up

- Patient received 6 cycles of EPOCH regimen.
- He had rapid clinical response within 3 months and was in complete remission after 6 months (at the end of therapy).
- He is on continuous clinical observation for the past 2 years and remains disease free.

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References

1. HHV-8-positive and EBV-positive intravascular lymphoma: an unusual presentation of extracavitary primary effusion lymphoma. Am J Surg Pathol. 2014 Mar;38(3):426-32.
2. Pan ZG, Zhang QY, Lu ZB, Quinto T, Rozenvald IB, Liu LT, Wilson D, Reddy V, Huang Q, Wang HY, Ren YS. Extracavitary KSHV-associated large B-Cell lymphoma: a distinct entity or a subtype of primary effusion lymphoma? Study of 9 cases and review of an additional 43 cases. Am J Surg Pathol. 2012 Aug;36(8):1129-40.
3. KSHV- and EBV-associated germinotropic lymphoproliferative disorder. Ming-Qing Du, and Peter G. Isaacson coworkers Blood 2002 100:3415-3418.
4. Guillet S, Gérard L, Meignin V, Agbalika F, Cuccini W, Denis B, Katlama C, Galicier L, Oksenhendler E. Classic and extracavitary primary effusion lymphoma in 51 HIV-infected patients from a single institution. Am J Hematol. 2016 Feb;91(2):233-7. doi: 10.1002/ajh.24251. PubMed PMID: 26799611.
5. Kim Y, Leventaki V, Bhaijee F, Jackson CC, Medeiros LJ, Vega F. Extracavitary/solid variant of primary effusion lymphoma. Ann Diagn Pathol. 2012 Dec;16(6):441-6. doi: 10.1016/j.anndiagpath.2012.03.004. Epub 2012 Apr 11. PubMed PMID: 22497739.

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CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Shanxiang Zhang, MD/PhD

Affiliation:
Indiana University School of Medicine, Indianapolis, Indiana, USA

E-mail:
sz5@iupui.edu

Clinical History:
45 year-old man with history of HIV and AIDS presented with weakness and paresthesia. Laboratory tests revealed pancytopenia with WBC 3.4 k/cumm and hemoglobin 8.4 g/dL with CD4-positive T cells around 150/cumm. Brain imaging studies (MRI, CT/PET) revealed multiple lesions with lymphoma and infection such as toxoplasmosis as the major differentials. Whole body imaging study revealed enlarged left axillary lymph node, up to 2.7 cm at the greatest dimension. There was no other lymphadenopathy, no hepatosplenomegaly and no pleural or abdominal effusion. There were increased lymphoid cells in the cerebral spinal fluid (CSF) smear, which were predominantly T cells with very rare B cells by flow cytometric analysis. The patient was treated for HIV with antiretroviral therapy (HARRT) and for presumptive toxoplasmosis with no improvement.

Biopsy Fixation Details:
An ultrasound-guided needle core biopsy of the left axillary lymph node, which was fixed in neutral buffered formalin overnight.

Description of Clinical Image if Any:
Not applicable.

Details of Microscopic Findings:
Histological sections showed needle core biopsy of lymph node with diffuse proliferation of large atypical lymphoid cells with distinct nucleoli admixed with cells resembling plasma cells and plasmablasts. Multiple small foci of predominantly small lymphocytes were present. There were no morphological features of multicentric Castleman disease, granuloma or geographic zone of necrosis.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
Immunohistochemical stains show the large atypical cells were positive for CD138 (partial), IgG, immunoglobulin kappa light chain, human herpes virus 8 (HHV8), and negative for CD3, CD20, CD56, CD30, Epstein-Barr virus (EBV) LMP1, IgM, immunoglobulin lambda light chain, pan-cytokeratin. However, EBV was positive in

these large atypical cells by in situ hybridization for EBV-encoded RNAs. Ki-67 stain revealed a high proliferative index (approximately 80%) for these atypical cells.

Flow cytometric analysis of the biopsy core sample was not informative.

Special Stains:
None

Cytogenetics:
None

Molecular Analysis:
None

Interesting Feature(s) of Submitted Case:
This EBV- and HHV8- positive large B-cell lymphoma share some morphological and immunophenotypical features of EBV+ plasmablstic lymphoma (PBL) and HHV8+ diffuse large B-cell lymphoma (DLBCL), which are also typically seen in HIV patients. However, PBL is usually negative for HHV8. While HHV8+ DLBCL, NOS is also commonly positive for EBV, the neoplastic cells are thought to arise from IgM, lambda-expressing naïve B cells instead of B cells expressing class switched immunoglobulin, such as IgG, kappa in the current case.

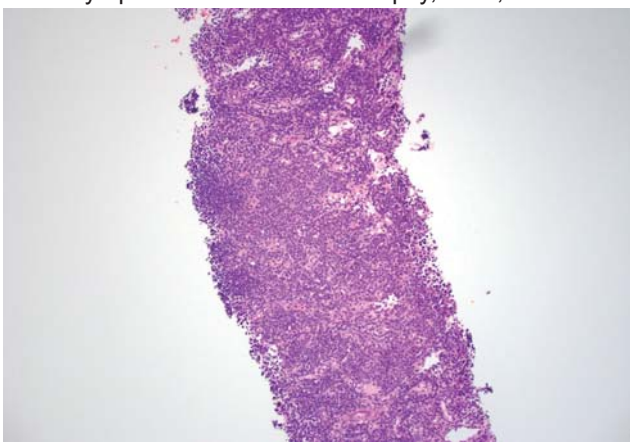
Proposed Diagnosis:
HHV8 and EBV-positive large B-cell lymphoma, most consistent with extracavitary primary effusion lymphoma.

Comments:
This is a very challenging case to diagnose, especially based on a very limited needle core biopsy sample, such as the current one. Being aware of the patient's history of HIV and immunodeficiency as well as types of HIV-associated large cell lymphoma is critical for us to perform a comprehensive but also highly discreet study. It is not sufficient to just define the lineage of large lymphoma cells, but also critical to study the status of HHV8 and EBV, as wells as types of immunoglobulin including the light chain restriction pattern.

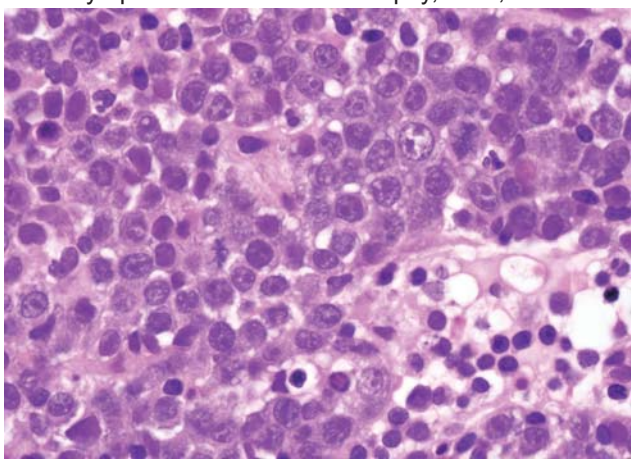
Extracavitary primary effusion lymphoma

Shanxiang Zhang, MD, PhD
Department of Pathology & Laboratory Medicine
Indiana University Health
Indiana, USA

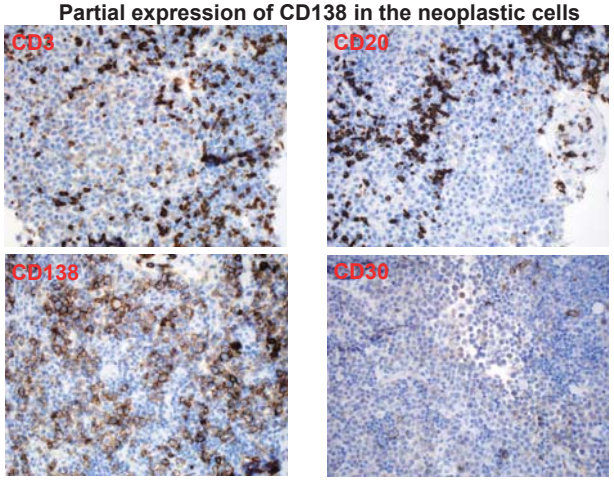
Lymph node needle core biopsy, H&E, 10X



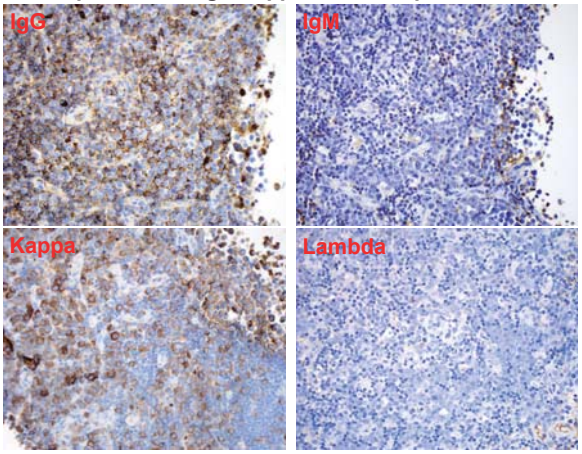
Lymph node needle core biopsy, H&E, 100X



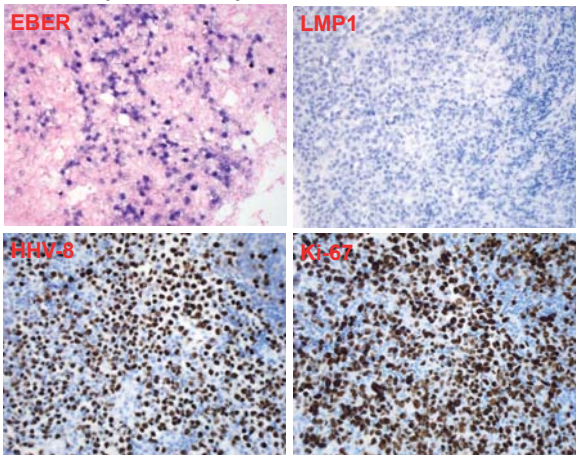
Partial expression of CD138 in the neoplastic cells



Expression of IgG kappa in the neoplastic cells



Neoplastic cells positive for EBV and HHV-8



Summary for the neoplastic cells

Positive: CD138, BCL-2, IgG, kappa, HHV-8, EBV (EBER), Ki-67 (~ 80%)

Negative: CD3, CD20, CD30, CD56, IgM, lambda, LMP1, pan-cytokeratin

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):

Ling Guo, MD, PhD, Daniel Jones, MD, PhD, Arwa Shana'Ah, MD, Robert Baiocchi, MD and Aharon G. Freud, MD, PhD.

Affiliation: Ohio State University Wexner Medical Center, Ohio 43210, USA

E-mail: ling.guo@osumc.edu

Title: Extracavitary primary effusion lymphoma with aberrant CD3 and CD4 expression.

1

CASE SUBMISSION FORM

Clinical History:
60 years old man with a history of HIV was admitted for gastrointestinal bleeding. Complete blood count showed anemia with hemoglobin of 6.7 and thrombocytopenia (37K/ul). Endoscopy revealed a 7cm ulcerated mass in the distal jejunum which was biopsied.

Biopsy Fixation Details: Fixed for 12 hours.

Description of Clinical Image if Any: On PET scan, there were multiple FDG-avid foci overlying small bowel loops in the midline of the abdomen and within the left pelvis, with a maximal SUV of 15.2. A dominant cluster of adjacent hypermetabolic possible lymph nodes showed a maximal SUV of 24.4.

2

CASE SUBMISSION FORM

Details of Microscopic Findings:
Sections of the biopsy show small bowel mucosa with a dense underlying neoplastic infiltrate within the lamina propria and submucosa. The neoplastic cells are composed of pleomorphic large lymphoid cells with vesicular nuclear chromatin, prominent nucleoli, and abundant amounts of eosinophilic cytoplasm.

3

CASE SUBMISSION FORM

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

- Neoplastic cells are positive for CD3, CD4, CD30 (diffuse and strong), CD45 (weak), kappa light chain (by in situ hybridization), MUM1, EBER, HHV8, P63, and Ki67 (>90%).
- Neoplastic cells are negative for AE1/3, CK7, CK20, CD20, CD79a, CD2, CD8, granzyme B, ALK, CD56, CD138, plasma C (aka VS38c), lambda light chain (by in situ hybridization), synaptophysin, chromogranin, P40, CK5/6, CDX2, TTF1, NKX3.1, PAX8, MITF1, MELANA, S100, DOG1, CD117, GATA3, and ERG.
- Flow cytometric analysis was not performed.

4

CASE SUBMISSION FORM

Special Stains: Not performed.

Cytogenetics: Not performed.

Molecular Analysis:

- IGH PCR shows a clonal rearrangement admixed with a reactive B cell pattern.
- TCRB PCR shows an oligoclonal pattern matching that seen in bone marrow aspirate.

5

CASE SUBMISSION FORM

Interesting Feature(s) of Submitted Case:
Extracavitary primary effusion lymphoma with aberrant T cell markers including CD3 and CD4 expression.

Proposed Diagnosis:
Extracavitary primary effusion lymphoma with aberrant CD3 and CD4 expression.

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Extracavitary primary effusion lymphoma with aberrant CD3 and CD4 expression

Ling Guo, Daniel Jones, Arwa Shana'Ah, Robert Baiocchi and Aharon G. Freud

Ohio State Wexner Medical Center, Columbus, Ohio 43210, USA

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Case history

- **60 yo male**
- **History of HIV and gastrointestinal bleeding**
- **A newly detected jejunal mass biopsied.**

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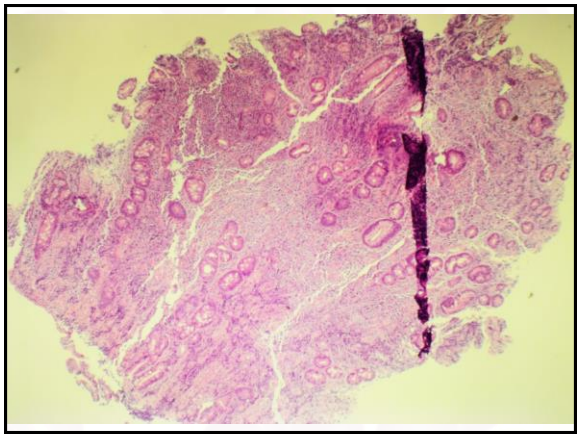

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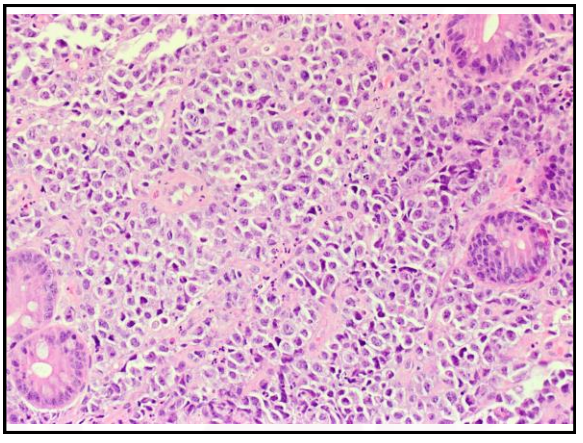

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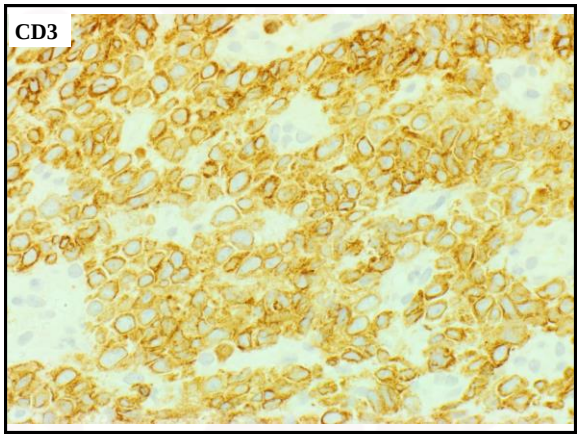

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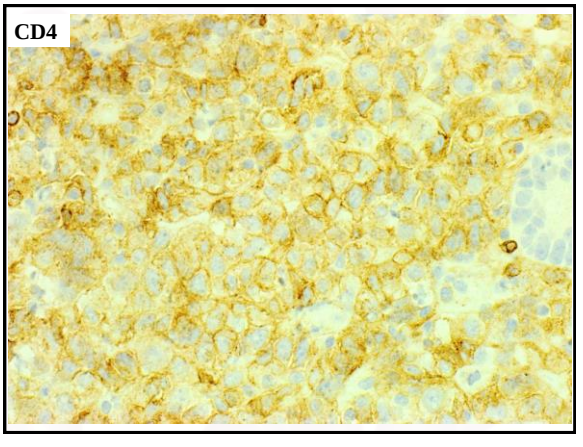

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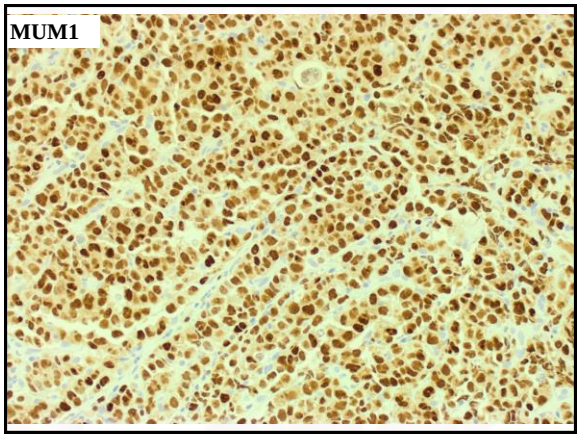

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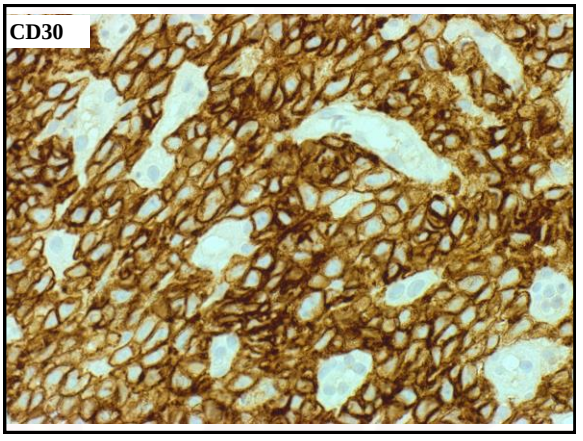

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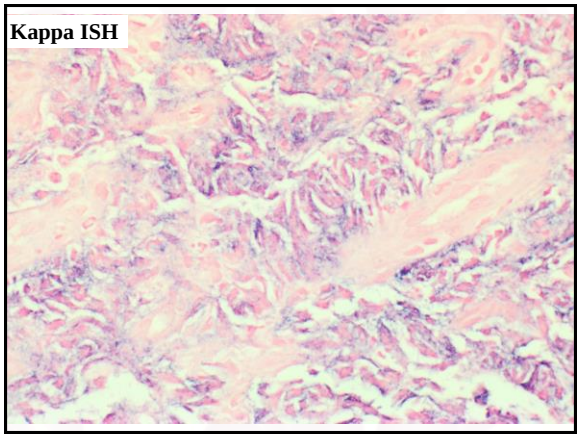

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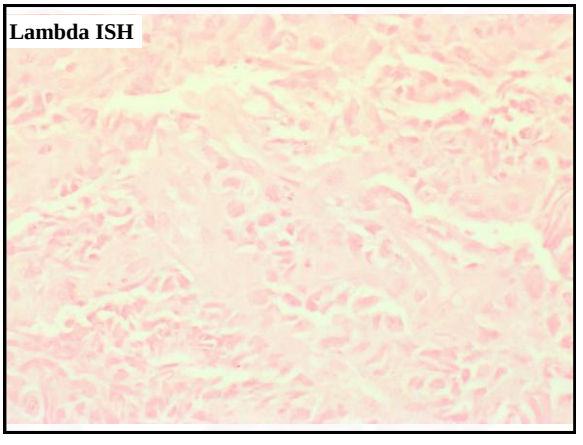

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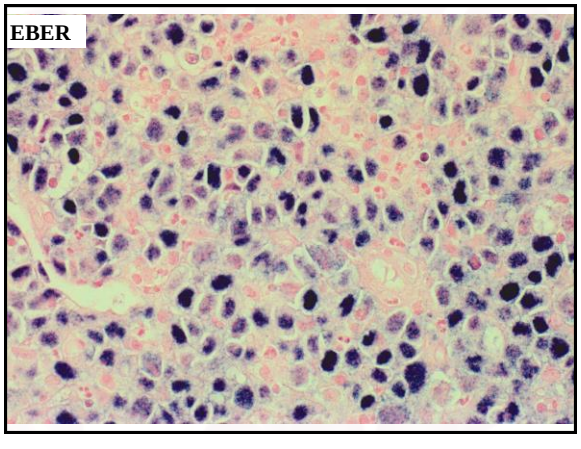

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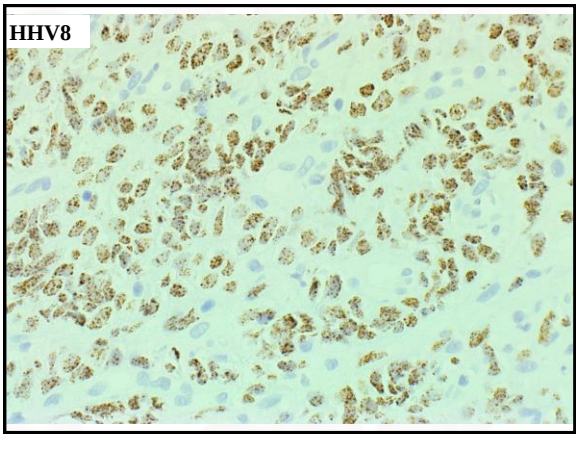

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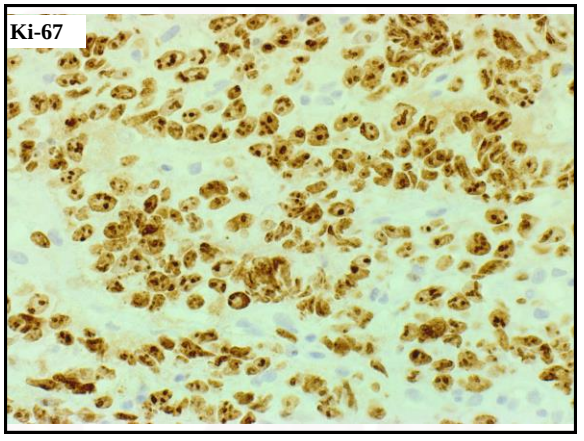

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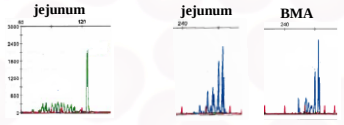
Summary of immunostains

- Positive for CD3, CD4, CD30, CD45 (weak), EBER, HHV8, MUM1, kappa ISH (weak) and Ki67 (>90%).
- Negative for AE1/3, CK7, CK20, S100, CD79a, CD20, ALK, CD2, CD8, CD56, CD138, granzyme B, Plasma C (aka VS38c), or lambda ISH.

20

Molecular tests

- PCR analysis for IgH rearrangements shows a clonal B-cell population.
- PCR analysis for TCR rearrangements shows an oligoclonal pattern that matches that seen in the negative bone marrow aspirate (BMA), likely related to HIV infection.



IGH (FR3) TCRB tube B

21

Additional information

- FISH analyses are negative for BCL2, BCL6 or MYC rearrangements.
- Bone marrow biopsy is negative for lymphoma involvement.

22

Proposed diagnosis

Extracavitary primary effusion lymphoma with aberrant CD3 and CD4 expression

23

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Qiongrong Chen, MD PhD

Affiliation:
Department of Pathology, Wuhan University Zhongnan Hospital

E-mail:
Qiongrongchen@hotmail.com

Clinical History:

The patient is a 53 years-old man. He complained of having discontinuous fever and oppression in the chest for 2 months and was hospitalized at our hospital on Feb 25, 2019. He was diagnosed HIV infection in 2003, HCV infection in 2009, tubercle bacillus infection in 1997. The patient had received antivirus treatment once a time, but he was allergic to the drug. So the treatment was stopped from then on. The CD4+ T cell counting is 10^6/ ul in July, 2018. The white cell counting is 13.10 x 10^9/ L and serum EBV-DNA load is 2.40 x 10^4 tested on Feb 26, 2019. Physical examination: the swollen right cervical lymph node was excised with the diameter of 2 cm.

Biopsy Fixation Details:

The excisional right cervical lymph node was fixed in 10% formalin neutral buffer solution for 24 hours.

Description of Clinical Image if Any:

No

Details of Microscopic Findings:

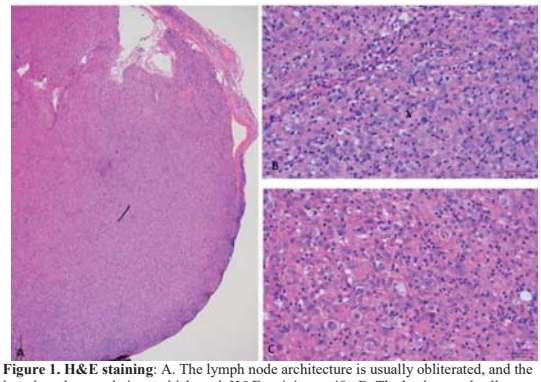


Figure 1. H&E staining: A. The lymph node architecture is usually obliterated, and the lymph node capsule is not thickened. H&E staining; x 40. B. The background cells consist of a mixture of cell types, including eosinophils, neutrophils, histiocytes, and plasma cells; x 400. C. The HRS cells in a mixed cellular infiltrate with lymphocytes, macrophages, and eosinophils are visible; H&E, x 400.

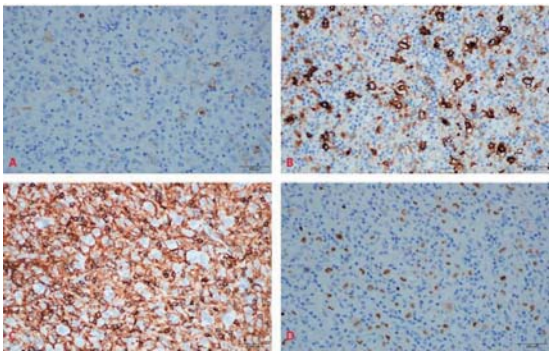


Figure 2. IHC staining: A. CD20 is negative or weakly positive in HRS cells. B. CD30 immunostaining highlights the presence of a large Reed-Sternberg cells and a small mononuclear variant. C. CD45 is negative in HRS cells and positive in the background cells. D. PAX5 is weakly positive in HRS cells. IHC, x 400.

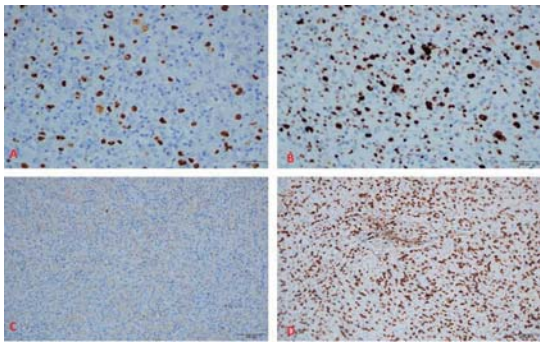


Figure 3. IHC staining: A. EBER is positive in HRS cells; . IHC, x 400. B. Ki-67 is positive in HRS cells; IHC, x 400. C. CD4 is negative in HRS cells and background cells; IHC, x 200. D. CD8 is positive in background cells; IHC, x 200.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

CD30(+), CD15(-), CD20(-), PAX5(weak +), CD3(-), Bcl-6(+), CD10(-), MUM1(+), ALK-1(-), CD5(-), CD68(KP1)(-), C-myc(+，40%), P53(Wild type), CK(-), CD23 and CD21(focal FDC meshwork +), CD79a(-), OCT2(-), BOB1(wek +), LCA(-), HHV-8(-), Ki-67(+). CD8 is positive in the background cells but CD4 is negative.

Cytogenetics: No.

Molecular Analysis:

In situ hybridization for EBV-encoded small RNA (EBER) is positive.

Interesting Feature(s) of Submitted Case:

HIV associated CHL is rare compared to DLBCL or Burkitt lymphoma.

Proposed Diagnosis:

Mixed-cellularity classic Hodgkin lymphoma associated with HIV infection.

Comments:

case presentation

Sufang Tian M.D., Ph.D
Department of Pathology
Wuhan University Zhongnan Hospital

武汉大学第二临床学院

1

Medical history

- A 43-year-old AIDS patient presented with intermittent fever with unknown cause for half a month.
- Laboratory test showed EBV DNA and CMV DNA copies were 6.73x103 and 2.03x103, respectively. Penicilliosis marneffei was detected in the blood culture and bone marrow.
- Excisional biopsy of cervix lymph node was performed.

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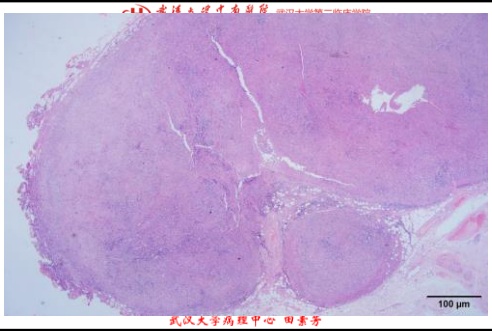

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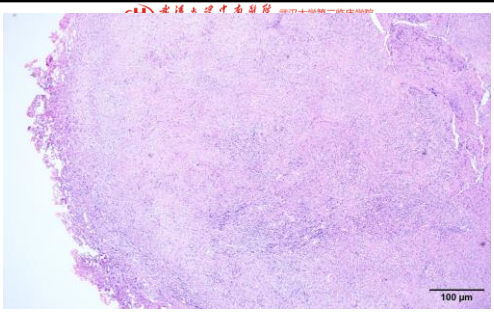

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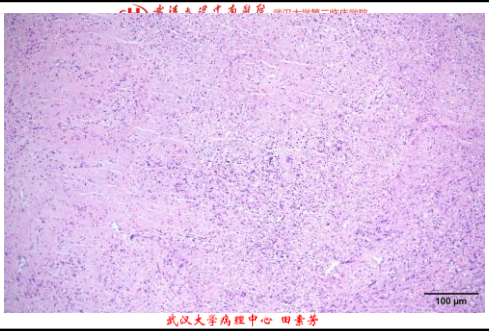

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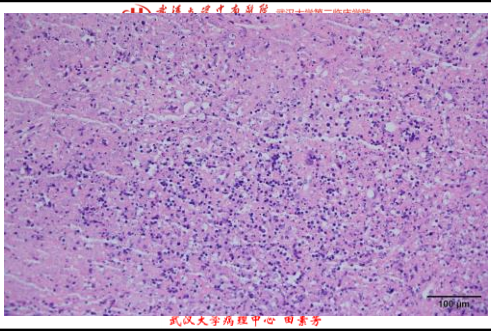

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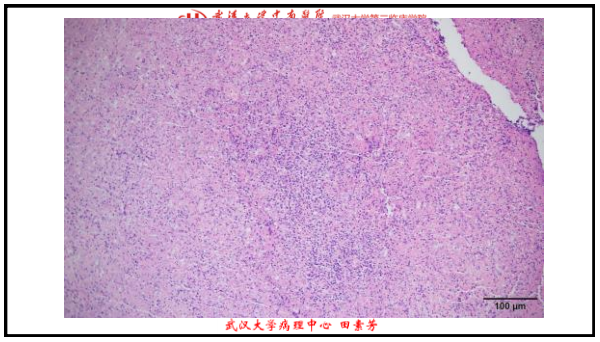
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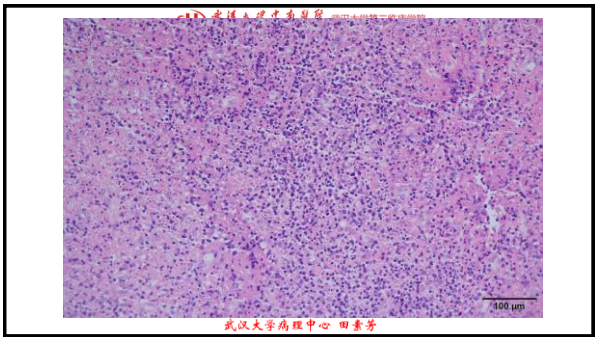
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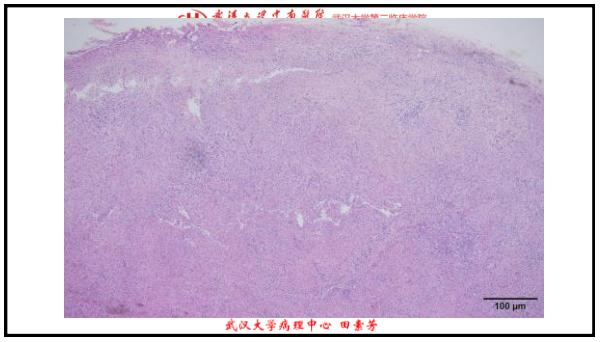
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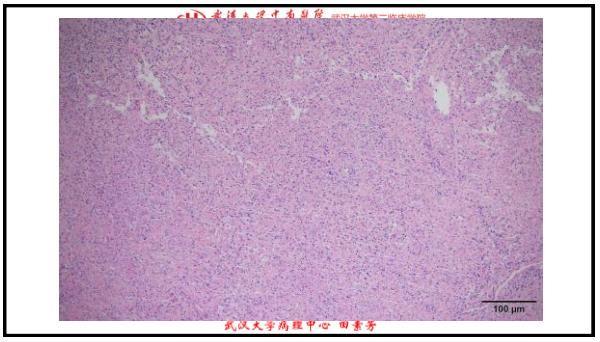
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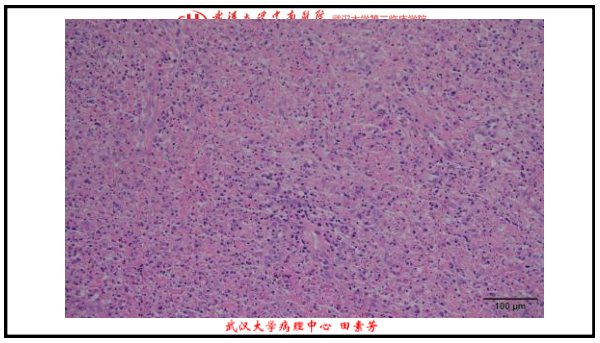
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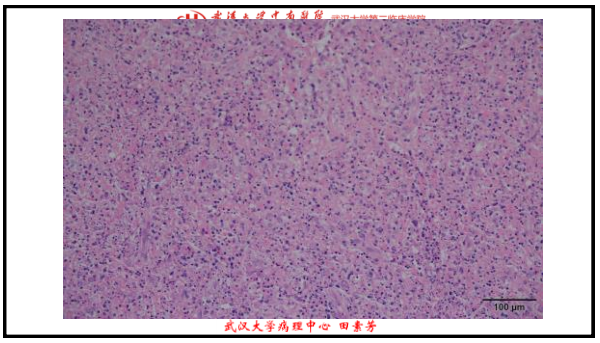
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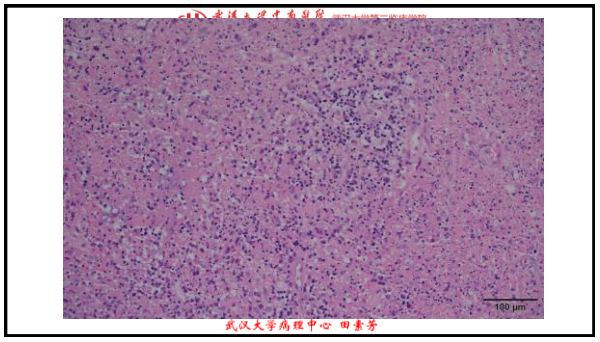

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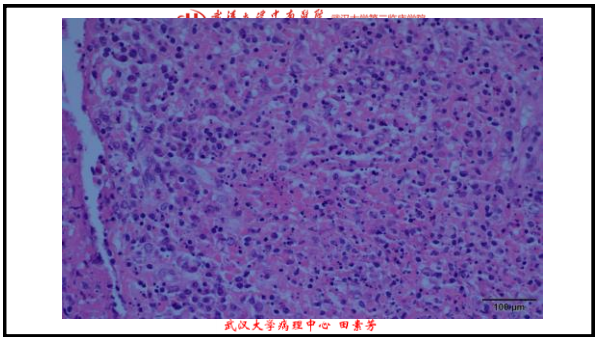

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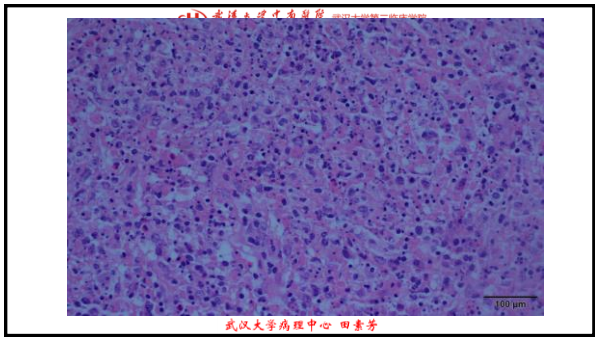

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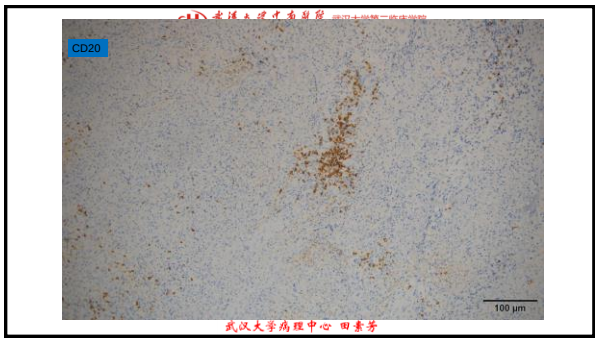

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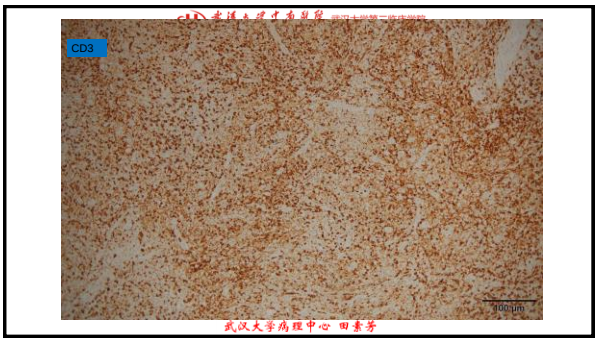

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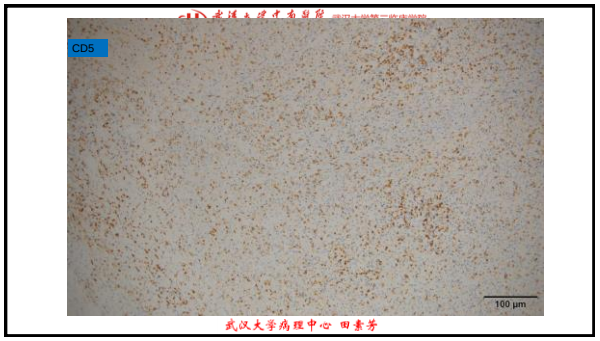

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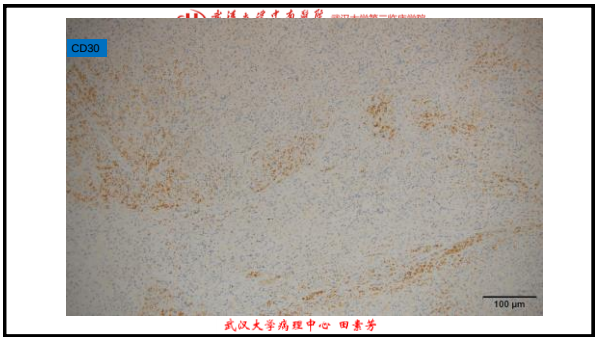

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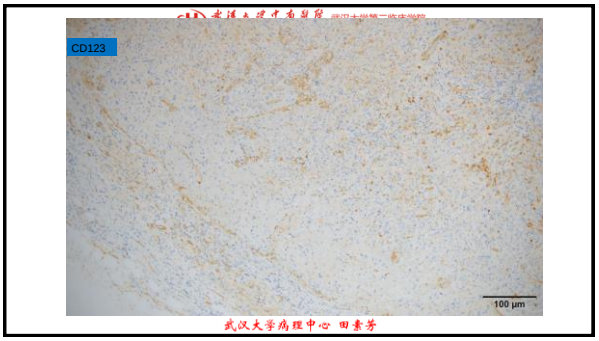

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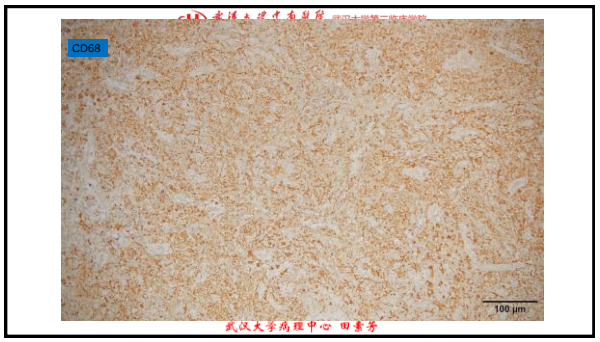

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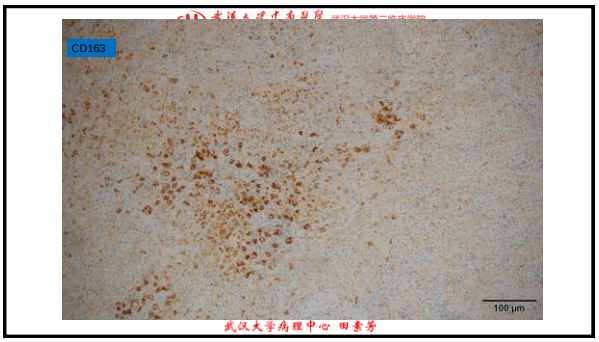

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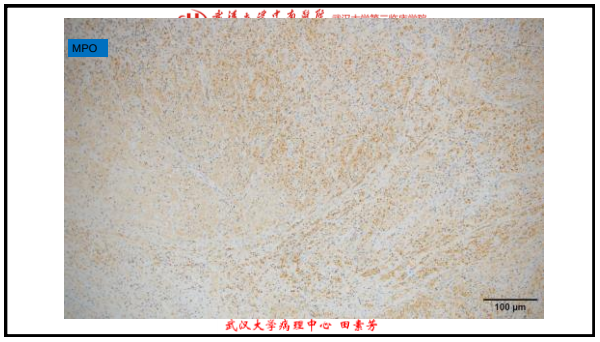
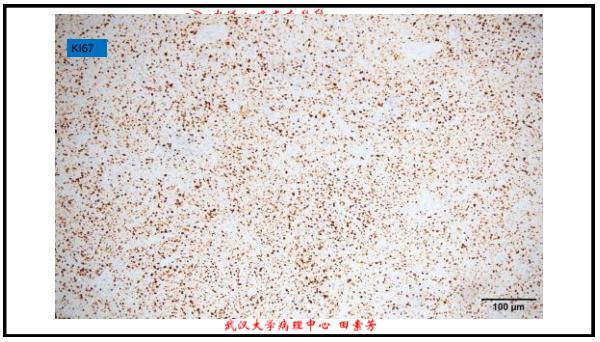

MPO
100 μm
武汉大学病理中心 田素芳

25


KI67
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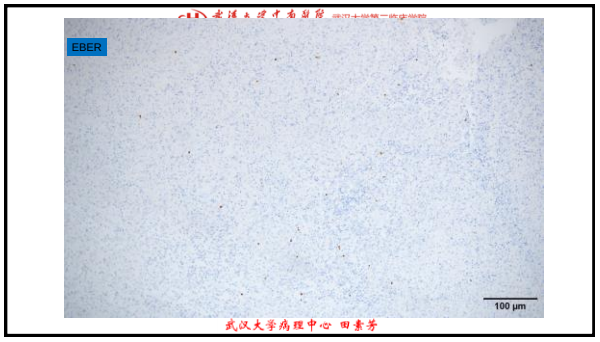
26


EBER
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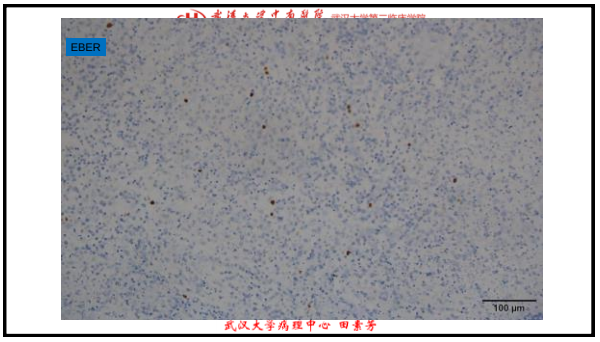
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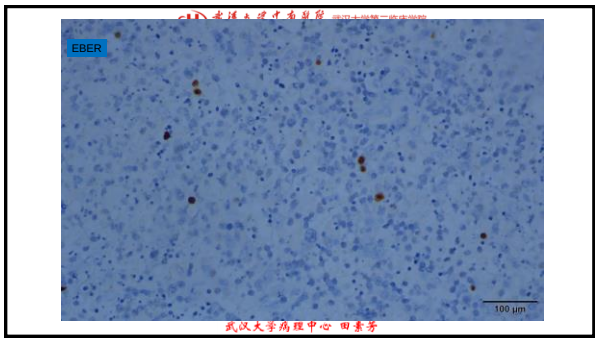
武汉大学病理中心 田素芳

28


EBER
100 μm
武汉大学病理中心 田素芳

29

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Christos Masaoutis MD[1], Dimitrios Sampaziotis MD[1], Aikaterini Choutri MD[2], Eleni Patsea MD[3], Lydia Abou-Asabeh MD[3], Dimitra Rontogianni MD PhD[1]

Affiliation: [1] Evangelismos General Hospital of Athens, [2] Nicosia General Hospital, [3] Metropolitan General Hospital of Athens

E-mail: masaoutis@hotmail.com

Clinical History: Case #1: A 66-year old woman with a history of breast cancer since 2015 presented with a solitary solid 4 cm splenic mass since 2017. Case #2: A 72-year old woman presented with a cystic 8 cm splenic mass, grown by ~20% over the last 8 months.

Biopsy Fixation Details: 10% phosphate-buffered formalin

Description of Clinical Image if Any: N/A

Details of Microscopic Findings: Both lesions were circumscribed, partly encapsulated and composed of a mixed population of mature plasma cells, small T- and B-lymphocytes forming few secondary follicles, rare eosinophils and bland spindle cells in short fascicles or storiform formations.

Immunophenotyping by Immunohistochemistry ~~and/ or Flow Cytometry~~: Spindle cells were diffusely SMA+, fascin+ and patchily CD35+, but CD21- and CD23-; there was minimal IgG4 production; no ALK expression was noted.

Special Stains: In situ hybridization highlighted EBERs in many inflammatory and spindle cells alike.

Cytogenetics: N/A

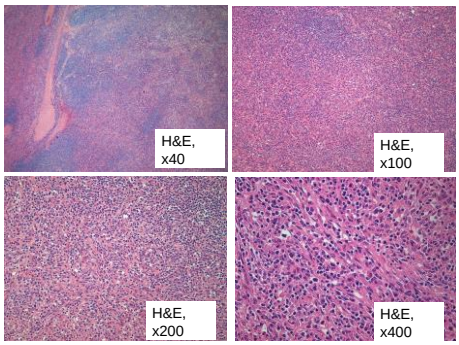
Molecular Analysis: N/A

Interesting Feature(s) of Submitted Case: A rare EBV-related tumour of the haematolymphoid tissues with an indolent behaviour.

Proposed Diagnosis: Inflammatory pseudotumour-like follicular dendritic cell sarcoma.

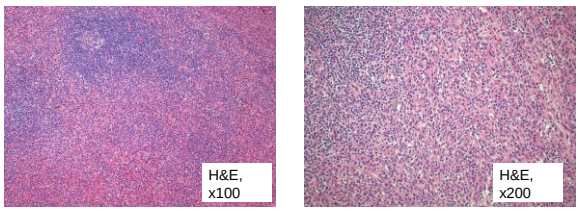
Comments:

Case #1:
circumscribed, partly encapsulated lesion, composed of a mixed population of mature plasma cells, small lymphocytes forming few secondary follicles, rare eosinophils and bland spindle cells in short fascicles or storiform formations



1

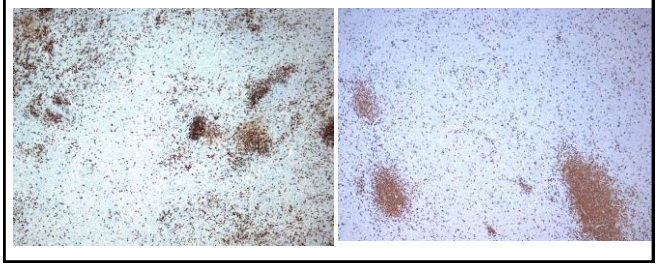
Case #2: similar histologic findings as in case #1



2

CD20, x40

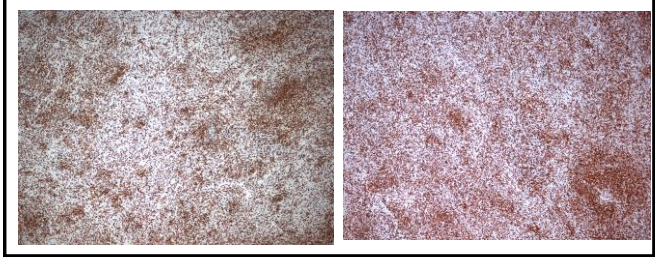
CASE #1 CASE #2



3

CD3, x40

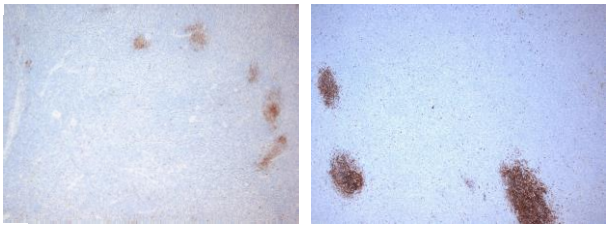
CASE #1 CASE #2



4

CD23, x40

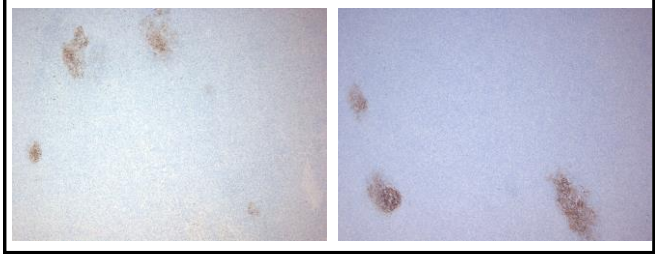
CASE #1 CASE #2



5

CD21, x40

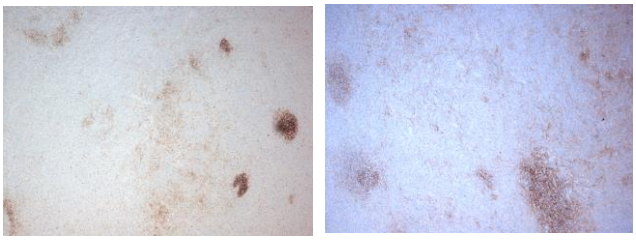
CASE #1 CASE #2



6

CD35, x40

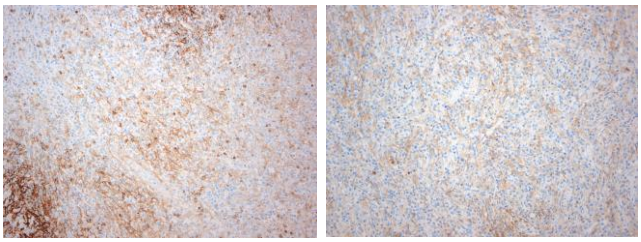
CASE #1 CASE #2



7

CD35, x200

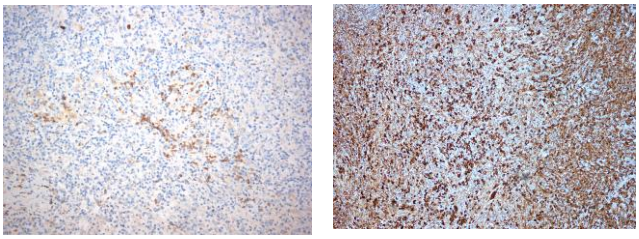
CASE #1 CASE #2



8

IgG, x400/x100

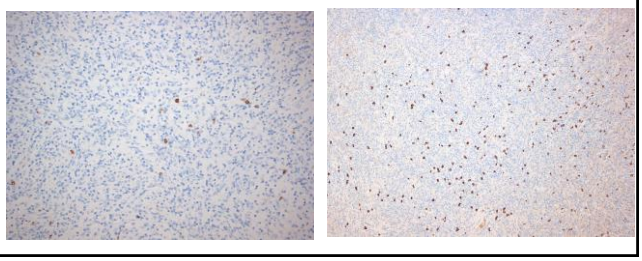
CASE #1 CASE #2



9

IgG4, x400/x100

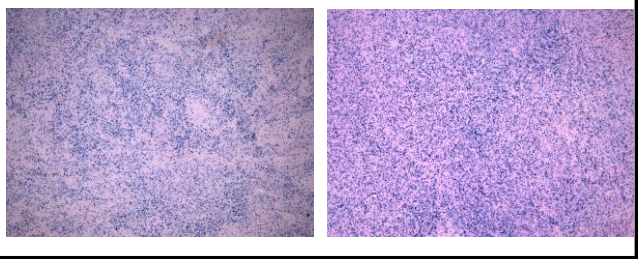
CASE #1 CASE #2



10

EBER-ISH, x40

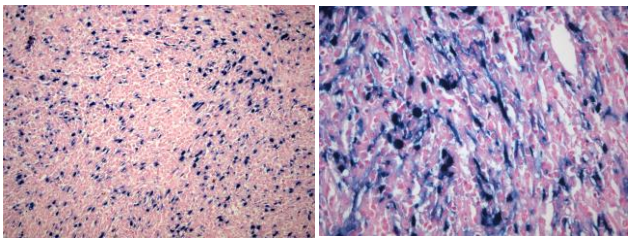
CASE #1 CASE #2



11

EBER-ISH, x200/x400

CASE #1 CASE #2



12

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Dan Li (李丹 PROF. MD PHD)

Affiliation:
Clinic Pathologic Diagnosis centre, Department of Pathology of Affiliated Hospitals & Basic College of Medicine, Chongqing Medical University

E-mail:
1219162617@qq.com

Clinical History:

- H20190534， 39/M， a consultation case in 03/2019
- **Clinical History**
  - He had abnormal sensation of his right thumb pulp two years ago (in 2017), then, felt swell and a mass formation with it.
  - He accepted laser therapy in one of Traditional Chinese Medicine Hospitals of Chongqing in 04/2018 and 12/2018. However, There was no visible improvement, the mass became bigger.
  - He accepted biopsy of the mass in the hospital on 1st March 2019, but the Pathologic diagnosis was not determined, then went to Pathology of Chongqing Medical University to consult on 5th March 2019.
- The others: (-)

Biopsy Fixation Details:
The specimens were isolated, fixed with 4% neutral formalin, dehydrated and embedded in paraffin.

Description of Clinical Image if Any:

- 22th April 2019 enhanced MRI of right hand :

1.Main discovery: A nodular lesion located in the metacarpal portion distal phalanx of right hand thumb, which was isointensity on T1WI, slight hyperintensity on T2WI, size 21× 25mm, a clear border, with visible membrane. It cannot be separated with the distal tendon of flexor pollicis longus, which was encased. and bone absorption can be seen in the distal phalanx. The lesion had slight heterogeneous enhancement in the postcontrast. There were no other abnormalities in the hand in the scanning area.
2. Diagnosis: The tumor lesion need to be considered, giant cell tumor of tendon sheath most probably.

- 29th April 2019 whole-body PET/CT scan：

1.Main discovery: A nodular lesion located in the metacarpal portion distal phalanx of right hand thumb, size 2.6× 1.8mm. Bone absorption can be seen in the distal phalanx. PET showed increased radioactivity uptake. SUVmax is 14.9.
2.Diagnosis：The malignant tumor lesion need to be considered.

Diagnosis: The tumor lesion need to be considered, giant cell tumor of tendon sheath most probably.

Details of Microscopic Findings:
Histology shows diffuse large lymphoid cells infiltrate the dermis and subcutaneous tissue, the tumor cells with nuclear atypia and many mitosis, Coagulation Necrosis in some area, but without angiocentric Angiodestructive.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
Immunophenotype: the lymphomatous infiltrate tumor cells belong to cytotoxic T-cell lineage, positive for CD2, CD3, CD8, GRB, etc.

IHC and EBER

➢ Positive

- CD45 most dim
- CD2 diffuse
- CD3 diffuse
- CD7 diffuse
- CD8 diffuse
- GRB most
- KI67 90%
- VIM diffuse
- EBER(twice) >80%

➢ Negative

- CD4
- CD5
- CD56
- TIA-1
- CD20
- EBNA2
- BCL2
- TDT
- CD30
- ALK
- TCRBF1
- CK
- EMA
- S100
- SOX10
- DESMIN
- CALDESMON
- SMA
- CD31
- CD34
- CD68

Special Stains:

Cytogenetics:

Molecular Analysis:
Antigen Receptor Gene Rearrangment Analysis by PCR：TCRγ +, IgH- IgK- .

Interesting Feature(s) of Submitted Case:
Location: solitary mass in the right thumb pulp instead of systemic symptoms.
The patient is young and strong.
EBER+.
HIV+.
The case is extremely Rare.

Proposed Diagnosis:
HIV- and EBV-related (cytotoxic) Peripheral T-cell Lymphoma.

Comments:
There were no effects with anti-inflammatory therapy, the mass of the right thumb pulp became bigger, necrosis and ulcer became more serious, combined with the former results of the morphology, IHC and Antigen Receptor Gene Rearrangment by PCR, it could support malignant tumor, EBV+ mature T-cell NK cell neoplasm, especially EBV+ cytotoxic PTCL should be considerable. The patient is young, strong, and only companied with solitary mass in the right thumb pulp instead of systemic symptoms, no fever, no weight loss, no lymphadenopathy, no hepatosplenomegaly, thus, the probability of CAEBV A3 also existed, MDT (Pathology, Hematology, Oncology, Surgery, Radiology) diagnosis and treatment is required.
We received important information from the MDT that the patient had concealed his history of HIV+ since the first pathologic diagnosis, so, our proposed diagnosis is HIV- and EBV-related (cytotoxic) Peripheral T-cell Lymphoma, which is extremely rare.

A case of HIV- and EBV-related (cytotoxic) Peripheral T-cell Lymphoma with the primary symptom of a mass in the right thumb pulp of a male adult

Li Dan (李丹 PROF. MD PHD)
Clinic Pathologic Diagnosis centre, Department of Pathology of Affiliated Hospitals & Basic College of Medicine, Chongqing Medical University
06/2019
Tel：18523065892 17782116247
Email: 1219162617@qq.com

1

Clinical Informations

➢H20190534， 39/M， a consultation case in 03/2019

➢**Clinical History**

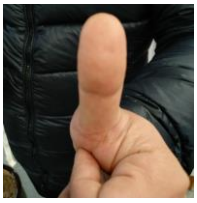
• He had abnormal sensation of his right thumb pulp two years ago (in 2017), then, felt swell and a mass formation with it.

•He accepted laser therapy in one of Traditional Chinese Medicine Hospitals of Chongqing in 04/2018 and 12/2018. However, There was no visible improvement, the mass became bigger.

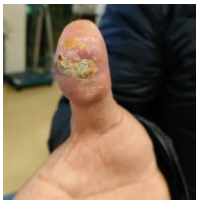
•He accepted biopsy of the mass in the hospital on 1st March 2019, but the Pathologic diagnosis was not determined, then went to Pathology of Chongqing Medical University to consult on 5th March 2019.

➢The others: (-)

2



The left thumb (normal thumb)



The mass of the right thumb (abnormal thumb) with necrosis and ulcer 1 week laterof biopsy (8th March 2019)

3

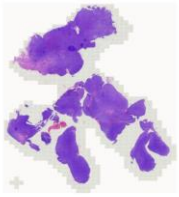
Pathologic findings

- **Gross: A pile of grey and soft tissue, d≈0.6cm.**
- **Microscopic Findings**

  Histology shows diffuse large lymphoid cells infiltrate the dermis and subcutaneous tissue, the tumor cells with nuclear atypia and many mitosis, Coagulation Necrosis in some area, but without angiocentric Angiodestructive.

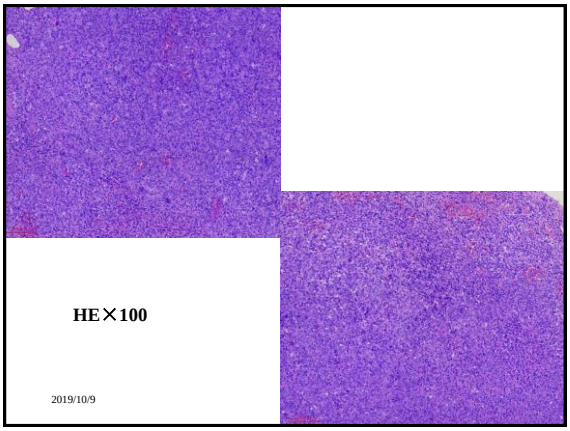
4

HE Stains



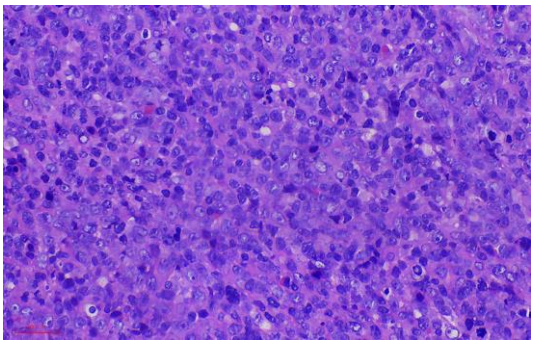
HE×3

5



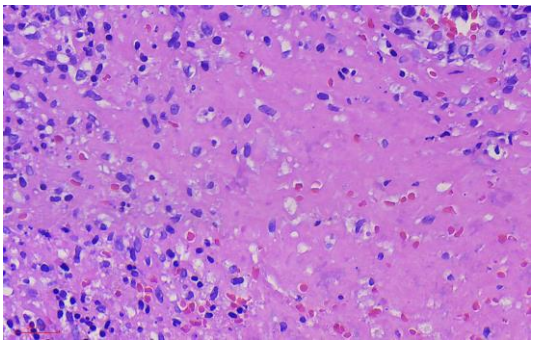
HE×100

6



Diffuse large cells hyperplasia with nuclear atypia and many mitosis. HE×400

7



Coagulation Necrosis area, but without angiocentric Angiodestructive lesion . HE×400

2019/10/9

8

IHC and EBER

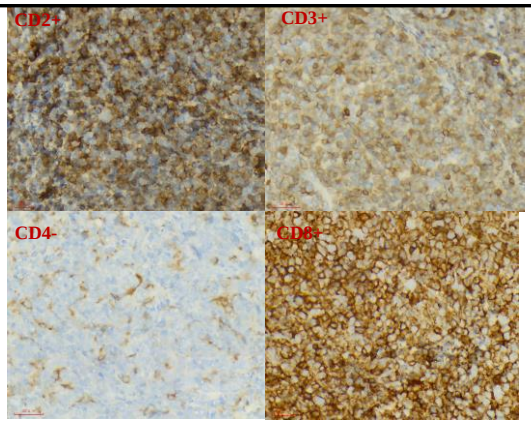
➢ **Positive**

- CD45 most dim
- CD2 diffuse
- CD3 diffuse
- CD7 diffuse
- CD8 diffuse
- GRB most
- KI67 90%
- VIM diffuse
- EBER(twice) >80%

➢ **Negative**

- CD4
- CD5
- CD56
- TIA-1
- CD20
- EBNA2
- BCL2
- TDT
- CD30
- ALK
- TCRBF1
- CK
- EMA
- S100
- SOX10
- DESMIN
- CALDESMON
- SMA
- CD31
- CD34
- CD68

9



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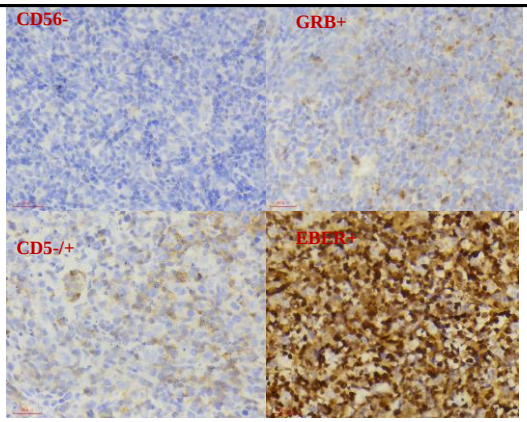

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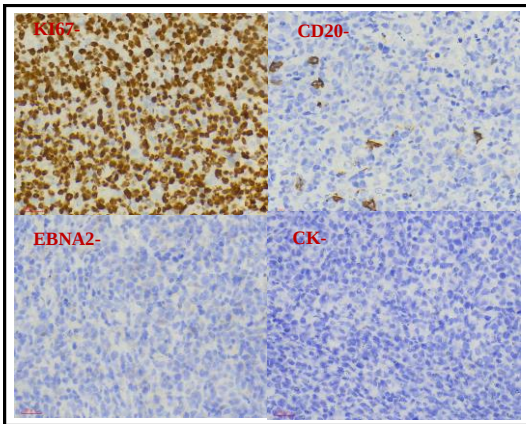


12

Antigen Receptor Gene Rearrangment Analysis by PCR

- TCRγ +
- IgH-
- IgK-

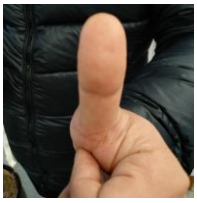
2019/10/9

13

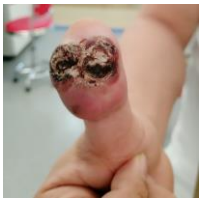
Preliminary Pathologic Diagnosis (11th March 2019)

- **Location:** the right thumb pulp biopsy
- **Microscopic Findings** :histology shows diffuse large lymphoid cells infiltrate the dermis and subcutaneous tissue, the tumor cells with nuclear atypia and many mitosis, Coagulation Necrosis in some area, but without angiocentric Angiodestructive.
- **Immunophenotype:** the lymphomatous infiltrate tumor cells belong to cytotoxic T-cell lineage, positive for CD2, CD3, CD8, GRB, etc.
- **EBER:** majority +.
- **Antigen Receptor Gene Rearrangment Analysis by PCR:** T-cell monoclonality (TCRγ +).
- **Preliminary Pathologic Diagnosis:**
  EBV-associated T-LPD, Monoclonality, but EBV+ PTCL need to be excluded.
- **Comments:** the morphology, results of IHC and Antigen Receptor Gene Rearrangment by PCR could support the EBV+ PTCL, but the patient is young, strong, and only companied with solitary mass in the right thumb pulp instead of systemic symptoms, no fever, no weight loss, no lymphadenopathy, no hepatosplenomegaly, etc.. Thus, Whole body imaging check, anti-inflammatory therapy, waiting, wacthing and Close follow-up are of importance to further diagnosis.

14



The left thumb (normal thumb)



The mass of the right thumb (abnormal thumb) became bigger with prominent necrosis and ulcer 6 weeks later of biopsy
(11th March 2019)

2019/10/9

15

Supplementary imaging examination (1)
(1st Affiliated Hospital of CQMU)

➢ **22th April 2019 enhanced MRI of right hand :**

- Main discovery: A nodular lesion located in the metacarpal portion distal phalanx of right hand thumb, which was isointensity on T1WI, slight hyperintensity on T2WI, size 21× 25mm, a clear border, with visible membrane. It cannot be separated with the distal tendon of flexor pollicis longus, which was encased. and bone absorption can be seen in the distal phalanx. The lesion had slight heterogeneous enhancement in the postcontrast. There were no other abnormalities in the hand in the scanning area.
- Diagnosis: The tumor lesion need to be considered, giant cell tumor of tendon sheath most probably.

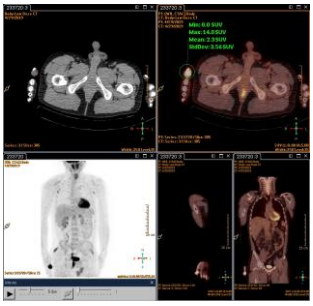
2019/10/9

16

Supplementary imaging examination (2)
(1st Affiliated Hospital of CQMU)

➢ **29th April 2019 whole-body PET/CT scan：**

- **Main discovery:** A nodular lesion located in the metacarpal portion distal phalanx of right hand thumb, size 2.6× 1.8mm. Bone absorption can be seen in the distal phalanx. PET showed increased radioactivity uptake. SUVmax is 14.9.
- **Diagnosis：** The malignant tumor lesion need to be considered.



2019/10/9

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Pathologic Diagnosis (26th April 2019)

- **Follow-up:** the mass of the right thumb pulp became bigger, necrosis and ulcer became more serious one and half month later.
- **Preliminary Pathologic Diagnosis:**
  EBV-associated T-LPD, Monoclonality, but EBV+ PTCL need to be excluded.
- **Comments:** there were no effects with anti-inflammatory therapy, the mass of the right thumb pulp became bigger, necrosis and ulcer became more serious, combined with the former results of the morphology, IHC and Antigen Receptor Gene Rearrangment by PCR, it could support malignant tumor, EBV+ mature T-cell NK cell neoplasm, especially EBV+ cytotoxic PTCL should be considerable. The patient is young, strong, and only companied with solitary mass in the right thumb pulp instead of systemic symptoms, no fever, no weight loss, no lymphadenopathy, no hepatosplenomegaly, thus, the probability of solitary CAEBV A3 also existed, MDT (Pathology, Hematology, Oncology, Surgery, Radiology) diagnosis and treatment is required.

18

MDT(30th April 2019)

- **Departments of Pathology, Hematology, Oncology, Surgery, Radiology**
- **The patient had concealed his history of HIV+ since the first pathologic diagnosis, it usually resulted in additional complication to the Pathologist in the medical practice.**
- **The final pathologic diagnosis:**

HIV- and EBV-related (cytotoxic) Peripheral T-cell Lymphoma.

2019/10/9

19

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CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Eric X. Wei, MD, PhD

Affiliation: Louisiana State University Health Science Center

E-mail: xwei1@lsuhsc.edu

Clinical History: A 55-year-old male with past medical history of classical Hodgkin lymphoma, HIV with CD4 count at 50, and pancytopenia presented to University Hospital emergency department with altered mental status and fever. Bone marrow biopsy was performed due to persistent pancytopenia and suspected disseminated histoplasmosis. Despite empirical antibiotic therapy, and blood transfusion of platelets and red blood cells, patient developed disseminated intravascular coagulation and later expired.

Biopsy Fixation Details: 10% formalin

Description of Clinical Image if Any:
CT head without contrast showed no acute intracranial processes. Chest X ray was negative for acute findings.

Details of Microscopic Findings:
Bone marrow examination showed hypercellular marrow with mild dyserythropoiesis, and multifocal involvement by a malignant lymphoma. The tumor cells were intermediate to large in size, with prominent nucleoli and abundant cytoplasm.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
The large atypical cells were positive for CD30 (weak), CD45, CD79a, BOB-1, OCT-2, PAX-5, MUM-1, c-Myc (20-30%), and negative for CD3, CD10, CD15, CD20, BCL-2, BCL-6, EBV-LMP1, EMA, Fascin, with Ki-67 proliferation index at about 50-60%.

CSHP/SH Workshop 2019

Special Stains:
AFB, GMS, PAS with diastase special stains were negative for microorganisms in bone marrow.

Cytogenetics: It was not performed as patient expired.

Molecular Analysis: It was not performed as patient expired.

Interesting Feature(s) of Submitted Case:
Patient previously had a history of nodular sclerosis type classical Hodgkin lymphoma in the right neck lymph node 9 years old. The Hodgkin and Reed-Sternberg cells were positive for CD30, and negative for CD15 and CD30. He had been on HAART therapy for his HIV infection. He has never received rituximab related therapy. He later developed the large B cell lymphoma that shared some features of classical Hodgkin lymphoma, in that the lymphoma cells were positive for CD30, and negative for CD20. It is possible his HIV associated diffuse large B cell lymphoma was transformed from previous classical Hodgkin lymphoma.

Proposed Diagnosis:
HIV associated diffuse large B cell lymphoma, non-germinal center B cell-like (non-GCB), possibly transformed from prior classical Hodgkin lymphoma.

Comments:
This is a unique case, and may be the first reported case of HIV associated diffuse large B cell lymphoma, possibly transformed from prior classical Hodgkin lymphoma.

HIV- Associated Diffuse Large B Cell Lymphoma, Possibly Transformed from Hodgkin Lymphoma

Eric X. Wei, MD, PhD
Lousiana State University Health Sciences Center
USA

1

Clinical History/Hospital Course

- A 55-year-old male with a past medical history of HIV associated classical Hodgkin lymphoma diagnosed 9 years ago, CD4+ T cell count at 50/µL, and pancytopenia presented to LSU University Hospital emergency department with altered mental status and fever.
- Bone marrow biopsy was performed due to persistent pancytopenia and suspected disseminated histoplasmosis.
- Despite empirical antibiotic therapy, and blood transfusion of platelets and red blood cells, patient developed disseminated intravascular coagulation and later expired.

2

Imaging

- CT head without contrast showed no acute intracranial processes.
- Chest X ray was negative for acute findings.

Tissue Processing

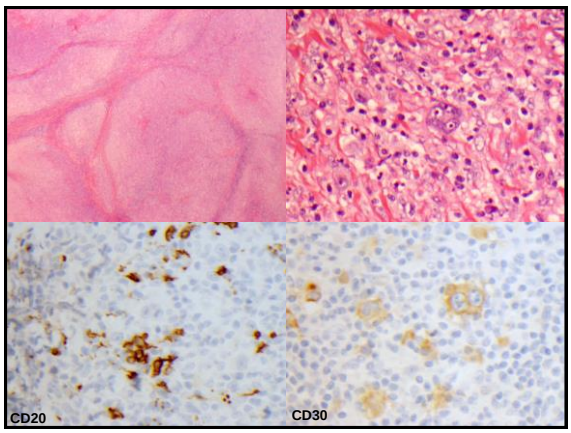
- The bone marrow biopsy and previous lymph node biopsy were fixed in 10% formalin.

3

Right Neck Lymph Node Biopsy

- Sections of the right neck lymph node revealed involvement by nodular sclerosis classical Hodgkin lymphoma. The Hodgkin and Reed-Sternberg cells expressed CD30 but did not express CD15 or CD20 on immunoperoxidase staining. Small reactive T and B lymphocytes were identified on the CD3 and CD20 stains. Background nodular sclerosis was noted.
- Patient had received treatment of Hodgkin lymphoma and HIV infection. The treatment of Hodgkin lymphoma was stopped one year after his initial diagnosis.

4



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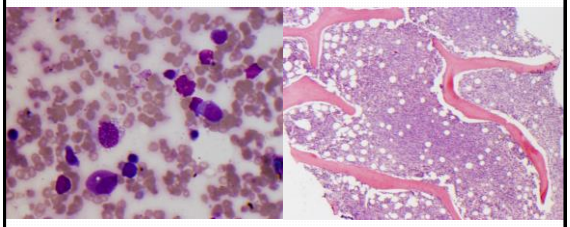
Bone Marrow Findings

- Bone marrow examination showed hypercellular marrow with mild dyserythropoiesis, and multifocal involvement by a malignant lymphoma, comprising about 35% medullary space.
- The tumor cells were intermediate to large in size, with prominent nucleoli and abundant cytoplasm.

6

Bone Marrow Aspirate and Core Biopsy



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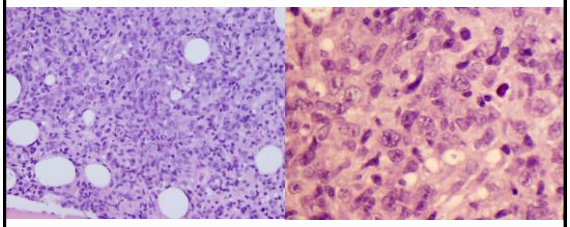
Focal Lymphoma Involvement



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Immunohistiochemical Studies

- The large atypical cells were:
  - positive for CD30 (weak), CD45, CD79a, BOB-1, OCT-2, PAX-5, MUM-1, c-MYC (20-30%);
  - negative for CD3, CD10, CD15, CD20, BCL-2, BCL-6, EBV-LMP1, EMA, Fascin;
  - Ki-67 proliferation index was approximately 50-80%.

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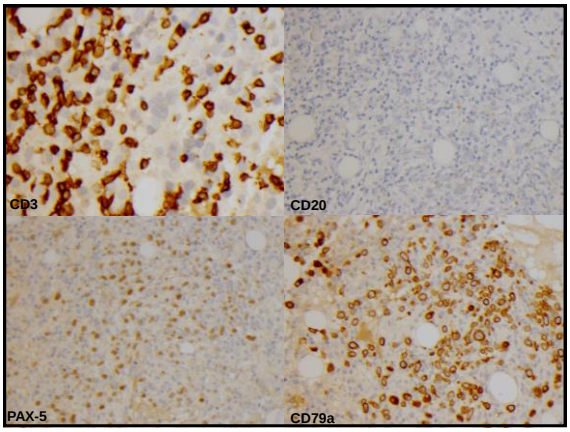

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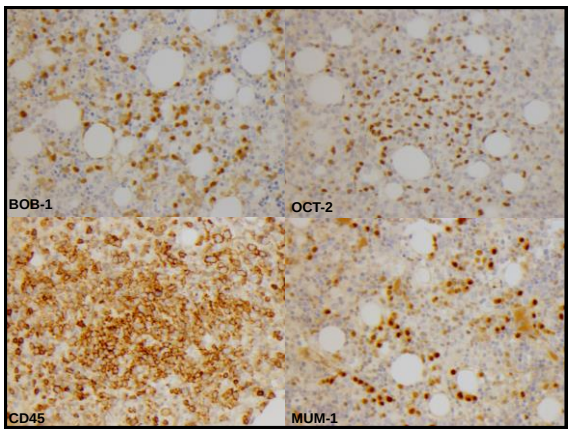

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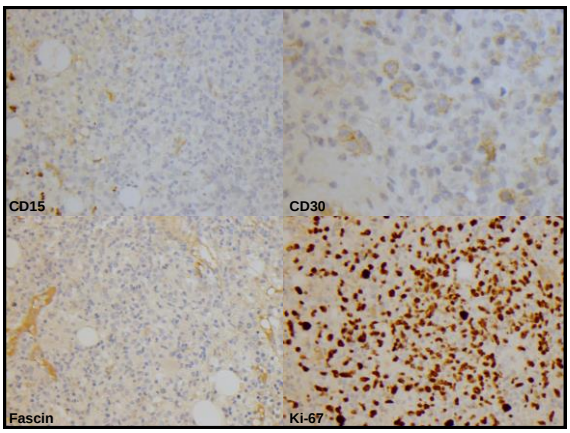

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Ancillary Studies

- **Special Stains:**
  - AFB, GMS, PAS with diastase special stains were negative for microorganisms in bone marrow.
- **Cytogenetics:** It was not performed as patient expired.
- **Molecular Analysis:** It was not performed as patient expired.

13

Interesting Features

- Patient previously had a history of nodular sclerosis type classical Hodgkin lymphoma in the right neck lymph node 9 years old, and had received therapy for Hodgkin lymphoma for one year. His Hodgkin lymphoma went into remission.
- The Hodgkin and Reed-Sternberg cells were positive for CD30, and negative for CD15 and CD20.
- He had been on HAART therapy for his HIV infection.
- He has never received Rituximab related therapy.
- He later developed the diffuse large B cell lymphoma, that shared some features of classical Hodgkin lymphoma, in that the lymphoma cells were weakly positive for CD30, and negative for CD20.
- It is possible his HIV associated diffuse large B cell lymphoma was transformed from previous classical Hodgkin lymphoma.

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Interesting Features

- The case is better not to be classified as B cell lymphoma, unclassifiable, with features intermediate between diffuse large B cell lymphoma and classic Hodgkin lymphoma.
- That type of grey zone lymphoma usually occurs in young men and children, with mediastinal, supraclavicular and intra-abdominal lymph nodes as the most common locations. It is not known to be associated with HIV infection or immunodeficiency. It may have strong expression of CD20, CD15, and CD30.

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HIV-associated DLBCL

- HIV-related diffuse large B cell lymphoma (DLBCL) tends to have extranodal or disseminated clinical presentations with less nodal involvements.
- It often have high disease stage, with central nervous system, gastrointestinal tract, bone marrow, and liver as the most sites.
- It may be germinal center cell type or activated B cell type. Morphologically, it can be centroblastic or immunoblastic. It usually expresses common B cell antigens such as CD19 and CD20. BCL-2, BCL-6, MYC pro-oncogenes may be involved.

16

HIV associated Hodgkin Lymphoma

- HIV associated classical Hodgkin lymphoma has been increasing, with nodular sclerosis type as the most common type since the era of combined anti-retroviral therapy.
- It tends to have advanced bone marrow or liver involvement at clinical presentations.
- Its Hodgkin and Reed-Sternberg cells are usually positive for EBER with type II EBV latency pattern showing expressions of EBNA1, LMP1, and LMP2.

17

Potential diagnostic pitfall

- This case presents a diagnostic challenge. If we had performed a limited immunohistochemical panel with only CD3, CD20, PAX-5, CD15 and CD30, the PAX5+, CD20+, CD15-, and CD30+ immunostaining might lead to misdiagnosis of bone marrow involvement of prior classical Hodgkin lymphoma.
- Extended B cell panel showed the large atypical B cells were positive for CD79a, BOB-1, OCT-2, which led to the correct diagnosis of HIV associated DLBCL.

18

Proposed Diagnosis

- HIV associated diffuse large B cell lymphoma, non-germinal center B cell-like (non-GCB), possibly transformed from prior classical Hodgkin lymphoma.

19

Conclusion

- This is a unique case, and may be the first reported case of HIV-associated diffuse large B cell lymphoma, possibly transformed from prior classical Hodgkin lymphoma.

20

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Kwun Wah Wen, MD PhD, Sonam Prakash, MD and Yi Xie, MD PhD

Unusual presentation of HHV-8 and EBV-positive lymphoproliferative disorder in a HIV-positive patient

Affiliation:
Department of Laboratory Medicine, University of California, San Francisco, San Francisco, CA, United States

E-mail:
kwun.wen@ucsf.edu, sonam.prakash@ucsf.edu, yi.xie@ucsf.edu

Clinical History:
A 55-year-old HIV-positive man who was well-controlled on highly active antiretroviral therapy (HAART) presented with a painless lump in right upper neck that was noted during dental appointment. He had no constitutional symptoms. His HIV was diagnosed 30 years ago with remote opportunistic infections as well as Kaposi sarcoma in the right leg that was treated twenty years ago. Laboratory values at presentation were within normal limits. The CD4 count was 496 x10E6/L and HIV viral load was undetectable. An ultrasound showed a heterogeneous hypervascular 2.7-cm lymph node adjacent to the submandibular gland. An excisional biopsy of the right level 1B lymph node was performed.

Biopsy Fixation Details:
Formalin fixed

Description of Clinical Image if Any:
Staging PET/CT scan was subsequently performed and revealed mildly enlarged right neck, bilateral subpectoral, axillary and possibly retroperitoneal lymph nodes with maximum SUV ranging from 2.3 to 6.1. No body cavity effusions, hepatosplenomegaly or extranodal sites of disease were detected.

Details of Microscopic Findings:
Histologic examination of hematoxylin-eosin (HE)–stained sections showed an enlarged lymph node with follicular hyperplasia. There were variably sized and shaped follicles with reactive germinal centers and largely intact mantles. In some interfollicular areas, there were scattered and aggregates of large atypical lymphoid cells, many exhibiting immunoblastic/plasmablastic features with round

to slightly irregular nuclei, a single centrally-placed nucleolus and moderate amount of amphophilic cytoplasm. Focal sinusoidal infiltration was present. Germinal centers were largely uninvolved. There were no distinct Castleman-like histopathological features.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
IHC staining showed the large atypical cells were diffusely positive for CD30, MUM1, c-myc and HHV-8. They were positive for EBER by in situ hybridization. Ki-67 stained approximately 90% of large cells. A small subset of the cells appeared dimly positive for OCT2 and CD45. They were negative for the other tested antibodies (CD3, CD20, EMA, CD79a, PAX-5, CD138, CD10, BCL6, CD15, TIA-1, CD4, CD8, and ALK-1). Kappa and Lambda IHC and ISH highlighted numerous polytypic plasma cells in the background but were negative in the large atypical cells.

Flow cytometry was performed on a portion of the excisional biopsy and showed no immunophenotypic evidence of an abnormal lymphoid population.

Special Stains:

Cytogenetics:
FISH studies were negative for *MYC*, *BCL6*, or *BCL2* rearrangement.

Molecular Analysis:

Interesting Feature(s) of Submitted Case:
Our patient had an unusually mild and indolent clinical course. Since he was asymptomatic and all organ functions were normal, he was monitored closely without specific treatment. Six months after the first biopsy, he underwent a repeat biopsy of a left neck lymph node, which demonstrated a small reactive lymph node with rare intrasinusoidal HHV-8-and EBV-positive atypical lymphoid cells.

The patient had been closely followed up for 26 months since the initial presentation. He remained asymptomatic with normal CBC and normal LDH. His previously noted right cervical lymph nodes were much diminished and no new adenopathy was detected.

Proposed Diagnosis:
HHV8-positive, EBV-positive lymphoproliferative disorder

Comments:
As illustrated in this case, precise classification of the HHV8+/EBV+ lymphoproliferative disorders (LPDs) can be challenging. Given the history of HIV infection and the findings of clusters of HHV8+/EBV+ large atypical cells in the interfollicular areas and sinusoids, we initially considered a diagnosis of nodal involvement by primary effusion lymphoma (PEL) or extracavitary PEL. However, the mild and indolent clinical course for 2 years in the absence of lymphoma therapy is unusual for PEL, which is generally an aggressive lymphoma with a median survival time of around 6 months. Similarly, although the clinical course can be compatible with germinotropic lymphoproliferative disorder (GLPD), the predominantly interfollicular/intrasinusoidal distribution of HHV8+/EBV+ immunoblasts is unusual for GLPD, which typically show predilection for germinal center involvement.

This unique case expands the diverse clinical and pathologic spectrum of HHV-8 and EBV associated lymphoproliferative disorders.

Unusual Presentation of HHV-8 and EBV-positive Lymphoproliferative Disorder in a HIV-Positive Patient

Kwun Wah Wen, Sonam Prakash and Yi Xie
Department of Laboratory Medicine
University of California, San Francisco

1

Clinical history

- A 55-year-old man with well-controlled HIV/AIDS on HAART presented with a painless lump in right upper neck that was noted during dental appointment.
- No systemic symptoms, no swelling elsewhere.
- Laboratory values were within normal limits. The CD4 count was 496 x10E6/L; HIV viral load was undetectable.
- An ultrasound showed a heterogeneous hypervascular 2.7-cm lymph node adjacent to the submandibular gland.

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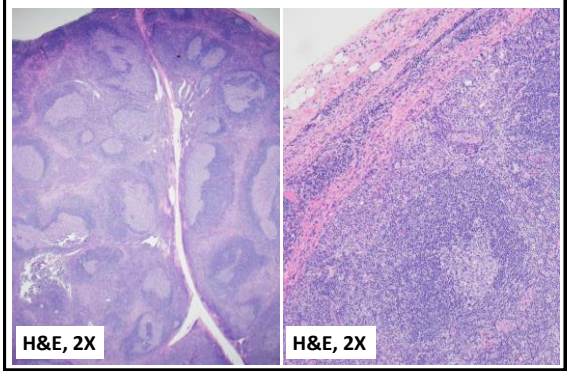
Right submandibular lymph node excision



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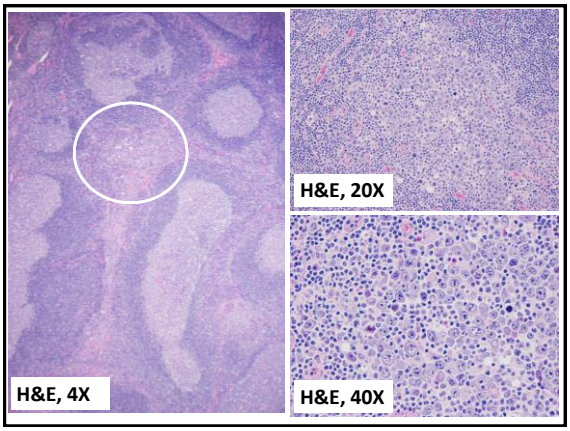

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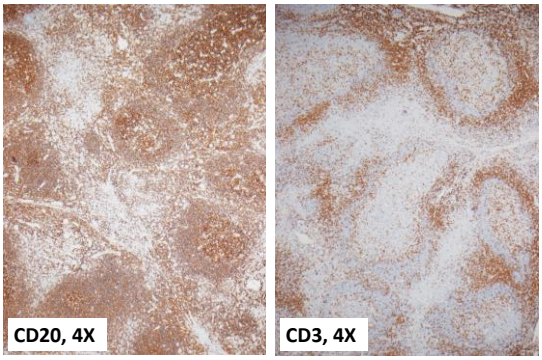

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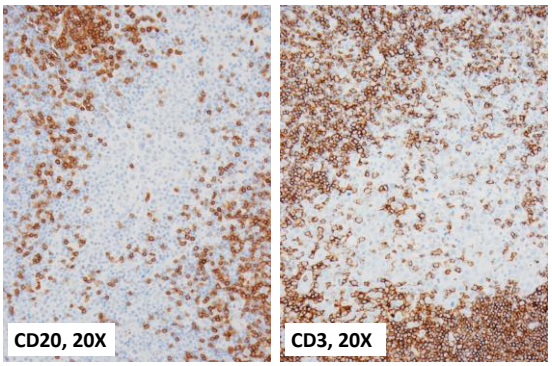

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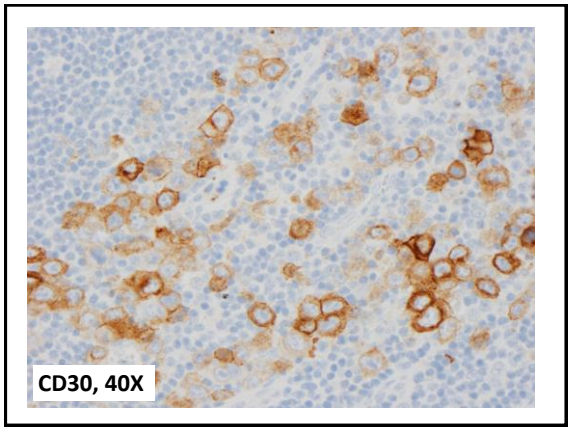
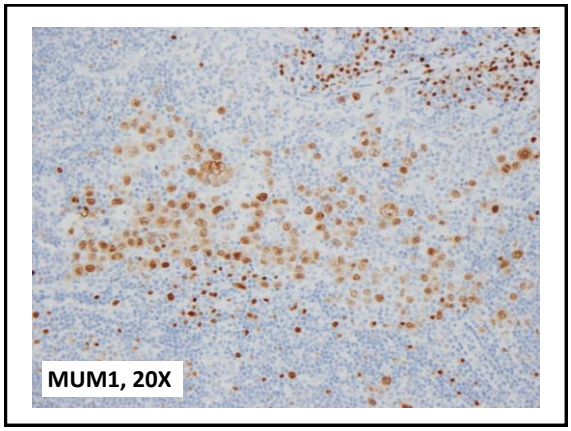
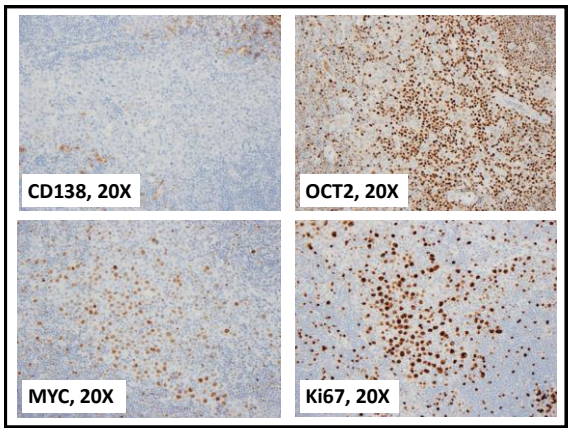

CD30, 40X

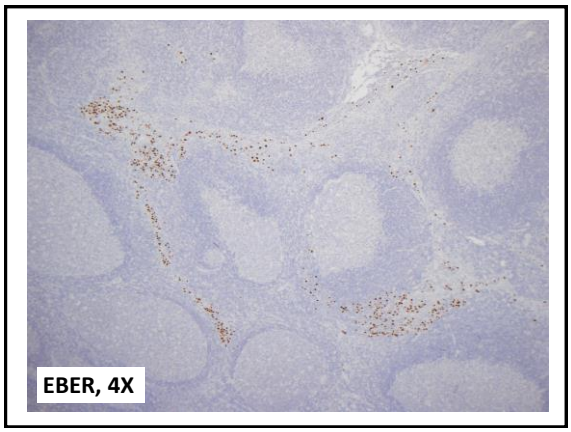
7


MUM1, 20X

8


CD138, 20X
OCT2, 20X
MYC, 20X
Ki67, 20X

9


EBER, 4X

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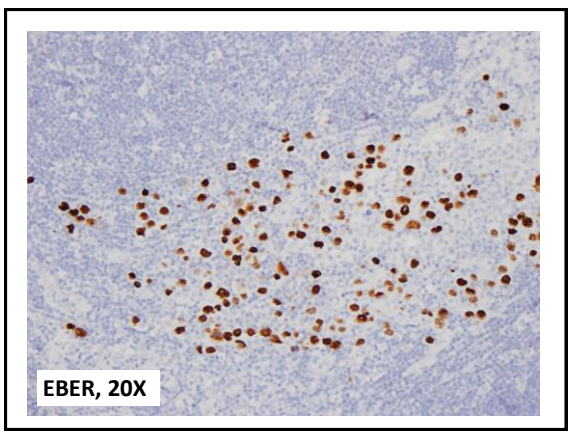
EBER, 20X

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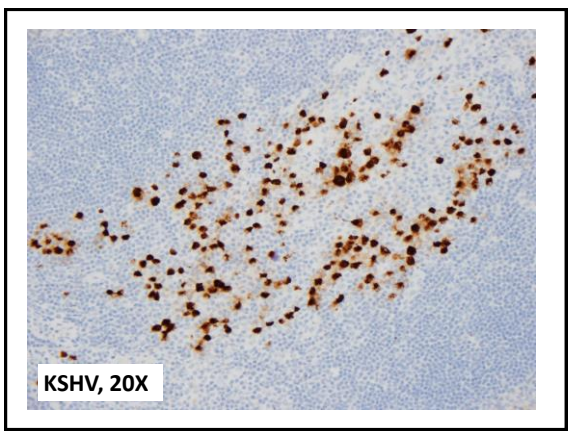

KSHV, 20X

12

Immunohistochemistry

- **Positive**
  - HHV8
  - EBER ISH
  - CD30
  - MUM1
  - C-MYC
  - Ki67
  - OCT2 (negative – partial dim)
- **Negative**
  - CD3, CD20, PAX5, CD79a, CD138, EMA, Kappa and Lambda IHC and ISH, ALK1, CD15, CD10, BCL6, CD4, CD8, TIA1, CD43

FISH

- Negative for MYC, BCL6, or BCL2 rearrangement

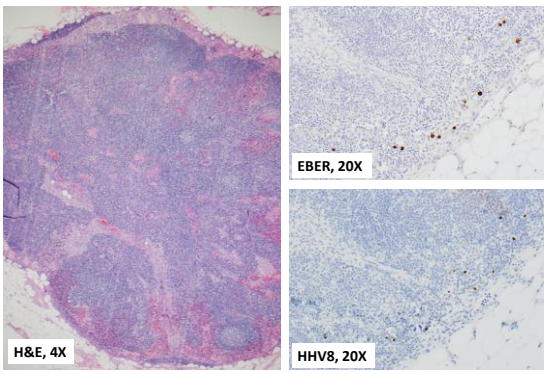
13

Clinical course

- The patient was monitored closely without specific treatment.
- Six months after the first biopsy, a repeat biopsy of a left neck lymph node was performed and demonstrated rare intrasinusoidal HHV-8+/EBV+ atypical lymphoid cells.

14

Left neck lymph node excision



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Clinical course

- The patient had been closely followed up for 26 months without treatment.
- He remained asymptomatic with normal CBC and normal LDH.
- His previously noted right cervical lymph nodes were much diminished and no new adenopathy was detected.

16

Proposed Diagnosis

- HHV8-positive EBV-positive Lymphoproliferative Disorder

17

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Victoria Alagiozian-Angelova, MD; Ismail Elbaz Younes, MD; Paul G. Rubinstein, MD

Affiliation: John H. Stroger Jr Hospital of Cook County

E-mail: vangelova@cookcountyhhs.org

Clinical History: 42 year old HIV+ male with prior history of cutaneous Kaposi sarcoma, treated more than 5 years prior to this presentation. Also treated for latent syphilis. Currently presenting with shortness of breath, bilateral leg edema and acute renal failure. Labs are significant for marked microcytic anemia. He was found to have moderate to severe pericardial effusion and bilateral pleural effusions. 2L of pleural fluid was tapped and sent to pathology for flow cytometric analysis.

Biopsy Fixation Details: Cytology specimen- Pap stain, Flow cytometry cytospin prep- Diff quick stain; Cell block of cytology of fluid, fixed in 10% buffered formalin.

Description of Clinical Image if Any: Images are of the pleural effusion that was tapped

Details of Microscopic Findings: The effusion contains large discohesive cells with plasmacytoid morphology and basophilic cytoplasm with ample cytoplasmic blebs. Some of the nuclei have central nucleoli.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry: Flow analysis showed a large population (~50% of cellular events) of dim to negative CD45 cells which did not express T-cell or B-cell lineage specific antigens (CD3-/CD20-) and no CD34 immunoreactivity.

Futher immunophenotyping by IHC on the cell block of the pleural effusion showed large hematopoietic neoplasm, CD45+ with variable immunoreactivity for CD138 (few cells), MUM1, CD30 and diffuse strong HHV8. EBER was non-immunoreactive.

Special Stains: None

Cytogenetics: Not performed

Molecular Analysis: Not performed

Interesting Feature(s) of Submitted Case: This is a classical morphology of primary effusion lymphoma. Notoriously the lineage categorization maybe difficult to make. These are HHV8 (+) with or without EBV expression proliferations that express activation markers, e.g. CD30. The diagnosis was made on morphologic grounds given the clinical history of the patient.

Proposed Diagnosis: Primary effusion lymphoma

Comments:

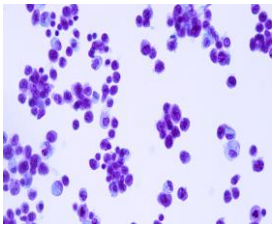
Case-1 History

- 42 y/o male with PMH of:
  - HIV
  - Cutaneous Kaposi sarcoma
  - Treated latent syphilis
  - Recent history of moderate to severe pericardial effusion
- Complains of:
  - bilateral lower extremities swelling
  - Acute kidney failure

CBC	Result
WBC	4
RBC	2.4
HGB	7.8
HCT	23.1
MCV	96.3
MCH	32.5
MCHC	33.8
RDW	22.8
PLT	221
Neutrophils	52
Lymphocytes	46
Monocytes	2
Eosinophils	0
Basophils	0

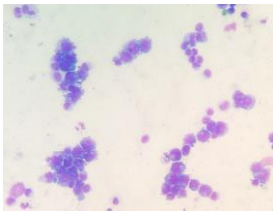
1

Pleural fluid- Diff Quick



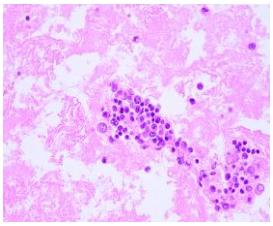
2

Pleural Fluid-Cytospin flow



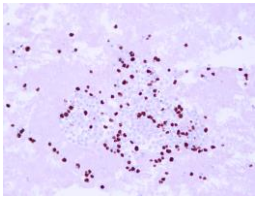
3

Pleural Fluid-Cell block H & E

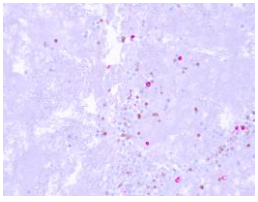


4

Immunohistochemistry



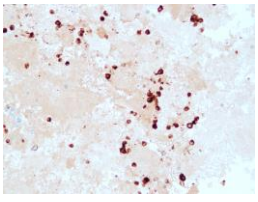
HHV-8



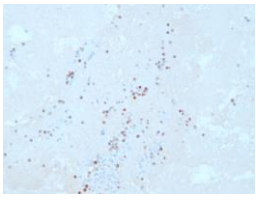
CD3/CD20

5

Immunohistochemistry-cont.



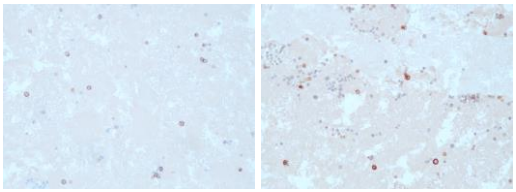
CD163



MUM1

6

Immunohistochemistry-cont.



CD138 CD30

7

Final diagnosis

- Large cell hematopoietic neoplasm, consistent with primary effusion lymphoma
- Cell block shows scattered large cells that are variably immunoreactive for:
  - CD30
  - CD138
  - MUM1
  - CD163
  - HHV8
- Note: In-situ hybridization for EBER is negative. No Kappa/Lambda surface chain expression present

8

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Carol Holman MD PhD

Affiliation:
University of Iowa

E-mail:
carol-holman@uiowa.edu

Clinical History:
A 55 year old previously healthy male presented with acute onset of double vision and right face/jaw numbness which began 2 weeks prior to presentation, and had progressed such that more recently (2-3 days prior to presentation) he also noted fatigue, generalized body aches, and jaw pain. He stated that his right testicle was painful and had doubled in size over the past several days. Physical examination showed left sixth nerve palsy. There was also palpable splenomegaly, an enlarged and tender right testicle, and a 1x1 cm soft, well-circumscribed nodule on the right chest wall between the sixth and seventh ribs. A CBC was performed which showed an elevated WBC count (20.1k/mm3), a slightly decreased platelet count (142 x10e9/L), and normal hemoglobin and MCV. The differential count showed an absolute neutrophilia (ANC of 15,000/mm3) with slight left shift and monocytosis (absolute monocyte count of 1,300/mm3). There were approximately 6% circulating abnormal lymphoid cells. Additional laboratory evaluation revealed elevated uric acid of 10.9 mg/dL (reference range 3.4-7.0 mg/dL), elevated phosphorus of 5.0 mg/dL (reference range of 2.5-4.5 mg/dL), normal potassium at 4.5 mEq/L (reference range of 3.5-5.0 mEq/L), and normal calcium at 9.8 mg/dL (reference range of 8.5-10.5 mg/dL), suggestive of early tumor lysis syndrome. An MRI of the brain revealed a left cavernous sinus mass which displaced the left cavernous internal carotid artery. CT of the chest, abdomen, and pelvis revealed diffuse lymphadenopathy in the mediastinum, mesentery, perinephric space and right pleural space. Soft tissue masses were observed along the right lateral chest wall and left rectus abdominis. Ultrasound of the testes revealed a right testicular mass. A needle core biopsy of the chest wall mass was performed and showed plasmablastic lymphoma. HHV8 immunostain and EBER in situ hybridization were both negative.

Given the association between plasmablastic lymphoma and HIV infection, the patient was tested for HIV, and was positive with 91,000 IU/mL by HIV quantitative PCR. His absolute CD4 count was 223/mm3 (reference range 263-

2045/mm3). Cerebrospinal fluid showed no evidence of lymphoma. EBV viral load was markedly elevated at 7,649,000 IU/mL by quantitative PCR.

He developed worsening tumor lysis syndrome, with potassium falling to 3.0 mEq/L ten days after admission. He was admitted to the intensive care unit where he received CVVH dialysis and was treated with Decadron and cyclophosphamide for initial debulking followed by hyper CVAD. Intrathecal chemotherapy was deferred until the patient was off dialysis (given the anticoagulation necessary while on dialysis). He was also started on Biktarvy (bictegravir, emtricitabine, and tenofovir alafenamide) for HIV approximately one week following the initiation of chemotherapy. Concurrent with starting HIV therapy, he had neutropenic fever and was found to have disseminated Cryptococcus. He is currently receiving isavuconazole therapy for the Cryptococcus, and remains on chemotherapy and HIV therapy.

Biopsy Fixation Details:
Formalin fixation

Description of Clinical Image if Any:

Left sixth nerve palsy (slide 1)

Details of Microscopic Findings:
Peripheral blood smear showed approximately 6% circulating abnormal lymphoid cells which were intermediate to large cells with abundant deeply basophilic cytoplasm, open chromatin with prominent nucleoli, and occasional eccentric nuclear placement (slide 2).

The chest wall mass showed a diffuse proliferation of intermediate to large cells with prominent eosinophilic nucleoli and abundant amphophilic cytoplasm. Numerous mitotic figures were identified (slides 3 and 4).

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

Flow cytometry performed on the chest wall mass showed a population of large cells which expressed bright CD4, CD33, bright CD38, and CD56 (slides 5 and 6). The differential diagnosis by flow cytometry included blastic plasmacytoid dendritic cell neoplasm as well as plasmablastic lymphoma.

Immunohistochemical stains were performed on the chest wall mass (slides 7-9) and showed that the neoplastic cells expressed CD43, CD45, CD30 (weak,

partial), CD79a (rare cells, weak), OCT2, BOB1, MUM1 (strong), and EMA (strong). They were lambda light chain restricted by kappa / lambda in situ hybridization. There were negative for CD138, PAX5, CD20, CD3, ALK, TdT, CD34, HHV8, and EBER ISH.

Special Stains:

EBER ISH (see Immunohistochemistry section)

Cytogenetics:
Not done

Molecular Analysis:

PCR for HIV and EBV performed on blood.

Interesting Feature(s) of Submitted Case:

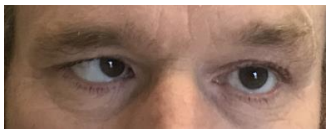
Flow cytometry alone in this case resulted in a differential diagnosis of plasmablastic lymphoma versus blastic plasmacytoid dendritic cell neoplasm. This case illustrates the utility of immunostains to provide additional information and lead to a definitive diagnosis.

Frequently plasmablastic lymphoma presents in patients with a known history of immunosuppression (such as HIV infection, iatrogenic immunosuppression, and in elderly patients with presumptive immunosenescence). This patient illustrates that a diagnosis of plasmablastic lymphoma in a patient with no known history of immunosuppression should trigger an evaluation for HIV infection.

Also, in situ hybridization for EBER is reported to be positive in 60-75% of cases of plasmablastic lymphoma. EBER ISH was negative in this case, but the very high EBV viral load in the blood suggests that the lymphoma may actually be EBV positive. The small sample size (needle core biopsy) and small area of viable tumor present on the EBER ISH slide may have resulted in a false negative.

Proposed Diagnosis:

Plasmablastic lymphoma



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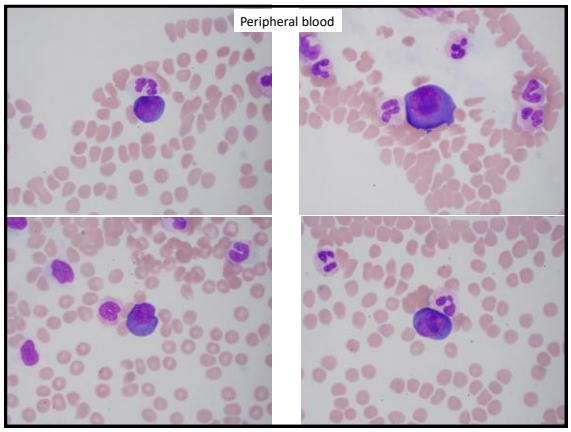
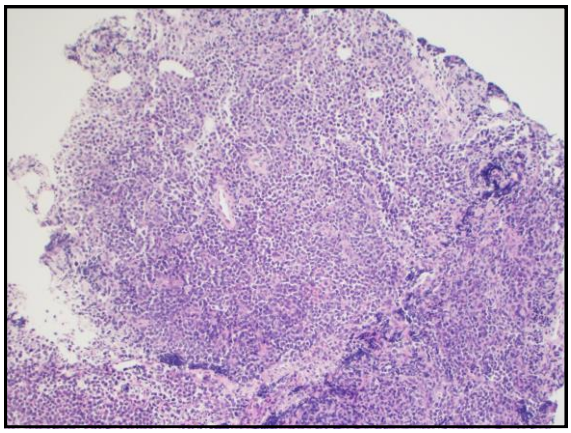

Peripheral blood

2



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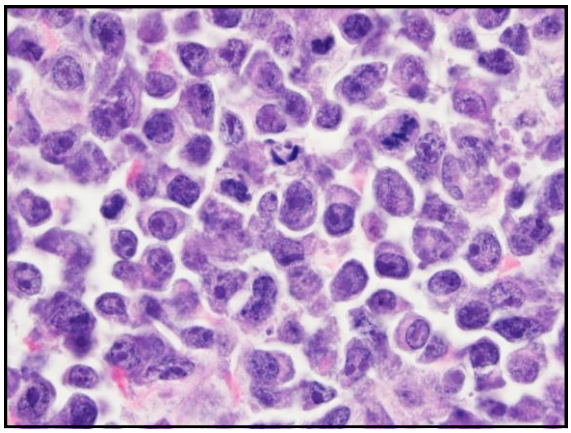

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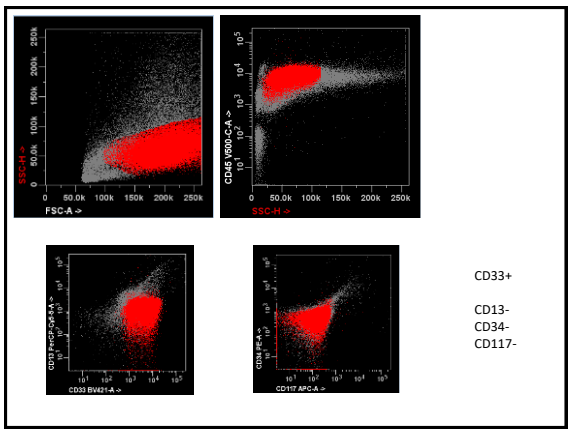
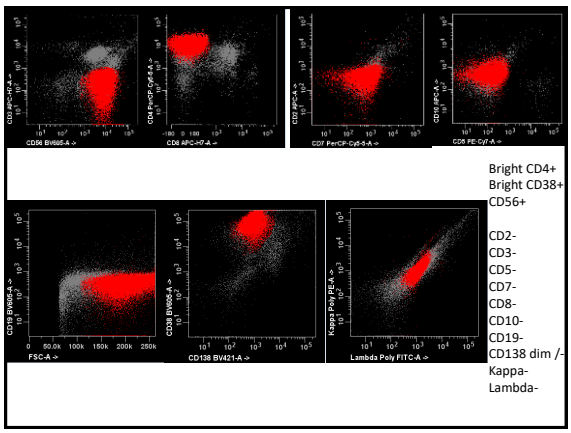
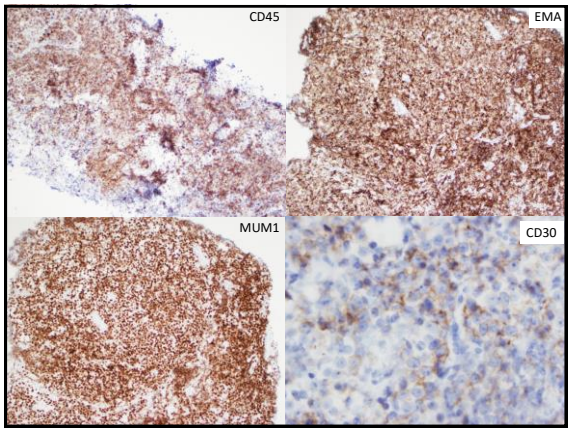

SSC-H
FSC-A
CD45 V500-C A
SSC-H
CD33 BV421-A
CD117 APC-A
CD33+
CD13-
CD34-
CD117-

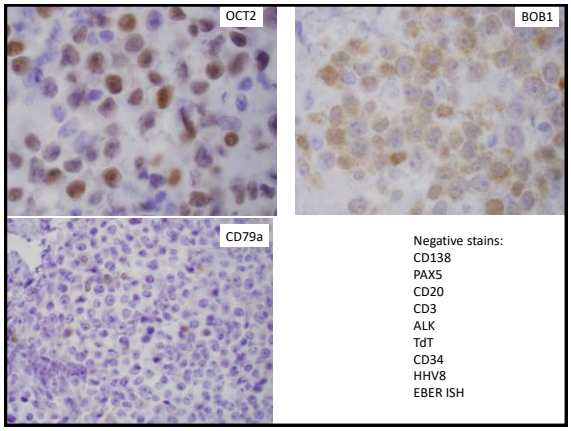
5


CD56 BV605-A
CD7 PerCP-Cy5-5-A
CD5 PE-Cy7-A
FSC-A
CD138 BV421-A
Lambda Poly FITC-A
Bright CD4+
Bright CD38+
CD56+
CD2-
CD3-
CD5-
CD7-
CD8-
CD10-
CD19-
CD138 dim /-
Kappa-
Lambda-

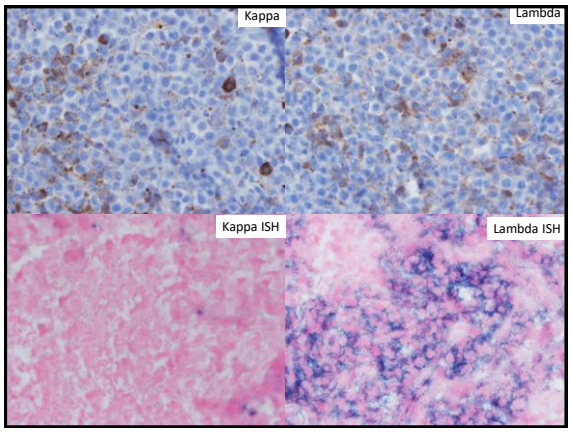
6


CD45
EMA
MUM1
CD30

7


OCT2
BOB1
CD79a
Negative stains:
CD138
PAX5
CD20
CD3
ALK
TdT
CD34
HHV8
EBER ISH

8


Kappa
Lambda
Kappa ISH
Lambda ISH

9

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Victoria Alagiozian-Angelova MD, Ismail Elbaz Younes MD, Paul G Rubinstein, MD

Affiliation: John H. Stroger Jr. Hospital of Cook County

E-mail: vangelova@cookcountyhhs.org

Clinical History: 31 year old HIV+ male with history of neurosyphilis, treated, anal squamous cell carcinoma in situ, resected in 2017 and asthma, presents with upper extremity numbness and weakness, and inability to walk (complete paralysis). Labs are non-contributory, except for high LDH (400U/L).

Biopsy Fixation Details: 10% buffered formalin

Description of Clinical Image if Any: MRI showed 4.5 cm prevertebral lesion at C7.

Details of Microscopic Findings: The biopsy of the prevertebral lesion shows a large cell pleomorphic neoplasm with some bilobed nuclei, some appearing anaplastic, admixed with small lymphocytes and histiocytes The cells appear to be cuffing around the vessels, foci of necrosis were present. Mitoses and apoptosis are easily noted.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry: IHC shows CD45+ CD20+ CD79a+ CD30+ EBER+ large cell hematopoietic neoplasm. Some of the large cells show dot-like immunoreactivity for CD15 (rare). There was no immunoreactivity for CD10 or CD5. Background of CD163+ histiocytes was present. Ki67 proliferation rate was ~70%. Additional immunostains were also performed to exclude carcinoma, melanoma and sarcoma- all negative.

CSHP/SH Workshop 2019

Special Stains: AFB and GMS are negative

Cytogenetics: Not done

Molecular Analysis: Not performed

Interesting Feature(s) of Submitted Case: The case was having features of both high grade DLBCL and Classical Hodgkin lymphoma (CD30+/CD15+).

Proposed Diagnosis: Grey zone lymphoma with features intermediate between DLBCL and classical Hodgkin lymphoma; EBER+

Comments: Rare entity in the setting of HIV+ lymphomas.

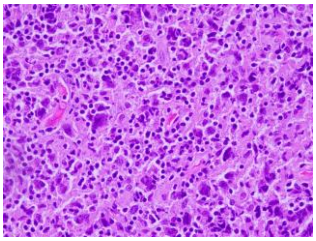
Case-2 History

- 31 y/o male with pmh of:
  - HIV
  - Neurosyphilis
  - Anal SCC in-situ resected in 2017
  - Asthma
- Complains of:
  - Inability to walk (complete paralysis)
  - Numbness
  - Upper extremity weakness
- MRI shows C7 4.5 cm vertebral lesion causing cord compression

CBC	Result
WBC	5.5
RBC	5.04
HGB	13.6
HCT	39.5
MCV	78.3
MCH	27
MCHC	34.4
RDW	16.2
PLT	221

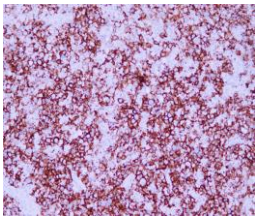
1

C7 prevertebral biopsy H&E

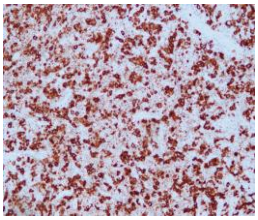


2

Immunohistochemistry



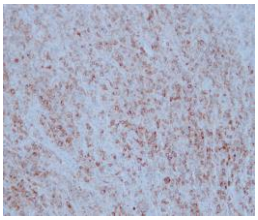
CD20



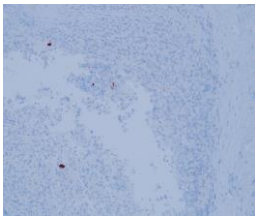
CD79a

3

Immunohistochemistry- cont.



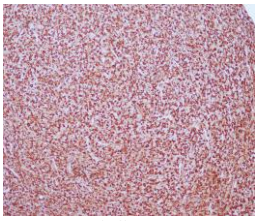
CD30



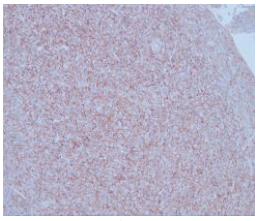
CD15

4

Immunohistochemistry-cont.



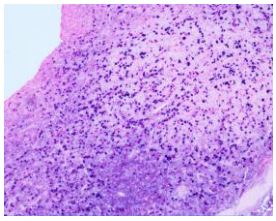
CD163



CD45

5

In situ hybridization EBV



EBER

6

Proposed diagnosis

- B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B cell lymphoma and classical Hodgkin lymphoma, EBER-positive

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Aarti Sidana, MBBS[1], Jagmohan S. Sidhu, MD[1], Ronald Harris, DO[2]

Affiliation:

1. United Health Services Hospitals, Johnson City, NY, USA

2. UHS Oncology, Johnson City, NY, USA

E-mail: jagmohansidhu@aol.com

Clinical History: 51-year-old male with a 30-year history of HIV infection and recent diagnosis of Kaposi sarcoma in gastric polyps presented in March 2008 with left-sided abdominal pain, fever, night sweats, and weight loss. His CD4 count was 297/ul (reference range: 600-1600/ul), HIV viral load was 88709 HIV1 RNA copies/ml (reference range: <50 copies/ml).Lymphadenopathy was palpable. CT scan showed cervical, mediastinal, axillary, and inguinal lymphadenopathy. Left posterior cervical lymph node was biopsied on 3/24/08. Extracavitary/Solid HHV8-associated large B-cell lymphoma of the lymph node was diagnosed. On 3/25/08 a colonoscopy was done because of abdominal pain and showed multiple small (2-3 mm) left colon polyps, which were biopsied. Extracavitary/Solid HHV8-associated large B-cell lymphoma of the colon was diagnosed. He was started on HAART (abacavir + lamivudine + efavirenz) after the diagnosis of lymphoma and 3 cycles of CHOP were given in 2008. Another colonoscopy was done due to abdominal pain in April 2009. Colonoscopy showed multiple small (2-3 mm) left and right colon polyps, which were biopsied. Persistent extracavitary/Solid HHV8-associated large B-cell lymphoma of colon was diagnosed. In July 2009, 2 days after completion of third cycle of CHOP he presented to the emergency department of a New York City Hospital with small bowel obstruction and was taken to the operating room for resection. Post-operative course was complicated by sepsis and severe fungal infection. He came back to the emergency department of our hospital in August 2009 and was admitted with high fever, bilateral neck swellings, difficulty in oral intake, abdominal pain, anemia, thrombocytopenia and worsening dyspnea. CD4 count in May 2009 was 264/ul (reference range: 600-1600/ul) and the viral Load in May 2009 was 60 HIV1 RNA copies/ml (reference range: <48 copies/ml). Chest x-ray showed bilateral lung infiltrates. CT scan showed bilateral upper lobe interstitial and alveolar lung infiltrates, and bilateral cervical, retroperitoneal/celiac axis/mesenteric lymphadenopathy. His cytopenias worsened and his respiratory condition deteriorated further. Despite aggressive measures, He died in August 2009. He died 17 months after the diagnosis of extracavitary HHV8-associated large B-cell lymphoma.

1

Biopsy Fixation Details: 10% Neutral Buffered Formalin

Description of Clinical Image if Any: None

Details of Microscopic Findings: Lymph node and colon specimens showed large sheets of large lymphoid cells with plasmacytoid appearance destroying the architecture. Numerous mitoses and apoptotic bodies were seen.
Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

Immunohistochemistry and In situ Hybridization for EBER: Most of the large lymphoid cells were positive for LCA, CD38, MUM1, OCT2, BOB1, CD30, MYC, and p53. Large cells were also positive for EBER and LANA (HHV-8). Ki67 labelling index was almost 100%. Lymphoid cells were negative for CD20, PAX5, CD79a, CD138, EMA, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD56, CD57, IgM, IgG, IgA, IgD, KAPPA, LAMBDA, BCL2, BCL1, BCL6, EBV-LMP1.

Special Stains: Not performed

Cytogenetics: Not performed

Molecular Analysis:

Clonality studies: B-cell clonality study showed presence of immunoglobulin heavy chain gene rearrangement and kappa light chain gene rearrangement..

Next Generation Deep Sequencing: Being performed

Interesting Feature(s) of Submitted Case:

CD138-negative extracavitary HHV8+/EBV+ large cell lymphoma involving lymph nodes and colonic mucosa without involvement of body cavities in an HIV-positive patient

Lack of CD138 expression by lymphoma cells could be the reason for the absence of involvement of serous cavities by this lymphoma.

Proposed Diagnosis: Extracavitary/solid EBV and HHV8-associated large B-cell lymphoma involving lymph node and colon

Comments: None

2

Extracavitary/Solid EBV and HHV8-Associated Primary Effusion Lymphoma involving Lymph Node and Colon

Aarti Sidana, MBBS[1]

Jagmohan S. Sidhu, MD[1]

Ronald P. Harris, DO[2]

1. Department of Pathology and Laboratory Medicine, UHS Hospitals, Johnson City, NY
2. Department of Hematology and Oncology, UHS Hospitals, Johnson City, NY

United Health Services

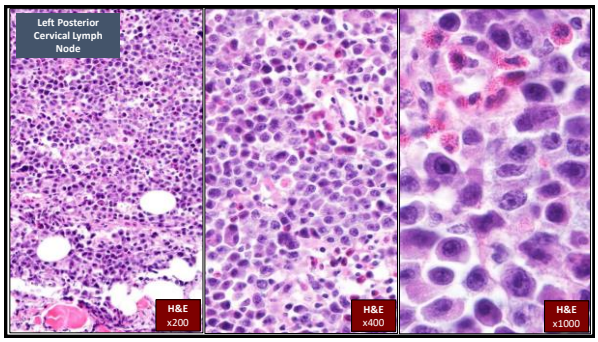
3

Clinical Data

- 51-year-old male with a 30-year history of HIV infection and recent diagnosis of Kaposi sarcoma in gastric polyps
- Presented in March 2008 with left-sided abdominal pain, fever, night sweats, and weight loss
- CD4 count: 297/ul (reference range: 600-1600/ul)
- Viral Load: 88709 HIV1 RNA copies/ml (reference range: <50 copies/ml)
- Left posterior cervical lymphadenopathy was palpable.
- CT scan showed cervical, mediastinal, axillary, and inguinal lymphadenopathy.
- Left posterior cervical lymph node was biopsied on 3/24/08.

United Health Services

4



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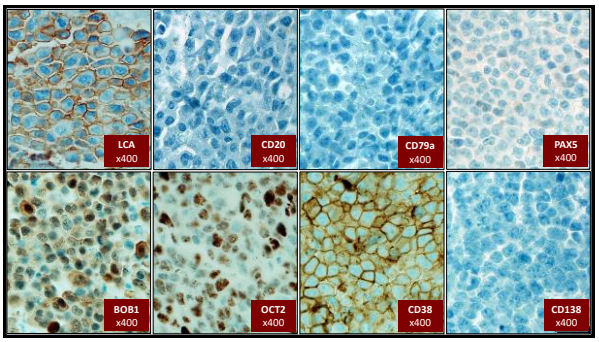

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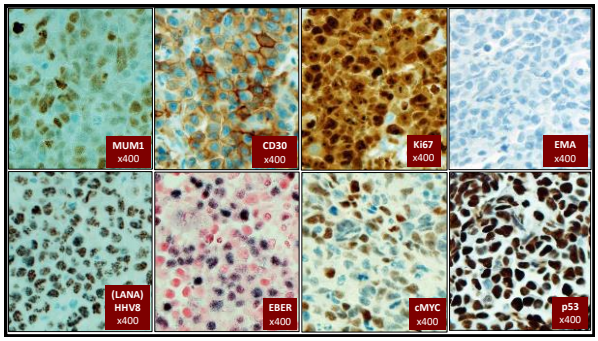


7

Negative Immunostains

CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD56, CD57, IgM, IgG, IgA, IgD, KAPPA, LAMBDA, BCL2, BCL1, BCL6, EBV-LMP1

Gene Rearrangement Studies

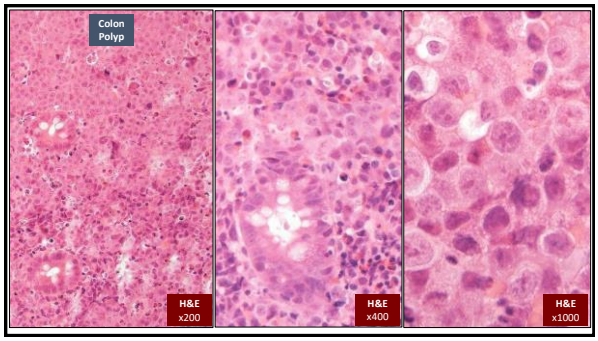
B-cell (IGH & IGK) Gene Rearrangement Analysis by PCR

INTERPRETATION:
Clonal immunoglobulin heavy chain (IGH) and kappa light chain (IGK) gene rearrangements were detected

Diagnosis and Follow-up

- Extracavitary/Solid HHV8-associated large B-cell lymphoma, lymph node
- On 3/25/08 a colonoscopy was done because of abdominal pain and showed multiple small (2-3 mm) left colon polyps, which were biopsied.

8



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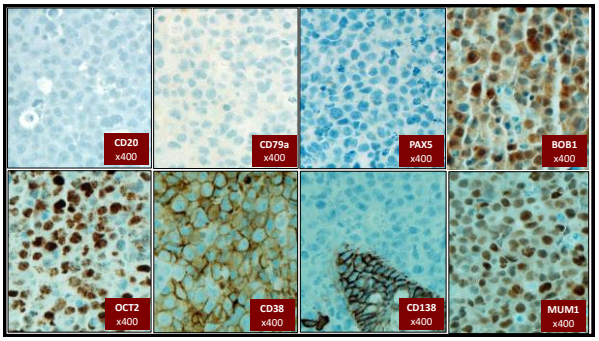

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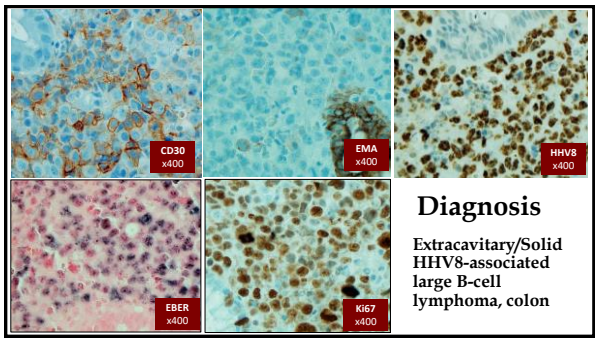


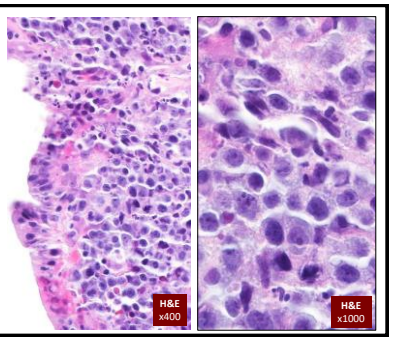
Diagnosis

Extracavitary/Solid HHV8-associated large B-cell lymphoma, colon

11

Clinical Course

- HAART (abacavir + lamivudine + efavirenz) started after the diagnosis of lymphoma
- 3 cycles of CHOP given in 2008
- Another colonoscopy was done due to abdominal pain in April 2009.
- Colonoscopy showed multiple small (2-3 mm) left and right colon polyps, which were biopsied.



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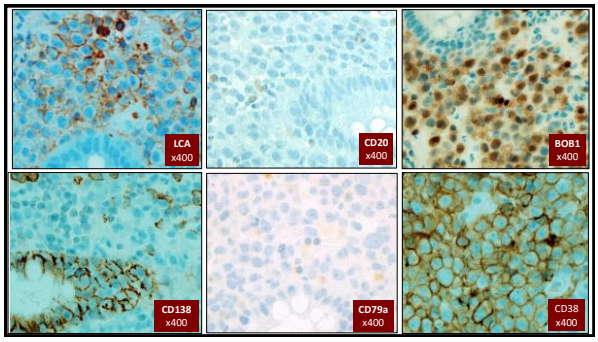

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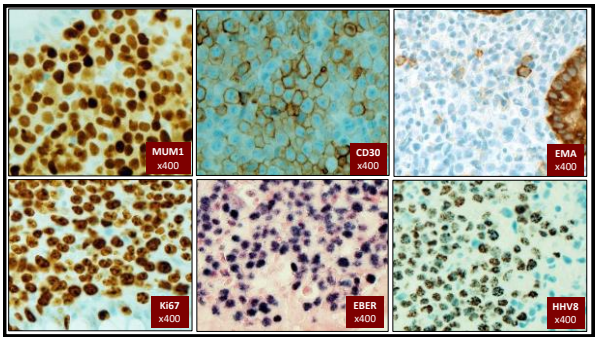

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Next Generation Sequencing

Deep sequencing of the lymphoma involving the lymph node is being done in Dr. Robert Ohgami's Lab at University of California, San Francisco, USA. The results will be available in a few weeks.

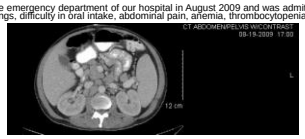
Diagnosis

Persistent extracavitary/Solid HHV8-associated large B-cell lymphoma, colon

15

Clinical Course

- In July 2009, 2 days after completion of third cycle of CHOP he presented to the emergency department of a New York City Hospital with small bowel obstruction and was taken to the operating room for resection. Post-operative course was complicated by sepsis and severe fungal infection.
- He came back to the emergency department of our hospital in August 2009 and was admitted with high fever, bilateral neck swellings, difficulty in oral intake, abdominal pain, anemia, thrombocytopenia and worsening dyspnea.



- CD4 count in May 2009: 264/ul (reference range: 600-1600/ul)
- Viral Load in May 2009: 60 HIV1 RNA copies/ml (reference range: <48 copies/ml)
- Chest x-ray showed bilateral lung infiltrates.
- CT scan showed bilateral upper lobe interstitial and alveolar lung infiltrates, and bilateral cervical, retroperitoneal/celiac axis/mesenteric lymphadenopathy
- His cytopenias worsened and his respiratory condition deteriorated further.
- Despite aggressive measures, he died in August 2009.

He died 17 months after the diagnosis of extracavitary HHV8-associated large B-cell lymphoma.

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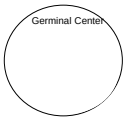
Interesting Features of Our Case

- CD138-negative extracavitary HHV8+/EBV+ large cell lymphoma involving lymph nodes and colonic mucosa without involvement of body cavities in an HIV-positive patient
- Lack of CD138 expression by lymphoma cells could be the reason for the absence of involvement of serous cavities by this lymphoma*

*Metcalf RA, Wang L, Deos PH, Chock E, Warnke RA, Natkunam Y. Extracavity primay effusion lymphoma presenting in a lymph node without lymphomatous effusions. Human Pathology 2014;xx-xxx-xxx (article in press)

17

EBV and HHV8 Co-infection of Lymphoid Cells



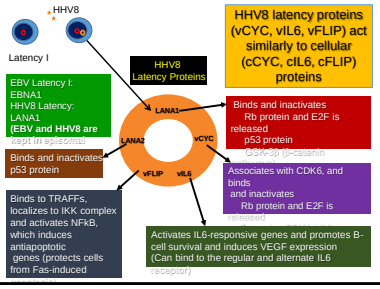
HHV8

Latency I

Latency III: EBNAs (1, 2, 3A, 3B, 3C, LP)
LMPs (1, 2A, 2B)
Viral miRNAs (2 groups)
EBER (non-coding)
Latency II : EBNA1, LMP1, LMP2, EBER
Latency 0: All latent genes downregulated, EBER
Latency I: Only EBNA1 protein switched on

18

EBV/HHV8 Lymphomagenesis



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References

1. Metcalf RA, Wang L, Deos PH, Chock E, Warnke RA, Natkunam Y. Extracavity primay effusion lymphoma presenting in a lymph node without lymphomatous effusions. Human Pathology 2014;xx-xxx-xxx (article in press)
2. Cesarman E, Mesri EA. Kaposi sarcoma-associated herpesvirus and other viruses in human lymphomagenesis. CTMI 2006;312:263-287
3. Vockerodt M, Yap L-F, Shannon-Lowe C, et al. The Epsein-Barr virus and the pathogenesis of lymphoma. J Pathol 2015;235:312-322

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Type 4

Other infectious agent-associated lymphomas

(H. pylori, HTLV-1, Hep B, Hep C, and others)

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD): Ocampo, Fernando A, MD; Ramos, Josean, MD; Ward, Nicholas, MD

Affiliation: Rush University Medical Center

E-mail: nicholas_d_ward@rush.edu

Clinical History:

A 47 year-old male with no significant past medical history was transferred from an outside institution with complaints of right sided chest pain, fevers (102°F) and severe neutropenia (absolute neutrophil value: 0.14 k/uL). Initial whole blood count showed a hemoglobin level of 13.9 g/dL, a white blood count of 0.81 K/uL (14% neutrophils, 3% metamyelocytes, 1 % myelocytes, 72% lymphocytes, 1% basophils and 9% monocytes) and a platelet count of 217,000/mm3. The initial peripheral smear was found to have leukopenia with many large, reactive appearing lymphocytes with occasional fine granules and increased large granular lymphocytes, suggestive of viral or other infectious reaction.

The following lab values were elevated: CRP (42.3 mg/dL), ferritin 28,518 (ng/dL), AST 60 mg/dL, ALT 44 mg/dL, glucose 125 mg/dL. Normal values: fibrinogen (388 mg/dL), TGL 74 (mg/dL), total cholesterol (149 mg/dL).

Bone marrow biopsy performed on the day of admission showed slightly hypocellular bone marrow (30% cellularity) with decreased myelopoiesis, adequate erythropoiesis and megakaryocytes, increased macrophages with erythrophagocytosis, and increased T-cells (CD3+ and CD8+). Concurrent flow cytometry showed a majority of lymphocytes were CD8+ and were much larger than CD4+ by forward scatter; they expressed TCR alpha/beta and retained pan-T-cell marker expression. TCR rearrangement was negative.

Serologies for HIV and hepatitis virus were negative. CT of chest/abdomen/pelvis was performed and showed some retroperitoneal and mesenteric lymphadenopathy as well as multiple bilateral pulmonary nodules. A respiratory pathogen panel identified Influenza B nucleic acid. Blood and urine cultures were negative after 5 days of incubation.

After 2 days of supportive measures and oseltamivir, patient's WBC rebounded to 10.6, but ANC remained low; after administration of Neupogen, ANC rebounded to 3,190. EBV DNA by PCR was performed at this time and was minimally positive (454 copies/mL). A tentative diagnosis at this time was made of acute myelosuppression secondary to viral infection (influenza B).

Patient was seen after 5 months with normal serum CRP and ferritin levels, negative EBV PCR, CBC with complete resolution and a control bone marrow biopsy showed a normocellular marrow (40% cellularity) with trilineage hematopoiesis, no increase in blasts, overt dysplasia or abnormal infiltrate identified. TCR rearrangement was again negative.

Biopsy Fixation Details:

Bone marrow biopsy cases submitted in Z5 fixative.

Description of Clinical Image if Any:

N/A

Details of Microscopic Findings:

Initial bone marrow biopsy: Slightly hypocellular bone marrow (30% cellularity) with markedly decreased myelopoiesis, adequate erythropoiesis and megakaryocytes and increased macrophages featuring erythrophagocytosis. No lymphoid aggregates are seen.

Recovery bone marrow biopsy: normocellular marrow (40% cellularity) with trilineage hematopoiesis, no increase in blasts, overt dysplasia or abnormal infiltrate identified. TCR rearrangement was negative. MISSING CD3

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

Initial bone marrow biopsy: Increased, scattered interstitially located T-cells (CD3+) that appear small and mature and show predominance of the CD8 subset when compared to CD4. EBER-ISH is negative. E-cadherin reveals appropriate erythroid precursors. MPO demonstrates decreased myeloid precursors. CD2, CD7 and CD5 highlight all the T-cells. CD30 is essentially negative. CD68 highlights increased numbers of macrophages with intracytoplasmic debris and rare red cells and erythroid precursors.

Flow cytometry: The majority of the lymphocytes (35%) of the total cells are CD8+ T cells that are much larger than the CD4+ T cells. They express TCR alpha/beta and retain pan-T cell marker expression.

Recovery bone marrow biopsy: Scattered small and mature appearing non-increased interstitially located T-cells are highlighted by CD3.

Flow cytometry: No diagnostic abnormalities identified. Approximately 7% of total cells are medium sized CD4/CD8-double negative T cells that express bright CD3 CD5 CD7 CD2 and TCR gamma delta. They do not express CD56 CD57 CD30 or TCR-alpha beta consistent with gamma-delta T-cells.

Special Stains:

Initial bone marrow biopsy: Minimal reticulin fibrosis (MF 0-1), iron stores present on aspirate with no ringed sideroblasts identified.

Recovery bone marrow biopsy: Minimal reticulin fibrosis (MF 0-1), iron stores present on aspirate with no ringed sideroblasts identified.

Cytogenetics:

Normal male karyotype (46, XY) at both time of admission and recovery bone marrow

Molecular Analysis:

T-Cell Receptor Gamma Gene Rearrangement Study: No clonal T-cell receptor gene rearrangement product was detected at time of initial marrow or recovery marrow sampling.

Interesting Feature(s) of Submitted Case:

The approach to an isolated neutropenia involves a consideration of a broad diagnostic differential including etiologies such as infectious, nutritional, medication related, immune, ethnic variation, and secondary to neoplasms, endocrinopathies and others. Particular to our case, large granular lymphocytosis and other T-cell lymphoproliferative entities were considered, but the lack of overt morphologic atypia, immunophenotypic aberrancy and no clonal T-cell receptor gene rearrangement essentially excluded these as possibilities. Influenza virus have been related to hemophagocytic lymphohistiocytosis (HLH) in some cases, however this patient did not meet clinical criteria for HLH i.e., no splenomegaly, normal triglycerides and fibrinogen and only a single lineage cytopenia (neutropenia). Overall, our case highlights the pertinent histologic, laboratory and clinical findings that can accompany an acute viral infection with influenza and the appropriate work-up to differentiate this from a true neoplastic lymphoproliferative disorder.

Proposed Diagnosis:

Acute myelosuppression secondary to viral infection (influenza B) with reactive lymphoproliferation.

Comments:

N/A

CSHP/SH Workshop 2019

Fernando A. Ocampo
Josean Ramos
Nicholas D. Ward

1

Clinical History

- 47 year old male
- Transferred from outside hospital with complaint of right chest pain and WBC of 0.81 (absolute neutrophil count: 0.14)

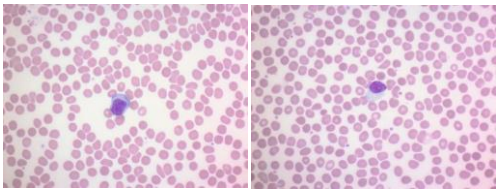
2

Laboratories

- Initial CBC: Hb 13.9, WBC 0.81 (14% PMN, 72% lymph, 9% mono, 3% metamyelocytes, 1% myelocytes and 1 % basophils), PLT 217k
- Peripheral smear: leukopenia with large granular lymphocytes; neutropenia with toxic granulation, no significant blasts.
- Elevated lab values: CRP (42.3 mg/dL), ferritin 28,518 (ng/dL), AST 60 mg/dL, ALT 44 mg/dL, glucose 125 mg/dL.
- Normal values: Fibrinogen (388 mg/dL), TGL 74 (mg/dL), total cholesterol (149 mg/dL)

3

PERIPHERAL BLOOD SMEAR



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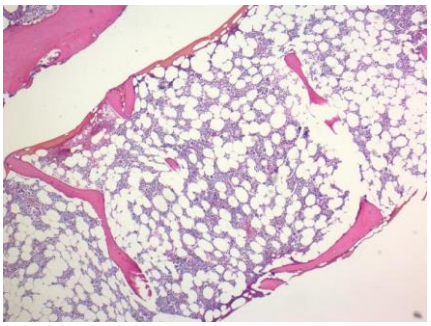
Radiology
CT of chest/abdomen/pelvis

- Retroperitoneal and mesenteric lymphadenopathy, as well as multiple bilateral pulmonary nodules.

5

BONE MARROW CORE BIOPSY



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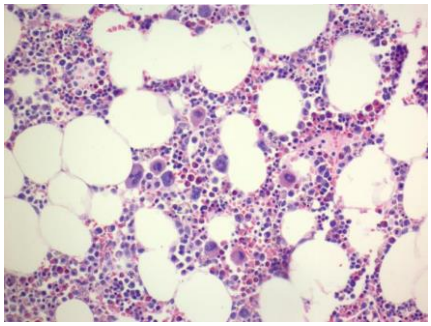
BONE MARROW CORE BIOPSY



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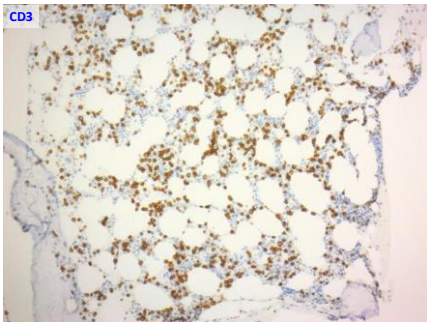

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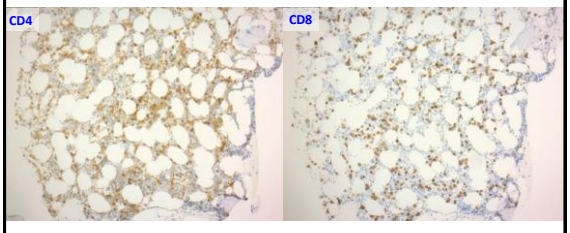

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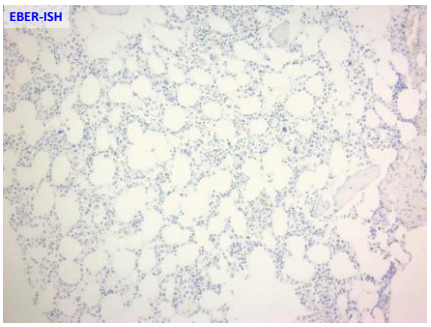

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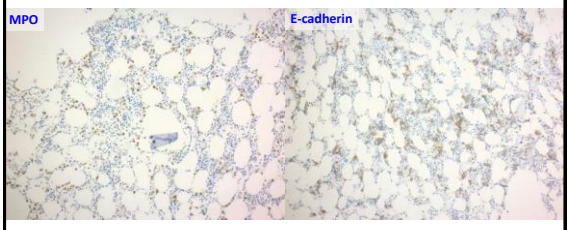

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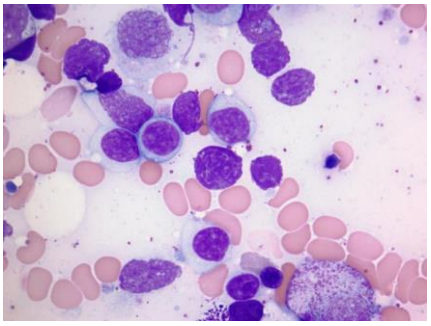
ASPIRATE SMEAR



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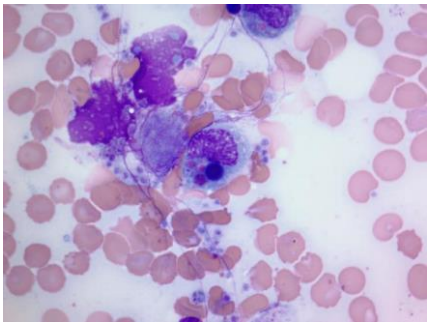

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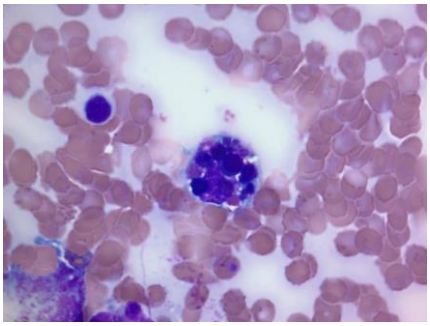

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Flow cytometry

Concurrent flow cytometry showed a majority of lymphocytes were CD8+ (CD4/CD8 ratio 0.32) and were much larger than CD4+ ones by forward scatter, they expressed TCR alpha/beta and retained pan-T-cell marker expression.

15

Additional Laboratory Studies

- Serology for HIV and hepatitis virus negative
- Respiratory pathogen PCR panel **positive** for **Influenza B**
- Blood and urine cultures negative after 5 days.
- Autoimmune panel negative

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Hospital course

- After 2 days with supportive measures and oseltamivir patient still with persistent neutropenia; post-Neupogen, ANC increased to 3.1.
- **EBV** DNA by PCR performed, minimally positive (454 copies/mL).
- **TCR rearrangement PCR** sent out; negative on follow-up 3 weeks later.

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Follow-Up (5 months)

- Normal ferritin, fibrinogen, negative EBV PCR and CBC with complete resolution.
- Bone marrow biopsy normocellular with trilineage hematopoiesis, with no morphologic or immunophenotypic findings concerning for malignancy.
- Molecular testing for TCR rearrangement was again negative.

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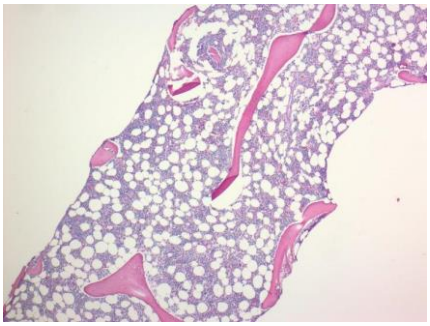
RECOVERY BONE MARROW BIOPSY



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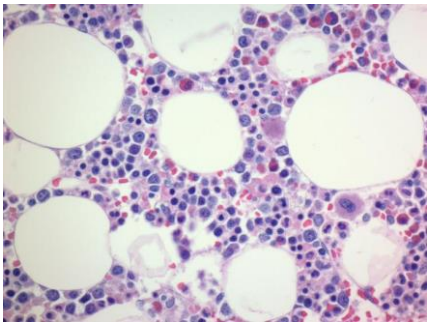
RECOVERY BONE MARROW BIOPSY



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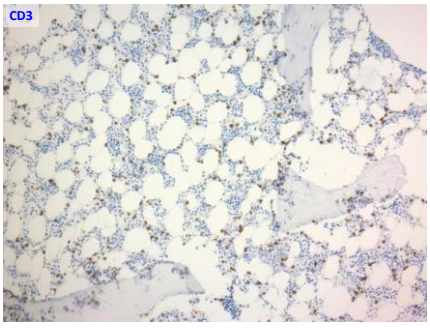
CD3



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Diagnosis

- Acute myelosuppression secondary to influenza infection with reactive lymphoproliferation

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CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Sophie Song, MD PhD

Affiliation:
Dept. of Pathology and Laboratory Medicine, UCLA Medical Center, Los Angeles, CA 90095-1732, U.S.A.

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ssong@mednet.ucla.edu

Clinical History:
52 year-old woman with no significant past medical history, presents to the Emergency Department at UCLA for two month history of progressive bilateral neck swelling, nasal airway obstruction, dysphagia, and dyspnea. The patient reports that she developed a cough and sore throat about two months ago, which were treated with antibiotics. She noted not much improvement, and developed additional symptoms, including progressive cervical lymphadenopathy and dysphagia. The patient reports about a 5-10 lb weight loss due to inability to eat, as well as sweats.

Social history reveals that the patient is from Jamaica, and has lived in the USA for 3 years, first in Maryland and now in Los Angeles. There is no other travel history.

Physical exam is remarkable for bulky bilateral cervical lymphadenopathy and palpable left axillary lymphadenopathy, with firm, non-mobile lymph nodes. In addition, there is shotty inguinal lymphadenopathy. There are no skin lesions and no hepatosplenomegaly.

Imaging studies reveal significantly enlarged bilateral high paratracheal, and cervical lymph nodes involving multiple stations, with the largest lymph node on the left side (level 5B) measuring 42 x 45 mm with necrotic center. In addition, there is left axillary lymphadenopathy and enlargement of the adenoids and tonsils is noted. Impression is suspicious for neoplasm or infection.

Laboratory studies show mild anemia (Hgb 11.3 g/dL) and mild thrombocytosis (Plt 424 x 10E3/uL). Ca is normal (9.4 mg/dL), LDH is elevated at 1,241 U/L. QuantiFERON-TB Gold Plus Elisa test is positive. All other infectious disease testing is negative.

Lymph node biopsy is performed and testing for HTLV I/II is pending at the time of the biopsy.

Biopsy Fixation Details:
Formalin and B5.

Description of Clinical Image if Any:
N/A.

Details of Microscopic Findings:
Histologic sections and touch preparations of the lymph node show a diffuse lymphomatous infiltrate, which is comprised of large pleomorphic lymphocytes with irregular to highly pleomorphic and often lobated nuclei, vesicular chromatin, prominent nucleoli, and moderate amounts of cytoplasm. Apoptotic bodies and mitoses are frequent. There are areas of necrosis.

Review of peripheral blood smear shows no atypical lymphocytosis.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
Limited flow cytometric studies of the lymph node are performed due to low quantity of recoverable cells. Findings indicate CD4+ mature T-cell neoplasm with predominantly large cells. The large neoplastic T-cells are positive for CD2, CD3, CD5 (bright), and CD45 (moderate to bright), with complete loss of CD7. No monotypic B-cells are detected.

Flow cytometric studies of peripheral blood reveal no support for lymphomatous involvement.

Immunochemical studies highlight lymphoma cells positive for CD2 (dim and moderate), CD3, CD4, CD5 (bright), CD25 (bright), CD52, BetaF1, and PD1, with complete loss of CD7 expression. The Ki67 proliferation index is about 80%. Staining for CD30 highlights occasional few neoplastic lymphocytes (about 5%). The lymphoma is negative for CD1a, CD8, CD10, CD20, CD21, CD23, CD56, CD57, ALK, EMA, TIA1, Granzyme B, Perforin, TCL1, BCL6, CXCL13, FOXP3, and TdT.

In-situ hybridization for EBV-EBER is negative.

Special Stains:
Acid fast stain is negative for microorganisms.

Cytogenetics:
N/A.

Molecular Analysis:
N/A.

Interesting Feature(s) of Submitted Case:

- The patient is recently immigrated to the US from Jamaica, where HTLV-1 infection is endemic;
- Unique clinical presentation of ATLL, lymphomatous variant

Proposed Diagnosis:
Adult T-cell leukemia/lymphoma, lymphomatous variant

Comments:

Joint CSHP and SH Workshop 2019

Case Two

Sophie Song, MD PhD

Dept. of Pathology and Laboratory Medicine
UCLA Medical Center
Los Angeles, CA, USA

1

Clinical Presentation

- 52 year-old woman with no significant PMH, presents to ED for two month history of progressive bilateral neck swelling, nasal airway obstruction, dysphagia, and dyspnea.
- Social history
  - recent immigrant from Jamaica
  - lived in the US for 3 yrs in Maryland and Los Angeles
  - no other travels
- Physical exam
  - bulky bilateral cervical lymphadenopathy
  - palpable left axillary lymphadenopathy
  - shotty inguinal lymphadenopathy
  - no skin lesions and no hepatosplenomegaly

2

Clinical Presentation

- Laboratory testing

Hgb	11.3 g/dL
Platelets	424 x $10^3/\mu L$
Calcium	9.4 mg/dL
LDH	1,241 U/L

- Infectious disease work-ups
  - QuantiFERON-TB Gold Plus ELISA is positive;
  - Pending HTLV I/II;
  - All other work-ups are negative

3

Imaging Studies

- CT whole body, with or without contrast:
  - significantly enlarged bilateral high paratracheal and cervical lymph nodes involving multiple stations, with the largest lymph node on the left side (level 5B) measuring 42 x 45 mm with necrotic center
  - left axillary lymphadenopathy
  - enlargement of the adenoids and tonsils

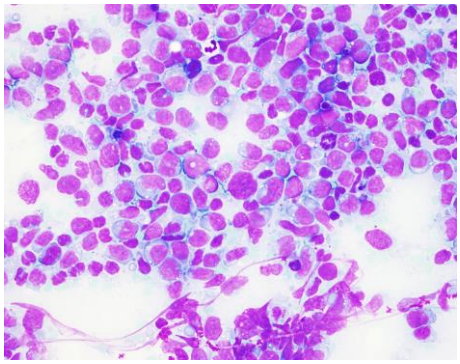
4

Specimens

- Left cervical lymph node biopsy
- Peripheral blood review

5

LN Biopsy (Touch Prep)



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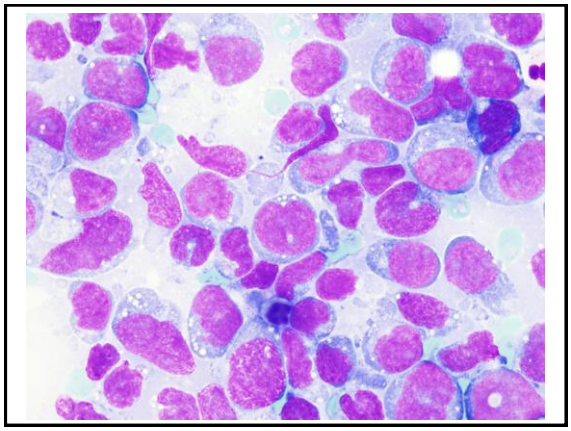

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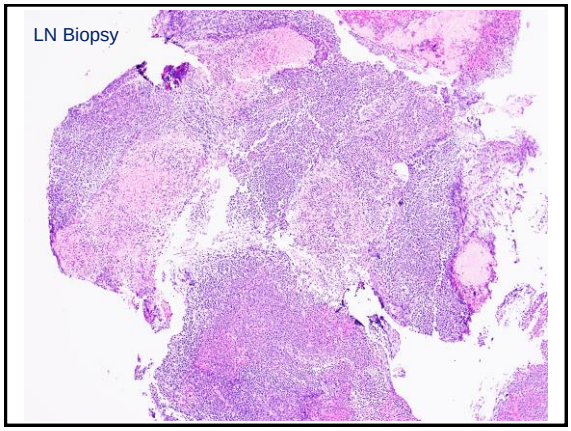

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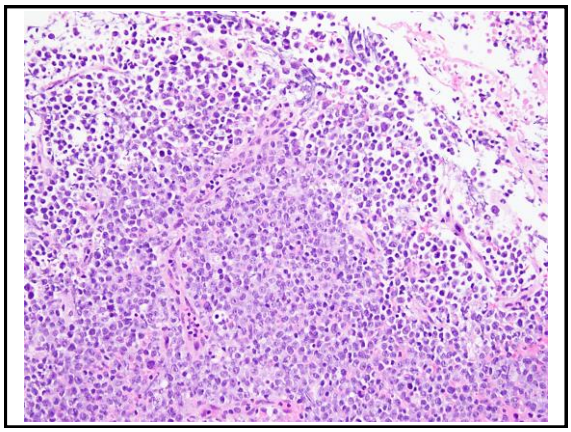

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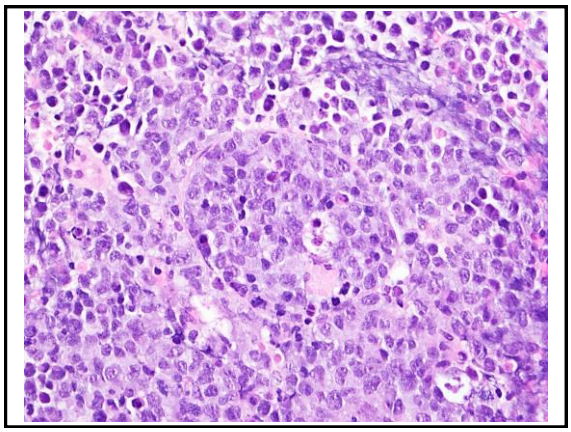

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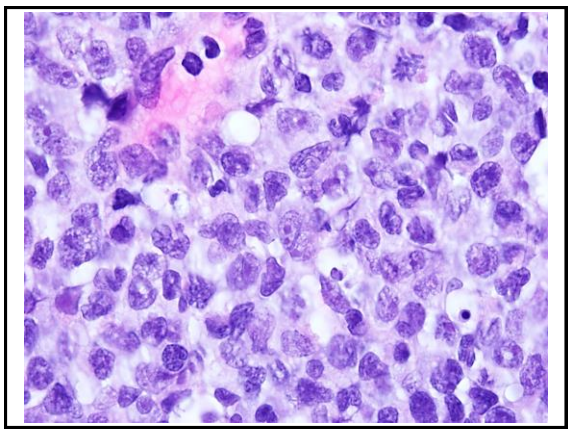

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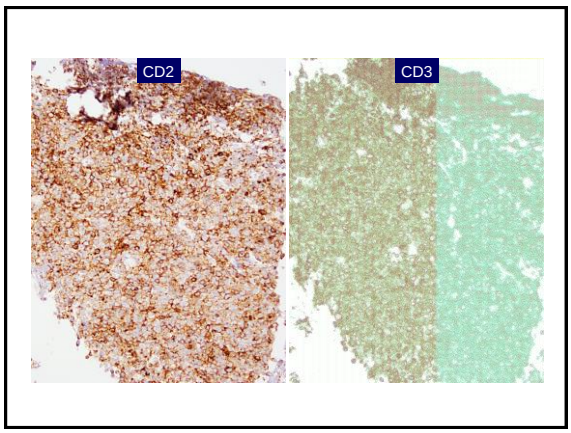

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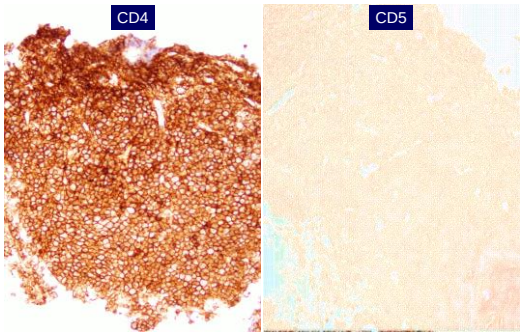

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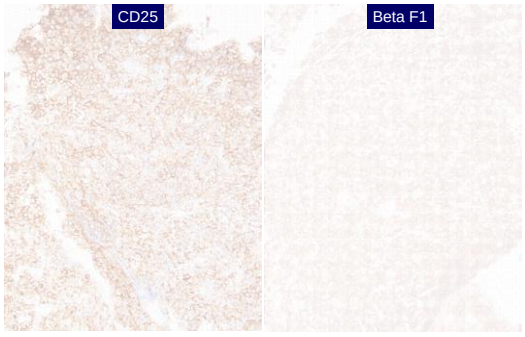

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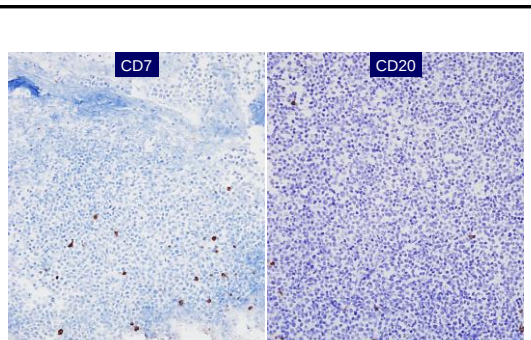

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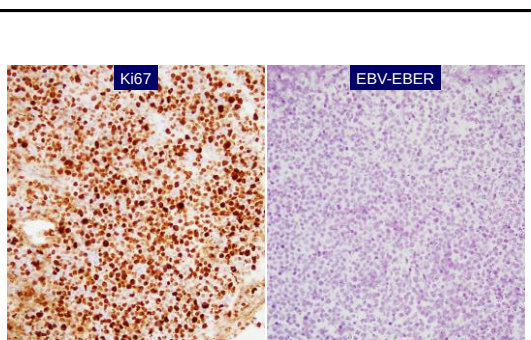

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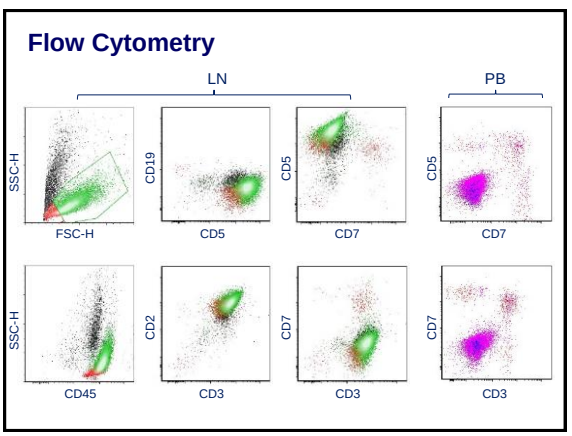


18

HTLV I/II Results

- HTLV-1 is detected by qualitative DNA test using real-time PCR

19

Proposed Diagnosis

Adult T-cell leukemia/lymphoma, lymphomatous variant

20

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):
Dachuan Zhang MD,PhD

Affiliation:
The Third Affiliated Hospital of Soochow University

E-mail:
zhangdachuan@suda.edu.cn

Clinical History:
cervical lymphaden swelling and fever

Biopsy Fixation Details:
Fixed in the neutral formalin liquid

Description of Clinical Image if Any:
No

Details of Microscopic Findings:
1. Lymph nodes are normally structurally present.
2. necosis of lymph node.
3. There are plasma cells, eosinophils, and proliferating reticulocytes in the interfollicular area.
4. The lumen is narrowed by swelling of small vascular endothelial cells.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
CD3, CD5 were positived in the interfollicular area.
CD20, CD79a were positived in the follicular area.
CD21 was positived on the FDC.
CD68 was positived on the histocyte.
CD30 was positived on the immunoblast.
EBER was negatived.

Special Stains:
No

Cytogenetics:
No

Molecular Analysis:

No

Interesting Feature(s) of Submitted Case:
A case of toxoplasma lymphadenitis

Proposed Diagnosis:
Toxoplasma lymphadenitis

Comments:

常州市第一人民医院
The First People's Hospital of Changzhou
苏州大学附属第三医院
The Third Affiliated Hospital of Soochow University

A case of toxoplasma lymphadenitis

The third affiliated hospital of Soochow University

The first people' s hospital of Changzhou

Dachuan Zhang

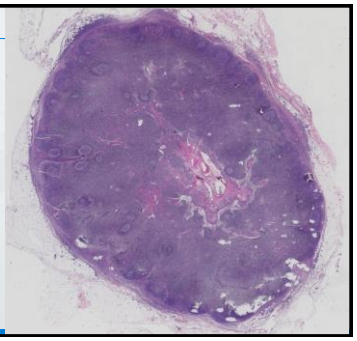
OUR DREAM FROM1918

1

H&E

- Huang**

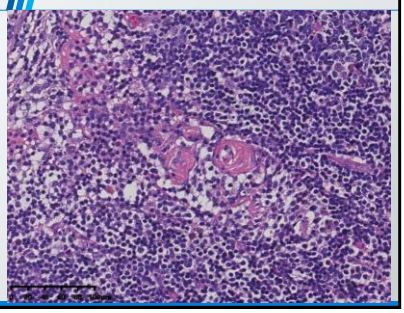
  Female, 55
  cervical lymph node swelling and fever



2

H&E

- The lumen is narrowed by swelling of small vascular endothelial cells.



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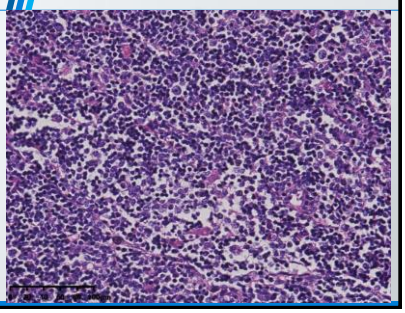
H&E

- plasmocyte



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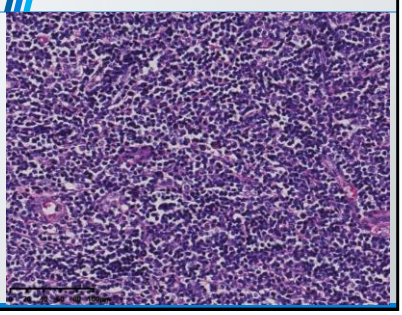
H&E

- Eosinophilic cell



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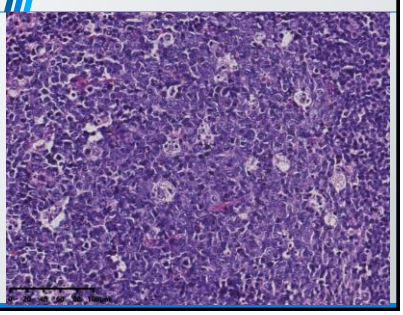
H&E

- necrosis



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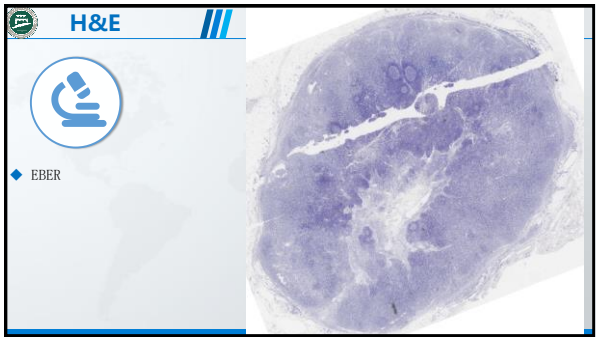

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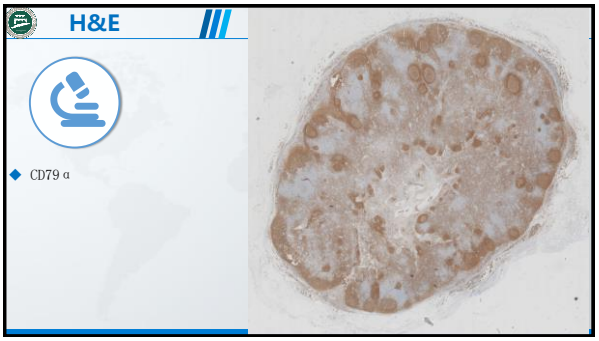

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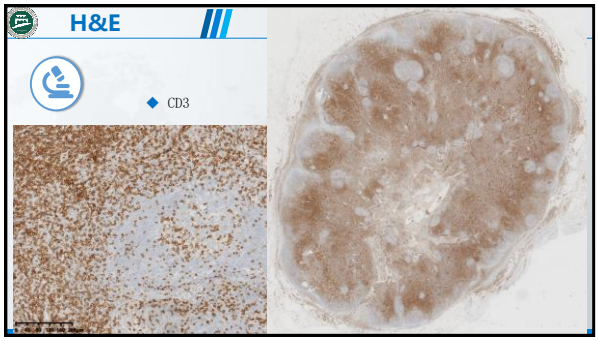

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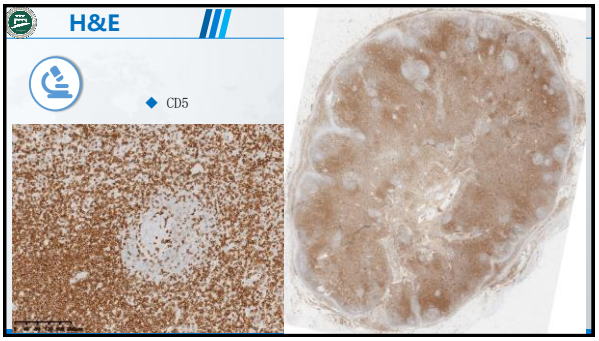

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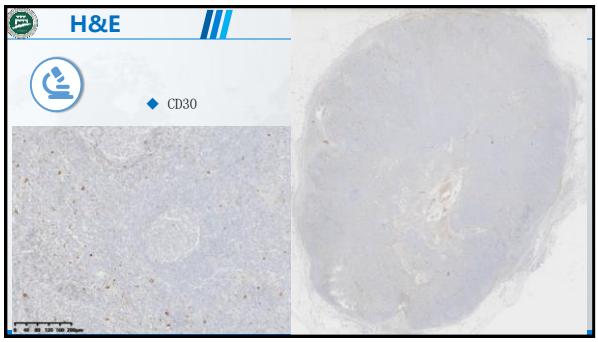

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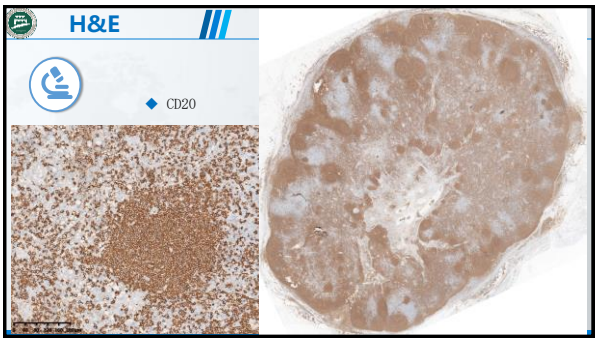

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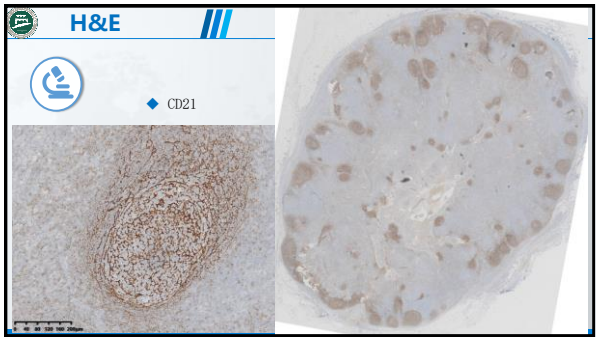
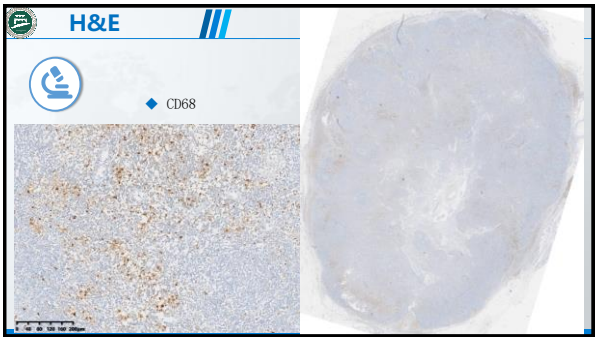

H&E
CD21

13


H&E
CD68

14


Thank You!
常州市第一人民医院
The First People's Hospital of Changzhou
苏州大学附属第三医院
The Third Affiliated Hospital of Soochow University

15

CASE SUBMISSION FORM

Submitter(s) and Titles (MD or MD PhD):

Yahya A. Al-Ghamdi, MD, Amy Chadburn, MD, Genevieve M. Crane, MD, PhD

Affiliation:

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Clinical History:

The patient is a 78 year old man with long history of untreated hepatitis C, which he contracted via a blood transfusion performed as part of hip replacement in 2008. He was found to have anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis in 2016 when he was found to have an elevated creatinine (9.5 mg/dL; normal 0.64 - 1.27 mg/dL) with a positive proteinase 3 IgG antibody (CANCA) and serum cryoglobulins. The vasculitis was treated with rituximab every 6 months with relative improvement of his creatinine (ranging from 1.6 to 2.5 mg/dL). He subsequently developed worsening anemia, thrombocytopenia and lymphadenopathy. A peripheral blood flow cytometry evaluation in 2016 showed no evidence of a lymphoproliferative process.

CBC 12/07/2018: WBC: 9.9 K/uL (Neut: 59.0 %, lymph: 35.5%, Mono: 4.0%, Eos: 1.0%, Baso: 0.5%), Hgb: 11.0 g/dL, Hct: 33.9%, MCV: 84.9 fL, Plt: 108 K/uL.

		Ref. range
Immunoglobulin G	2,304 (h)	610 - 1,616 mg/dl
Immunoglobulin A	684 (h)	85 - 499 mg/dl
Immunoglobulin M	56	35 - 242 mg/dl
Kappa, free light chain	15.42 (h)	0.33 - 1.94 mg/dl
Lambda, free light chain	13.12 (h)	0.57 - 2.63 mg/dl
Kappa/Lambda FLC ratio	1.18	0.26 - 1.65

Urine immunofixation at an outside facility reportedly showed monoclonal kappa immunoglobulin, but was not quantified.

HCV quantitative PCR (2016)	Not detected
Hepatitis C Antibody	Reactive

Biopsy Fixation Details:

Bone marrow trephine biopsy Bouin's fixative. All other specimens 10% formalin.

Description of Clinical Image if Any:

Not applicable

Details of Microscopic Findings:

Bone marrow clot section and biopsy (2018): The bone marrow is hypercellular and contained multiple lymphoid aggregates. There is a background of active trilineage hematopoiesis. Megakaryocytes are occasionally seen in small clusters but are overall unremarkable.

Bone marrow aspirate smears/touch preps:

BONE MARROW ASPIRATE SMEAR DIFFERENTIAL CELL COUNT: 500 CELLS

TYPE	%	TYPE	%
BLAST	2	PRONORMOBLAST	1
PROMYELOCYTE	5	NORMOBLAST	14
MYELOCYTE	14	MONOCYTE	3
METAMYELOCYTE	10	LYMPHOCYTE	8
BAND / NEUTROPHIL	25	PLASMA CELLS	10
EOSINOPHIL	7	OTHERS	
BASOPHIL	1	M:E RATIO	4.3

COMMENTS: The bone marrow aspirate is hypercellular for the patient's age. Megakaryocytes are present, clustered in the spicules and dysplastic (>10%), and include forms that are multinucleated and/or hyperlobulated. Full maturation of the myeloid and erythroid lineages is present with an increase in the myeloid lineage. An increase in blasts or evidence of significant dysplasia is not seen. Occasional erythroid cells with megaloblastoid features are noted (<10%). A plasmacytosis with cytologic atypia is noted. Occasional Mott cells are seen. The residual cellular elements present are composed primarily of small lymphocytes and monocytes. Neither lymphoid aggregates nor atypical lymphoid cells are identified.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:

Immunohistochemistry: The lymphoid aggregates are predominantly positive for CD20, Pax5 and CD5 with a smaller number of CD3+ T cells. The atypical lymphoid cells express cyclin D1, which is absent from the plasma cells by a BCL1/CD138 double stain. The CD138+ plasma cells are polytypic based on cytoplasmic kappa and lambda light chain expression. Immunostaining for CD10 highlights scattered marrow elements and is equivocal in the lymphoid

aggregates. Immunostaining for Sox11 shows some background, but appears positive in at least a subset of the lymphoid cells within the aggregates. LEF1 is expressed by the background T cells.

Flow cytometry: The lymphocytes account for 28% of total viable events, of which 54% are CD19, CD20+ B-cells. The vast majority of B cells are surface kappa immunoglobulin light chain positive and are CD5+ (47%) with partial, dim CD10 expression. The CD2+ T-cells comprise 18% of total lymphocytes and show a CD4:CD8 of ~1. Only rare CD3 negative, CD7+ NK cells are present. The T-cells show no significant loss of pan-T cell antigens. The CD38 bright positive plasma cells comprise ~0.5% of total viable events and are polytypic.

Special Stains:

Reticulin stain shows no increase in bone marrow fibrosis. Iron stores are markedly increased with no ring sideroblasts seen.

Cytogenetics:

Karyotype: 45,X,-Y[8]/46,XY[12]

FISH analysis: CCND1-IGH gene rearrangements were identified in 10% of 200 interphase nuclei evaluated indicating the presence of a t(11;14) translocation. FISH did not detect TP53 gene deletion in this sample, but detected a heterozygous deletion of the 13q14 region in 8.5% of 200 interphase nuclei evaluated.

Molecular Analysis:

Molecular evaluation of the peripheral blood (12/21/2018) was performed at Genoptix (Carlsbad, CA) using their standard lymphoid panel.

Pathogenic alterations are DETECTED in the ATM (VAF 22%) and RB1 (VAF 9%) genes and a probable pathogenic alteration is DETECTED in the MEF2B gene (VAF 18%). Genomic alterations of uncertain significance are also DETECTED in the BCL2 (VAF 55%), DNMT3A (VAF 9%) and EZH2 (VAF 16%) genes. The specific mutations are:.

MEF2B (c.68A>G; p.K23R): Previously observed in six primary mantle cell lymphomas [Bea S, et al. 2013. Proc Natl Acad Sci 110:18250-18255), and is expected to be likely pathogenic.

ATM (c.6514_6522delACACTTAGCinsT; p.T2172*): Expected to be pathogenic.

BCL2 (c.95C>G; p.A32G): Uncertain clinical significance.

DNMT3A (c.2477A>G; p.K826R). Uncertain clinical significance.

EZH2 gene (c.2075C>T; p.A692V). Uncertain clinical significance.

RB1 (c.1654C>T; p.R552*). Expected to be pathogenic

Interesting Feature(s) of Submitted Case:

Mantle cell lymphoma in the setting of hepatitis C virus infection and ANCA-associated vasculitis

Proposed Diagnosis:

Mantle cell lymphoma.

Comments:

Several studies have established an association between Hepatitis C virus (HCV) infection and certain non-Hodgkin B cell lymphoma subtypes, particularly marginal zone lymphoma and diffuse large B-cell lymphoma[1, 2]. The range of B cell lymphomas reported in this setting is broad; however, the association between HCV infection and mantle cell lymphoma is less clear. The mechanism of lymphomagenesis in this setting is still unknown. One possibility is direct infection of B-cells and alteration of cell signaling[3]. Chronic immune stimulation rather than a direct role in the lymphoma cells has also been suggested. To this end, some bias in *IGHV* gene usage and stereotyped *HCDR3* sequences have been found in HCV-associated splenic marginal zone lymphomas suggesting the potential for an antigen-driven process[4]. Furthermore, reports have demonstrated the efficacy of antiviral therapies in the management of HCV induced lymphoma and mantle cell lymphoma in particular[5]. The lack of evidence of active HCV in this case, however, could argue against this as a potential strategy.

The patient's presentation of ANCA-associated vasculitis is also of interest. Chronic HCV has been associated with detection of ANCA in the serum, although this was not necessarily related to clinical symptoms[6]. ANCA-associated vasculitis has also been associated with an increased risk of malignancy including B cell lymphomas in one series[7].

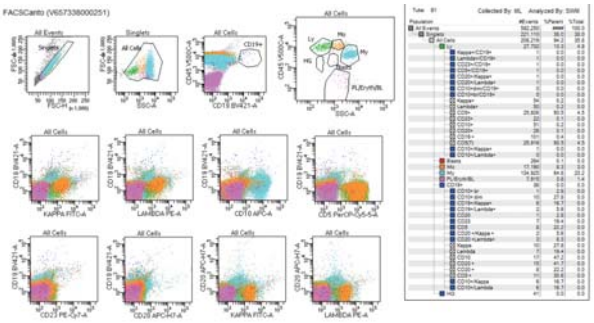
Patient follow up: The patient was treated with bendamustine and rituximab which was well tolerated, and he appears to have achieved complete remission after 6 cycles.

References:

1. de Sanjose, S., et al., *Hepatitis C and non-Hodgkin lymphoma among 4784 cases and 6269 controls from the International Lymphoma Epidemiology Consortium.* Clin Gastroenterol Hepatol, 2008. **6**(4): p. 451-8.
2. Peveling-Oberhag, J., et al., *Hepatitis C-associated B-cell non-Hodgkin lymphomas. Epidemiology, molecular signature and clinical management.* J Hepatol, 2013. **59**(1): p. 169-77.
3. Dai, B., et al., *Hepatitis C virus upregulates B-cell receptor signaling: a novel mechanism for HCV-associated B-cell lymphoproliferative disorders.* Oncogene, 2016. **35**(23): p. 2979-90.
4. Zibellini, S., et al., *Stereotyped patterns of B-cell receptor in splenic marginal zone lymphoma.* Haematologica, 2010. **95**(10): p. 1792-6.
5. Levine, A.M., S. Shimodaira, and M.M. Lai, *Treatment of HCV-related mantle-cell lymphoma with ribavirin and pegylated interferon Alfa.* N Engl J Med, 2003. **349**(21): p. 2078-9.
6. Cojocaru, M., I.M. Cojocaru, and S.A. Iacob, *Prevalence of anti-neutrophil cytoplasmic antibodies in patients with chronic hepatitis C infection associated mixed cryoglobulinemia.* Rom J Intern Med, 2006. **44**(4): p. 427-31.
7. Pankhurst, T., et al., *Malignancy is increased in ANCA-associated vasculitis.* Rheumatology (Oxford), 2004. **43**(12): p. 1532-5.

Mantle cell lymphoma, ANCA-associated vasculitis and longstanding hepatitis C

Peripheral blood flow evaluation in 2016: Rare CD19+ B cells without light chain restriction



Lymphoid Molecular Profile

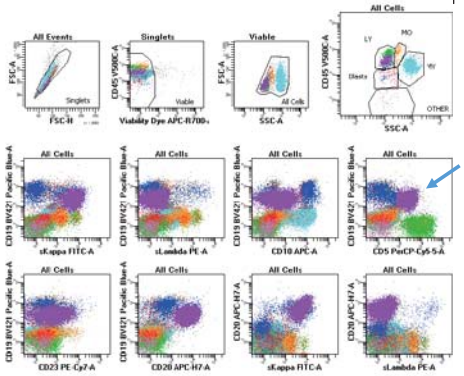
NGS RESULTS
Pathogenic alterations are DETECTED in the ATM and RB1 genes.
Likely pathogenic alteration is DETECTED in the MEF2B gene.
Genomic alterations of uncertain significance are DETECTED in the BCL2, DNMT3A and EZH2 genes.

Gene	Exons Tested	Genomic Alteration(s)	Mutation Effect	Allele Frequency	Pathogenic
ATM	ALL	c.6514_6522delACACTTAGCinsT; p.T 2172*	NONSENSE	22%	YES
BCL2	ALL	c.95C>G; p.A32G	MISSENSE	55%	UNCERTAIN
DNMT3A	8-23	c.2477A>G; p.K826R	MISSENSE	9%	UNCERTAIN
EZH2	ALL	c.2075C>T; p.A692V	MISSENSE	16%	UNCERTAIN
MEF2B	2-8	c.68A>G; p.K23R	MISSENSE	18%	LIKELY
RB1	ALL	c.1654C>T; p.R552*	NONSENSE	9%	YES

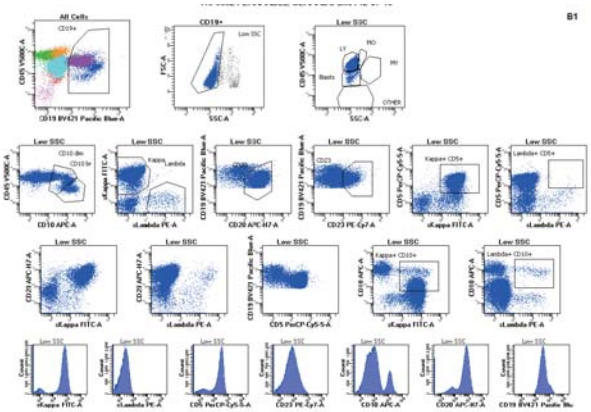
For a complete list of the genes analyzed by NGS, please refer to the assay summary in this report.

Lymphoid panel completed at Genoptix (peripheral blood 12/2018)

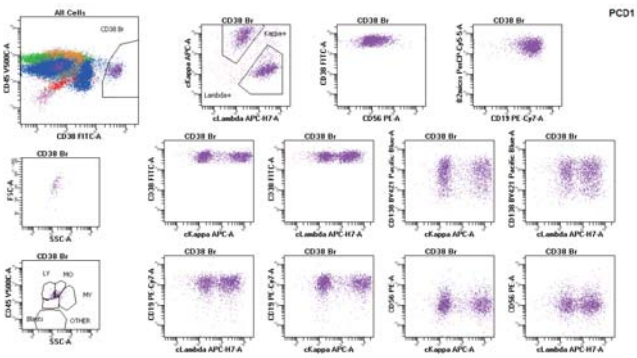
Flow cytometry bone marrow aspirate 12/2018



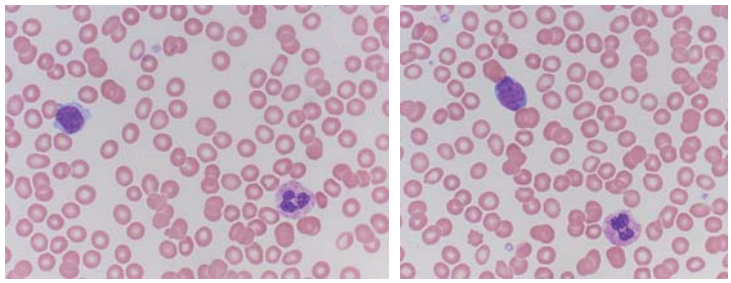
- CD5+, CD19 (dim) CD20+ kappa-restricted B cell population
- 47% of lymphoid cells
- 13% of all viable events



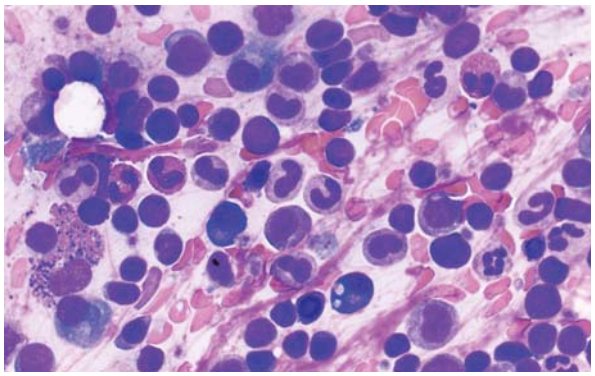
CD38 bright plasma cells are polytypic



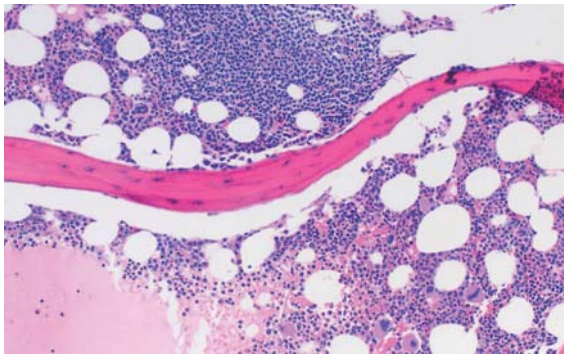
Circulating atypical lymphoid cells

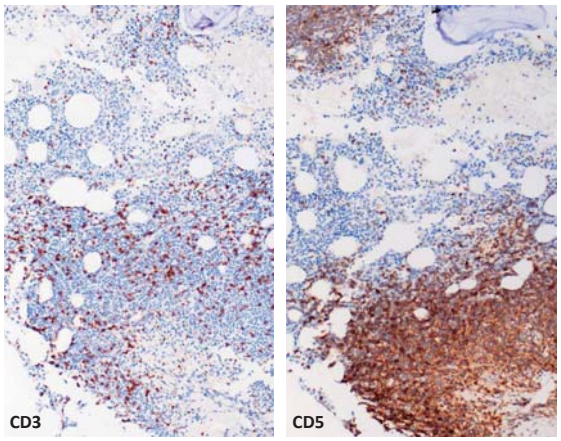


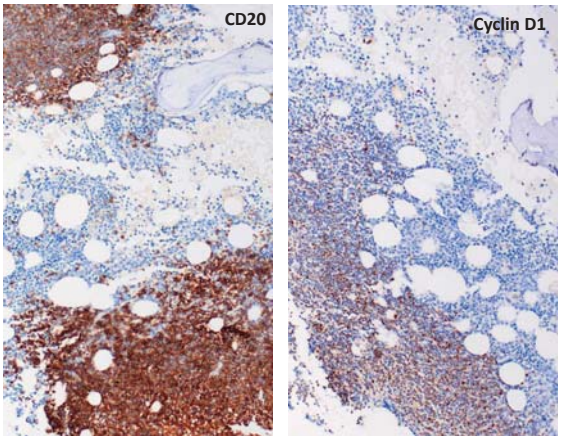
Bone marrow aspirate



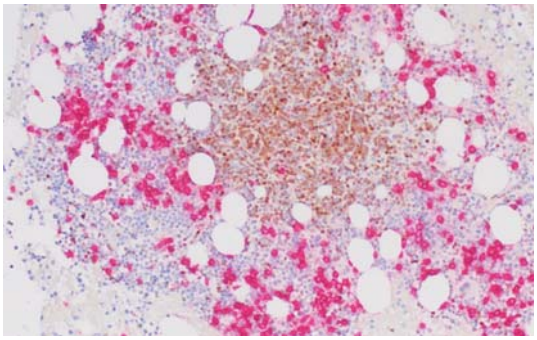
Trephine biopsy







Slight plasmacytosis, plasma cells are negative for cyclin D1 in contrast to the lymphoid aggregates



CASE SUBMISSION FORM

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Clinical History:
The patient is a 61 year old male who was incidentally found to have hepatitis C virus (HCV) infection five years prior to BM biopsy. The patient did not receive treatment at the time due to other co-morbidities. Two years prior to the BM biopsy, the patient developed petechial skin lesions, migratory arthralgias, and fatigue and workup identified mixed cryoglobulinemia (type II). He was treated symptomatically, but noted that his symptoms worsened during the winter months. One month before BM biopsy the patient was hospitalized due to dehydration and acute kidney injury. Hematology was consulted for neutropenia and an outpatient BM biopsy was performed after discharge.

The CBC at BM biopsy was WBC 2.91 x10^9/L (62% neutrophils, 25% lymphocytes, 9% monocytes, 3% eosinophils, 0% basophils, 1% plasma cells), hemoglobin 8.7 g/dL (88.3 fL), and platelet count 19 x10^9/L. Hepatitis C antibody was positive and reflex hepatitis C viral load was positive at 820,000 IU/mL. Genotyping studies showed the HCV infection was genotype 1a. Hepatitis B surface antigen, surface antibody, and core antibody were negative. Serum protein electrophoresis showed polyclonal hypergammaglobulinemia with no evidence of a monoclonal protein. Cryoglobulin was present at 818 µg/mL (RR: 0-50 µg/mL). Immunofixation of the cryoglobulin showed abnormal matching homogeneous bands in the IgM and kappa regions as well as polyclonal IgG. Rheumatoid factor activity was markedly elevated at 621 IU/mL (RR: <20 IU/mL). Overall, the findings were consistent with a mixed cryoglobulinemia (type II).

Biopsy Fixation Details:
The bone marrow aspirate was taken from the right posterior superior iliac crest. Bone marrow biopsies were performed bilaterally. The aspirate smears were air-dried and Wright-Giemsa stained. The biopsies and clot were submitted in zinc formalin and embedded in paraffin.

Description of Clinical Image if Any:
N/A

Details of Microscopic Findings:
The peripheral blood showed pancytopenia and a rare circulating plasma cell. Bone marrow aspirate smears were aspicular and hemodilute, but demonstrated increased lymphocytes (26%) and mature-appearing plasma cells (5%). There was trilineage maturation with no morphologic evidence of dysplasia. The bone marrow core biopsy was normocellular (50%) with trilineage hematopoiesis and no identifiable lymphoid aggregates.

Immunophenotyping by Immunohistochemistry and/ or Flow Cytometry:
Immunohistochemical stains CD3, CD20, CD138, and kappa and lambda light chains were performed on formalin fixed, paraffin-embedded tissue with appropriately staining controls. Plasma cells were increased (10%), but polytypic for kappa and lambda light chains. CD20 highlighted increased numbers of interstitial B-cells including small clusters of lymphocytes. CD3 highlighted scattered interstitial T-cells.
Flow cytometry performed on the BM aspirate showed that 25% of total events were lymphocytes with 48% of the lymphocytes representing monotypic B-cells expressing CD19, CD20 (bright), CD22 (dim), CD11c, CD45, FMC-7, CD79b, and kappa light chains. The B-cells were negative for CD5, CD10, CD25, CD103, and CD123.

Special Stains:
N/A

Cytogenetics:
Conventional cytogenetics were performed on the bone marrow and showed 46,XY[25].

Molecular Analysis:
N/A

Interesting Feature(s) of Submitted Case:
This patient presented with characteristic symptoms of cryoglobulinemia including purpuric skin lesions, arthralgias, and fatigue. Immunofixation of the cryoprecipitate demonstrated monoclonal IgM kappa and polyclonal IgG. Additionally, the rheumatoid factor activity was markedly elevated, which in combination with the immunofixation results, is diagnostic of type II mixed cryoglobulinemia. Over 90% of cases of type II mixed cryoglobulinemia are associated with current or antecedent HCV infection. In the rare HCV-negative cases, an autoimmune disorder (Sjögren's syndrome, systemic lupus erythematosus, or systemic sclerosis) or other infection (HBV) can be identified in most cases. Although generally thought of as a benign B-cell lymphoproliferative disorder, approximately 10% of patients with type II mixed cryoglobulinemia go on to develop overt lymphoma. These lymphomas consist predominately of marginal zone lymphoma and diffuse large B-cell lymphoma.

Although the bone marrow was morphologically unremarkable in this case, flow cytometry identified a kappa-restricted B-cell population that was negative for CD5 and CD10. Immunohistochemistry demonstrated polytypic plasmacytosis (10%) and increased B-cells with an interstitial distribution. On physical examination, there was no evidence of organomegaly or lymphadenopathy, but the patient was lost to follow-up before imaging studies were performed to asses for systemic disease.

In the absence of clinical or histologic features to suggest an overt lymphoma, this can be considered as an HCV-associated monoclonal B-cell lymphocytosis. If splenomegaly or lymphadenopathy was identified on imaging studies then tissue sampling would be warranted to evaluate for lymphoma.

Proposed Diagnosis:
Mixed cryoglobulinemia (type II)-associated B-cell lymphoproliferative disorder (HCV-associated monoclonal B-cell lymphocytosis)

Comments:

CSHP/SH

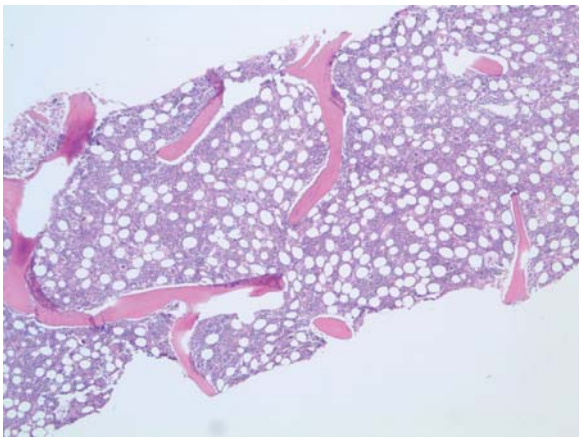
Daniel Socha MD
Eric Hsi MD

Peripheral Blood

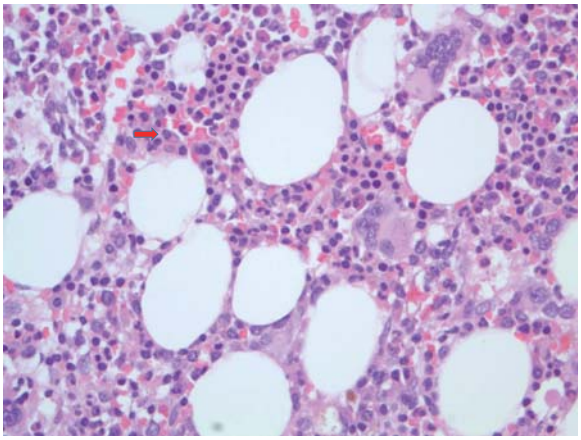
- CBC at BM biopsy
  - WBC: 2.91 x 10^9/L
    - 62% neutrophils
    - 25% lymphocytes
    - 9% monocytes
    - 3% eosinophils
    - 0% basophils
    - 1% plasma cells
  - Hemoglobin: 8.7 g/dL (88.3 fL)
  - Platelet count: 19 x 10^9/L

BM Biopsy

BM Biopsy (40x):
At low power the bone marrow is normocellular (50%) with no identifiable lymphoid aggregates.

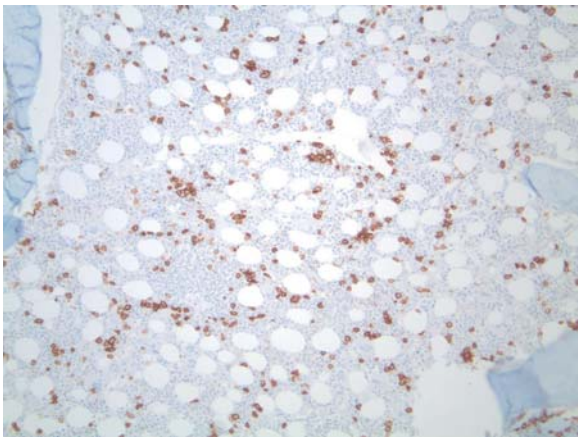


BM Biopsy (400x):
At high power the bone marrow shows maturing trilineage hematopoiesis. Scattered plasma cells are present (arrows).



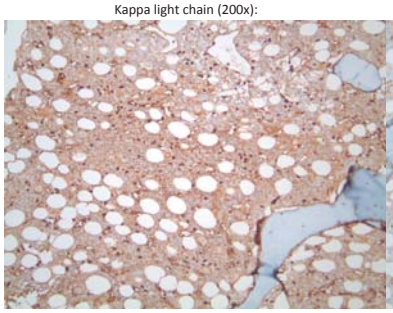
BM Biopsy IHC

CD138 (200x):
CD138 highlights increased plasma cells (10%) with perivascular clusters.

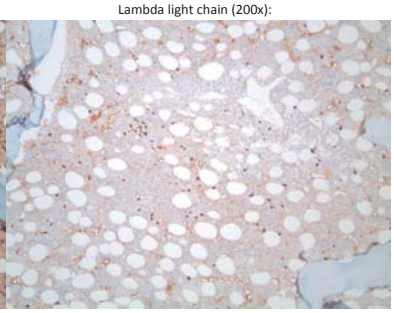


Kappa and lambda light chains highlight increased, but polytypic plasma cells.

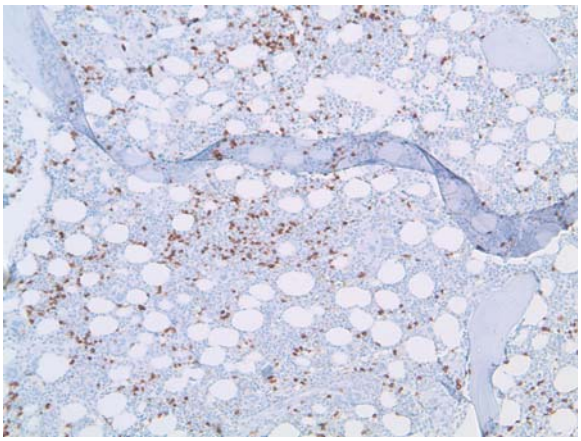
Kappa light chain (200x):



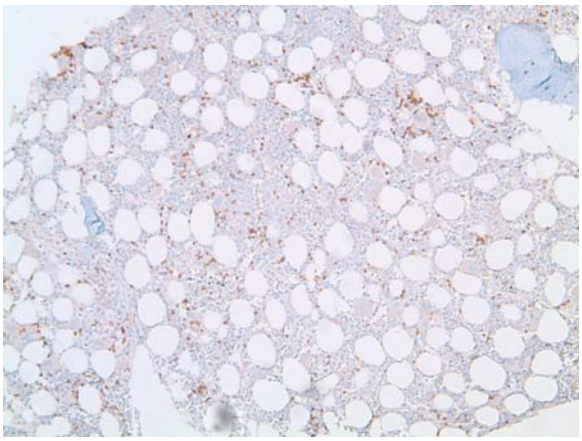
Lambda light chain (200x):



CD20 (200x):
CD20 highlights increased interstitial B-cells with occasional clusters of lymphocytes.

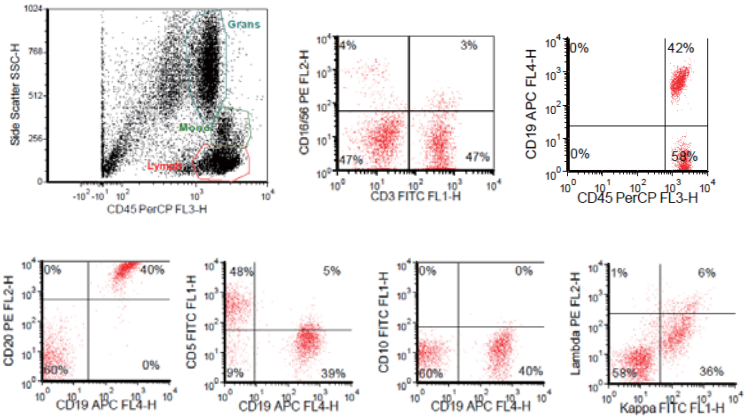


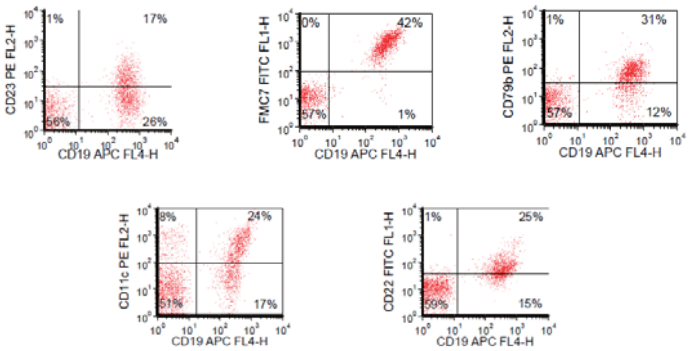
CD3 (200x):
CD3 highlights scattered interstitial T-cells.



Flow Cytometry

- Lymphocytes (25% of total events):
  - 45% T-cells (CD4:CD8 ratio: 1.2)
  - 3% NK cells
  - 48% monotypic B-cells
    - Positive: CD19, CD20 (bright), CD22 (dim), CD11c, CD45, FMC-7, CD79b, and kappa light chains
    - Negative: CD5, CD10, CD25, CD103, and CD123





Conventional Cytogenetics

- 46,XY[25]
  - -Normal male karyotype