Chronic Active Epstein-Barr Virus Infection of T/NK-Cell Type Mimicking Classic Hodgkin Lymphoma

Clinicopathologic and Genetic Features of 8 Cases Supporting a Variant With "Hodgkin/Reed-Sternberg-like" Cells of NK Phenotype

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EBV感染的疾病状态

急性感染（反应性）	慢性活动性感染（增殖性疾病）	淋巴瘤（肿瘤性）
传单	老年性EBV+LPD	Burkitt
EBV淋巴结炎	淋巴瘤样肉芽肿	HL
EBV咽/鼻炎	EBV+LPD:	NK/T淋巴瘤
	(CAEBV-B细胞型)	ANKL
	(CAEBV-T/NK细胞型)	等等
	(种痘样水疱病/淋巴瘤)	
	(蚊叮超敏反应)	

参考周小鸽教授课件

EBV+淋巴增殖性疾病临床生物学特征

疾病	细胞种类 克隆性	发病年龄	流行病学特征	临床特征	相关疾病和说明
CAEBV B细胞型	B细胞 多/单克隆	儿童 年轻人	罕见，多见于西方国家	发烧，全身症状伴器官受累：肺炎，葡萄膜炎，肝炎，脾淋巴结大，Ig低	慢性/持续性传单伴器官受累
EBV+LBCL (老年EBV LPD)	B细胞 单克隆	成人，>60岁	无种族地域差异	常见结外：皮肤，消化道，肺。侵袭过程	中年以上EBV LPD
淋巴瘤样肉芽肿	B细胞 寡/单克隆	成人 中位~40岁	多见于西方	结外。主要在肺，也可在胃，肝，CNS,皮肤	也可伴发免疫缺陷病
CAEBV T/NK细胞型 (含下面特殊疾病)	T或NK细胞 单->寡/多克隆	儿童 少数年轻人	亚洲人 中南美洲土著美洲人	发烧，肝脾LN大，血细胞减少，也有水疱病，蚊叮超敏，CSEBV-T-LPD	NK细胞预后好于T细胞
种痘样水疱病 种痘水疱病(HV)样淋巴瘤	T细胞 寡/多克隆	儿童 少数年轻人	同上	丘疱疹伴溃疡 成年可消退或进展为CSEBV-T-LPD	部分严重HV可能是单克隆，与种痘水疱病(HV)样淋巴瘤有重叠
蚊叮超敏反应	NK细胞 克隆性未定	儿童 少数年轻人	同上	蚊叮超敏反应伴溃疡坏死	较HV更惰性
儿童系统性EBV+T细胞淋巴增殖性疾病(CSEBV-T-LPD)	T细胞 单克隆	儿童 少数年轻人	同上	发烧，肝脾淋巴结肿大，HPS, DIC,肝衰。侵袭过程	严重CAEBV~75% SCAEBV是单克隆与CSCAEBV有重叠

CAEBV=慢性活动性EBV感染；LPD=淋巴增殖性疾病；HPS=噬血细胞综合征； Ann Oncol. 2009;20(9):1472-82.

参考周小鸽教授课件

形态特征

- 形态学表现谱系较广
- 根据淋巴结结构破坏程度和细胞异型性，将CAEBV分为三级：

  - I级：无破坏，无细胞异型性/轻微异型（非瘤）
  - II级：部分破坏，细胞轻/中度异型（交界）
  - III级：完全破坏，中/重度异型（肿瘤）

参考周小鸽教授课件

Chronic active Epstein-Barr virus (EBV) infection of T-cell and NK-cell type, systemic form (CAEBV-T/NK-S)

慢性活动性EBV感染（CAEBV）-T/NK细胞型

- 儿童及青少年多见，T/NK细胞型多见于亚洲人群；
- 病程超过3或6个月；
- 常表现为高热、肝脾肿大、淋巴结大等全身症状；
- 血清EBV抗体滴度增高或EBV DNA拷贝数（负荷）升高；
- 主要脏器受累的组织学证据，如淋巴结炎、持续性肝炎、脾大、间质性肺炎等，组织中EBV阳性的T/NK细胞数量增多；
- 排除其他疾病。

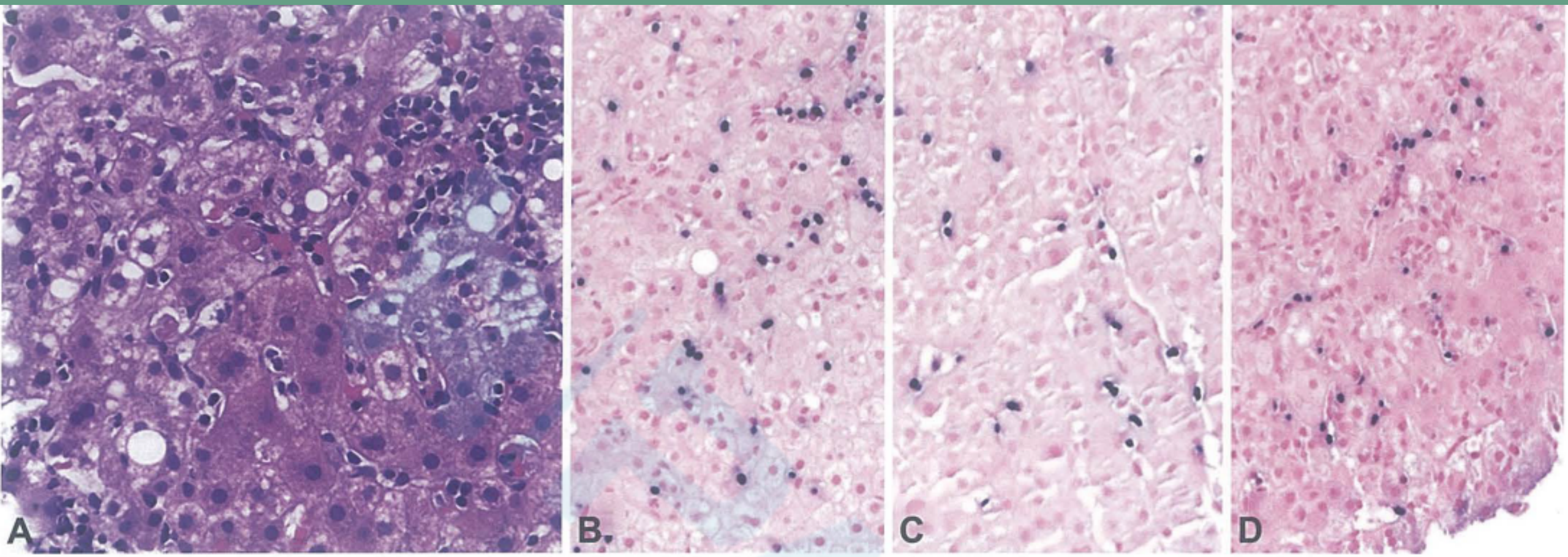


Fig. 14.22 Chronic active EBV infection of T-cell type in the liver. Sequential liver biopsies demonstrating stable disease without progression. **A** Liver biopsy shows single cell necrosis and a sinusoidal lymphocytic infiltrate. Lymphocytes (CD3+) do not show cytological atypia. **B**,**C**,**D** EBER in situ hybridization of sequential biopsies obtained over a period of four years shows no increase in EBER-positive cells over time.

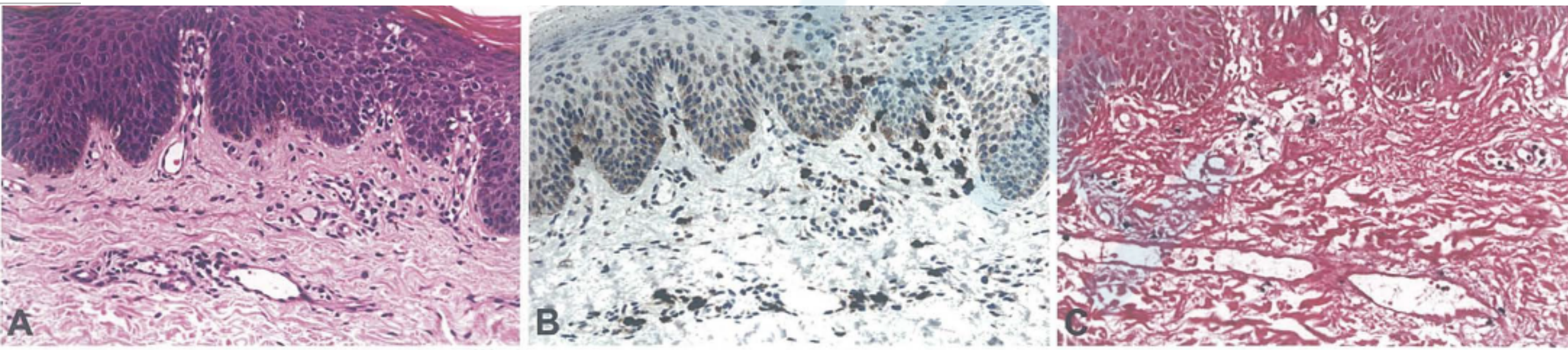


Fig. 14.23 Chronic active EBV infection in skin. **A** Skin biopsy shows a discrete lymphoid infiltrate without atypia in the dermis surrounding blood vessels, extending to the epidermis. **B** The lymphoid cells are positive for CD8. **C** The relatively discrete lymphoid infiltrate is positive for EBV as demonstrated by in situ hybridization for EBV-encoded small RNA (EBER).

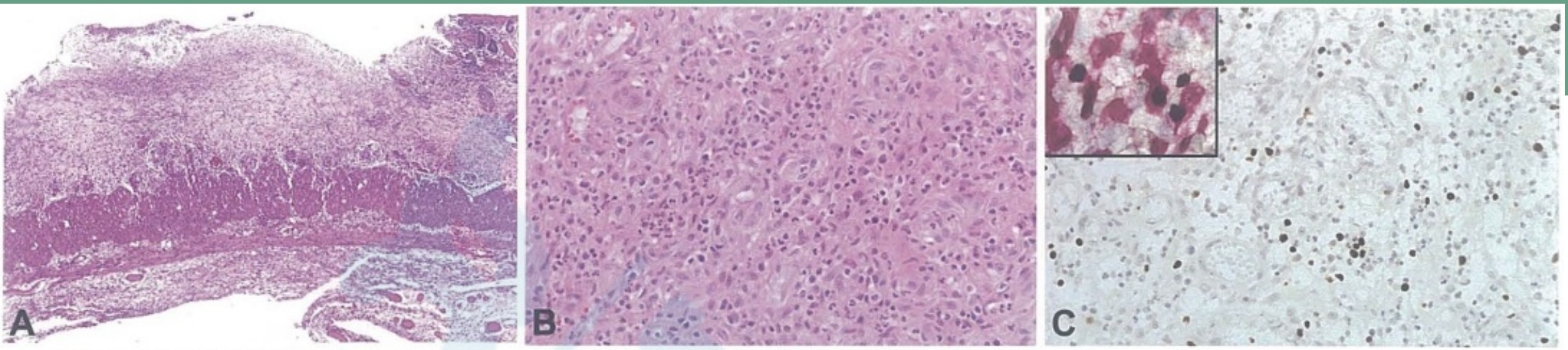


Fig. 14.24 Chronic active EBV infection of probable NK-cell type in the intestine of a 4-year-old girl with recurrent bowel perforation and NK-cell lymphocytosis. **A** Colon resection with ulceration of the mucosa. **B** The submucosa shows granulation tissue and a subtle lymphoid infiltrate without atypia. **C** In situ hybridization for EBV-encoded small RNA (EBER) shows scattered positive cells. Inset: Double staining shows that the EBER+ cells (brown) are CD3-positive (red). CD56 was positive in fewer cells (not shown).

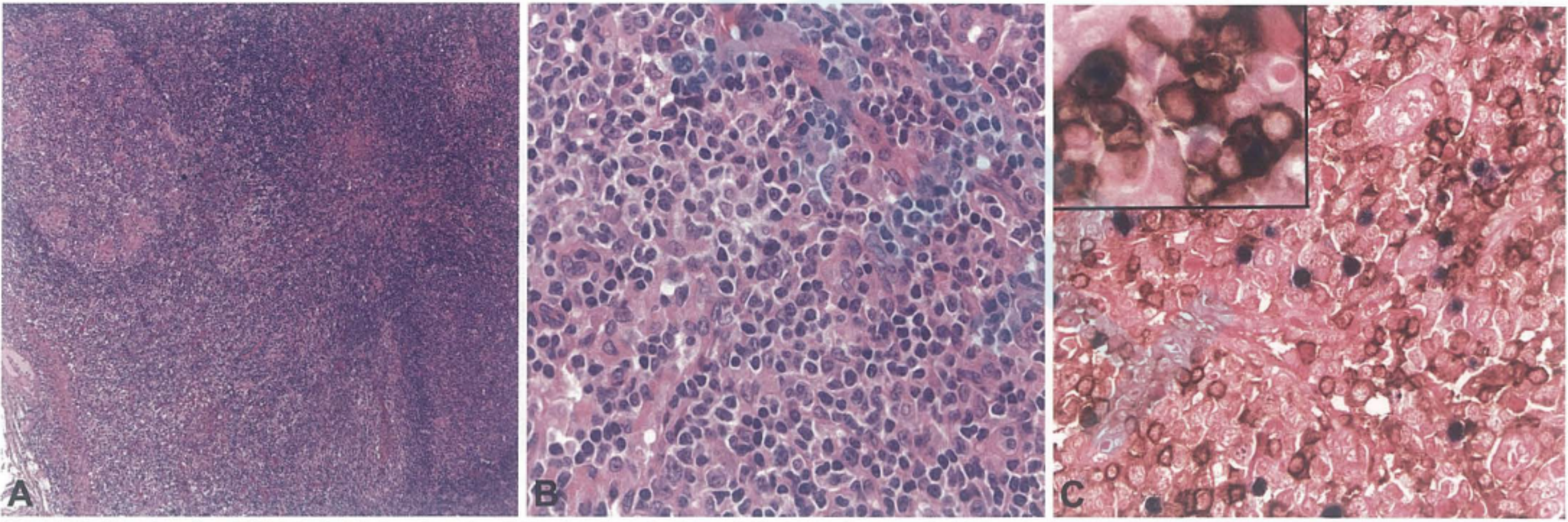


Fig. 14.25 Chronic active EBV infection in lymph node. **A** The lymph node shows follicular and paracortical hyperplasia. **B** At high magnification, the interfollicular areas show a polymorphic infiltrate lacking cytological atypia. **C** In situ hybridization for EBV-encoded small RNA (EBER) shows scattered positive cells. Inset: Double staining shows that the EBER+ cells (black) are CD4-positive (brown).

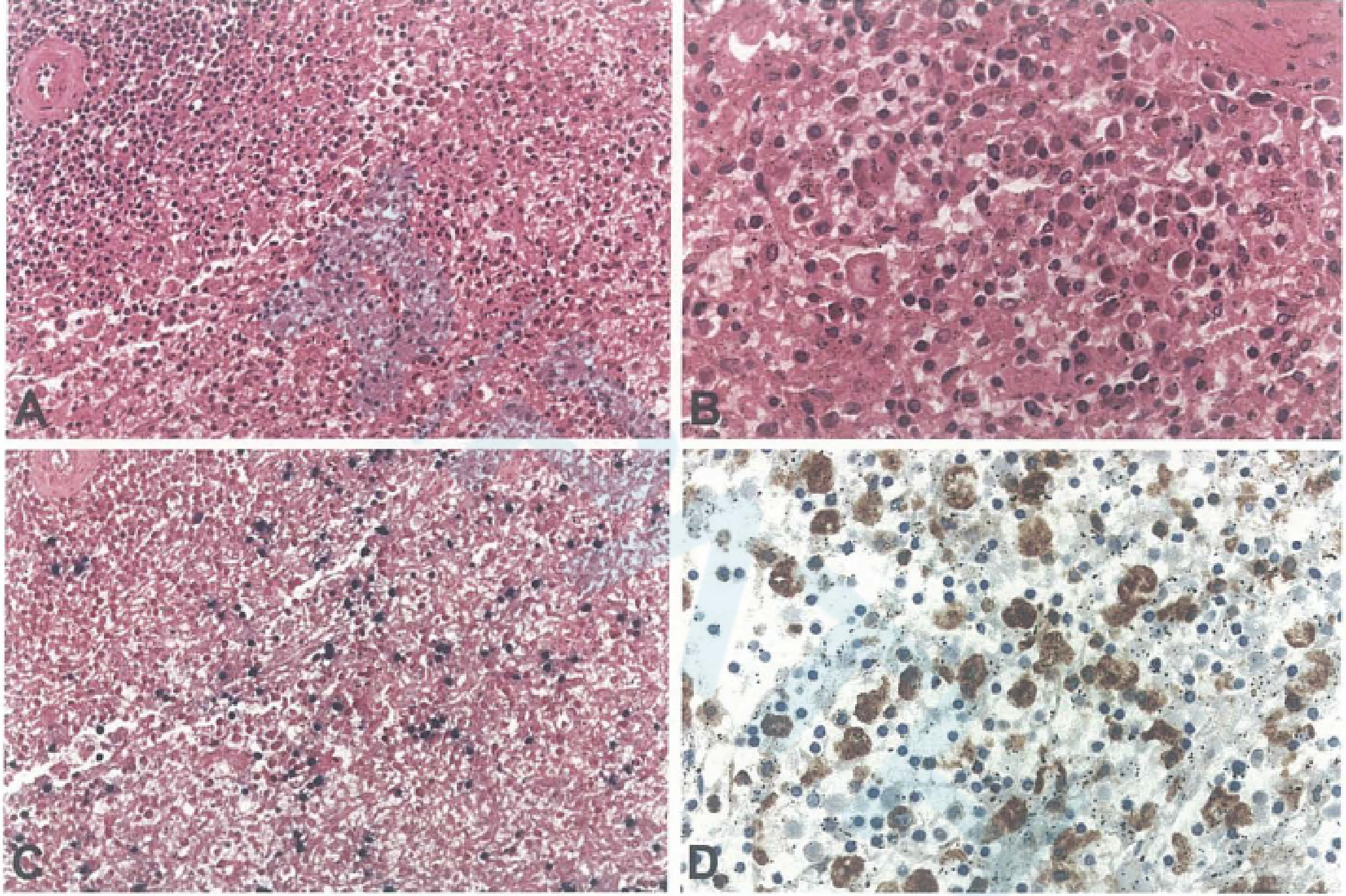


Fig. 14.26 Chronic active EBV infection of NK-cell type in spleen. Haemophagocytic syndrome. **A** The spleen shows white pulp atrophy with congestion of the red pulp. **B** Higher magnification shows that the red pulp is congested with a subtle lymphoid infiltrate and numerous histiocytes, some with erythrophagocytosis. **C** In situ hybridization for EBV-encoded small RNA (EBER) shows scattered positive cells. **D** CD4 staining highlights the abundant histiocytes with erythrophagocytosis. Note that most of the lymphoid cells are CD4-negative.

EBV Related T/NK Lymphproliferative Disease

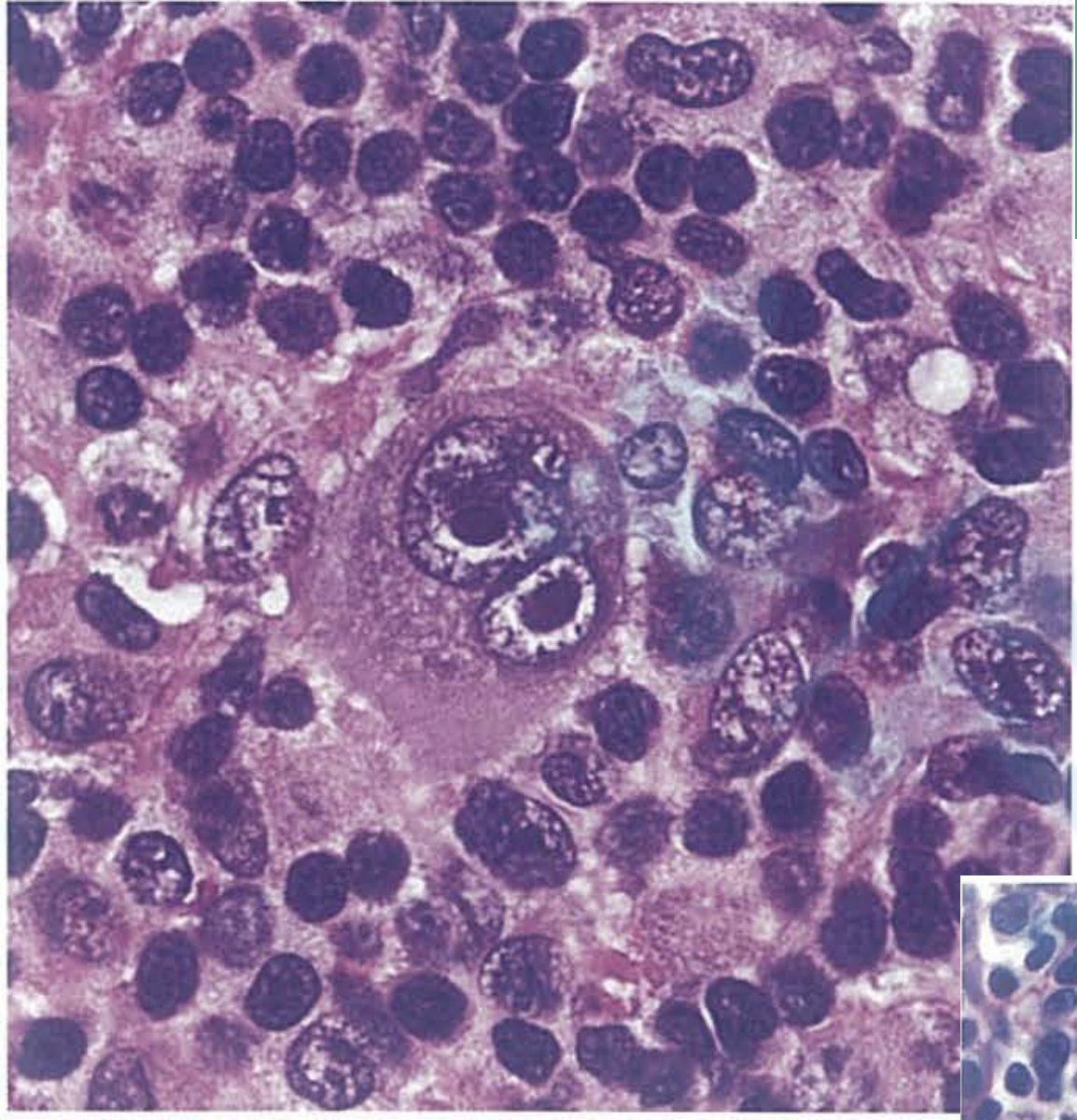
- Extranodal NK/T cell lymphoma, nasal type
- Aggressive NK cell leukemia
- Systemic EBV+ T-cell Lymphoma of childhood
- Chronic active EBV infection
  - Systemic CAEBV of T or NK cell type
  - Hydroa vacciniforme (Hydroa vacciniforme-like T-cell lymphoma)
  - Severe mosquito bite allergy
- Nodal EBV+ cytotoxic T cell lymphoma (EBV+ PTCL-U)
- Hemophagocytic lymphohistiocytosis
  - Familial and Acquired
    - X-linked lymphoproliferative disease
    - X-linked familial hemophagocytic lymphohistiocytosis

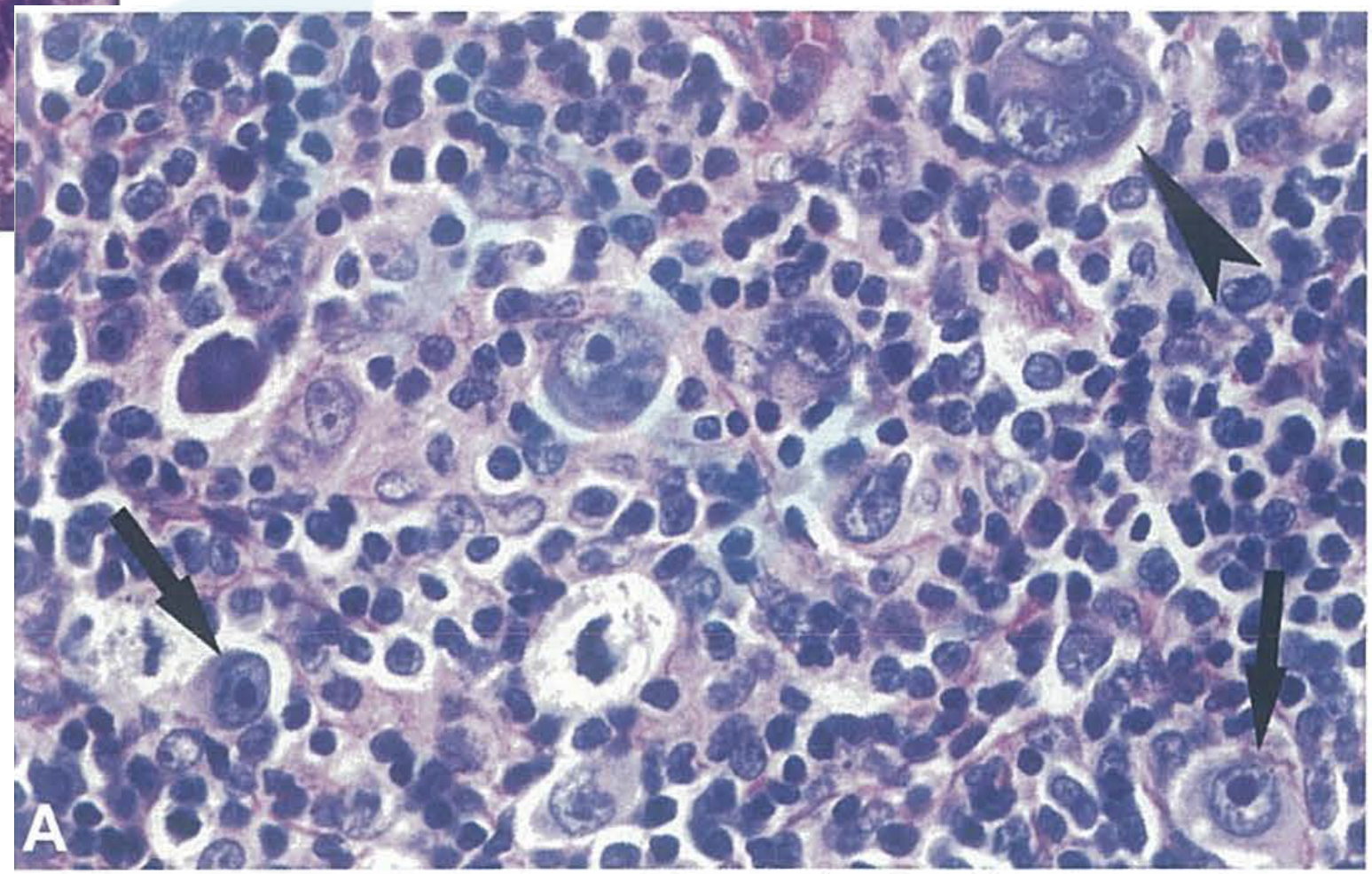
Diagnostic information of CAEBV

- Clinical relevant
- Blood anti-EBV titer and EBV DNA copy number
- Morphology of atypical T cell proliferation
- EBER + in T cells
- TCR rearrangment

Hodgkin/Reed-Sternberg (HRS)

- Hodgkin/Reed-Sternberg (HRS) cells are large, abnormal, mononuclear, or multinuclear/multilobed cells that were first described as hallmark cells in classic Hodgkin lymphoma (cHL)
- The distinct morphologic appearance of HRS cells established them as a key diagnostic feature
- HRS-like cells of B-cell lineage have also been detected in a spectrum of lymphoproliferative disorders from B-cell malignancies such as CLL/SLL, FL, and MCL to T-cell malignancies such as AITL and PTCL, NOS

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MATERIALS AND METHODS

- Case Selection
  - Department Pathology, West China Hospital, Sichuan University, for a period of 5 years (2013-2017)
- Histologic Assessment
  - H&E
- Immunohistochemistry
  - CD2, CD3p, CD4, CD5, CD7, CD8, CD15, CD20, CD30, CD45, CD56, PAX-5, TIA-1, GrB, mum-1, and Ki-67
- PCR for TCR Gene Rearrangements
- EBV Studies
  - EBER-1, EBER-2 and LMP-1
- Next-Generation Sequencing Assay(6/8)

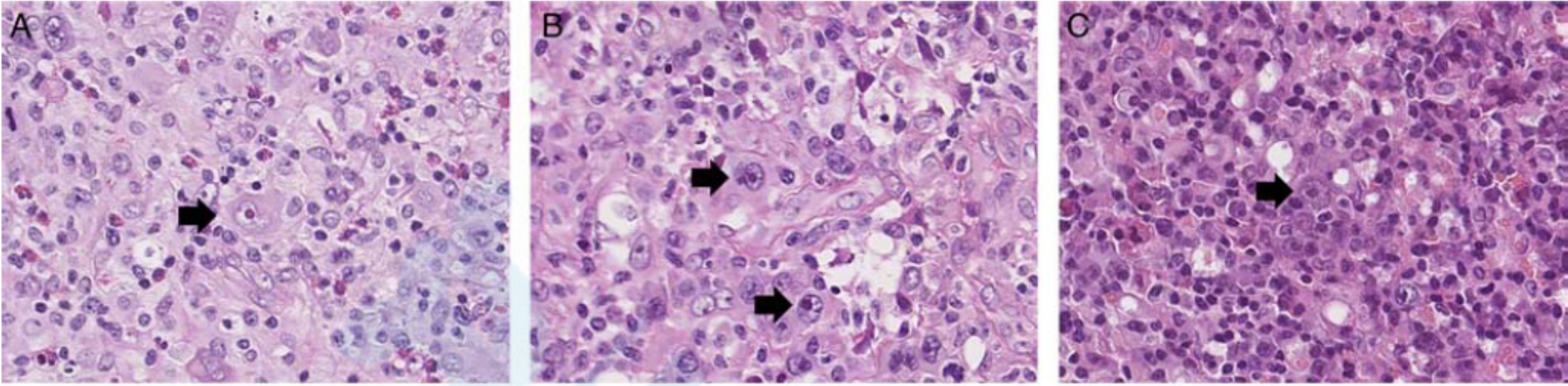
Diagnostic Criteria

- (1) sustained or recurrent infectious mononucleosis (IM)-like symptoms (fever, sore throat, and lymphadenopathy) persists for >3 months
- (2) elevated EBV genome load in the peripheral blood or the tissue lesion
- (3) EBV infection of T or NK cells in the affected tissues or the peripheral blood
- (4) exclusion of other possible diagnoses: systemic acute EBV infection (including IM), autoimmune diseases, congenital immunodeficiencies, HIV, and other immunodeficiencies requiring mmunosuppressive therapies or underlying diseases with potential immunosuppression

TABLE 1. Clinical Characteristics of the Patients of CAEBV-T/NK-S With HRS-like Cells of NK Phenotype

Case	Age/ Sex	Clinical Presentation	Imaging Findings*	HLH	LDH (IU/L)	EBV-DNA (Copies/ mL)	BM Test	Treatment	Follow-up
1	32/M	Fever, headache, and weakness	Multiple lymphadenopathy in both the cervical region and the thorax	−	232	1.97×10^3	Normal BM (biopsy) ND (FCM)	IFN-γ +Acyclovir; CHOP; GED	Progress to ENKTL (19 mo) Died (28 mo)
2	29/F	Fever and weakness	Multiple lymphadenopathy in fossa axillaris, thorax, and abdomen; hepatosplenomegaly	+	1255	6.01×10^3	$EBER^+$ T/NK cells were detected (biopsy) Normal phenotype (FCM)	Vp16+DXM	Died (12 mo)
3†	18/M	Fever and cough	Bilateral pleural effusion; mediastinal and hilar lymphadenopathy; hepatosplenomegaly	+	751	2.03×10^5	$EBER^+$ T/NK cells were detected (biopsy) Normal phenotype (FCM)	DXM; symptom-atic treatment	Died (6 mo)
4	41/M	Fever, sore throat, and weakness	Cervical lymphadenopathy hepatosplenomegaly	+	671	1.84×10^3	$EBER^+$ T/NK cells were detected (biopsy) 1% large NK-cell (FCM)	GLIDE	Died (20 mo)
5	66/F	Fever and cough	Mediastinal and hilar lymphadenopathy; pericardial effusion	−	1260	2.71×10^4	$EBER^+$ T/NK cells were detected (biopsy) 2.2% large NK-cell (FCM)	Symptomatic treatment	Died (8 mo)
6	18/M	Fever and facial edema	Axillary and inguinal lymphadenopathy; splenomegaly	−	614	4.38×10^5	Normal BM (biopsy) ND (FCM)	GED	Loss of follow-up (35 mo)
7	45/F	Fever, cough, and headache	Multiple lymphadenopathy in bilateral cervical region, fossa axillaris, thorax, abdomen, and bilateral inguinal region	−	113	6.02×10^2	Normal BM (biopsy) ND (FCM)	IFN-γ	Alive (23 mo)
8	28/M	Fever, cough, and weakness	Bilateral cervical and mediastinal lymphadenopathy; splenomegaly	+	404	4.41×10^2	$EBER^+$ T/NK cells were detected (biopsy) Normal phenotype (FCM)	GED	Died (16 mo)

*Radiological testing includes computed tomography, magnetic resonance imaging, and positron emission tomography-computed tomography.
†This patient had a history of thalassemia intermedia and tuberculosis.
BM indicates bone marrow; CHOP, cyclophosphamide+ hydroxydaunorubicin+ vincristine+ prednisone; DXM, dexamethasone; F, female; FCM, flow cytometry; GED, gemcitabine+etoposide+ dexamethasone; GLIDE, gemcitabine+ L-asparaginase + ifosfamide+ dexamethasone+ etoposide; LDH, lactate dehydrogenase; M, male; ND, not done.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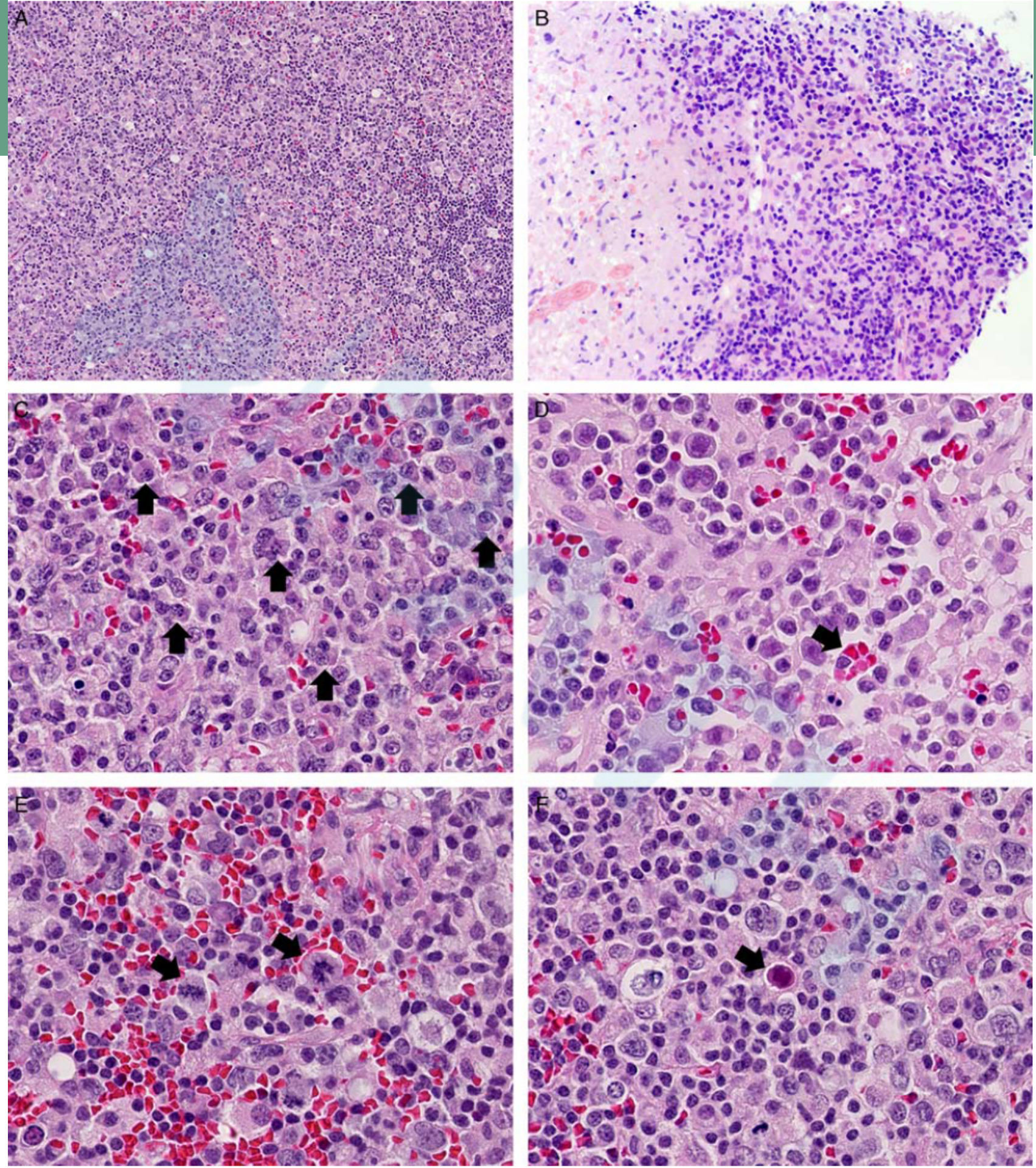
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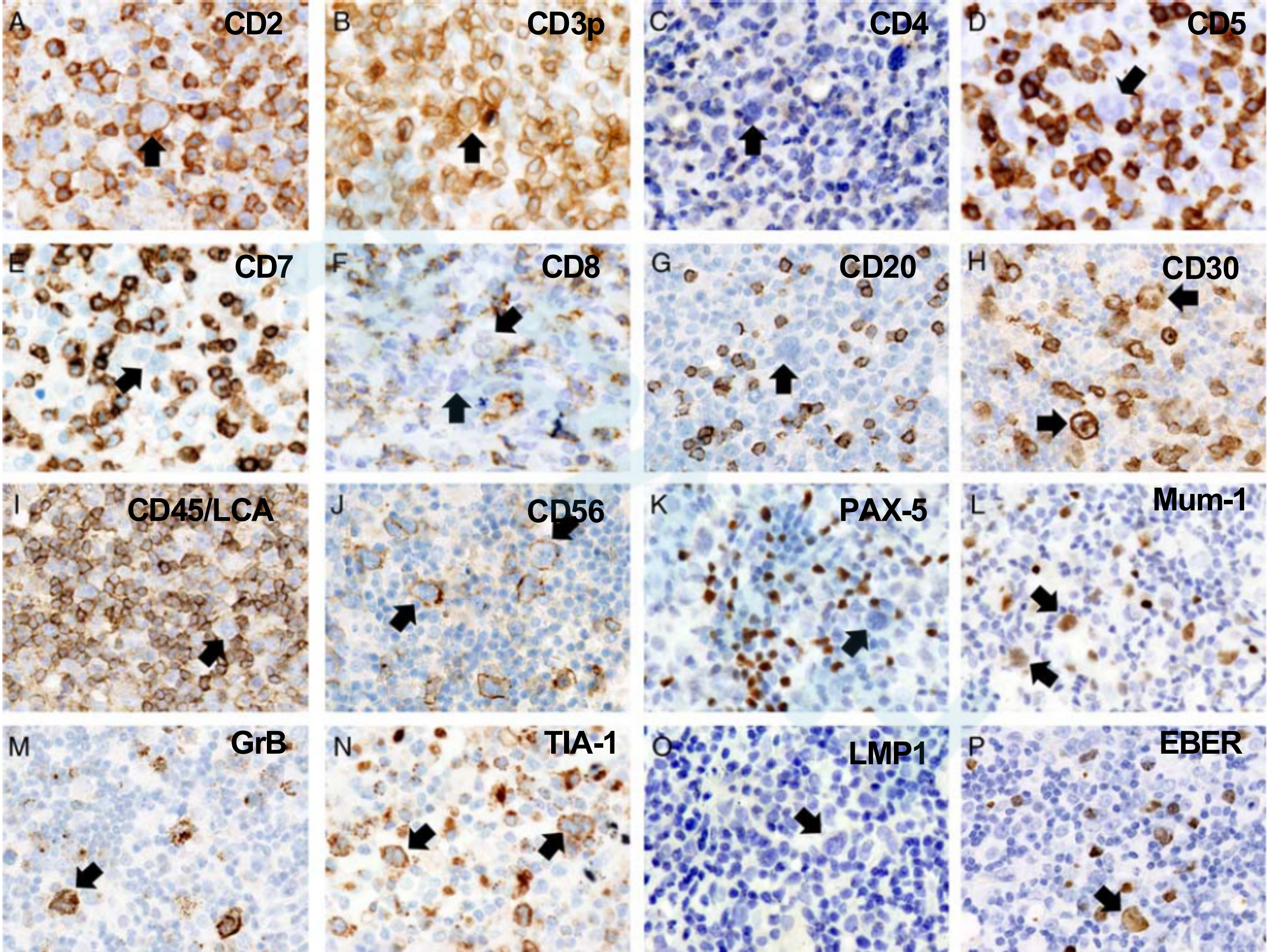
TABLE 2. Morphology of the CAEBV-T/NK-S With HRS-like cells of NK Phenotype (11 Samples)

					HRS Cells			Surroundings							
								Lymphocytes							
Case	Site	ECI	Structure	Necrosis	Main Type	Apoptosis	Mitosis	Size	Atypia	Apoptosis	Mitosis	PC	EOS	MØ	EPC
1	LN	+	Preserved architecture with paracortical hyperplasia	—	Mononuclear	+	−	Medium	−	+	+	+	−	Cluster	−
	LN	+	Deformed structure with diffused hyperplasia	Focal	Mononuclear	+, F	−	Small-medium	−	+	+	+	−	Cluster	−
2	LN	+	Preserved architecture with paracortical hyperplasia	—	Mononuclear/binuclear (bilobed)	−	+	Small	−	+	+	+	−	Cluster	−
	LN	+	Preserved architecture with paracortical hyperplasia	—	Mononuclear/binuclear (bilobed)	+	+, P	Medium	−	+	+	+	−	Cluster	−
	LN	+	Preserved architecture with paracortical hyperplasia	—	Mononuclear/binuclear (bilobed)	+	+, P	Medium	−	+	+	+	−	Sheet	+
3	LN	−	Preserved architecture with paracortical hyperplasia	—	Mononuclear/binuclear (bilobed)	+, F	−	Small	−	+,F	+,F	+	−	Sheet	+
4	LN	−	Preserved architecture with paracortical hyperplasia	—	Mononuclear	−	+	Small	−	+	+	+	−	Cluster	+
5	LN	+	Deformed structure with diffused hyperplasia	Focal/patchy	Mononuclear/multinuclear (multilobed)	+, F	−	Small	−	+,F	+,F	+	−	Cluster	−
6	LN	−	Preserved architecture with paracortical hyperplasia	Focal	Multinuclear (multilobed)	−	+	Small-medium	−	+	+	+	+	Cluster	−
7	LN	−	Preserved architecture with paracortical hyperplasia	—	Mononuclear/multinuclear (multilobed)	+	+	Small	−	+,F	+,F	+	−	Cluster	−
8	LN	+	Preserved architecture with paracortical hyperplasia	Focal	Mononuclear	+	+	Medium	−	+	+	+	+	Sheet	+

ECI indicates extracapsular infiltration; EOS, eosinophilia; EPC, erythrophagocytosis; F, a few (apoptosis and/or mitosis can be seen but ≤1/high-power field); LN, lymph node; MØ, macrophage; P, partial (1/high-power field < n ≤ 3/high-power field); PC, plasma cell.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CD20
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CD30
I
CD45/LCA
J
CD56
K
PAX-5
L
Mum-1
M
GrB
N
TIA-1
O
LMP1
P
EBER

TABLE 3. Phenotype, EBER-ISH, and TCR Rearrangement of the CAEBV-T/NK-S With HRS-like Cells of NK Phenotype

		Immunophenotype (HRS-like Cells)									Immunophenotype (Surrounding T Cells)			EBER-ISH		
Case	Site	CD3p	CD5	CD20	CD30	CD45	CD56	PAX5	GB	LMP-1	CD4	CD8	CD4/CD8	HRS-like cell	Bystander T Cell (%)	TCR
1	LN	+	−	−	+	+	+, v	−	+	−	−	+, p	—	+	20	P
	LN*	+	−	−	−	ND	+, w	−	+	ND	−	-	—	+	60	P
2	LN	+, w	−	−	+	−	+, v	−	+	−	ND	ND	—	+	10	ND
	LN†	+	−	−	+, p	−	+	−	+	−	−	+	—	+	20	P
	LN†	+	−	−	+	+, p	+, v	−	+	−	-	+	—	+	20	P
3	LN	+	−	−	+	+, p	+, v	−	+	−	+	+,f	3/1	+	15	P
4	LN	+, p	−	−	+	−	+	−	+	−	+, f	+,p	1/3	+	30	P
5	LN	+	−	−	+	+	+, v	−	+	−	+	+	1/3	+	30	P
6	LN	+, p	−	−	+, p	−	+, v	−	+, w	+	−	+	—	+	40	P
7	LN	+, w	−	−	+	−	+, p	−	−	−	+, w	+	1/2	+	30	P
8	LN	+	−	−	+	+, p	+	−	+	−	+	+	1/2	+	30	P

*The second biopsy was performed 1 year later.

†Concurrent biopsies (within 3 mo).

f indicates a few (< 25% positive cells); LN, lymph node; ND, not done; p, partial; P, polyclonal; v, variable (the cells express such markers in various degrees; strong, weak, or partial positive); w, weak.

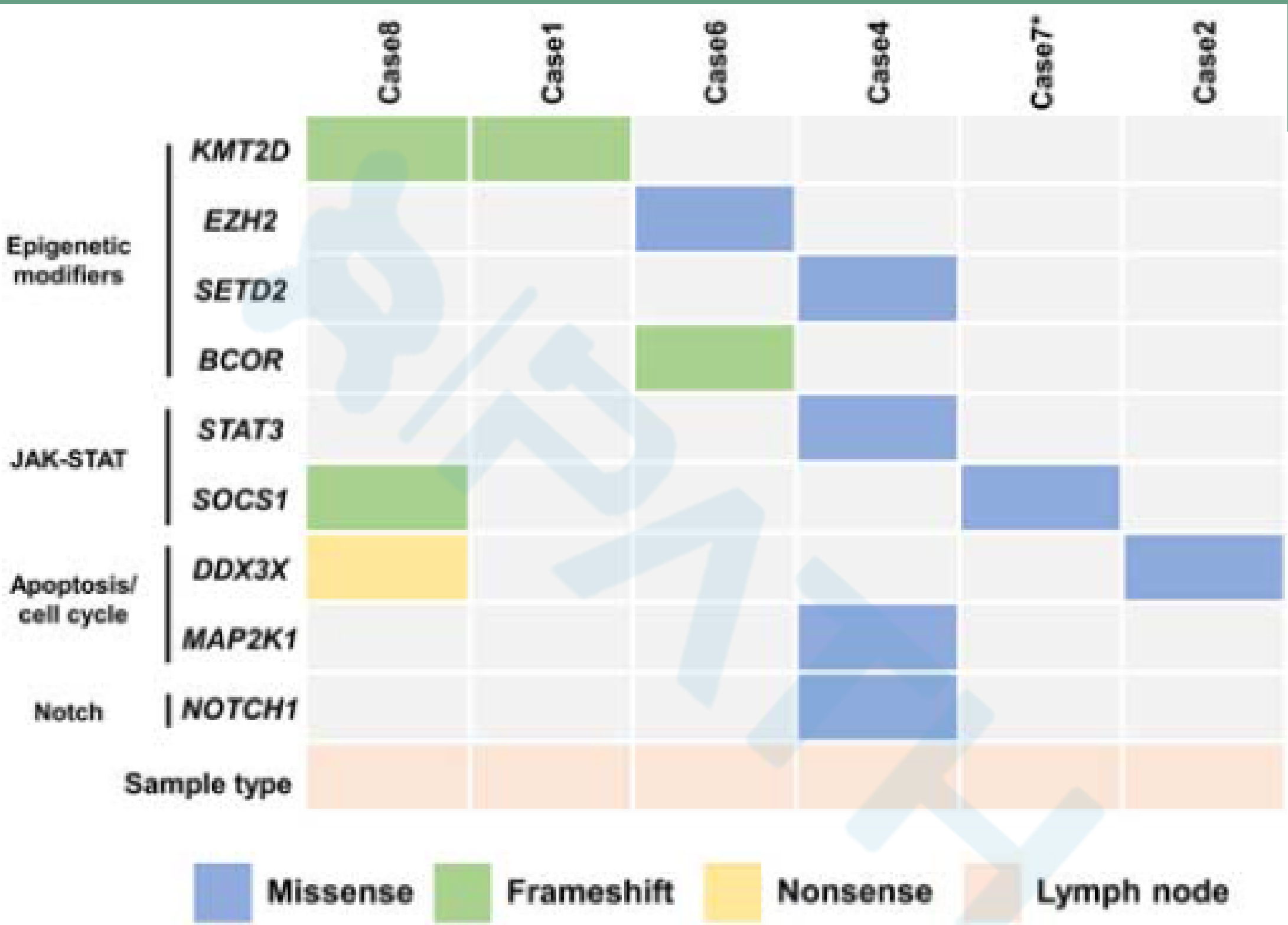

FIGURE 4. Alterations identified by targeted sequencing (112 genes) arranged on the basis of recurrence and biological significance

Differential Diagnostic

TABLE 4. The Difference Between CAEBV-T/NK-S With HRS-like Cells of NK Phenotype and Mixed Cellular cHL[20–30]

Characteristics	CAEBV-T/NK-S With HRS-like Cells of NK Phenotype	Mixed Cellular cHL
Clinical features		
Fever	+	+/−
Lymphadenopathy	Multiple lymphadenopathy	Localized lymphadenopathy
Hepatosplenomegaly	+	−/+
Hemophagocytic lymphohistiocytosis	Often presented	Rarely presented
EBV-DNA in peripheral blood	Elevated	Negative
Other	All patients presented with persistent or recurrent systemic symptoms	Can be asymptomatic
Morphology		
HRS cell/HRS-like cell		
Binuclear cells	Not common	Frequently seen
Other variants	Mononuclear and Multinuclear cells in predominance	Can be seen
Surroundings		
Eosinophils	Not commonly detected	Commonly detected
Erythrophagocytosis	Often detected	Rarely detected
Necrosis	Focal or patchy necrosis in paracortical area	Not common
Immunophenotype		
HRS cell/HRS-like cell		
Positive	CD2, CD3p, CD30, CD56, TIA-1, GrB, mum-1	CD15, CD30, PAX-5, mum-1
Negative	CD5, CD7, CD15,CD20, PAX-5	CD2, CD5, CD7, CD20*, CD56, TIA-1, GrB
Surrounding lymphocytes	Frequently $CD8^+$ T cells outnumber $CD4^+$ T cells	Often $CD4^+$ T cells outnumber $CD8^+$ T cells
EBV Status		
HRS cell/HRS-like cell		
LMP-1	− (usually)	+/−
EBER-ISH	+	+/−
Consistency of LMP-1 and EBER-ISH	Unmatched	Matched
Surroundings		
LMP-1	−	−
EBER-ISH	+(T cells in various number)	−
Frequent mutations	*SOCS1*, *KMT2D*, *DDX3X*, *STAT3*, *BCOR*	*STAT6*, *GNA13*, *XPO1*, *ITPKB*, *CIITA*, *TNFAIP3*, *TNFRSF14*, *CD58*

*CD20 is usually negative in cHL, but can only be weakly expressed on a subset of the neoplastic cells.

Differential Diagnostic

TABLE 5. The Difference Among CAEBV-T/NK-S With HRS-like Cells of NK Phenotype, ANKL, and Primary EBV$^+$ Nodal T-Cell or NK-Cell Lymphoma[17,31–35]

Characteristics	CAEBV-T/NK-S With HRS-like Cells of NK Phenotype	ANKL	Primary EBV$^+$ Nodal T-Cell or NK-Cell Lymphoma
Clinical features			
High-risk populations	Young adults	Young to middle age adults	Elderly or immunocompromised patients
Lymphadenopathy	Multiple	Multiple	Multiple
Hepatosplenomegaly	+	+	+
HLH	Often detected	Frequently detected	Not common
Extranodal involvement*	Not detected	Can be detected	Not common
Morphology	cHL-mimicking appearance	Diffuse or sinus infiltration of monotonous cells	Monomorphic pattern of centroblastoid cells
	Necrosis can be detected	Necrosis can be detected	Lacking the necrosis
Immunophenotype	Typical NK phenotype (HRS-like cells) Cytotoxic T-cell phenotype (Surrounding T-cell)	Typical NK phenotype	Most cases show cytotoxic T-cell phenotype Typical NK phenotype is not common
TCR rearrangement	Polyclonal	Polyclonal	Most cases are monoclonal (T-cell type) A minority show polyclonal (NK-cell type)
Frequent mutations	*SOCS1*, *KMT2D*, *DDX3X*, *STAT3*, *BCOR*	DDX3X JAK-STAT pathway (*STAT3*, *JAK2*) RAS-MAPK pathway (*KRAS*, *NRAS*)	Not reported
Prognosis	Better	Poor	Poor

*Extranodal involvement: bone marrow, liver, and spleen are not included.

Summary

- We strongly argue that the accumulated evidence here supports that “HRS-like cells of NK phenotype” is a variant of CAEBV-T/NK-S
- Clinically, all the cases presented with persistent or recurrent systemic symptoms and met the diagnostic criteria of CAEBV
- Morphologically, this study, first, reported a special group of CAEBV-T/NK-S cases having HRS-like cells of NK phenotype, which broadens the spectrum of cHL mimics

Summary

- Genetically, the harbored genetic changes were similar to those of aggressive EBV-associated T/NK cell neoplasm, but with the absence of some recurrent driver mutations, which provides a deep understanding of this rare disease
- Prognostically, the overall survival was also significantly longer than other more aggressive T/NK-cell neoplasms, which indicates that the current group is a less aggressive variant of EBV-associated T/NK-cell lymphoproliferative disorders
- Furthermore, we summarized the outlines for differential diagnosis, especially for cHL, ANKL, and primary EBV+ nodal T-cell or NK-cell lymphoma, which could help in avoiding misdiagnosis

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

Thank You !