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Epstein–Barr virus-positive mucocutaneous ulcer resulting in severe methotrexate intoxication: a case report

Kazutoshi Ebisawa[1*], Toshihide Iwashita[2], Kohdai Uchiyama[3], Yasuhiko Kitayama[4] and Takahiro Takeuchi[1]

Abstract

Background Epstein–Barr virus-positive mucocutaneous ulcer is one of the mature B-cell lymphoproliferative diseases occurring in patients with immune dysfunction including those with immunosuppressive treatment such as methotrexate.

Case presentation A Japanese elderly man in his 80s with rheumatoid arthritis on methotrexate was admitted to our hospital complaining persistent pharyngeal pain. Laboratory tests revealed severe pancytopenia, elevated C-reactive protein, and increased creatinine levels. An otolaryngological examination showed ulceration of the right tonsil, from which diagnostic biopsy was performed. The diagnosis of Epstein–Barr virus-positive mucocutaneous ulcer was made and bone marrow aspiration revealed hypocellularity and megaloblastic changes. Pancytopenia was improved after discontinuing methotrexate, and repeated bone marrow aspiration test revealed recovery of normal cellularity and disappearance of dysplasia, confirming the diagnosis of methotrexate intoxication. Tonsil ulcer was improved only with discontinuation of methotrexate, which strongly supported the diagnosis of EBV-MCU.

Conclusion Our case suggested that even this best prognosis form of lymphoproliferative disease could lead to fatal complications if not appropriately managed.

Keywords Rheumatoid arthritis, Methotrexate, EBV-positive mucocutaneous ulcer

Background

Methotrexate (MTX) is considered as a safe and effective agent for autoimmune diseases including rheumatoid arthritis [1, 2]. MTX directly inhibits dihydrofolate reductase, which is an essential enzyme for thymidylate synthesis, resulting in megaloblastic anemia [3]. Although rare, severe pancytopenia and febrile neutropenia could also occur as a severe adverse event of MTX [4–6]. In addition, long-term exposure to MTX induced chronic immunosuppressive conditions and reactivation of Epstein–Barr virus (EBV), resulting in the development of lymphoid proliferations and lymphomas associated with immune deficiency and dysregulation [7–9]. EBV-positive mucocutaneous ulcer (EBV-MCU) is one of such mature B-cell lymphoproliferative diseases characterized with localized ulcerative lesions in skin or mucosa and proliferation of EBV-positive atypical B lymphocytes [9, 10]. Initial treatment for EBV-MCU is to discontinue of MTX just as other immunodeficiency-associated lymphoproliferative disorders, and complete spontaneous

*Correspondence:
Kazutoshi Ebisawa
k181251@siz.saiseikai.or.jp
[1] Department of Hematology, Shizuoka Saiseikai General Hospital, 1-1 Oshika, Suruga-ku, Shizuoka 422-8021, Japan
[2] Department of Regenerative & Infectious Pathology, Hamamatsu University School of Medicine, Hamamatsu, Japan
[3] Department of Otorhinolaryngology, Shizuoka Saiseikai General Hospital, 1-1 Oshika, Suruga-ku, Shizuoka 422-8021, Japan
[4] Department of Pathology, Shizuoka Saiseikai General Hospital, 1-1 Oshika, Suruga-ku, Shizuoka 422-8021, Japan

regression is attained in most cases without further therapy [8].

We describe a case of pharyngeal EBV-MCU complicated with acute kidney injury and severe febrile neutropenia, in contrast to extremely favorable clinical courses generally considered to follow [8, 11],

Case presentation

A Japanese elderly man in his 80 s with rheumatoid arthritis treated with 8 mg MTX every week combined with folic acid supplementation in other clinic for 11 years was admitted to our hospital for further examination of pharyngeal pain lasting 2 months. On general examination, blood pressure was 98/42 mmHg, pulse rate was 61 beats per minute, oxygen saturation was 93%, and body temperature was 36.3 ℃. Superficial lymph nodes were not swelling, and spleen was not enlarged. Otolaryngological examination revealed ulceration of the right tonsil with adhesions of white massive moss (Fig. 1A), from which diagnostic biopsy was performed. Laboratory tests detected severe neutropenia (0.24×10^9/L), thrombocytopenia (13×10^9/L) and anemia (6.1 g/dL), and elevated C-reactive protein (CRP; 19.76 mg/dL) and creatinine levels (2.08 mg/dL). Other results were summarized in Table 1. Computed tomography test revealed the right tonsil swollen, but other lymph nodes were not enlarged.

Table 1 Results of laboratory tests at admission

Parameters	Values	Reference values
White blood cells ($\times10^9$ cells/L)	0.94	3.3–8.6
Neutrophils (%)	25.5	38.5–80.5
Lymphocytes (%)	50.0	16.5–49.5
Monocytes (%)	3.2	2.0–10.0
Eosinophils (%)	20.2	0.0–8.5
Basophils (%)	1.1	0.0–2.5
Red blood cells ($\times10^9$ cells/L)	1.66	4.35–5.55
Hemoglobin (g/dL)	6.1	13.7–16.8
Platelet ($\times10^9$ cells/L)	13	158–348
BUN (mg/dL)	63	8–20
Creatinine (mg/dL)	2.08	0.65–1.07
Lactate dehydrogenaze (LDH)	154	124–222
C-reactive protein (CRP) (mg/dL)	19.760	0.00–0.14
Procalcitionin (ng/mL)	0.70	0.00–0.05
Soluble interleukin-2 receptor (U/mL)	1539	121–613
EBV-DNA (LogIU/mL)	3.63	not detected

Biopsy from this lesion showed proliferating EBV-encoded RNA (EBER)-positive atypical cells, but no evidences of lymphoma were observed (Fig. 1C, D). The level of EBV-DNA in whole blood was significantly high at 3.63 logIU/mL. Although clinical signs of infection associated with neutropenia existed on the basis of the

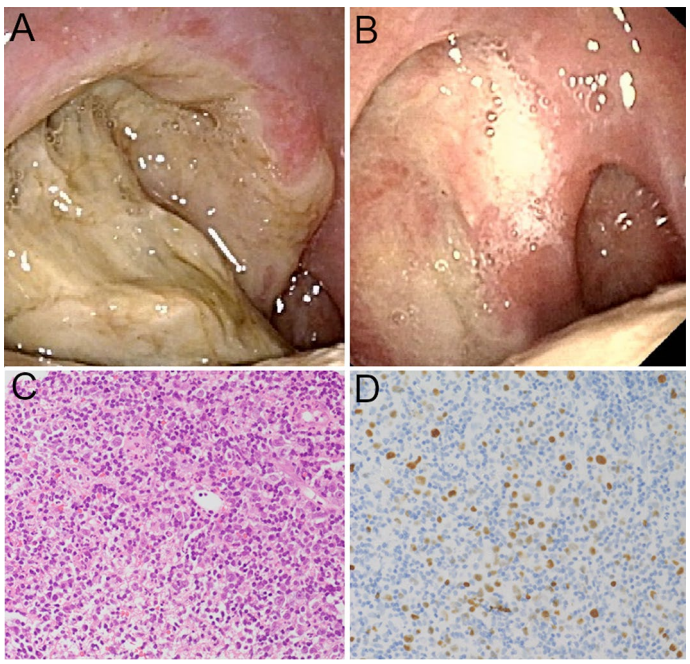

Fig. 1 Macroscopic and microscopic findings of EBV-MCU. Massive ulcerative lesions of the right tonsil at diagnosis (**A**) could not be observed after MTX discontinuation (**B**). In hematoxylin–eosin (HE) stain, ×200 magnification (**C**) and EBER *in situ* hybridization (ISH), ×200 magnification (**D**), EBER-positive atypical cells were observed

atypical EBER-positive lymphocytes attained from the oral ulcer the diagnosis of EBV-MCU was made.

To investigate the etiology of pancytopenia, bone marrow aspiration was performed immediately to reveal hypocellular marrow with remarkable megaloblastic changes in erythroid precursors (Fig. 2A, B), which was a typical finding observed in MTX poisoning. The serum concentration of MTX measured 48 hours after the last dose was 0.12 μmol/L. From these findings, pancytopenia by MTX intoxication was suspected.

Thereafter, MTX was discontinued, and 15 mg of calcium folinate every 6 hours according to the protocol of high dose MTX therapy for malignant lymphoma was initiated until the serum MTX level was decreased below 0.1 μmol/L [12]. Antibiotic therapy with piperacillin–tazobactam in combination with filgrastim was immediately initiated. Three days later, CRP level was still high (15.026 mg/dL), and thus teicoplanin and micafungin were added empirically. Blood culture tests attained repeatedly before initiating these antibiotics were positive for *Enterococcus faecium,* which was resistant to piperacillin–tazobactam but sensitive to glycopeptide antibiotics.

A total of days later, his neutrophil count was recovered to 17.4×10^9/L. Bone marrow aspiration was repeatedly performed to demonstrate normal cellularity and disappearance of megaloblastic changes (Fig. 2C, D), confirming that MTX-induced myelosuppression was responsible for the pancytopenia and erythroid dysplasia. Although G-banding analysis in the first bone marrow aspiration could not be performed because no dividing cells were attained, it was successfully performed in the follow-up bone marrow test to show normal karyotype. In both tests, no abnormal lymphocytes suggesting the marrow infiltration of lymphoma or lymphoproliferative diseases were observed.

Tonsil ulcer was gradually improved after discontinuation of MTX (Fig. 1B). EBV-DNA level was decreased by approximately one-tenth (2.73 logIU/mL) in 1 month. After approximately 3 months of hospitalization for intensive rehabilitation, he was discharged home. Three months later since then, multiple occurrences of joint pain by rheumatoid arthritis recurred, which was ameliorated by iguratimod 25 mg/day. EBV-MCU was still in remission and EBV-DNA was decreased to below the lower limit of quantification.

Discussion and conclusions

Although MTX is accepted as a safe and effective treatment for rheumatoid arthritis, severe pancytopenia is reported to occur in 0.3% to 2.1% patients, especially in patients with low renal function [2, 4, 13]. Bone marrow morphology in such cases is characterized with decreased cellularity and significant dysplasia including megaloblastic changes [5, 6, 14, 15], as observed in our case. In this case, megaloblastic anemia owing to cobalamin or folate deficiency was less likely because it was characterized with hypercellular bone marrow [16]. Other major diseases that could cause bone marrow failure were myelodysplastic neoplasms (MDS) or

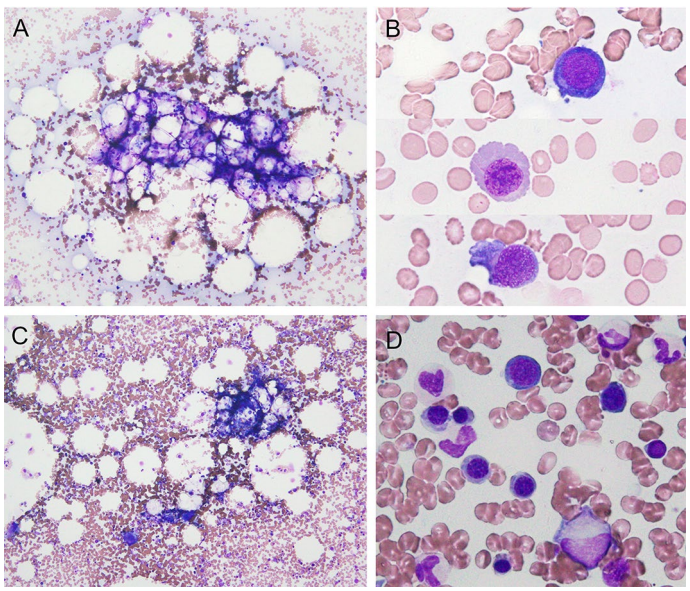

Fig. 2 Bone marrow findings of methotrexate-induced myelosuppression. Wright–Giemsa stain, ×100 magnification (**A**, **C**) and ×600 magnification (**B**, **D**). Hypocellular marrow (**A**) with dysplastic erythroid cells with megaloblastic changes (**B**) was observed at diagnosis. Recovery of normal cellularity (**C**) and disappearance of dysplastic erythroid cells (**D**) were observed after MTX discontinuation

aplastic anemia (AA). In our case, these diseases were ruled out by repetitive bone marrow aspiration showing recovery of normal hematopoiesis and disappearance of significant megaloblastic change. As a principle, these hematologic disorders could be diagnosed only when other conditions like MTX intoxication were excluded. Thus, further evaluation for these disorders should have been performed if cytopenia did not improved even after discontinuing MTX.

EBV-MCU is a newly recognized disease characterized by circumscribed ulcerative lesions occurring in skin or mucosa with infiltration of atypical EBV-positive B cells [8]. It has similarities to MTX-associated lymphoproliferative disorders (MTX-LPD), which are lymphoma-like diseases that develop after long-term exposure to MTX [7], in that both diseases occur in patients with immune dysfunction including those with immunosuppressive treatment such as MTX [8, 11]. However, approximately 50% cases of MTX-LPD are negative for EBV [17], and spontaneous regression is observed in only a half of MTX-LPD cases after MTX discontinuation [18], while it is attained in most cases in EBV-MCU [7, 8, 11]. Considering that the diagnosis of EBV-MCU was based on the histological findings attained from punched biopsy, it might not be completely ruled out that more aggressive diseases such as MTX-LPD were detected if more extensive excisional biopsy had been performed. However, due to pancytopenia and sepsis, this procedure might carry an unacceptable risk. Further, considering the ulcerative lesions confined to the oral mucosa with EBV-positive cells and spontaneous regression, additional invasive procedure to suspect the presence of lymphoma might not be necessary.

In our case, in accordance with these previous reports, the large ulcerative lesion of EBV-MCU disappeared without therapy such as rituximab. However, in our patient, the clinical course of EBV-MCU, which should normally follow favorable course, was complicated with febrile neutropenia and sepsis by *Enterococcus faecium* subsequent to MTX intoxication and acute kidney injury. All of these complications were triggered by EBV-MCU. Namely, undiagnosed for a few months, the lesion of EBV-MCU gradually progressed with pain while MTX was continued. This worsening pain led to insufficient food and fluid intake, which in turn led to deteriorated renal function and chronically increased serum methotrexate concentration. Under the background of severe neutropenia, *Enterococcus faecium*, one of the commonly detected resident microbiota in the oral cavity [19], presumably entered into the bloodstream through the ulcerative lesion of EBV-MCU resulting in bacteremia, as was often the case with neutropenic patients with stomatitis who received stem cell transplantation or intensive chemotherapy for leukemia.

There were several reports in which clinical courses of EBV-MCU were complicated with various conditions such as bone necrosis [20, 21] or erosion [22] and bowel perforation [23, 24] or intestinal obstruction [25]. Theodoros *et al.* reported a case with postoperative would infection by *Streptococcus anginosus* possibly related to immunosuppressive state [23], and Nakauyaca *et al.* reported a case with postoperative complications of transient mild neutropenia (0.7×10^9/L) without signs of infection and fatal hemorrhage from the ulcerative lesion in the absence of thrombocytopenia [26]. However, there were no reports like our case in which clinical course of EBV-MCU was complicated with profound neutropenia by MTX and subsequent opportunistic infection owing to direct entry of resident bacteria from the lesion of EBV-MCU. Our case underscores the importance of immediate biopsy and MTX discontinuation when patients on low-dose MTX therapy complain of stomatitis, especially in case with decreased oral intake. Further, our case strongly suggests that even this best prognosis form of lymphoproliferative disease could lead to fatal complications if not appropriately managed.

From a cost-effective standpoint, considering that oral ulcers or stomatitis were relatively common side effect of MTX, which were reported to appear approximately 10% of MTX treated patients [27, 28], it seemed not realistic to perform biopsy on all cases. In clinical practice, simple stomatitis by MTX were sometimes managed with folate supplementation or topical steroids with MTX continued [27], but there would be no improvement if the lesion was EBV-MCU, for which discontinuation of MTX was mandatory. Therefore, it would be reasonable to limit immediate biopsy to severe or massive ulcerations like our case, or cases in which MTX was advisable to be continued unless withdrawal was truly necessary.

In conclusion, we experienced a case of EBV-MCU resulting in severe MTX-induced myelosuppression and fatal febrile neutropenia. As the serum concentration of MTX increases in presence of renal failure, when patients treated with MTX complain of oral ulcerative lesion, clinicians should perform biopsy and discontinue MTX to gain the accurate diagnosis and to prevent the development of MTX intoxication.

Abbreviations

MTX	Methotrexate
EBV	Epstein–Barr virus
EBV-MCU	EBV-positive mucocutaneous ulcer
CRP	C-reactive protein
EBER	EBV-encoded RNA
MDS	Myelodysplastic neoplasms

AA Aplastic anemia
MTX-LPD MTX-associated lymphoproliferative disorders

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Author contributions
KE and TT treated the patient and provided clinical information. KU performed biopsy and TI, YK and KE reviewed the pathological specimen. KE wrote the manuscript. TI, KU, YK and TT advised and helped writing the manuscript.

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Availability of data and materials
Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication
Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing interests
All the authors declared that they had no competing interests to disclose.

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