Clinicopathological evaluation of methotrexate-associated lymphoproliferative disorders with special focus on Epstein-Barr virus-positive mucocutaneous lesions

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Some patients diagnosed with methotrexate-associated lymphoproliferative disorder (MTX-LPD) develop spontaneous regression upon the discontinuation of MTX, whereas others require chemotherapy. The mechanisms underlying this differential response and the capacity to spontaneously regress are not clearly understood. We evaluated numerous clinicopathological features in 63 patients diagnosed with MTX-LPD, with a special focus on those with Epstein-Barr virus (EBV)-positive mucocutaneous lesions (EBVMCL). The diagnosis of EBVMCL included cases of both EBV-positive mucocutaneous ulcers (EBVMCU) and diffuse gingival swelling associated with proliferation of EBV-positive large B-cells. Of the four subgroups of MTX-LPD, one-year treatment-free survival (TFS) after the discontinuation of MTX was achieved among those with EBVMCL (100%), diffuse large B-cell lymphoma (57%), Hodgkin-like lesions (60%), or classical Hodgkin lymphoma (29%); a significant difference in TFS was observed when comparing the responses of patients with EBVMCL to those diagnosed with other subtypes. Multivariate analysis revealed predictive factors for prolonged TFS that included EBV-positive lesions and comparatively low levels of serum LDH. Taken together, our study suggests that a diagnosis of EBVMCL is related to the overall clinical outcome after the discontinuation of MTX.

Keywords: Methotrexate-associated lymphoproliferative disorder, Epstein-Barr virus, Mucocutaneous ulcer, Lymphoma, Prognosis

INTRODUCTION

Methotrexate-associated lymphoproliferative disorder (MTX-LPD) was initially described by Ellemans et al. in 1991, who documented a patient who developed lymphoma during immunosuppressant treatment for autoimmune disease. MTX-LPD is now recognized as an important clinical entity and includes many lymphomas that develop in patients undergoing MTX treatment for rheumatoid arthritis (RA). In 2017, the World Health Organization (WHO) subclassified this diagnosis within “other iatrogenic immunodeficiency-associated lymphoproliferative disorders” as part of a larger category of immunodeficiency-associated lymphoproliferative disorders.

MTX has been used as a first-line drug for RA in Japan since 1999; this drug is currently regarded as the most effective treatment for 0.6–1 million RA patients. RA patients are likely to develop lymphoma at a 2–4-times higher rate than that observed in the general population; RA patients undergoing treatment with MTX are 1.7-times more likely to develop LPD than those not treated using this immunomodulatory drug. Although MTX itself is not believed to promote the development of LPDs in patients with RA, disease activity associated with this diagnosis together with MTX-mediated immune suppression may induce its development.

There are several published reports documenting the clinicopathological characteristics of MTX-LPD in Japan. MTX-LPD includes several characteristic histological...
subtypes and is associated with a high rate of EBV infection affecting many extra-nodal sites.6-11 Diffuse large B-cell lymphoma (DLBCL) was the most common histological subtype identified in these studies, followed by classic Hodgkin lymphoma (CHL).12-14 Although the disease can regress in some MTX-LPD patients after discontinuation of the drug, one recent study reported comparatively poor regression rates for patients who developed CHL compared with those with DLBCL.14

EBV-positive mucocutaneous ulcer (EBVMCU) is a newly recognized disease2 that is characterized by ulcerative lesions at cutaneous and mucosal sites, including the oropharynx and gastrointestinal tract. Histologically, these lesions are notable for proliferation of EBV-positive large-sized B-cells including Hodgkin and Reed-Sternberg (HRS)-like cells.15,16 EBVMCUs develop most frequently in patients with age-related immune-senescence or who have experienced iatrogenic immunosuppression; this condition is associated with a good prognosis. Although EBVMCU is a common subtype of MTX-LPD and is likely to regress after discontinuation of the drug,17 its definition is unclear.11

We occasionally treat patients with MTX-LPD who do not develop ulcers but exhibit diffuse tumorous swelling of the gingiva, as previously described by Ishida et al.18 Gingival histology included the proliferation of EBV-positive large-sized B-cells, similar to the histology typically associated with EBVMCU. These patients were not included among those diagnosed with EBVMCU due to the absence of mucosal ulcerative lesions; however, such tumor-type lesions may be included among the diagnoses associated with EBVMCU as they typically regressed after the discontinuation of MTX therapy.

In this study, we evaluated the clinicopathological characteristics of 63 patients diagnosed with MTX-LPD over a 10-year period (2007 to 2017) at Tokai University Hospital, with a specific focus on EBV-positive mucocutaneous lesions (EBVMCL), including EBVMCU and gingival swelling. We demonstrated that EBVMCL are common and typically regress after the discontinuation of MTX.

MATERIALS AND METHODS

Patients

Sixty-three patients diagnosed with MTX-LPD between January 2007 and December 2017 at the Department of Pathology of Tokai University Hospital, Japan, were enrolled in this study. This retrospective study had a maximum follow-up time of 117 months (median, 24 months). Primary diagnoses included RA in 60/63 (95%), combined RA and Sjögren’s syndrome (SjS) in 2/63 (3%), and mixed connective tissue disease in 1/63 (2%). As shown in Figure 1, our final analysis included 48 patients, including those diagnosed with EBVMCL, DLBCL, Hodgkin-like lesions (HLL), or CHL.

Diagnostic criteria

In this study, MTX-LPD was defined as LPD that developed in patients with a primary autoimmune disease who were undergoing treatment with MTX. MTX-LPD was subclassified into 4 diagnostic subgroups as follows: (1) EBVMCL, (2) DLBCL, (3) HLL, and (4) CHL; all other

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**Fig. 1.** Patient selection and classification of MTX-LPD

Sixty-three patients were classified into 4 subtypes: EBV-positive mucocutaneous lesions (EBVMCL), diffuse large B-cell lymphoma (DLBCL), Hodgkin-like lesions (HLL), and classic Hodgkin lymphoma (CHL) by the 2017 WHO classification, and 15 with other subtypes were excluded. In addition, our study classified EBV-positive mucocutaneous lesions (EBVMCL) including EBVMCU and gingival swelling.
subtypes were excluded from the analysis (Figure 1). All categories except EBVMCL were as defined by the 2017 WHO classification.

Definition of EBVMCL: EBVMCL included all EBVMCU patients with localized ulcers of the oral mucosa, including the tonsils, tongue, skin, and digestive tract, and those with diffuse tumorous swelling of the gingiva. Proliferation of EBV-positive large-sized cells and occasional HRS-like cells were observed in both.

Histology, immunohistochemistry, and in situ hybridization

Resected tissues were fixed in 10% formaldehyde and embedded in paraffin; some sections were stained with hematoxylin & eosin (H & E), and others were used for immunohistochemistry and in situ hybridization. Immunohistochemical analysis used primary monoclonal antibodies (mAbs), including anti-human CD3 (non-glycosylated epsilon chain, clone LN10, 1:200 dilution, Novocastra [NC], Leica Microsystems K.K., Tokyo, Japan), anti-CD5 (4C7, 1:400, NC), anti-CD10 (56C6, 1:100, NC), anti-CD20 (L26, 1:200, NC), anti-BCL-2 (bcl-2/100/D5, 1:400, NC), anti-BCL-6 (LN22, 1:100, NC), anti-MUM-1 (EAU32, 1:100, NC), anti-CD15 (BY87, 1:50, NC), anti-CD30 (JCM182, 1:100, NC), anti-PAX5 (1EW, 1:100, NC), anti-LMP1 (CS1, CS2, CS3, and CS4, 1:200, NC), and anti-EBNA2 (PE2, 1:50, Vector Laboratories, Burlington, CA, USA). Positive signals were detected using the Leica Bond-Max fully automatic IHC system with the Leica Bond Polymer Refine Detection kit and Bond Epitope Retrieval Solution 2 (EDTA based pH 9.0) for antigen retrieval according to the manufacturer’s instructions (DS9800 and AR9640, Leica Microsystems).

Detection of latent Epstein-Barr virus (EBV) genome was performed by in situ hybridization for EBV-encoded mRNA using the EBER Probe, Anti-Fluorescein Antibody and Bond Polymer Refine Detection kits (Bond ISH EBER Probe, #BP0589; Bond Ready-to-Use primary antibody and anti-fluorescein antibody, #AR0833; Leica Microsystems). EBER-positive samples were evaluated further by immunostaining with anti-LMP1 and anti-EBNA2 as a means to characterize EBV latency; these designations included latency type I (LMP1 EBNA2+), latency type II (LMP1‘ EBNA2+), and latency type III (LMP1‘ EBNA2+).

Clinical information

Clinical information for each patient was obtained from medical records maintained at Tokai University Hospital. Clinical stage (CS) was determined using the patient history, physical examination, presence of B (systemic) symptoms, serum levels of both lactate dehydrogenase (LDH) and soluble IL-2 receptor (IL-2R), in addition to information obtained from positron emission tomography (PET)/computed tomography (CT) and bone marrow biopsies. Tumor responses were evaluated by contrast CT or PET/CT; patients were classified by optimal tumor response according to the reaction criteria defined for malignant lymphoma. The clinical features of patients diagnosed with EBVMCU, DLBCL, HLL, or CHL were analyzed together with immunohistochemical and cytogenetic evaluations of the tumor tissues. Chemotherapy included R-CHOP, or weekly rituximab or CHOP-like regimens; some patients diagnosed with Hodgkin type MTX-LPD were treated using ABVD.

Statistical analysis

The clinicopathological features among groups were compared with the Pearson’s chi-square test. Progression was considered when the patients did not achieve regression after MTX discontinuation (within 1 year). Relapse was considered when the patients exhibited tumor increase after 1 year or chemotherapy. Treatment-free survival (TFS) was evaluated only in patients who discontinued MTX treatment at the time of MTX-LPD onset and who remained untreated during a follow-up period of at least 2 weeks thereafter. Overall survival (OS) was defined as the time from the date of diagnosis of MTX-LPD to the date of death or final follow-up. Patients were excluded if the efficacy of MTX withdrawal was unable to be ascertained. Survival was evaluated using the Kaplan-Meier method and compared using the log-rank test. P-values < 0.05 were used to assess significance. All statistical analyses were performed using EZR (Easy R) software for medical statistics.

RESULTS

Subgrouping by pathological diagnosis

Subgroups of the 63 patients diagnosed with MTX-LPD included (1) EBVMCL, n=17 (27%); 15 with EBVMCU and 2 with gingival swelling (Figures 2 and 3); (2) DLBCL, n=18 (29%); (3) HLL, n=5 (8%); (4) CHL, n=8 (13%); and others n=15 (24%). The uncategorized subtypes included 8 cases of non-Hodgkin lymphoma other than DLBCL and 7 cases identified as reactive conditions, including reactive follicular hyperplasia of the lymph node; no cases of lymphoblastic lymphoma or extra-nodal NK/T-cell lymphoma, nasal type were detected among the otherwise uncategorized subtypes. As such, our final analysis included 48 patients, including those diagnosed with EBVMCL (35%), DLBCL (38%), HLL (10%), or CHL (17%).

Patient characteristics

Clinical characteristics are summarized in Table 1, and clinical information for each patient with EBVMCL or DLBCL is included in Table 2. Among the four subgroups, we observed no significant differences with respect to median age (EBVMCL at 72; DLBCL at 69; HLL, at 64; CHL at 63 years). However, with respect to gender, the CHL subgroup included a significantly higher ratio of men/women than that observed among the DLBCL patients. The duration of MTX therapy in patients who developed CHL ranged from 6.1 to 15.2 years, with a median of 9.9 years; for HLL, the mean duration of MTX therapy ranged from 1.6 to 10.6 years, with a median of 4.1 years. The time to onset of MTX-LPD in patients diagnosed with HLL was shorter than that associated
with the development of CHL. Similarly, extra-nodal involvement was observed significantly more frequently among patients diagnosed with DLBCL than among those who developed CHL.

This series included 2 patients diagnosed with composite lymphoma, specifically, a case of pharyngeal EBVMCU together with EBV-negative DLBCL with systemic lymphadenopathy; this patient was included in the DLBCL subgroup (Table 2, Case 6). We also noted a case in which a patient initially diagnosed with HLL developed peripheral T-cell lymphoma (PTCL-not otherwise specified [NOS]) 3 years later. This patient was included in the HLL group.

Immunohistochemical and cytogenetic findings

The immunohistochemical and cytogenetic findings associated with 48 cases of MTX-LPD subgroups, including EBVMCL, DLBCL, HLL, and CHL, are summarized in Table 3. Expression of CD10, CD20, CD30, and EBER was detected in 18% (7/39), 83% (38/46), 66% (25/38), and 77% (37/48) tissue samples, respectively. Immunostaining of the EBER-positive tissue samples was performed using anti-LMP1 anti EBNA2 antibodies to define latency type in 32
cases. LMP1 and EBNA2 were detected in 88% (28/32) and 22% (7/32) of these cases, respectively. Among these, 13% (4/32) were diagnosed as latency type I (EBER+, LMP-1−EBNA2−), 66% (21/32) were type II (EBER+, LMP-1+EBNA2−), and 22% (7/32) were type III (EBER+, LMP-1+EBNA2+).

Among the EBVMCL cases (n=17), immunohistochemical studies included detection with anti-CD3, 0/15; anti-CD10, 0/14; anti-CD15, 0/11; anti-CD20, 13/15 (87%); anti-BCL-2, 10/11 (91%); anti-BCL-6, 10/12 (83%); and anti-MUM-1, 12/12 (100%). All cases were EBER+ (17/17), with 8% (1/13) identified as latency type I, 69% (9/13) as type II, and 23% (3/13) as type III. Both cases of gingival swelling were CD20-negative, CD79a-positive, and EBER-positive.

Among the DLBCL cases (n=18), immunohistochemical studies included detection with anti-CD3, 0/18; anti-CD10, 7/18 (39%); anti-CD15, 0/12; anti-CD20, 18/18 (100%); anti-BCL-2, 14/18 (78%); anti-BCL-6, 15/18 (83%); and anti-MUM-1, 12/12 (100%). All cases were EBER+ (17/17), with 8% (1/13) identified as latency type I, 69% (9/13) as type II, and 23% (3/13) as type III. Both cases of gingival swelling were CD20-negative, CD79a-positive, and EBER-positive.

Clinical outcomes

Detailed treatment responses were available for 43 patients with a median follow-up of 24 months (range 0.2–120 months); the remaining 8 patients were transferred to another hospital after diagnosis and detailed treatment information was unavailable.

All patients discontinued MTX after diagnosis; patients in all four subgroups included those diagnosed with EBVMCL (n=17), DLBCL (n=18), HLL (n=5), or CHL (n=8). Of these, 27 (56%) exhibited disease regression, including those with EBVMCL (n=13), DLBCL (n=9), HLL (n=3), and CHL (n=2). Sixteen patients (33%) developed disease progression, including those with DLBCL (n=9), HLL (n=2), and CHL (n=5); the median period from diagnosis to initiation of chemotherapy was 2 months (range, 0.5–22 months). The median TFS was unable to be estimated accurately in this patient cohort, although the estimated one-year TFS was 69%. The one-year TFS was significantly different when comparing EBVMCL with other subtypes (Figure 4A). Patients diagnosed with DLBCL and HLL had indistinguishable TFS rates (one-year TFS of 57% and 60%, respectively). Those diagnosed with CHL had the most unfavorable prognosis, with a one-year TFS of 29%. The median overall survival was unable to be estimated, although the estimated five-year OS was 77.7%. OS rates were not significantly different among the groups (Figure 4B).

### Table 1. Clinical characteristics of patients diagnosed with MTX-LPD

|                  | EBVMCL | EBVMCU | DLBCL | HLL | CHL | Other |
|------------------|--------|--------|-------|-----|-----|-------|
| Gingival swelling| n=2    | n=15   | n=18  | n=5 | n=8 | n=15  |
| Median age, (range), year | 79 (74–83) | 70 (55–90) | 69 (44–81) | 64 (51–70) | 63 (41–83) | 68 (47–83) |
| Male: Female     | 0:2 | 5:10 | 2:16 | 1:4 | 4:4 | 6:9 |
| Extra-nodal lesion, n (%) | 2 (100) | 9 (60) | 12 (67) | 1 (20) | 2 (25) | 5 (33) |
| High LDL > 245 U/L, n (%) | 0 | 0 | 11 (61) | 5 (100) | 3 (38) | 5 (41) |
| High sIL2R > 1500 U/mL, n (%) | 0 | 1 (7) | 8 (50) | 3 (60) | 3 (38) | 1 (17) |
| Stage > III, IV, n (%) | 0 | 0 | 15 (83) | 3 (60) | 3 (38) | 5 (33) |
| B symptoms, n (%) | 0 | 0 | 3 (17) | 3 (60) | 3 (38) | 3 (20) |
| Duration, median, year | 1.9 | 20.7 (3.8-32.6) | 14.9 (0.8-52.6) | 6.2 (1-9-21.0) | 12.2 (6.5-35.4) | 10.4 (1.0-20.5) |
| LD1 onset from autoimmune disease diagnosis | 1.9 | 10.4 (3.6-17.4) | 5.6 (0.8-14.6) | 4.1 (1.6-10.6) | 9.9 (6.1-15.2) | 5.1 (1.0-18.9) |
| Regression / progression after discontinuation of MTX, n (%) | 2(100) / 0 | 11 (73) / 0 | 9 (50) / 9 (50) | 3 (60) / 2 (40) | 2 (25) / 5 (62) | 9 (60) / 3 (20) |
| Unknown outcome | 0 (0) | 4 (27) | 0 (0) | 0 | 1 (13) | 3 (20) |
| Relapse, n (%) | 0 | 0 | 4 (24) | 2 (40) | 0 | 2 (15) |

Values shown are the median (interquartile range) unless otherwise indicated. Data are shown from 17 (gingival swelling, 2; ulcer, 15) EBVMCL, 18 DLBCL, 5 HLL, 8 CHL, and 15 other subtype patients. The clinical outcomes of 10 patients remained unknown. EBVMCL, EBV-positive mucocutaneous lesions; DLBCL, diffuse large B-cell lymphoma; HLL, Hodgkin-like lesions; CHL, classical Hodgkin lymphoma; MTX, methotrexate; LPD, lymphoproliferative disease; BA, biologics.
| Patient Number | Age | Sex | Primary immune disease | Immunomodulatory agent | Site | Stage | EBER | EBV latency | Disease progression after discontinuation of MTX |
|---------------|-----|-----|------------------------|------------------------|------|-------|------|-------------|--------------------------------------------------|
| 1* | 74 | F | RA | MTX | gingiva (swelling) | I | + | NT | regression |
| 2* | 83 | F | RA | MTX | gingiva (swelling) | I | + | II | regression |
| 3 | 59 | F | RA | MTX, salazosulfapyridine | tonsil | I | + | II | NR |
| 4 | 70 | M | RA | MTX | skin | I | + | II | regression |
| 5 | 90 | F | RA | MTX, infliximab | skin | I | + | NT | regression |
| 6 | 72 | M | RA | MTX, PSL | tongue | I | + | NT | regression |
| 7 | 55 | F | RA, SjS, UCTD | MTX, infliximab | tonsil | I | + | II | NR |
| 8 | 84 | F | RA | MTX, infliximab | skin | I | + | II | NR |
| 9 | 59 | F | RA | MTX, tocolizumab | pharynx | I | + | III | regression |
| 10 | 79 | F | RA | MTX | pharynx (ulcer) | I | + | II | regression |
| 11 | 83 | F | RA | MTX, infliximab | nasal septum | I | + | III | regression |
| 12 | 79 | F | RA | MTX, infliximab | pharynx | I | + | II | regression |
| 13 | 61 | M | RA | MTX, PSL, salazosulfapyridine | tonsil | I | + | II | NR |
| 14 | 67 | M | RA | MTX, PSL | oral cavity | I | + | III | regression |
| 15 | 61 | F | RA | MTX, bucillamine | tonsil | I | + | NT | regression |
| 16 | 70 | M | RA | MTX | gingiva (ulcer) | I | + | II | regression |
| 17 | 77 | F | RA | MTX, golimumab | pharynx | I | + | I | regression |
| 18 | 69 | M | RA | MTX, tacrolimus | lymph node | I | + | I | regression |
| 19 | 74 | F | RA | MTX, PSL | lymph node | III | + | I | regression |
| 20 | 49 | F | RA | MTX, PSL | bone marrow | IV | + | I | progression |
| 21 | 46 | F | RA | MTX | uterus, lung | IV | + | III | regression |
| 22* | 81 | F | RA | MTX | pharynx, skin, lymph node | IV | + | III | progression |
| 23 | 72 | F | RA | MTX, bucillamine, PSL, salazosulfapyridine | lung | IV | + | III | regression |
| 24 | 81 | F | RA | MTX, salazosulfapyridine | lung | IV | + | III | regression |
| 25 | 63 | F | RA | MTX, abatacept | bone | IV | + | II | regression |
| 26 | 77 | F | RA | MTX | mediastinal | I | – | – | progression |
| 27 | 57 | F | RA | MTX, abatacept | thyroid gland | II | – | – | regression |
| 28 | 81 | F | RA | MTX, bucillamine, etanercept | lymph node | III | – | – | progression |
| 29 | 54 | F | RA | MTX | lymph node | III | – | – | regression |
| 30 | 79 | F | RA | MTX | bone, lung | IV | – | – | progression |
| 31 | 64 | F | RA | MTX, PSL | soft tissue | IV | – | – | progression |
| 32 | 70 | F | RA | MTX, etanercept | bone | IV | – | – | progression |
| 33 | 69 | M | RA | MTX, etanercept | stomach | IV | – | – | regression |
| 34 | 44 | F | RA, SjS | MTX, PSL | ileum | IV | – | – | progression |
| 35 | 62 | F | RA | MTX, abatacept | bone | IV | – | – | progression |

Cases 1 and 2 exhibited only diffuse neoplastic swelling of the gingiva. Case 22 is composite lymphoma of EBVMCU in the pharynx and EBV (−) DLBCL with systemic lymphadenopathy. RA, rheumatoid arthritis; SjS, Sjogren’s syndrome; UCTD, undifferentiated connective tissue disease; MTX, methotrexate; PSL, prednisolone; EBV, Epstein-Barr virus; EBER, EBV-encoded small RNA; NT, not tested; NR, not recorded
EBV-positive and EBV-negative MTX-LPD

Our patient cohort included 37 who were EBV-positive and 11 who were EBV-negative. Among the 4 subgroups, those who were EBV-positive included 17 with EBVMCL, 8 with DLBCL, 5 with HLL, and 7 with CHL. Of note, all EBVMCL and HLL evaluated were EBV-positive. The median one-year TFS rates were significantly higher for patients with EBV-positive lesions than for those with EBV-negative lesions (79% vs. 33%, \( P = 0.001 \); Figure 5A). Similarly, when comparing the median one-year TFS among those diagnosed with DLBCL, patients with EBV-positive lesions had a higher TFS than EBV-negative patients (83% vs. 38%, \( P = 0.028 \); Figure 5B). No differences in TFS associated with EBV latency types were observed.

Analysis of Prognostic Factors

Univariate analyses of subgroups, including EBVMCL, DLBCL, HLL, and CHL, revealed that high levels of serum LDH (\( P = 0.027 \)) and IL-2R (\( P = 0.006 \)), clinical Stage III-IV (\( P = 0.086 \)), and EBV-positive status (\( P = 0.032 \)) were all significant factors related to TFS. Multivariate analysis revealed that EBV-positive status (\( P = 0.006 \)) and high serum LDH (\( P = 0.011 \)) were significant factors predicting TFS (Table 4). Based on these results, we evaluated survival using the log-rank test focused on patients with both EBV-positive lesions and high levels of serum LDH. We found that EBV-positive patients with low serum LDH values had a significantly prolonged TFS compared with those who were EBV-negative with high serum LDH values (\( P = 0.000002 \);
DISCUSSION

In this study, we evaluated the clinicopathological characteristics of MTX-LPD. We divided 48 patients into four subgroups, including the newly proposed group of EBVMCL together with those diagnosed with DLBCL, HLL, or CHL. Patients diagnosed with EBVMCL (including EBVMCU and gingival tumorous lesions) were encountered on a relatively frequent basis and responded positively to discontinuation with good clinical outcomes. Common clinical characteristics of old onset, low LDH, low sIL-2R, limited stage, and no B symptoms were found in patients with EBVMCU or gingival swelling.

Several previous studies of EBVMCU in the context of MTX-LPD reported good clinical outcomes associated with this diagnosis.17,19-21 Of note, the incidence of EBVMCU in association with MTX-LPD is higher than that of age-related EBVMCU.17 The mechanism underlying the markedly high rate of spontaneous regression in response to drug discontinuation among patients in this specific MTX-LPD cohort remains unclear. Among the possibilities, EBV-positive MTX-LPD may be associated with significant impairment of cytotoxic T-cell function due to the combined effects of RA and MTX. As such, this combination of disease and drug therapy may result in the reactivation of EBV and the development of LPD.22,23 This hypothesis is consistent with the findings of Feng et al.24 who also suggested that the administration of MTX promotes reactivation of latent EBV. EBV typically establishes lifelong and persistent infections in the oral cavity in the majority of adults worldwide. EBV copy numbers specifically within tissues in the oral cavity are significantly higher in immunocompromised patients, including those with periodontitis.25,26 Thus, EBV reactivation may be tolerated in the oral cavity during local immunosuppression; these observations may be related to the disease regression typically observed among patients with EBVMCL. For this reason, it is important to recognize that EBV-positive cases with gingival tumorous lesions should be included as EBVMCL. Similar tumorous swelling may be found at other mucocutaneous sites in MTX-LPD patients, but none was noted in our cohort.

We reported one case of composite lymphoma associated with MTX-LPD that included both EBVMCU in the pharynx and EBV-negative DLBCL with systemic lymphadenopathy (Table 2B, Case 6).27 The pharyngeal lesion disappeared after the discontinuation of MTX, although systemic lymphadenopathy remained. Identification of the site of proliferation of EBV-positive large B-cells in the setting of MTX-LPD is another important feature. As such, the diagnosis of EBVMCL, included within the modified criteria of EBVMCU, remains important for predicting the overall clinical outcome and the expected impact of discontinuing MTX.
EBV-positive malignancies are characterized by one of three modes of EBV-latency; these develop in relation to the host immunity and tumorigenesis.\(^2\) For example, post-transplant lymphoproliferative disorder (PTLD) is another iatrogenic LPD that is typically associated with type III latency; under these conditions, host immunity may be reduced. Of note, most of EBV-positive cases of MTX-LPD are associated with type II latency.\(^2\) In our study, many EBVMCL cases were type II, whereas many of the EBV-positive cases of DLBCL were type III. This may also be related to the distinct sites of disease proliferation and immunity patterns of the host.

We demonstrated that EBV-negative status and high levels of serum LDH were both factors leading to a poor prognosis. These findings are consistent with previous studies that reported that EBV-positivity,\(^2\)\(^,\)\(^2\)\(^3\) high levels of serum IL-2R,\(^3\) absolute lymphocyte count,\(^3\)\(^0\)\(^-\)\(^3\)\(^2\) and International Prognostic Index (IPI) risk\(^2\) are all important factors associated with the likelihood of spontaneous regression in patients diagnosed with MTX-LPD.

In conclusion, the new proposed diagnostic category of EBVMCL, which includes both EBVMCU and diffuse gingival swelling, is important regarding our ongoing understanding of MTX-LPD as it is associated with an excellent prognosis. Further analysis and validation of EBVMCL, including differential diagnosis between EBVMCL and EBV-positive DLBCL at mucocutaneous sites, is needed.

**CONFLICT OF INTEREST**

The authors declare no conflicting interests.

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