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Original Article

Cytopathologic findings of cell block materials from the vitreous: Diagnostic distinction between intraocular lymphoma and non-lymphomatous diseases

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Running title: **cell block materials from the vitreous**

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Abstract

   Intraocular lymphoma is a rare neoplasm that occurs only in the eyes and/or central

nervous system. Diagnosis of intraocular lymphoma is difficult because its clinical

manifestations mimic chronic uveitis. Pathological examination of the vitreous is one of the

main diagnostic tools for intraocular lymphoma, but this is challenging due to the sparse

cellularity and specimen degeneration.

   Here, we reviewed 33 cell block preparations from vitreous perfusion fluid in order to

examine the significance of cytopathological findings for differential diagnosis using vitreous

samples. The cases comprised 12 intraocular lymphomas and 21 non-lymphomatous diseases.

Cytologically, vitreous samples from non-lymphoma cases showed lower cellularity than the

lymphoma cases. Whereas vitreous material from cases with infectious endophthalmitis

showed prominent neutrophilic infiltration, material from sarcoidosis cases showed

infiltration of small lymphoid cells, especially CD4-positive T cells. On the other hand,

lymphoma cases showed higher cellularity, with large, irregular and atypical lymphoid cells,

frequent necrotic cells in the background, and less pronounced neutrophil infiltration.

Immunocytochemically, 11 of the 12 lymphoma cases were of the B-cell phenotype and the

remaining case was of the T/NK-cell phenotype.

In conclusion, careful cytopathological examination or immunocytochemistry of vitreous

material facilitates appropriate diagnosis of intraocular lymphoma.

Introduction

  Intraocular lymphoma is a rare neoplasm manifesting as either primary intraocular

lymphoma (PIOL) or secondary intraocular lymphoma[1]. In PIOL, only the eyes and/or central

nervous system are affected, whereas in secondary intraocular lymphoma, a lymphoma

located outside the eyes or central nervous system metastasizes to the eye. Approximately

42-92% of PIOL cases eventually lead to intracranial lymphoma, which means that the

neoplasm is closely associated with primary central nervous system lymphoma (PCNSL)[1-4].

  Intraocular lymphoma account for 1-2% of all extranodal lymphomas, and <1% of intraocular

neoplasms [1, 5]. It is estimated that the incidence of intraocular lymphoma has been increasing

in parallel with an increase in PCNSL [6]. Histologically, most intraocular lymphomas are

classified as diffuse large B-cell lymphomas (DLBCLs) [7].

  Frequent symptoms of intraocular lymphoma at presentation include deterioration of

visual acuity and blurred vision, which masquerade as chronic uveitis. Examination of the

posterior segment reveals vitreous cells and haze or subretinal lesions, which are classically

creamy lesions with orange-yellow infiltrates. Because biopsy from the retina is difficult and

risky due to its anatomical and functional characteristics, vitrectomy is commonly

undertaken for diagnosis of intraocular lymphoma. We previously reported the diagnostic

efficacy of cell block preparation of vitreous samples compared to conventional smear cytology

[8]. Still, diagnosis using vitreous material poses a challenge to pathologists owing to the sparse cellularity and possible degeneration of the samples.

In this study, we added further histological examination of various types of non-lymphoma cases to explore the significance of pathological diagnosis using vitreous materials.

Furthermore, detailed immunocytochemical examination was performed to elucidate the immunophenotype of intraocular lymphoma.

Materials and Methods

Patients

We retrospectively reviewed 37 samples from 33 patients obtained by vitrectomy at our

hospital between June 2006 and March 2014. The medical records and pathology database

were searched for the patients' age, gender, clinical diagnosis, history of lymphoma, presence

of CNS lesion, the levels of IL-6 and IL-10 in vitreous fluid, and immunoglobulin heavy chain

(IgH) gene rearrangement in vitreous fluid. This study was approved by the Institutional Review

Board on Ethical Issues of Hokkaido University Hospital, Sapporo, Japan.

The patients included 13 men and 20 women with a mean age of 64.6 years (range, 17-82

years). A final diagnosis of malignant lymphoma was made in 12 cases, and non-lymphoma in

21 cases, based on cytopathological review, clinical course, IL-10/IL-6 ratio in vitreous fluid

and IgH gene rearrangement. Eleven of 12 malignant lymphoma cases were included in our

previous study [8]. One T/NK-cell lymphoma (case 12) was previously reported [9].

Of the 12 lymphoma cases, 8 had a previous history or coexistence of extraocular lymphoma.

Of these, 5 cases had a history or coexistence of CNS lymphoma, 1 case had a history of

DLBCL of the right inguinal lymph node, 1 had a history of splenic DLBCL, and 1 had a

coexisting bone marrow lesion (as detailed below). In 4 of the 5 cases of CNS lymphoma,

biopsy had not been undertaken due to the patients' poor physical status. These latter 4 cases

were diagnosed as CNS lymphoma on the basis of positron emission tomography (PET),

magnetic resonance imaging (MRI) and vitreous fluid examination. The remaining case of

CNS lymphoma was histologically proven CNS-DLBCL. The final clinicopathologic diagnoses

of the non-lymphoma cases included infectious endophthalmitis in 6, uveitis of uncertain

etiology in 6, epiretinal membrane in 2, sarcoidosis in 1, Behçet's disease in 1, familial

amyloidosis in 1, vitreous hemorrhage in 1, acute retinal necrosis in 1, vitreomacular traction

syndrome in 1, and retinal detachment in 1.

Cytopathological examination

Cell block preparations were performed as follows. Vitreous perfusion fluid was centrifuged

at 3,000 rpm for 3 min and the supernatant was discarded. The dry pellet was fixed in 10%

neutral buffered formalin overnight, and then the samples were processed for paraffin

embedding and sectioning as usual.

For cytopathological examination, we examined cellularity, background appearance, cell

types, and cell atypia. The extents of necrosis, neutrophils, histiocytes, small lymphocytes and

atypical lymphocytes were scored as absent (-), mild (+), moderate (++) or marked (+++).

In lymphoma cases or possible lymphoma cases, immunocytochemical staining was

performed using antibodies against CD3 (clone LN10, Leica, RTU), CD5 (Clone 4C7, Leica,

RTU), CD20 (clone MJ1, Leica, RTU), CD10 (clone 56C6, Leica, RTU), CD56 (clone CD564,

Leica, RTU), Bcl-2 (Clone bcl-2/100/D5, Leica, RTU), Bcl-6 (clone LN22, Leica, RTU), MUM-1

(clone EAU32, Leica, RTU) and Granzyme B (clone GrB-7, MONOSAN, 1:50). For in situ

hybridization, a Bond™ Ready-to-Use ISH EBER Probe (Leica, Catalog No: PB0589probe)

was used.

Results

Cytopathological examination of non-lymphoma cases

Representative non-lymphoma cases are shown in Figures 1 and 2. Non-lymphoma cases

showed low cellularity and mixed cellular infiltration including neutrophils, histiocytes and

small lymphocytes. In the case of retinal detachment, histiocytes with pigment deposition

were frequent in a fibrin-like, eosinophilic background (Fig. 1A). Neutrophils were sparse and

lymphocytes were rarely seen (Fig. 1B). The case of infectious endophthalmitis showed

prominent neutrophils in a dirty, bluish background (Fig. 1C). Histiocytes were also observed

while lymphocytes were rare (Fig. 1D). In the case of sarcoidosis, the cellularity was low, and

the cells consisted of small lymphocytes without atypia and a few histiocytes in a clean

background (Fig. 2A).   In the case of uveitis of uncertain etiology, the cellularity was also low

and small lymphocytes without atypia were frequent in a clean background, similar to the

H&E appearance of the sarcoidosis case (Fig. 2F). The cytopathological features of the 21

non-lymphoma cases are summarized in Table 1. In the non-lymphoma group, no case showed

necrotic material in the background.

Of 21 cases, immunocytochemical staining was performed in 8 cases and was evaluable in 7

cases.   In remaining 1 case, there was no evaluable cell in immunocytochemical staining.

Most lymphocytes were positive for CD3 and negative for CD20 in all 7 cases (Fig. 2B, 2C, 2G,

2H). In the case of sarcoidosis, those positive for CD4 outnumbered those positive for CD8,

although the number of evaluable cell was very low (Fig. 2D, 2E). On the other hand, both

CD4-positive cells and CD8-positive cells were observed in equal numbers in the case of

uveitis of uncertain etiology, (Fig. 2I, 2J).

Cytopathological examination of lymphoma cases

 A representative lymphoma case is shown in Figure 3. In the background, ghost-like necrotic

cells were prominent (Fig. 3A). Cellularity was relatively higher than in non-lymphoma cases.

The majority of cells were atypical lymphocytes with hyperchromatic irregular nuclei, being

larger than small lymphocytes (Fig. 3B). Immunocytochemistry demonstrated positivity for

CD20 in atypical cells and necrotic cells (Fig. 3D). CD3 was negative in atypical lymphocytes

but positive in small lymphocytes (Fig. 3C). CD10 was negative atypical lymphocytes, but

MUM-1 was positive (Fig. 3E, 3F).

The cytopathological features of the 12 lymphoma cases are summarized in Table 2. Ten

of the 12 lymphoma cases (83.3%) showed atypical lymphocytes; however, we were unable to

identify definitive atypical lymphocytes in 2 cases, due to sparse cellularity and cellular

degeneration.   On the other hand, necrotic material in the background was observed in 11

out of 12 lymphoma cases (91.7%), being the most characteristic feature. Eleven of the 12

cases showed a B-cell phenotype (91.7%), while the remaining case showed a T/NK-cell

phenotype. One B-cell lymphoma case with a history of splenic DLBCL was negative for CD20,

probably due to previous rituximab treatment; however, the alternative B-cell markers,

CD79a and PAX5, were positive in lymphoma cells. In 9 of the 11 B-cell lymphoma cases,

immunocytochemical staining for CD10, Bcl-6 and MUM-1 was performed, and all 9 cases

were negative for CD10. The immunocytochemical phenotypes of Bcl-6 and MUM-1 were as

follows: 4 cases were Bcl-6-positive/ MUM-1-positive, 3 cases were Bcl-6-negative/ MUM-1-

positive, and 2 cases were Bcl-6-negative/ MUM-1-negative, all of which corresponded to the

non-germinal center type. Furthermore, CD5 was positive in 1 of 5 cases, and Bcl-2 was

positive in 4 of 4 cases.　EBER-ISH was performed in only one B-cell lymphoma case; the

result was positive in lymphoma cells although the staining was obscure due to non-specific

staining of neutrophils.

　The case of T/NK-cell lymphoma is shown in Figure 4. In the background, eosinophilic

material was prominent, probably due to retinal detachment (Fig. 4A). Necrotic material was

not evident, and there were various cell types, including lymphoid cells, neutrophils,

eosinophils and histiocytes. Immunocytochemical staining revealed that most lymphoid cells

were positive for CD3 (Fig. 4B) and negative for CD20 (data not shown). We were unable to

reach a diagnosis from this vitreous sample, but biopsy from the bone marrow showed large

atypical lymphoid cells (Fig. 4F), which were positive for CD3, CD56, Granzyme B and

EBER-ISH (Fig. 4G-4J). Clinicopathologically, this patient was diagnosed as having a mature

NK/T-cell neoplasm. Retrospective immunocytochemical staining of vitreous material

revealed a few CD56-positive, Granzyme B-positive and EBER-ISH-positive lymphocytes (Fig.

4C-4E). Together, the findings obtained from the vitreous sample were consistent with involvement of a mature NK/T-cell neoplasm.

Assessment of cytokines and IgH gene rearrangement using vitreous fluid

Measurement of IL-6 and IL-10 levels in vitreous fluid was performed in 18 cases. Among them, 8 cases showed an IL-10/IL-6 ratio higher than 1, while 10 cases showed a ratio lower than 1. Of the 11 lymphoma cases, 8 (72.7%) showed an IL-10/IL-6 ratio higher than 1. On the other hand, all non-lymphoma cases showed a ratio lower than 1. Information about IgH gene clonal rearrangement was available in 16 cases; 9 showed IgH gene rearrangement while 6 were negative for IgH gene rearrangement. The rearrangement analysis was unsuccessful in 1 case due to DNA degradation. Out of 11 B-cell-type lymphomas, 9 (81.8%) were positive for IgH gene rearrangement. All non-lymphoma cases and the T/NK-cell lymphoma case were negative for IgH rearrangement.

Discussion

Although intraocular lymphoma is a rare intraocular neoplasm, its incidence is considered to

be increasing along with that of PCNSL. Pathological diagnosis using samples of vitreous is

important for reaching a definitive diagnosis of intraocular lymphoma, and is valuable in

terms of its relatively low invasiveness. Also, CNS lymphoma with intraocular lesions is

sometimes diagnosed based on vitreous material because brain biopsy is invasive. In this

study, we reviewed 33 vitreous samples from 12 lymphoma cases and 21 non-lymphoma cases

in order to examine the significance of pathological diagnosis using vitreous materials.

There have been several previous reports on cytologic diagnosis of intraocular lymphoma

using vitreous material [8, 10-12]. However, cytologic appearance of non-lymphoma cases has not

been fully elucidated. We investigated the characteristic appearance of intraocular lymphoma

by reviewing not only lymphoma cases but also non-lymphoma cases, including retinal

detachment, infectious endophthalmitis, sarcoidosis and uveitis of uncertain etiology. Our

study revealed that necrotic material in the background, predominance of lymphocytes and

cellular atypia were the key features for making a pathological diagnosis of intraocular

lymphoma.

In addition to cytomorphological examination, cell block preparation makes it possible to

examine a variety of immunocytochemical stains retrospectively. In our research,

immunocytochemical staining for CD10, Bcl-6 and MUM-1, as well as the routinely stained

CD3 and CD20, was performed in 9 B-cell lymphoma cases. As reported previously, all 9 cases

were negative for CD10 [7].   Other immunophenotypes of intraocular lymphoma have not been

fully elucidated, but it is known that most cases of PCNSL are positive for Bcl-6 and MUM-1,

corresponding to the non-germinal center type of systemic DLBCL [13, 14]. In our study, all B-cell

lymphoma cases corresponded to the non-germinal center type. Furthermore, CD5 was

positive in 1 out of 5 cases, and Bcl-2 was positive in 4 out of 4 cases. EBER-ISH was

performed in only one of 11 B-cell lymphoma cases, and the lymphoma cells were positive

although the staining was obscure due to non-specific staining of neutrophils. These data may

be of help when trying to understand the origin of intraocular lymphoma. Although most

intraocular lymphoma have been considered DLBCL, further immunocytochemical

examination including Ki-67 and MYC is needed in order to exclude other B-cell lymphoma

and explore malignant potential. It's important to prepare enough unstained sections in

advance to avoid sample loss.

　　Additionally, we performed immunocytochemical staining in 1 case of sarcoidosis, and most

lymphocytes were found to be positive for CD3, of which CD4-positive cells outnumbered

CD8-positive cells. This result is consistent with a previous report that demonstrated the high

diagnostic value of the CD4/CD8 ratio determined by flow cytometry of vitreous material in

cases of ocular sarcoidosis [15]. It is suggested that immunocytochemical staining using cell

block preparations could also be useful for diagnosis of non-lymphoma cases.

Although we found cell block preparations to be diagnostically significant, pathological

diagnosis may sometimes be challenging due to specimen paucity and cell degeneration.

Accordingly, several adjunctive diagnostic tests are required to supplement pathologic

diagnosis. Intraocular lymphoma is reported to exhibit a high IL-10/IL-6 ratio (>1) with

approximately 81% sensitivity and 100% specificity [16, 17]. In our study, 8 out of 11 lymphoma

cases (72.7%) exhibited a high IL-10/IL-6 ratio (>1) (data were not available in one case). One

case of mature NK-cell neoplasm showed a low IL-10/IL-6 ratio, which indicates that different

cytokine pathways are activated in T/NK-cell lymphoma. Furthermore, B-cell lymphoma

cases with a low IL-10/IL-6 ratio may indicate the presence of different subtypes with

different etiology in the DLBCL group.

In addition to the IL-10/IL-6 ratio, clonality assay of B lymphocytes using IgH is useful for

diagnosis of intraocular lymphoma. In this study, 9 out of 11 B-cell lymphomas (81.8%)

showed IgH rearrangement. It is known that the IgH rearrangement test does not have 100%

sensitivity. Furthermore, PCR-based IgH rearrangement assay may give a false-positive

result [18]. Together, comprehensive assessment of all tests is required for accurate diagnosis of

intraocular lymphoma.

Our study included one T/NK-cell lymphoma that showed a distinctive appearance compared to B-cell lymphoma cases. In this case, various types of cells were observed, including lymphoid cells, neutrophils, eosinophils and histiocytes. Necrotic material in the background was not prominent, unlike the B-cell lymphoma cases. We were able to reach a diagnosis of T/NK-cell lymphoma by retrospective immunocytochemical staining after examination of bone marrow biopsy specimens. Because the IL-10/IL-6 ratio and IgH rearrangement are not reliable for diagnosis of T/NK-cell lymphoma, cytopathological examination of vitreous material is essential.   Pathologists should consider T/NK-cell lymphoma when encountering various types of cell, including atypical lymphoid cells, in vitreous samples, especially in cases with an aggressive clinical course.

In conclusion, we have reviewed 33 cell blocks of vitreous material from cases including both lymphoma and non-lymphomatous diseases. Our findings indicate that cell block preparations of vitreous material can be diagnostic for intraocular lymphoma when combined with careful cytomorphological and immunocytochemical examination, in addition to assessment of the IL-10/IL-6 ratio and IgH rearrangement.

Acknowledgement

Disclosure Statement

None Declared

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Table 1. Pathological findings of vitreous specimen in 21 non-lymphoma cases.

	age	sex	Diagnosis	H&E section					IgH	IL-10/IL-6
				necrosis	neutrophil	histiocyte	small lymphocyte	atypical lymphocyte		
1	60	M	Infectious endophthalmitis	-	+++	+++	++	-	ND	ND
2	70	F	Infectious endophthalmitis	-	+++	++	+	-	ND	ND
3	39	M	Infectious endophthalmitis	-	+++	++	+	-	ND	ND
4	77	F	Infectious endophthalmitis	-	++	+++	++	-	ND	ND
5	78	F	Infectious endophthalmitis	-	++	++	+	-	ND	ND
6	32	M	Infectious endophthalmitis	-	+++	+	+	-	ND	ND
7	45	M	Uveitis of uncertain etiology	-	-	++	++	-	ND	ND
8	81	F	Uveitis of uncertain etiology	-	-	++	+	-	ND	<0.1
9	70	F	Uveitis of uncertain etiology	-	-	+	++	-	-	<0.1
10	78	F	Uveitis of uncertain etiology	-	-	++	++	-	-	<0.1
11	81	M	Uveitis of uncertain etiology	-	-	+	-	-	ND	ND
12	69	M	Uveitis of uncertain etiology	-	-	++	++	-	-	<0.1
13	79	M	Epiretinal membrane	-	-	-	-	-	ND	ND
14	17	F	Epiretinal membrane	-	-	-	-	-	ND	ND
15	76	F	Sarcoidosis	-	-	++	++	-	ND	<0.1
16	30	M	Behcet's disease	-	-	++	++	-	ND*	ND
17	79	F	Familial amyloidosis	-	-	-	-	-	ND	ND
18	78	F	Vitreous hemorrhage	-	-	+	+	-	ND	<0.1
19	48	M	Acute retinal necrosis	-	-	++	+	-	ND	ND
20	62	F	Vitreomacular traction syndrome	-	-	+	-	-	ND	<0.1

| 21 | 68 | F | Retinal detachment | - | + | +++ | ++ | - | ND | ND |

* The rearrangement analysis was unsuccessful in 1 case due to DNA degradation.

Table 2. Pathological findings of vitreous specimen in 12 malignant lymphoma cases.

	age	sex	Diagnosis	H&E section					Immunocytochemistry and in situ hybridization								IgH	IL-10/IL-6	History of lymphoma
				necrosis	neutrophil	histiocyte	small lymphocyte	atypical lymphocyte	CD20	CD3	CD10	MUM-1	Bcl-6	CD5	Bcl-2	EBER-ISH			
1	81	F	BCL	+++	+	++	++	-	+	-	ND	ND	ND	ND	ND	ND	+	2.85	none
2	71	M	BCL	+	+	++	++	-	+	-	ND	ND	ND	ND	ND	ND	-	ND	CNS
3	69	F	BCL	++	+	++	++	++	+	-	-	+	-	+	+	+	+	373.45	CNS
4	82	F	BCL	+++	+	++	++	+++	+	-	-	-	-	-	+	ND	+	<0.1	none
5	79	F	BCL	+++	+	++	++	+++	+	-	-	+	-	ND	ND	ND	-	1.27	DLBCL (right inguinal lymph node)
6	76	M	BCL	+++	+	++	++	++	+	-	-	+	+	-	+	ND	+	6.45	none
7	74	F	BCL	+++	+	++	++	+++	+	-	-	-	-	-	+	ND	+	35.52	CNS
8	74	F	BCL	+	+	++	+	+	+	-	-	+	-	-	ND	ND	+	<0.1	CNS
9	44	M	BCL	++	+	++	++	+	-	-	-	+	+	ND	ND	ND	+	70.07	DLBCL (Spleen)
10	65	M	BCL	+++	+	++	++	++	+	-	-	+	+	ND	ND	ND	+	1.84	DLBCL (CNS)
11	49	F	BCL	+	+	++	++	++	+	-	-	+	+	ND	ND	ND	+	5.08	none
12	50	F	T/NK	+/-	++	+++	++	+	-	+	-	-	-	ND	ND	+	-	0.03	T/NK-cell lymphoma (Bone marrow)

Figure legends

Fig.1　Cell block samples of vitreous from a case of retinal detachment (A, B) and a case of

infectious endophthalmitis (C, D).

A. Fibrin-like, eosinophilic material is present in the background. The cellularity is low. (H&E

staining, original magnification x20. Scale bar, 1000 μm)

B. Histiocytes with pigment deposition are frequent. Some neutrophils are also present. (H&E

staining, original magnification x400. Scale bar, 50 μm)

C. Background shows dirty, bluish material. The cellularity is low. (H&E staining, original

magnification x40. Scale bar, 500 μm)

D. The majority of cells are neutrophils. Some histiocytes are also present. (H&E staining,

original magnification x400. Scale bar, 50 μm)

Fig. 2 Cell block samples of vitreous from a case of sarcoidosis (A-E) and a case of uveitis of

 uncertain etiology (F-J).

A-E.   Background is clean. The majority of cells are small lymphoid cells. Some histiocytes

are also present (A: H&E staining). CD20-positive cells are rare (B: CD20). CD3-positive (C:

CD3), CD4-positive (D: CD4) and CD8-negative (E: CD8) cells are frequent. (A-E: original

magnification x400. Scale bar, 50 μm)

F-J: The background is clean. The majority of cells are small lymphoid cells. Neutrophils and

histiocytes are infrequent (F: H&E staining). Most lymphoid cells are positive for CD3 and

negative for CD20 (G: CD20, H: CD3). Among them, both CD4-positive cells (I: CD4) and

CD8-positive (J: CD8) cells are present in equal numbers. (A-E: original magnification x400.

Scale bar, 50 μm)

Fig. 3 Cell block sample of vitreous from a case of diffuse large B-cell lymphoma.

A. Low-power view shows prominent ghost-like necrotic cells. In addition to the necrotic

background, viable lymphoid cells are shown. (H&E staining, original magnification x100.

Scale bar, 100 μm)

B. High-power view shows atypical lymphoid cells with a high N/C ratio and hyperchromatic

nuclei. (H&E staining, original magnification x400. Scale bar, 50 μm)

C. CD3 is positive in small lymphocytes and negative in atypical lymphoid cells. (CD3,

original magnification x400. Scale bar, 50 μm)

D. Both atypical lymphoid cells and ghost-like necrotic cells are positive for CD20. (CD20,

original magnification x400. Scale bar, 50 μm)

E. CD10 is negative in atypical lymphoid cells. (CD10, original magnification x400. Scale bar,

50 μm)

F. MUM-1 is positive in atypical lymphoid cells. (MUM-1, original magnification x400. Scale

bar, 50 μm)

Fig. 4 Cell block sample of vitreous (A-E) and a bone marrow biopsy material (F-J) from a case

of T/NK-cell lymphoma.

A. The background shows eosinophilic material; however, necrotic cells are not prominent.

Various types of cells are seen including lymphoid cells, neutrophils, eosinophils and

histiocytes. (H&E staining, original magnification x400. Scale bar, 50 μm)

B. Most lymphoid cells are positive for CD3. (CD3, original magnification x400. Scale bar, 50

μm)

C. Lymphoid cells are partially positive for CD56. (CD56, original magnification x400. Scale

bar, 50 μm)

D. Lymphoid cells are partially positive for Granzyme B. (Granzyme B, original magnification

x400. Scale bar, 50 μm)

E. A few lymphoid cells are positive for EBER-ISH. (EBER-ISH, original magnification x400.

Scale bar, 50 μm)

F. Bone marrow biopsy material is 60% cellular and shows proliferation of large atypical

lymphoid cells. (H&E staining, original magnification x400. Scale bar, 50 μm)

G. Most atypical lymphoid cells are positive for CD3. (CD3, original magnification x400. Scale

bar, 50 μm)

H. CD3-positive cells are partially positive for CD56. (CD56, original magnification x400.

Scale bar, 50 μm)

I. CD3-positive cells are positive for Granzyme B. (Granzyme B, original magnification x400.

Scale bar, 50 μm)

J. Most CD3-positive cells are positive for EBER-ISH. (EBER-ISH, original magnification
x400. Scale bar, 50 μm)