Virchows Archiv (2019) 474:219–226
https://doi.org/10.1007/s00428-018-2498-7

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

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 (8/9, 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.

Keywords Extranodal NK/T cell lymphoma · Human herpesvirus 4 · Infectious mononucleosis · Upper aerodigestive tract · Immunophenotyping

Introduction

Epstein–Barr virus (EBV) was initially discovered in 1964 through detection of viral particles in cultured Burkitt lymphoma cell lines [1]. The virus is not only widespread among healthy people but is also associated with several non-neoplastic and neoplastic diseases, such as infectious mononucleosis (IM), chronic active EBV infection (CAEBV), NK/T cell lymphoma, and Burkitt lymphoma. Extranodal NK/T cell lymphoma nasal type (ENKTL) accounts for 11% of non-Hodgkin's lymphoma (NHL) in China [2], whereas CAEBV T cell and NK cell type, systemic form (CAEBV-T/NK) is rarely reported [3, 4]. Some benign lymphoproliferative disorders in the upper aerodigestive tract, such as infectious mononucleosis, are occasionally misdiagnosed as malignancy because the histopathologic features of these lesions resemble

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lymphoma [5]. Here, we report nine cases of lymphoproliferative disorder caused by EBV-positive cytotoxic T cell proliferation in the upper aerodigestive tract; we designated this as acute EBV-positive cytotoxic T cell lymphoid hyperplasia (EBV+TLH). Notably, EBV+TLH is easily misdiagnosed as ENKTL, because both share similar features in histology and immunohistochemistry, including a cytotoxic T cell phenotype and EBV positivity. However, based on its clinical characteristics, i.e., spontaneous regression within a short time, EBV+TLH should be recognized as a reactive process.

Materials and methods

Case selection and morphologic review

We retrospectively reviewed hematoxylin and eosin (H&E), immunohistochemical, and EBV-encoded small RNA (EBER) in situ hybridization slides from 1213 cases diagnosed as lymphocytic-related diseases in the upper aerodigestive tract during the period from January 2006 to November 2015. These were obtained from the files of the Department of Pathology, Beijing Friendship Hospital, Capital Medical University, which is a large lymphoma diagnosis research center that is located in Beijing, China. Among them, there were nine cases of EBV+TLH that had the following common features: (1) T cell predominant lymphoproliferation in the upper aerodigestive tract; (2) EBV infection mainly of T cells; (3) EBV-positive cells of > 50/high-power field (HPF); (4) exclusion of ENKTL and CAEBV-T/NK; (5) no radiotherapy or chemotherapy; and (6) follow-up for more than 12 months without recurrence.

The clinical data of these nine cases, including gender, age at diagnosis, onset symptoms, localization at presentation, computed tomogram manifestation, disease duration, serum EBV detection, treatment, and follow-up, were collected. All cases were reviewed for H&E morphologic evaluation, and the following features were recorded: surface lining epithelium, mucous and serous glands, necrosis, karyorrhectic debris, angiocentric growth, lymphoid cells, and background cells.

Immunohistochemistry and EBER in situ hybridization

Immunohistochemistry (IHC) staining was performed manually on formalin-fixed, paraffin-embedded (FFPE) tissue for immunophenotypic analysis. Sections were stained with antibodies against CD2, cytoplasmic CD3, CD4, CD5, CD7, CD8, CD20, CD56, PD-1, PD-L1, CD79a, TIA-1, Granzyme B, EBNA-2, and Ki-67 (Maxim-Bio, Fuzhou, China). The MaxVision™ 2 kit obtained from Maxim-Bio (Cat. No. KIT-5910/5931) was used for the detection of all used antibodies. Positive and negative controls were analyzed according to the manufacturer's instructions. The EBV Probe In Situ Hybridization Kit (Triplex International Biosciences (China) Co. Ltd., Fuzhou, China) was used to detect EBERs. Details of this procedure are described in our previous report [6]. Observers counted only definite EBV-positive cells, and the field selection was from an area with a high positive rate. The total number of EBV-positive cells per HPF was recorded.

Double staining

An immunohistochemical plus EBER in situ hybridization dual-staining technique was performed using the Leica Bond MAX autostainer (Leica, Melbourne, Australia). Sections (2-μm thick) were first stained for EBER with DAB staining (brown), then for CD3 (LN10, RTU; Lecia), CD20 (L26, RTU; Lecia), CD4 (4B12, RTU; Lecia), and CD8 (4B11, RTU; Lecia) followed by visualization of the red staining after application of amino-ethylcarbazole (Bond Polymer Refine Red Detection kit, Leica) as chromogen.

T cell receptor gene clonality analysis

T cell receptor (TCR) gene rearrangement analysis was performed using polymerase chain reaction (PCR) based on the "Biomed-2" primers (InVivoScribe Technologies, San Diego, CA, USA) [7]. DNA was extracted from FFPE tissue samples using the TIANamp FFPE DNA Kit (DP331) (TIANGEN, Beijing, China). For the gene rearrangement assay, PCR was carried out in a 25-μl volume containing 22.5 μl of master mix, 0.13 μl of AmpliTaq Gold DNA polymerase, and 100 ng of genomic DNA. The cycling profile used for all reactions was as follows: 95 °C for 7 min; 35 cycles of 95 °C for 45 s, 60 °C 45 s, 72 °C 90 s; and a 10-min final extension at 72 °C. After amplification, PCR products were denatured at 94 °C for 5 min followed by a quick chill to reanneal the PCR products at 4 °C for at least 60 min. The PCR products were electrophoresed in 6% polyacrylamide gels (BioRad) in 1 × TBE buffer at 120 V for approximately 65 min. The gel was then soaked for 20 min in 100 ml of 0.1 M NaCl solution containing 10 μl of 10 mg/ml Gel Red (Biotium, USA) and photographed using ultraviolet illumination.

Results

Clinical features

All nine patients with EBV+TLH (five males and four females with a median age of 16 years, range 5–29) were Chinese. All patients had been healthy, notably without a history of EBV-associated disease; however, they presented with an acute onset of symptoms. Eight (89%) patients presented with nasopharyngeal symptoms, including stuffy nose, pharyngeal

discomfort, and rhinorrhea. The remaining patient lacked nasopharyngeal symptoms but had a fever and cervical lymphadenopathy. A computed tomogram (CT) scan showed a nasopharyngeal mass and pharyngeal crypt disappearance. CT scan data were available for three of the nine patients. There was no obvious tissue damage or bone destruction. Other clinical presentations included lymph node enlargement, fever, headache, and hepatosplenomegaly. Nasopharyngoscopy revealed masses or polypoid protrusions in the nasal and pharyngeal mucosae, with an average maximum diameter of 0.9 cm, of which biopsy specimens were taken. The average interval from onset to biopsy was 22 days (range, 10–30 days). The main clinical characteristics of all patients are listed in Table 1.

Laboratory data were reviewed from several patients. Whole blood count data were obtained in only three cases; in these cases, leukocyte count ($10.43–15.7 \times 10^9$/L) and lymphocyte count ($5.18–6.50 \times 10^9$/L) were higher than normal. All cases in this series were consultation cases, and information regarding EBV serology was unattainable. Serum anti-EBV antibody detection was only assayed in one patient (case 8), who was positive for serum anti-EBV capsid antigen IgM antibody but negative for anti-EBV early antigen IgG and anti-EBV nuclear antigen IgG antibodies.

Morphological features

In all cases, lymphocytes infiltrated diffusely in the submucosa with reduction (4 cases) or disappearance (5 cases) of mucosal glands, a pseudostratified ciliary columnar epithelium covered the mucosa in eight of the nine cases. Necrosis was observed in four cases, two of which showed surface mucosal erosion with necrosis. The other two cases presented with focal necrosis and significant nuclear debris in the lesions. Intravascular fibrin deposition and peripheral tissue necrosis were found in two cases. A pattern of peripheral vascular growth was noted in one case (Fig. 1). Scattered eosinophils and plasma cells were found, whereas neutrophils and histocytes were rare. In seven cases, the lymphocytes were predominantly small-sized, with irregularly folded, twisted nuclei, granular chromatin, non-obvious nucleoli, and a narrow rim of pale cytoplasm. One case was predominantly composed of medium-sized cells and a mixture of several large and small lymphocytes. In another case, the lesion consisted of an infiltration of intermediate-sized lymphocytes with round or irregular nuclear contours and abundant pale cytoplasm.

Immunohistochemical and in situ hybridization findings

In all cases, many infiltrating lymphocytes were cytotoxic T cells with as immunophenotype co-expression of CD2, cytoplasmic CD3, CD5, CD7, and granzyme B/TIA-1 (except for one case that was CD5-negative (Fig. 1)) while negative for CD20, CD79a (Fig. 2h), and CD56. The Ki67 index ranged from 40 to 80%. In all five cases tested for these parameters, CD8-positive cells were more abundant than CD4-positive cells, PD-L1 was positive (Fig. 2g), and only scattered reactive PD-1 positive T cells were noted. Scattered cells showed EBNA-2 positive nuclei in two of the three cases tested.

All cases were positive for EBERs with a proportion of positive nuclei ranging from 100 to 200 cells per HPF. EBER in situ hybridization and CD3 immunohistochemistry double staining showed that EBER-positive cells were also CD3-positive (Fig. 3a, b), and admixed with fewer CD20-positive B cells (Fig. 3c, d). EBER in situ hybridization with CD4 and CD8 double staining showed that there were more $EBER^+/CD8^+$ cells (Fig. 3e, f) than $EBER^+/CD4^+$ cells in all five cases tested.

PCR for TCR gene rearrangements

PCR analysis could be performed on only five cases due to poor DNA quality of the remaining four. PCR analysis results revealed no clonal band of the TCR in any of the amplified samples, even though the DNA quality of all analyzed samples was > 300 bp. Specific bands were found in the positive control, whereas the negative control yielded no bands.

Follow-up

A total of nine patients were followed up successfully until January 15, 2017. The mean follow-up interval was 72.44 months (19–124 months). Three patients underwent antiviral therapy, and the others received anti-inflammatory treatment. All patients achieved complete remission spontaneously within 1 month after pathological diagnosis and lived asymptomatically.

Discussion

EBV-associated lymphoproliferative diseases encompass a group of disorders that include reactive processes and malignant tumors. EBV can infect B cells, T cells, and NK cells [8]. One group of EBV-associated T cell lymphoid hyperplasias (e.g., EBV+TLH) primarily present with a mass in the upper aerodigestive tract and share several histopathological and immunophenotypical features similar to those of ENKTL. Here, we report nine such cases with self-limiting lesions, all of which of sudden onset (duration of symptoms less than 1 month), and complete remission in a short time without the need for radiotherapy or chemotherapy. Differentiating between EBV+TLH and ENKTL by morphology and

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	10/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	13/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	18/M	Nasal mucosa	30	N	N	N	N	Y
9	5/F	Nasal mucosa	10	Y	N	N	N	Y

Case no.	Clinical presentation		Observation of nasopharyngoscopy	Computed tomogram manifestation	Treatment	Outcome	Length of follow-up (months)
	Lymph node enlargement	Hepatosplenomegaly					
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

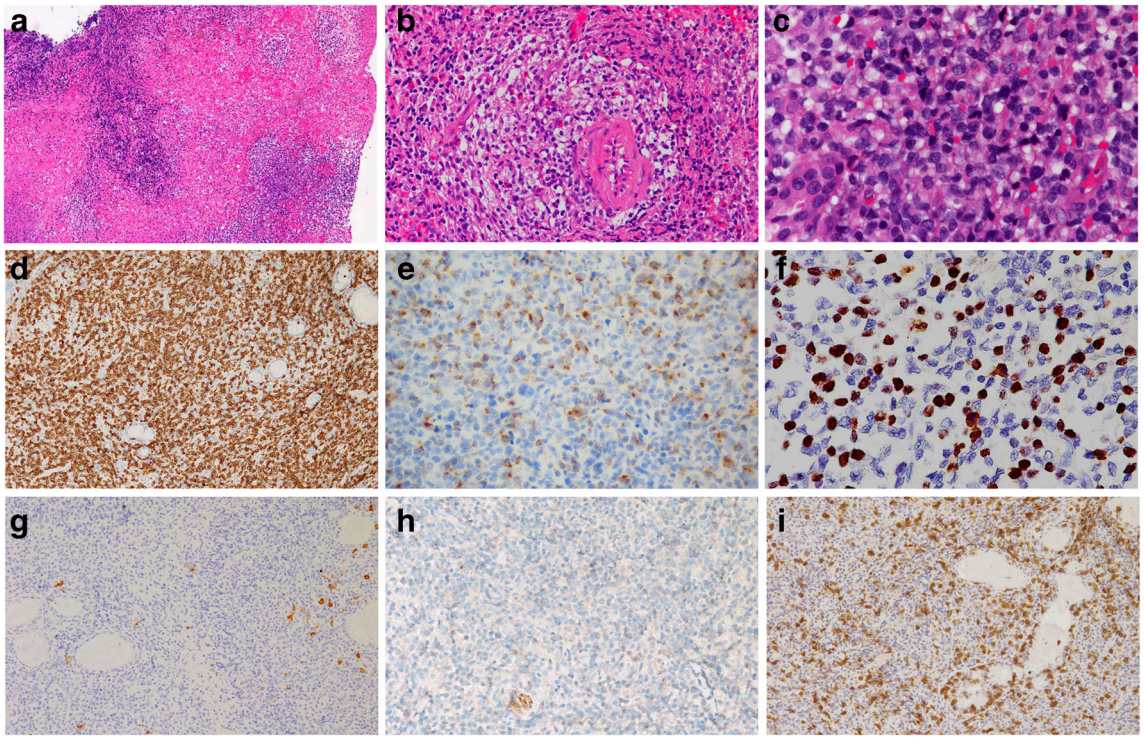

Fig. 1 Histologic and immunohistochemical presentations in the case of a CD5-negative EBV-positive T cell lymphoid hyperplasia (case 9). **a** The mucosa showed extensive ulceration and coagulative necrosis (H&E, × 40). **b** Lymphocytes surrounding the vessel (H&E, × 200). **c** Lesion with an infiltration 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

immunophenotype is always difficult; however, the self-limiting character of EBV+TLH is an important clue. For conditions with this presentation, anti-inflammatory or antiviral therapies or follow-up only are likely the best options for management of the case.

In China, ENKTL is the most common T/NK cell lymphoma occurring in the upper aerodigestive tract, and it is also prevalent throughout Asia [9]. According to the diagnostic criteria from the World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues, tumor cells

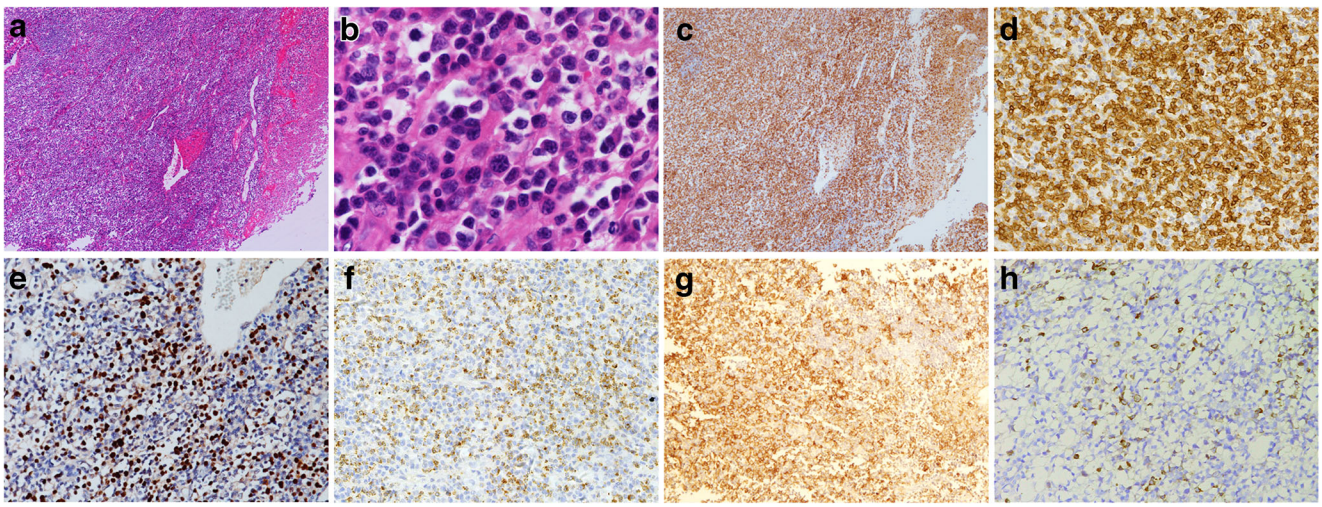

Fig. 2 Findings from case 7. Histopathology (HE) (**a**, **b**), immunohistochemical staining (**c**–**d**, **f**–**h**), and EBERs (EBV-encoded RNAs) (**e**) of case 7. **a** Mucosa with diffusely infiltrated lymphoid cells and erosion of the surface epithelium (× 40). **b** Lymphoid cells of various sizes, ranging from small to large (× 630). **c**–**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)

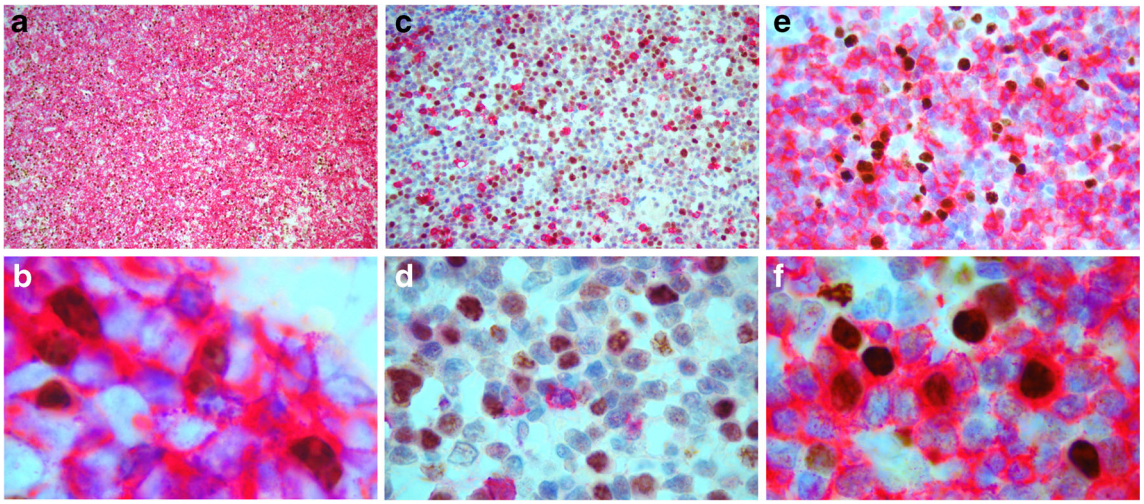

Fig. 3 Double staining from case 6. **a**–**f** Double staining of combined in situ hybridization for EBV-encoded RNAs (EBERs) with immunostaining against CD3 (**a**, × 100; **b**, × 1000), CD20 (**c**, × 100; **d**, × 1000), and CD8 (**e**, × 100; **f**, × 1000). EBER-positive cells (brown) with expression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

in ENKTL are positive for cytoplasmic CD3ε$^+$, CD56$^+$, and cytotoxic molecules, and they are infected with EBV. Lesions that express cytotoxic molecules and are EBV-positive, but lack CD56 expression, can also be diagnosed as ENKTL. In the case of a nasal lesion presenting with EBV-infected NK or T cells, the possibility of ENKTL needs to be seriously considered. The cytologic spectrum of ENKTL is broad, with lymphoid cells ranging from medium and large up to the size of anaplastic cells. However, ENKTL may be misdiagnosed as inflammation when the infiltrated cells are predominantly small lymphocytes without irregularly folded nuclei [10]. Therefore, the morphologic differential diagnosis of ENKTL is difficult. All nine cases in our study were initially misdiagnosed as ENKTL or suspicion of lymphoma, owing to the lesions present in the nasal cavity and pharynx that displayed large patches of necrosis with obvious nuclear debris and infiltration of atypical T lymphocytes. EBV+TLH shares some histopathologic features with ENKTL, but the EBV+TLH cases in this report occurred most frequently in adolescents and presented with rapidly developing acute nasopharyngeal symptoms. CT scans revealed a nasopharyngeal mass lacking adjacent bone destruction. All patients in our series achieved complete remission within 1 month, without chemoradiotherapy, with a clinical course mimicking IM [11]. Although some cases of EBV+ TLH histologically showed necrosis, angiodestructive growth was uncommon. Vascular invasion is one of the major features of ENKTL, and it may also be a new prognostic indicator [12]. Immunophenotypically, CD56-positive and CD5-negative cells almost always appear in ENKTL cases [13], but rarely in EBV+TLH cases. Finally, in ENKTL with cytotoxic T cell derivation, TCR gene rearrangements can be found [14]. The negative results of TCR gene rearrangements analysis (performed in five of our cases) were inconsistent with ENKTL. These clinical, pathomorphological, immunophenotypic, and molecular biological characteristics allowed us to rule out the possibility of ENKTL.

In addition to ENKTL, CAEBV of T cell and NK cell type, systemic form (CAEBV-T/NK) can also involve the upper aerodigestive tract [15]; this systemic disease is prevalent in East Asian as well as Central and South American countries. The most commonly involved sites are liver, spleen, and lymph nodes, but nasopharynx involvement has been observed in a few cases. Approximately 50% of patients present with recurrent or persistent IM-like symptoms persisting for more than 3 months [16, 17]. Furthermore, progression to ENKTL or cytotoxic T cell lymphoma has been reported in some cases [18, 19]. Therefore, the differential diagnosis of ENKTL, CAEBV-T/NK, and EBV+TLH can be problematic when on the basis of morphology and immunophenotype only [20]. Unlike ENKTL or CAEBV-T/NK, patients with EBV+ TLH are usually young and have an acute course lacking repeated onset of clinical symptoms. Polyclonal TCR gene rearrangements are the rule in EBV+TLH.

The clinical manifestations of our cases were all similar to those of IM, which is a type of infectious disease that occurs more often in adolescents and young adults and with as typical symptoms sore throat, cervical lymphadenopathy, and fever [21]. Primary infection with EBV is the major cause. Normal nasopharyngeal mucosae have several EBV-infected T cells around crypts, but B cells are more common [22]. Furthermore, EBV usually infects B cells in IM, only rare cases showing EBV-infected T or NK cells [23]. In the nine patients with IM involving tonsils reported by Anagnostopoulos et al., most EBER-positive cells were B cells, with several scattered EBER-positive T cells in the background [24]. The histopathological characteristics of B cell-IM in lymph nodes and tonsils have been well described [25]; however, the features of EBV-infected T cell lymphoid hyperplasia occurring in the upper

aerodigestive tract have not been well reported. The double labeling of EBER and CD20 in a patient reported by He et al., who had IM presenting with atypical T lymphocyte proliferation in the nasopharynx and recovered 1 month later after antibiotic treatment, indirectly demonstrates that EBV might infect T cells [5]. However, the hypothesis that T cells can be infected by EBV has not been accepted because of a lack of specific double staining for EBER and CD3. Ha et al. reported one case of T lymphocytes infected with EBV, but the number of EBV-positive T cells was less than 4/HPF [26]. Literature on EBV-infected T cell lymphoid hyperplasia is rare. The results of our double labeling EBER and CD3 experiments showed many EBER-positive CD3-positive T cells in all cases; moreover, in three cases, elevated lymphocyte counts were found similar to what is seen in IM. Furthermore, EBNA-2 was expressed in two cases with scattered positive nuclei, one of which had increased anti-EBV VCA IgM antibodies. EBNA2 is always expressed in patients who have a primary immune response or who are immunocompromised [25], and IM patients usually have EBNA2 positive cell. Combining clinical and pathological presentations, we hypothesize that two of our cases are consistent with T cell-IM. However, a definitive diagnosis of IM could not be made because results of serum EBV tests were not available. Therefore, we designate this disorder as EBV+ TLH.

The pathogenesis of T cell-IM or EBV+TLH is not well known. Even though > 90% of the population is EBV infected, only a small number of people present IM or other EBV-related diseases, which are closely related to the immune dysfunction of T lymphocytes [27]. In B cell-IM, a large number of activated $CD8^+$ T cells are observed [28], and most of which have the ability to attack EBV-positive B cells [29]. Compared with EBV-infected B cells in traditional IM, in our cases, a large number of EBV-positive T cells were found, both with a high number of $CD8^+$ T cells. Therefore, our cases provide indirect evidence for the existence of T cell-IM. How EBV infects non-neoplastic T cells is still unclear. The presence of weakly expressed CD21 on the surface of mature T cells in normal human peripheral blood may mediate EBV binding to T cells [30]. One report showed that PD-L1 can help EBV-positive B cells escape from immune attack [31]. Our immunohistochemical analysis also revealed a high level of PD-L1 expression in all five tested cases, which may help suppress local host immunity and allow proliferation of EBV-positive T cells. In addition, there were more $EBER^+/CD8^+$ cells than $EBER^+/CD4^+$ cells in our cases, which might influence the function of cytotoxic T cells. In earlier experiments, we found EBV-type 1 to be predominant in healthy Chinese, EBV-positive classic Hodgkin lymphoma and nasopharyngeal carcinoma [32]. EBV-type2 was found in immunodeficiency-associated lymphomas. All nine patients were still alive at last follow-up with no evidence of immunodeficiency; we speculate that the type of EBV strain in our cases is likely type1.

In summary, we report patients in China with EBV+TLH in the upper aerodigestive tract. The clinical presentation of EBV+TLH is similar to that of IM, which is more common in adolescents or young adults, with short disease duration and acute onset, and whose symptoms spontaneously regress in a short period of time. It is difficult to differentiate EBV+TLH from ENKTL and CAEBV-T/NK-lymphoproliferative diseases based only on morphology and phenotype. Thus, a combination of clinical presentation and molecular biological characteristics is necessary to make the proper diagnosis.

Acknowledgements The authors thank Drs Sun Jirui and Ma Yin for providing clinical follow-up details, the technical staff of the immunohistochemistry laboratories at the Capital Medical University, the Beijing Friendship Hospital and the Beijing Chaoyang Hospital for performing additional double staining studies.

Funding information This work was supported by grants from the Internal Start-up Science Foundation of Beijing Friendship Hospital, China (No. Yyqdkt2013-15) and by grants from the Internal Talent Development Foundation of Beijing Chaoyang Hospital, China (No. CYMY-2017-01).

Compliance with ethical standards

All patients provided written informed consent for use of tissue samples for research and were audited by the Medical Ethics Committee of Beijing Friendship Hospital, Capital Medical University (approval number 2017-P2-129-01).

Conflict of interest The authors declare that they have no conflicts of interest.

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