Clinicopathological analysis of 34 Japanese patients with EBV-positive mucocutaneous ulcer

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Abstract

Epstein-Barr virus (EBV)-positive mucocutaneous ulcer (EBVMCU) is a unifocal mucosal or cutaneous ulcer that is histologically characterized by proliferating EBV-positive atypical B cells. While EBVMCU demonstrates a histology similar to that of EBV-positive diffuse large B-cell lymphoma (DLBCL), their clinical behavior differs. Thus, characterizing distinguishing features of EBVMCU and EBV-positive DLBCL is critical. To identify unique characteristics between EBVMCU and lymphoma, we analyzed the clinicopathological and genetic features of 34 Japanese patients with EBVMCU and compared them to those of 24 EBV-positive DLBCL patients and 25 EBV-negative DLBCL patients. All patients with EBVMCU had localized ulcerative lesions, and 31 patients (91%) were using immunosuppressants, such as methotrexate (MTX) or hydroxycarbamide. All patients that were followed up with exhibited good prognosis following immunosuppressant reduction or chemotherapy. Additionally, 17 EBV-positive DLBCL patients, and 15 EBV-negative DLBCL patients, received chemotherapy ($P < 0.001$, $P < 0.001$, respectively). Our data showed that EBVMCU did not increase indicators associated with lymphoma prognosis, such as soluble interleukin 2 receptor (sIL-2R) and lactate dehydrogenase (LDH) compared to those in the EBV-positive DLBCL or EBV-negative DLBCL groups (sIL-2R, $P < 0.001$, $P =$

0.025; LDH, *P* = 0.018, P = 0.038, respectively). However, histologically, EBVMCU exhibited EBV-positive, variable-sized, atypical B-cell proliferation. Thus, EBVMCU was histologically classified as: (1) polymorphous; (2) large cell-rich; (3) classic Hodgkin lymphoma-like; and (4) mucosa-associated lymphoid tissue lymphoma-like. Moreover, genetic analysis showed that immunoglobin heavy chain (IGH) gene rearrangement did not differ significantly between EBVMCU and EBV-positive DLBCL (44% vs. 32%; *P* = 0.377), or between EBVMCU and EBV-negative DLBCL (44% vs. 58%; *P* = 0.280). Therefore, it is difficult to distinguish EBVMCU from EBV-positive DLBCL using only pathological and genetic findings, suggesting that clinical information is important in accurately distinguishing between EBVMCU and EBV-positive DLBCL.

Introduction

Epstein-Barr virus (EBV)-positive mucocutaneous ulcer (EBVMCU) was first described as a distinct clinicopathological entity in 2010, when Dojcinov *et al.* reported 26 patients with ulcerative lesions confined to the oropharynx, skin and gastrointestinal tract (1). These patients were immunosuppressed, with either age-related immunosenescence or by iatrogenic immunosuppression. Subsequently, EBVMCU has been reported in patients with primary immunodeficiencies (2-4), solid organ or bone marrow transplant recipients (5-8) and human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) (9). In general, patients with EBVMCU exhibit good prognosis with spontaneous regression or complete remission following reduced immunosuppression (10). EBVMCU was later described by the World Health Organization as a new disease concept, which was distinct from lymphomas, and recognized as a specific type of iatrogenic immunodeficiency-associated lymphoproliferative disorders (LPD) (11).

Histologically, EBVMCU is characterized by EBV-positive, polymorphous, atypical proliferating B cells that can resemble Hodgkin and Reed-Sternberg (HRS) cells. These atypical cells accompany dense polymorphic inflammatory cell infiltration, such as plasma cells, eosinophils and histiocytes. The EBV-positive cells demonstrate B-cell

immunophenotypes, i.e., CD20 expression. Thus, it is difficult to distinguish EBVMCU from lymphomas (1, 10, 11). To clarify clinical, pathological and molecular characteristics between EBVMCU and diffuse large B-cell lymphoma (DLBCL), we examined clinicopathological features and gene rearrangements for immunoglobulin heavy chain (IGH) and T cell receptor (TCR).

Materials and methods

Patient samples

We analyzed the clinicopathological features of 34 patients with EBV-positive mucocutaneous ulcers. All cases were retrieved from the surgical pathology consultation files of the Department of Pathology at Okayama University between 2009 to 2019. Of the 34 EBVMCU patients, 33 were biopsy specimens and one was a resected specimen. We excluded patients with a history of lymphoma; other lesions identified by radiation diagnosis i.e., positron emission tomography and computed tomography; as well as samples with inappropriate material for analysis via polymerase chain reaction (PCR), such as microtissues or necrotic tissues. We also selected 24 patients with EBV-positive DLBCL and 25 patients with EBV-negative DLBCL as control groups, all of which were not on active therapy by immunosuppressants. All EBVMCU, EBV-positive DLBCL and EBV-negative DLBCL cases were reviewed independently by three pathologists (TI, TY and YS) to confirm the diagnosis and immunophenotype.

This study was approved by the Institutional Review Board of Okayama University.

Histological examination and *in situ* hybridization

Specimens were fixed in 10% formaldehyde and embedded in paraffin. Serial, 3-μm thick sections were cut from paraffin-embedded tissue blocks for staining procedures. Sections were stained with hematoxylin and eosin or were immunohistochemically stained with antibodies specific for CD3 (clone: LN10, 1:200; Novocastra Laboratories, Ltd., Newcastle upon Tyne, UK), CD5 (clone: 4C7, 1:100; Novocastra Laboratories, Ltd.), CD10 (clone: 56C6, 1:100; Novocastra Laboratories, Ltd.), CD15 (clone: Carb-3, 1:50; DAKO, Glostrup, Denmark), CD20 (clone: L26, 1:100; DAKO), CD30 (clone: Ber-H2, 1:40; DAKO), CD79a (clone: JCB117, 1:50; DAKO) and Ki-67 (clone: MIB-1, 1:2500; DAKO). Staining was performed using automated Bond Max Stainer (Leica Biosystems, Wetzlar, Germany) according to manufacturer's instructions. Immunoglobulin kappa or lambda light chains (Igκ or Igλ) were detected by *in situ* hybridization with Kappa and Lamda probes (PB0645, PB0669, respectively; Leica Biosystems) using automated Bond Max Stainer (Leica Biosystems). EBV was detected by *in situ* hybridization for EBV-encoded small RNA (EBER, fluorescein-conjugated oligonucleotide probe: PB0589; Leica Biosystems) using automated Bond Max Stainer (Leica Biosystems).

PCR assays of the IGH locus and TCR gene rearrangements

Tissue sections were scraped from the lesion and placed in AmpliTaq Gold Buffer (Applied Biosystems, Inc., Foster City, CA, USA). DNA was extracted by incubating at 94°C for 45 minutes in an automated GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Inc.). DNA was quantified using NanoDrop ND1000 spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). All gene rearrangement analyses were performed by a method that has been previously described (12). All primers were purchased from Sigma-Aldrich (Sigma-Aldrich Japan, Tokyo, Japan). PCR products were analyzed using ABI PRISM 310 Genetic Analyzer with GeneScan Analysis and GeneMapper software (Applied Biosystems, Inc.) (12). For IGH rearrangements, DNA was amplified using Framework Region II and III primers. JH consensus primer was fluorescently labeled (6-Carboxyfluorescein) (12). For EBVMCU cases, additional IGK rearrangement assays were performed to investigate rearrangements involving Vk loci. IGH, IGK, IGL and TCR gene rearrangements were analyzed and evaluated using BIOMED-2 protocol (12). The results of fragment analysis were interpreted as monoclonal, oligoclonal, or polyclonal. If the exponential amplification of PCR is a single high peak, cell samples are monoclonal. In the same way, two high peaks indicate oligoclonal samples and multiple peaks indicate

polyclonal samples. If peaks were not visible, the samples were deemed to have an undetectable expression.

Statistical analysis

Statistical differences between EBVMCU, EBV-positive DLBDL and EBV-negative DLBCL were determined using the Mann-Whitney test and the Chi-squared (χ^2) test. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS for Windows software version 14.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinical features

The clinical features of 34 EBVMCU patients have been summarized in Table 1. The median age of the 34 cases was 73 years (range: 54 – 91 years). The male-to-female ratio was 0.89:1. A total of 30 patients were treated with MTX for rheumatoid arthritis (RA) or polymyalgia rheumatica. One patient was treated for polycythemia using hydroxycarbamide. Further, one patient was treated with tacrolimus (TAC) as an immunosuppressant in addition to MTX. Another three patients were not on active immunosuppression therapy. The median soluble interleukin 2 receptor (sIL-2R) value was 652 U/mL (range: 263 – 2786 U/mL, n = 22). The median lactate dehydrogenase (LDH) value was 212 U/mL (range: 151 – 397 U/mL, n = 28) and the median serum albumin (SA) value was 3.8 g/dL (range: 2.9 – 4.6 g/dL, n = 25). All patients had mucosal or cutaneous ulcers with no apparent mass lesions [gingiva (n = 13, 38%), tonsil (n = 7, 21%), pharynx (n = 3, 9%), tongue (n = 2, 6%), oral cavity (n = 3, 9%), buccal mucosa (n = 1, 3%), nasal cavity (n = 2, 6%) and skin (n = 3, 9%)] (Figure 1) . Multiple ulcers were identified in the oral mucosa or bilateral tonsils in three cases. (Case No.4, 13, 15) Patients did not have lesions in other regions.

After the diagnosis, MTX or hydroxycarbamide was discontinued in 29 patients. In 28 patients (97%), lesion remission occurred and treatment was discontinued. No additional chemotherapy was found to be necessary. Only one patient did not present with resolved lesions. This patient then received chemotherapy (R-THP-COP; combined rituximab, pirarubicin, cyclophosphamide, vincristine and prednisolone) and had complete remission (Case No.8). Since the disease concept of EBVMCU was not established at the time of diagnosis, one patient was treated with chemotherapy without reducing MTX (Case No.4). For the same reason, another one patient with no history of immunosuppression was treated with chemotherapy (R-CHOP; combined rituximab, cyclophosphamide, vincristine, Adriamycin and prednisolone) and was in complete remission (Case No.7).

Among the 24 patients with EBV-positive DLBCL, the median age was 77 years (range: 33 – 94 years) and the male-to-female ratio was 3:1. One patient had a nodular skin lesion with no ulcer and another 23 patients had no mucocutaneous lesions. The median sIL-2R value was 4585 U/mL (range: 460 – 18600 U/mL, n = 22), the median LDH value was 265 U/mL (range: 146 – 903 U/mL, n = 22) and the median SA value was 3.3 g/dL (range: 2.0 – 4.9 g/dL, n = 17). Of the 17 patients who received

chemotherapy, four exhibited complete remission after treatment and eight patients died of the primary disease.

Among the 25 patients with EBV-negative DLBCL, the median age was 73 years (range: 33 – 92 years) and the male-to-female ratio was 1.5:1. The median sIL-2R value was 2392 U/mL (range: 199 – 18832 U/mL, n = 25), the median LDH value was 277 U/mL (range 142 – 1576 U/mL, n = 25) and the median SA value was 3.9 g/dL (range: 2.8 – 4.4 g/dL, n = 20). Of the 15 patients who received chemotherapy, nine exhibited complete remission after chemotherapy and three died of the primary disease. We have shown the clinical features of EBVMCU, EBV-positive DLBCL and EBV-negative DLBCL in Table 2 and Figure 2. The median follow-up durations were 20.4 months, 30.9 months and 30.3 months for patients with EBVMCU, EBV-positive DLBCL and EBV-negative DLBCL, respectively.

Histological and immunohistochemical features

EBVMCU presented with localized mucosal or cutaneous ulcers. The disease is characterized by the presence of atypical lymphoid cells of various sizes and is accompanied by dense polymorphic infiltration with variable inflammatory cells such as plasma cells, histiocytes and granulocytes. The large cells often resemble HRS cells.

Angioinvasion was observed in 14 of the 34 EBVMCU patients (Figure 3). Atypical lymphoid cells did not infiltrate into the epithelium. Accordingly, we classified EBVMCU into the following morphological types:

Polymorphous

Polymorphous refers to cases with various small to large atypical EBER-positive lymphoid cells. Some cases include few HRS-like cells. Atypical lymphoid cells are found in various densities such as dense and scattered. This type is often associated with necrosis and angioinvasion. Of the 34 EBVMCU patients, 20 cases (59%) were classified as polymorphous (Figure 4).

Large cell-rich

The atypical lymphoid cells primarily consist of large and monomorphic, with dense proliferation observed similar to those in monomorphic proliferation DLBCL. Seven of the 34 EBVMCU patients (21%) were classified as large cell-rich (Figure 5).

Classic Hodgkin lymphoma (CHL)-like

This type consists of many HRS cells and various sized atypical lymphoid cells. The aggregation of CD30-positive HRS cells is highlighted similar to those in CHL, however EBER-positive small to medium sized atypical lymphoid cells also are seen. Some cases also contain epithelioid granulomas or eosinophil infiltration. Of the 34

EBVMCU patients, 4 cases (12%) were classified as CHL-like (Figure 6). Interestingly, two cases of these cases exhibited spontaneous regression. This clinical outcome differed from the CHL type of other iatrogenic immunodeficiency-associated LPD (13).

Mucosa-associated lymphoid tissue (MALT) lymphoma-like

The atypical lymphoid cells show small to medium sized, centrocytic-like features, and/or plasmacytic features, which proliferate in the expanded interfollicular zone. One case showed mature plasmacytic differentiation with Russell bodies (Figure 7). All cases exhibited light chain restriction and had no lymphoepithelial lesions. Of the 34 EBVMCU patients, three (9%) were classified as MALT lymphoma-like, two of which exhibited spontaneous regression.

Immunohistochemistry

Positive CD3, CD5, CD10, CD20, CD30 and EBER staining was observed in 0% (0/34), 0% (0/12), 10% (1/10), 94% (32/34), 92% (11/12) and 100% (34/34) of the atypical lymphoid cells in samples, respectively. Two cases were negative for CD20 and positive for CD79a.

Atypical B cells exhibited diffuse or scattered infiltration primarily in subepithelial tissue. The background consisted mainly of numerous CD3-positive T cells (Figure 4).

Similarly, in EBV-positive DLBCL, B cells exhibited a wide range of infiltration patterns, from diffuse to scattered.CD3-positive T cells did not clearly form a rosette around HRS-like cells.

All three cases of MALT lymphoma-like EBVMCU showed Igκ-monotype by *in situ* hybridization.

Molecular features

Table 3 shows the IGH and TCR rearrangements. In patients with EBVMCU, IGH (FR2) PCR analysis was successful in 23 cases and monoclonality was detected in four patients (17%). IGH (FR3) PCR analysis was successful in 32 cases and monoclonality was detected in 13 patients (41%). In summary, IGH rearrangement was detected in 14 of the 32 patients (44%). TCR rearrangement was detected in nine of the 28 patient cases (32%) that were successfully analyzed by PCR.

We also investigated light chain rearrangements in EBVMCU. As summarized in Table 3, IGK monoclonality was detected in ten of the 25 patients (40%) that were successfully assayed for IGK by PCR. Of the seven patients with a successful IGL PCR analysis, six (86%) showed an IGL rearrangement.

Table 3 also shows the results of EBV-positive DLBCL and EBV-negative DLBCL cases: EBV-positive DLBCL [IGH: monoclonal/oligoclonal (32%, n = 7), polyclonal (68%, n = 15); TCR: n = 20, monoclonal/oligoclonal (10%, n = 2) and polyclonal (90%, n = 18)]. EBV-negative DLBCL [IGH: monoclonal/oligoclonal (58%, n = 14), polyclonal (42%, n = 10); TCR: n = 20, monoclonal/oligoclonal (15%, n = 3) and polyclonal (85%, n = 17)].

We then compared the IGH rearrangement in EBVMCU, EBV-positive DLBCL and EBV-negative DLBCL, and observed that the IGH rearrangement occurred in 44% (14/32), 32% (7/22, $P = 0.377$) and 58% (14/24, $P = 0.280$) of the cases, respectively. In addition, the TCR rearrangement occurred in 32% (9/28), 10% (2/21, $P = 0.060$) and 15% (3/20, $P = 0.176$) of the cases, respectively.

Discussion

EBVMCU was first described by Dojcinov *et al.* in 2010 as a distinct clinicopathological entity occurring in immunosuppressed patients demonstrating either age-related immunosenescence or iatrogenic immunosuppression (1). Before EBVMCU was defined, several reports described mucosal ulcerations occurring as a side effect of MTX therapy. However, most of these reports did not perform histological evaluation (14). Soon after, EBVMCU was reported in patients with primary immunodeficiencies (3, 4), solid organ or bone marrow transplant recipients (5-8) and in patients with HIV/AIDS (9). EBVMCU was later described as a new disease type by the World Health Organization (11). Here, it should be noted that other disease concepts, such as MTX-LPD and EBV-positive marginal zone lymphoma (MZL), overlap with EBVMCU (15, 16). Given that most EBVMCU do not require chemotherapy, we believe that prioritizing diagnosis of EBVMCU over other immunodeficiency-associated LPD will benefit those patients with overlapping disease concepts.

In this study, we investigated the clinicopathological differences as well as the IGH and TCR gene rearrangements between EBVMCU and DLBCL. Overall, more female patients developed EBVMCU, which may be related to the fact that diseases involved in

RA tend to be more common in women (17). Our data showed that EBVMCU did not increase lymphoma prognosis indicators, such as sIL-2R and LDH (18), compared to that in EBV-positive DLBCL and EBV-negative DLBCL. Since lesions in EBVMCU are local, patients tend to show relatively low sIL-2R or LDH levels. Particularly, the level of sIL-2R exhibited significant differences (Figure 2). However, it is necessary that the clinical significance of LDH and sIL-2R levels be ultimately entrusted to each clinician. Of note, many EBVMCU patients had autoimmune diseases that caused chronic inflammation. Therefore, hemoglobin and SA values were not found to differ significantly between EBVMCU and EBV-positive DLBCL, EBVMCU and EBV-negative DLBCL cases.

In this study, one patient with age-related EBVMCU received chemotherapy. The lesions in 24 patients with iatrogenic EBVMCU achieved complete or partial remission after discontinuation of the immunosuppression therapy. One patient suffered relapse after hydroxycarbamide re-administration. These results indicate that EBVMCU relapse is associated with repeated immunosuppression. Given that patients with EBVMCU generally reach complete remission, we suggest that further long-term observations may be required. Moreover, even if the ulcers improved, EBVMCU often destroys normal tissues, making clinical complete remission difficult to determine.

Herein, we classified EBVMCU into four morphological types as described above. It is necessary to be aware of this histological variety in EBVMCU to accurately distinguish it from lymphomas.

In the best of our knowledge, MALT lymphoma-like EBVMCU has not been previously reported. However, some reports have proposed EBV-positive MZL occurring in immunosuppressed patients (15, 16). These reports found that most patients had clinically indolent disease with response to reduced immune suppression. In this regard we consider the disease concept of EBV-positive MZL to overlap with MALT lymphoma-like EBVMCU. However, clinical findings differ between MALT lymphoma-like EBVMCU, and EBV-positive MZL. For instance, in previous reports, EBV-positive MZL was described as being extraoral with non-ulcerative tumoral lesions, while MALT lymphoma-like EBVMCU is characterized by oral and ulcerative lesions. Considering the clinical findings, MALT lymphoma-like EBVMCU should be distinguished from EBV-positive MZL.

Few previous reports have investigated the clonality in EBVMCU. Dojcinov *et al.* first reported that 38% of the cases exhibited IGH rearrangements and 31% exhibited TCR rearrangements, however, there were no control groups included in this study (1). In 2011, relatively low frequency of the clonal IGH rearrangements were described in

patients with age-related EBVMCU compared to those in EBV-positive DLBCL and EBV-negative DLBCL (19).

In the current study, the clonal IGH rearrangements in EBVMCU and EBV-positive DLBCL patients tended to occur less frequently than in patients with EBV-negative DLBCL (44%, 32%, 58%). However, there were no significant differences between the three groups. Thus, IGH rearrangements are not useful for distinguishing between EBVMCU and DLBCL.

Previous reports have shown that most EBV-positive cases exhibit polyclonal multiplication (13, 20). EBV-positive DLBCL showed a relatively low frequency of clonal IGH rearrangements, although this may be due to possible false-negative PCR results, which can be attributed to the presence of EBV-positive blastocytes. Since EBV-positive cases tend to have a low IGH monoclonality, the IGH rearrangement was also found to be of low frequency in EBV-positive DLBCL (13, 20).

A previous report also demonstrated that serum CD8-positive T cells were elevated after MTX reduction (21). Meanwhile, another report showed that B-cell post-transplant LPD were associated with clonal expansion of CD8-positive T cells (22). Thus, we considered that EBVMCU also may be associated with T cell clonal expansion as a result of reduced immune surveillance. Another report showed the presence of TCR

beta chain variable restriction in more than half of the elderly patients (23). In our study, clonal TCR rearrangements in EBVMCU tended to occur more frequently than in EBV-positive DLBCL and EBV-negative DLBCL cases (32%, 10%, 15%). Our data, therefore, supports that T cell clonal expansion in EBVMCU is associated with immunosuppression, as with other immunodeficiency-associated LPD (21, 22). In addition, T cell clonality does not indicate EBVMCU or the possibility of T cell lymphoma.

While T cell clonality was observed, the HRS-like cells may not have an immune evasion mechanism. For example, previous reports have shown the presence of PD-L1 in most EBV-positive DLBCL cases (24-26), however, PD-L1 was absent in all EBVMCU cases (27). These results suggest that there may be no immune evasion mechanism as seen in EBV-positive DLBCL.

In summary, EBVMCU in the Japanese population represents a wide pathological spectrum, similar to that exhibited by neoplastic lesions, that includes polymorphic LPD, sometimes, DLBCL, CHL and MALT lymphoma. Furthermore, the IGH rearrangement in EBVMCU has been confirmed, though at a lower frequency than that observed in EBV-negative DLBCL. Regardless of the histological feuture and the IGH rearrangement, EBVMCU shows good prognosis.

In our study, EBVMCU was histologically and genetically difficult to distinguish from lymphoma, suggesting that the collection of clinical information, particularly that related to medical history, lesion location and laboratory data may lead to more accurate diagnosis.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure legends

Figure 1. Macroscopic findings of Epstein-Barr virus-positive mucocutaneous ulcer in buccal mucosa (Case No. 31). The ulcer appeared while the patient was undergoing methotrexate treatment (A). After reducing methotrexate, the lesion spontaneously disappeared (B).

Figure 2. Comparison of sIL-2R and LDH in EBVMCU, EBV-positive DLBCL and EBV-negative DLBCL

(A) Differences and distribution of sIL-2R in EBVMCU, EBV-positive DLBCL and EBV-negative DLBCL. (B) Differences and distribution of LDH in EBVMCU, EBV-positive DLBCL and EBV-negative DLBCL. Box plot explanation: upper horizontal line of box, 75th percentile; lower horizontal line of box, 25th percentile; horizontal bar within box, median; upper horizontal bar outside box, 95th percentile; lower horizontal bar outside box, 5th percentile.

Figure 3. Pathologic findings of angioinvasion in EBVMCU.

Various sized atypical lymphoid cells infiltrating the wall of blood vessels. These atypical lymphoid cells are positive for EBER. (HE, EBER, ×400)

Figure 4. Pathologic findings of polymorphous EBVMCU

(A) A gingival ulcer of a 71-year-old-female undergoing methotrexate treatment (Case No. 28). Atypical polymorphous lymphoid cells with few HRS-like cells are seen with granulocytes (×400). Atypical lymphoid cells infiltrated and destroyed the blood vessel wall. The atypical cells infiltrating the vessel wall are positive for CD20 and EBER. The background consists primarily of numerous CD3-positive cells without atypia (B: HE, CD20, CD3, EBER, ×200).

Figure 5. Pathologic findings of large cell-rich EBVMCU

A gingival ulcer of an 85-year-old female resembling DLBCL (Case No. 27). The lesion shows a monomorphic and dense proliferation of atypical large lymphoid cells. *In situ* hybridization shows atypical cells positive for EBER. (HE, EBER, ×400)

Figure 6. Pathologic findings of CHL-like EBVMCU

A tonsillar ulcer of an 83-year-old male undergoing methotrexate treatment (Case No. 26). (A) Lymphoid cells infiltration with epithelioid granuloma observe under the ulcer (×100). (B) This lesion includes small to large sized atypical lymphoid cells and many

HRS cells with epithelioid granuloma (×200). (C) Hodgkin cell. (D) Reed-Sternberg cell with epithelioid cells. The HRS cells and other polymorphous atypical lymphoid cells are positive for EBER. The HRS cells are also positive for CD30 (C, D, CD30, ×400, EBER, ×200).

Figure 7. Pathologic findings of MALT lymphoma-like EBVMCU

A lingual ulcer in a 66-year-old female undergoing methotrexate treatment (Case No. 14). (A) This lesion shows ulceration and dense lymphoid cell proliferation (×40). (B) The atypical lymphoid cells show centrocytic-like feature with plasmacytic differentiation proliferating in the expanded interfollicular zone (×100). (C) Atypical plasmacytic cells with Russell bodies are seen (HE). The atypical lymphoid cells are positive for EBER. (EBER, ×400)

Table 1 Clinical and pathological findings of patients with Epstein-Barr virus-positive mucocutaneous ulcer

Case No.	Age	sex	Site	Cause of immunosuppression	Management	sIL-2R (U/ml)	LDH (IU/L)	WBC (/μl)	Ly (/μl)	Hb (g/dl)	Alb (g/dl)	Histological type	Angioinvasion	Spontaneous regression	Response	Outcome (months)
1	71	M	Tonsil	IS (MTX for RA therapy)	Reduced IS	N.D.	N.D.	5500	897	12	3.9	Large	−	YES	CR	109
2	66	M	Tonsil	IS (MTX for RA therapy)	Reduced IS	2786	209	2700	864	9.8	4.1	Large	−	YES	PR	101
3	61	F	Nasal cavity	IS (MTX for RA therapy)	Reduced IS	N.D.	173	N.D.	N.D.	N.D.	N.D.	Large	+	YES	CR	100
4	91	M	Gingiva	IS (MTX for RA therapy)	R-THPCOP	N.D.	203	5550	1249	11.8	4.1	CHL	+	N.D.	N.D.	26
5	80	F	Nasal cavity	IS (MTX for RA therapy)	Reduced IS, R-THPCOP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Large	−	NO	CR	63
6	80	M	Gingiva	Age	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Poly	−	N.D.	N.D.	N.D.
7	69	M	Gingiva	Age	R-CHOP	263	151	8100	2236	13.3	4.1	MALT	−	NO	CR	56
8	81	M	Oral cavity	IS (MTX for RA therapy)	Reduced IS, R-THPCOP	477	269	7990	320	11.2	3.6	Large	+	NO	CR	28
9	79	M	Angle of mouth	IS (Hydroxycarbamide for polycythemia)	Reduced IS	493	397	10570	423	10.9	3.6	Poly	−	YES	PR	7
10	73	M	Tonsil	Age	N.D.	851	249	4250	1305	12.9	4.3	Poly	−	N.D.	N.D.	49
11	73	F	Oral cavity	IS (MTX for RA therapy)	Reduced IS	N.D.	286	4450	979	10.9	4.2	CHL	−	YES	CR	34
12	74	F	Nasopharynx	IS (MTX for RA therapy)	Reduced IS	1000	277	11600	1508	10.7	3.6	Poly	−	YES	CR	19
13	65	F	Tonsil	IS (MTX for RA therapy)	Reduced IS	551	211	5200	2132	10.8	3.8	CHL	−	YES	CR	1
14	66	F	Tongue	IS (MTX for RA therapy)	Reduced IS	373	152	4800	629	10.7	4.1	MALT	−	YES	PR	23
15	70	F	Gingiva	IS (MTX for RA therapy)	Reduced IS	744	195	7500	975	11.5	3.4	Large	+	YES	CR	33
16	76	F	Gingiva	IS (MTX for RA therapy)	Reduced IS	894	206	7500	1875	12.8	N.D.	Poly	+	YES	CR	33

17	71	M	Skin of leg	IS (MTX for PMR therapy)	Reduced IS	466	273	4110	1278	12.5	4.3	Poly	−	YES	CR	21
18	74	F	Tonsil	IS (MTX for RA therapy)	Reduced IS	N.D.	281	N.D.	N.D.	N.D.	N.D.	Poly	+	YES	CR	21
19	78	F	Gingiva	IS (MTX and TAC for RA therapy)	Reduced IS	N.D.	236	4830	1159	10.2	3.5	Poly	−	YES	PR	10
20	75	F	Tongue	IS (MTX for RA therapy)	Reduced IS	N.D.	260	4060	889	11	3.5	Poly	+	YES	PR	11
21	78	M	Oral cavity	IS (MTX for RA therapy)	Reduced IS	628	185	6310	1660	13.4	3.9	Poly	+	YES	PR	3
22	72	F	Skin of leg	IS (MTX for RA therapy)	Reduced IS	1157	327	4150	506	11.3	3.1	Poly	+	YES	PR	2
23	70	F	Tonsil	IS (MTX for RA therapy)	Reduced IS	1771	221	4800	336	11.6	3.4	Poly	−	N.D.	N.D.	N.D.
24	67	M	Pharynx	IS (MTX for RA therapy)	Reduced IS	666	200	6950	514	12	3.8	Poly	−	YES	CR	26
25	81	M	Pharynx	IS (MTX for RA therapy)	Reduced IS	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Poly	−	YES	CR	13
26	83	M	Tonsil	IS (MTX for RA therapy)	N.D.	655	154	7090	N.D.	12.7	N.D.	CHL	−	N.D.	N.D.	N.D.
27	85	F	Gingiva	IS (MTX for RA therapy)	Reduced IS	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Large	+	YES	CR	15
28	71	F	Gingiva	IS (MTX for RA therapy)	Reduced IS	385	200	6710	570	13.7	4.6	Poly	+	N.D.	N.D.	N.D.
29	67	M	Gingiva	IS (MTX for RA therapy)	Reduced IS	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Poly	+	YES	CR	11
30	65	F	Gingiva	IS (MTX for RA therapy)	Reduced IS	500	233	8800	1602	12.5	4.3	Poly	+	YES	CR	3
31	54	M	Buccal mucosa	IS (MTX for RA therapy)	Reduced IS	648	212	7560	1875	11.4	3.8	Poly	−	YES	PR	1
32	67	F	Gingiva	IS (MTX for RA therapy)	Reduced IS	270	225	8420	1372	14.3	4.2	MALT	−	YES	CR	2
33	75	M	Gingiva	IS (MTX for RA therapy)	Reduced IS	1224	180	6840	903	11.6	2.9	Poly	−	YES	CR	5

34	86	F	Gingiva	IS (MTX for RA therapy)	Reduced IS	921	187	7000	3983	9.4	3.5	Poly	+	YES	CR	4

CHL, classic Hodgkin lymphoma-like feature; CR, complete remission; Large, large cell rich feature; F, female; IS, immunosuppression; M, male; MALT, mucosa-associated lymphoid tissue lymphoma-like feature; MTX, methotrexate; N.D., not done; PMR, polymyalgia rheumatica; Poly, polymorphous feature; PR, partial response; RA, rheumatoid arthritis; TAC, tacrolimus; UD, undetectable.

Table 2 Comparison of laboratory findings in EBVMCU, EBV-positive DLBCL and EBV-negative DLBCL

	EBVMCU n=34	EBV-positive DLBCL n=24	EBV-negative DLBCL n=25
sIL-2R (U/ml)	652 (263-2786)	4584 (460-18600)	2392 (199-18832)
	n=22	n=22	n=25
		P<0.001*	*P*=0.025*
LDH (U/ml)	212 (151-397)	265 (146-903)	277 (142-1576)
	n=28	n=22	n=25
		P=0.018*	*P*=0.038*
WBC (/μl)	6710 (2700-11600)	6395 (2700-12670)	5730 (2700-9170)
	n=27	n=20	n=24
		P=0.714	*P*=0.540
Lymphocytes (/μl)	1069 (423-3983)	1010 (313-2361)	1041 (247-2322)
	n=26	n=17	n=20
		P=0.747	*P*=0.690
Hemoglobin (g/dl)	11.6 (9.3-14.3)	11.7 (8.3-17.8)	12.7 (7.7-16.1)
	n=27	n=19	n=23
		P=0.823	*P*=0.052
Albumin (g/dl)	3.8 (2.9-4.6)	3.3 (2.0-4.9)	3.9 (2.8-4.4)
	n=25	n=17	n=20
		P=0.004*	*P*=1.000

sIL-2R: soluble interleukin 2 receptor, LDH: lactate dehydrogenase, WBC: white blood cell count

Table 3 Comparison of molecular findings in EBVMCU, EBV-positive DLBCL and EBV-negative DLBCL

	EBVMCU (n=34)	EBV-positive DLBCL (n=24)	EBV-negative DLBCL (n=25)
IGH (FR2) clonality			
mono/oligo	4 (17%)	1 (13%)	10 (53%)
poly	19 (83%)	7 (88%)	9 (47%)
IGH (FR3) clonality			
mono/oligo	13 (41%)	6 (30%)	10 (42%)
poly	19 (59%)	14 (70%)	14 (58%)
IGH clonality			
mono/oligo	14 (44%)	7 (32%)	14 (58%)
poly	18 (56%)	15 (68%)	10 (42%)
		P=0.377	*P*=0.280
IGK clonality			
mono/oligo	10 (40%)	Not done	Not done
poly	15 (60%)		
IGL clonality			
mono/oligo	6 (86%)	Not done	Not done
poly	1 (14%)		
TCR clonality			
mono/oligo	9 (32%)	2 (10%)	3 (15%)
poly	19 (68%)	18 (90%)	17 (85%)
		P=0.060	*P*=0.176

mono: monoclonal, oligo: oligoclonal, poly: polyclonal

Figure 1

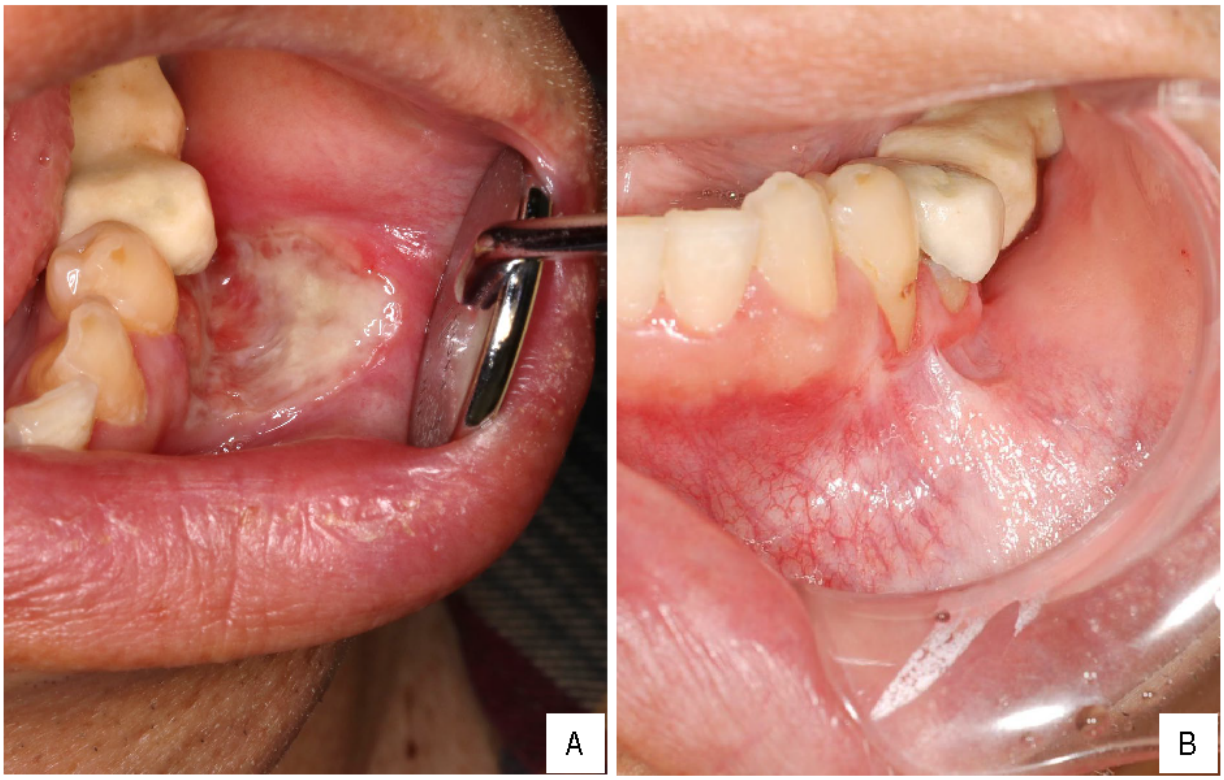
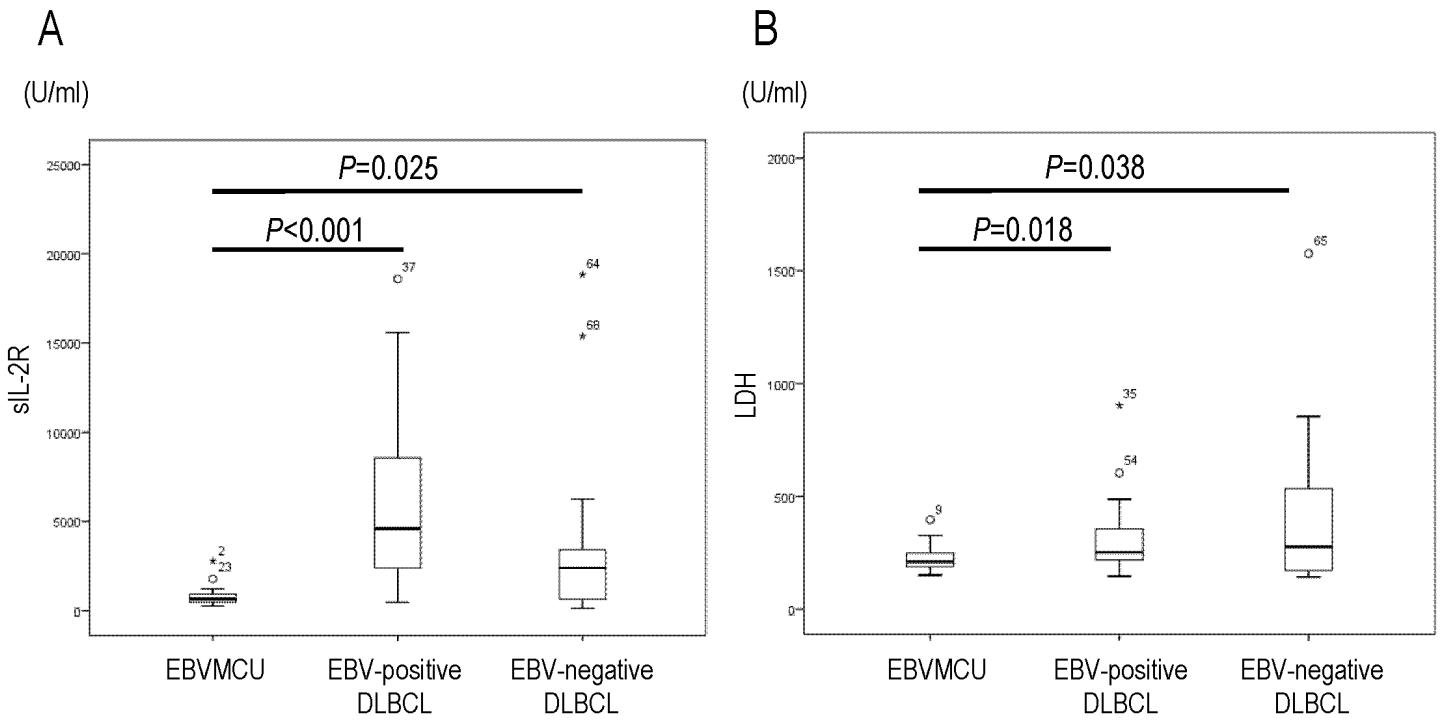


Figure 2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(U/ml)
sIL-2R
25000
20000
15000
10000
5000
0
P=0.025
P<0.001
37
64
68
2
23
EBVMCU
EBV-positive DLBCL
EBV-negative DLBCL
B
(U/ml)
LDH
2000
1500
1000
500
0
P=0.038
P=0.018
65
35
54
9
EBVMCU
EBV-positive DLBCL
EBV-negative DLBCL

Figure 3

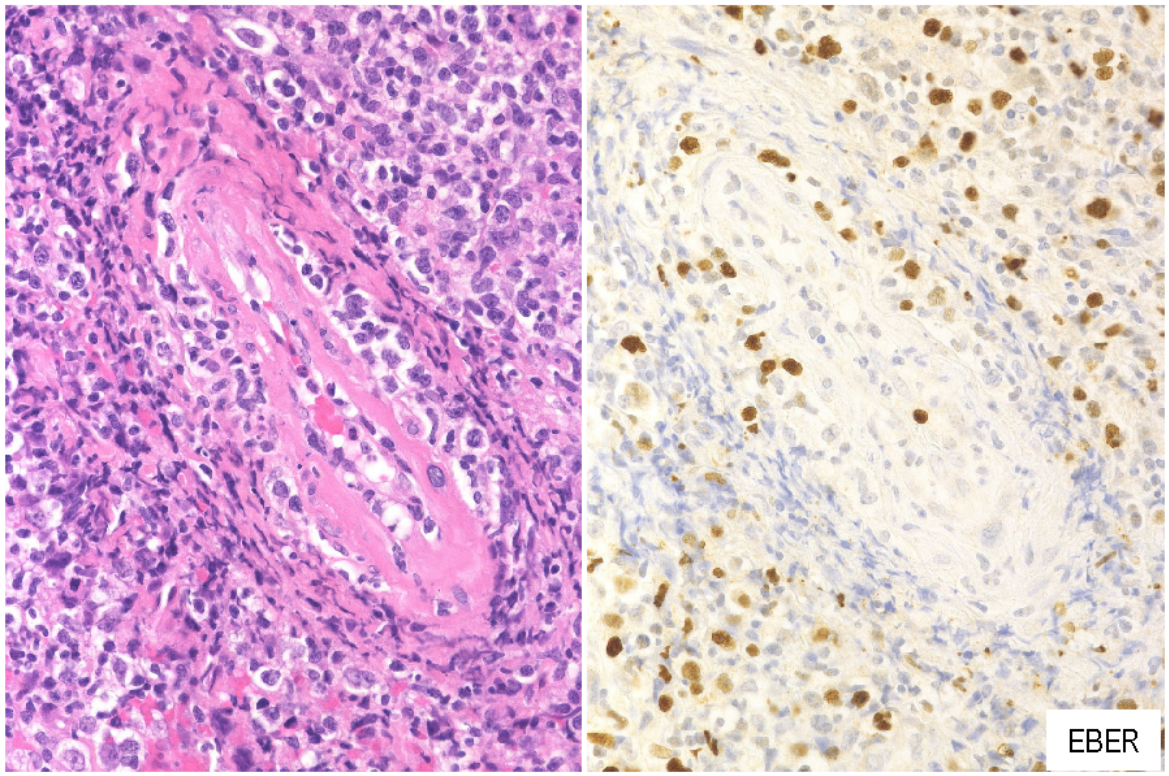

Figure 4

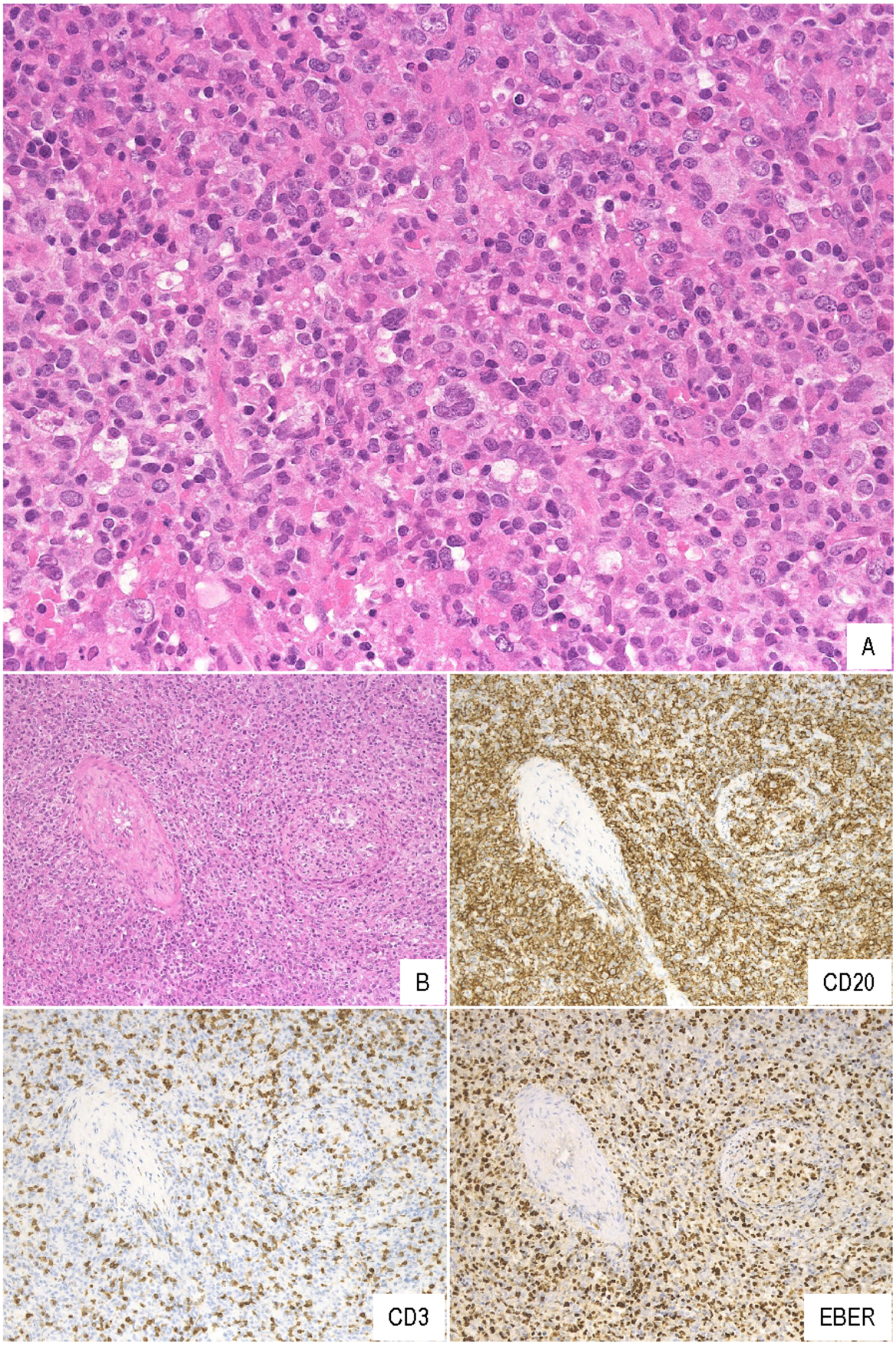

Figure 5

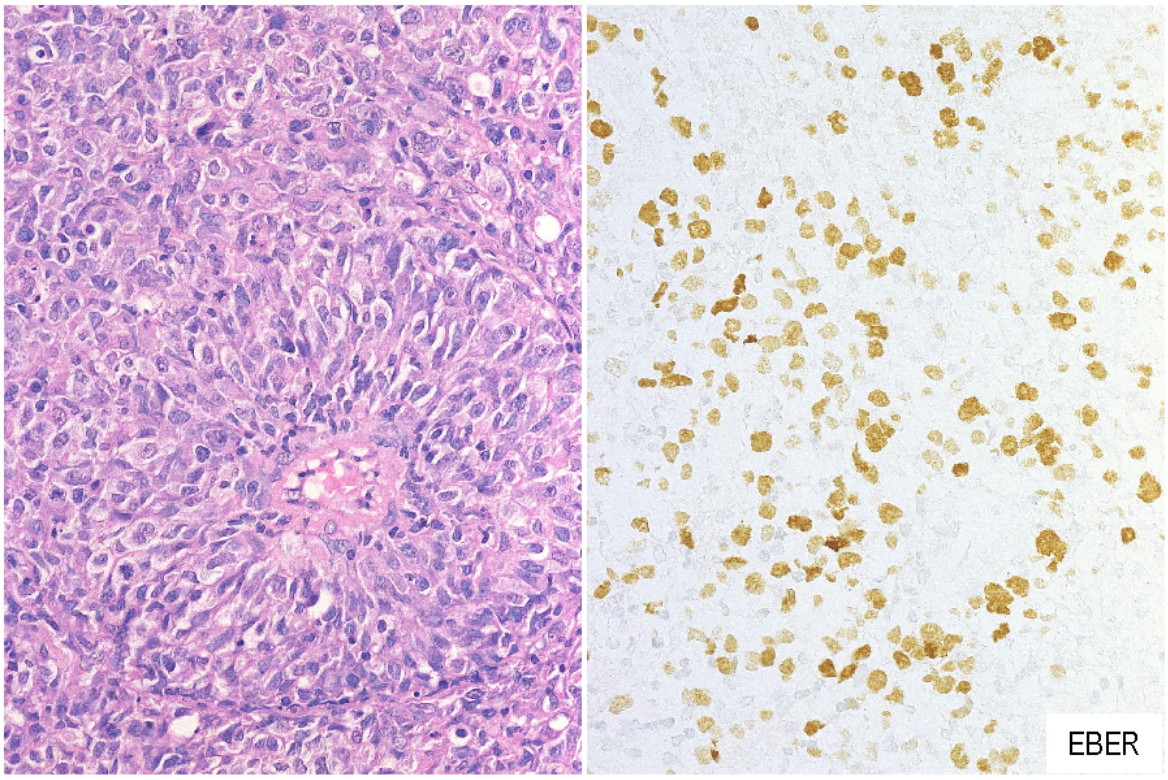

Figure 6

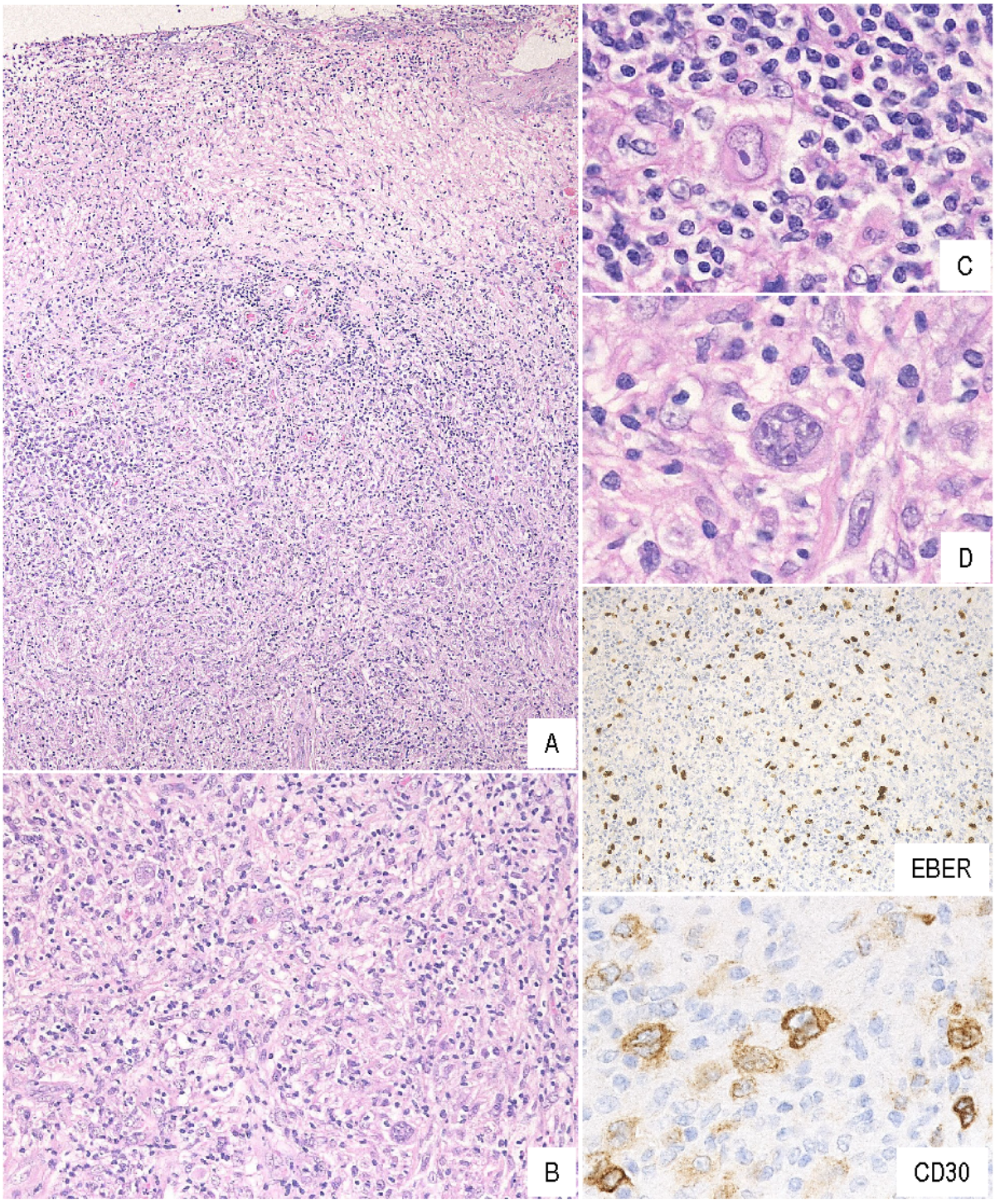

Figure 7