Successful Treatment of Intracranial Methotrexate-associated Lymphoproliferative Disorder without Epstein-Barr Virus Infection Using Rituximab, Methotrexate, Procarbazine, and Vincristine: A Case Report

Makoto Mizushima,1 Yukitomo Ishi,1 Hiroshi Ikeda,1 Ikuma Echizenya,1 Takuya Otsuka,2 Tomoko Mitsuhashi,2 Shigeru Yamaguchi,1 and Miki Fujimura1

1Department of Neurosurgery, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, Japan
2Surgical Pathology, Hokkaido University Hospital, Sapporo, Hokkaido, Japan

Abstract

Methotrexate-associated lymphoproliferative disorder (MTX-LPD) occurs in patients with rheumatoid arthritis (RA) treated with methotrexate (MTX). MTX-LPD is typically associated with Epstein-Barr virus (EBV) infection and regresses with MTX discontinuation. On the other hand, EBV-negative MTX-LPDs are less common and are more likely to show partial or no regression after MTX discontinuation. There were no standard chemotherapeutic options for refractory MTX-LPD. We present a case of EBV-negative MTX-LPD in the central nervous system (CNS) that was successfully treated with rituximab, methotrexate, procarbazine, and vincristine (R-MPV), followed by reduced-dose whole-brain radiotherapy (rdWBRT), following the same treatment protocol as primary CNS lymphoma. A 59-year-old woman with RA treated with MTX presented with gradually developing staggered gait, memory deficit, and disorientation. Multiple lesions with heterogeneous contrast enhancement were discovered using brain magnetic resonance imaging. The patient was suspected of having MTX-LPD, but discontinuing MTX did not result in regression of the brain lesions. She underwent a biopsy from the left parietal lesion. The tissue was pathologically diagnosed as diffuse large B-cell lymphoma. Furthermore, pathological examination through EBV-encoded ribonucleic acid in situ hybridization demonstrated a lack of EBV infection. She was ultimately diagnosed with EBV-negative CNS MTX-LPD. We applied chemotherapy with R-MPV and rdWBRT. The patient achieved a complete response. In the case of CNS MTX-LPD without EBV infection, chemotherapy with R-MPV followed by rdWBRT may be considered.

Keywords: methotrexate-associated lymphoproliferative disorder (MTX-LPD), methotrexate, rituximab, methotrexate, procarbazine, and vincristine (R-MPV), rheumatoid arthritis, primary central nervous system lymphoma (PCNSL)

Introduction

Methotrexate (MTX) has been widely administered to patients with autoimmune diseases, including rheumatoid arthritis (RA). On the other hand, patients receiving MTX have an increased risk of lymphoproliferative disorder (LPD) due to the immunosuppressive state induced by MTX administration. The majority of methotrexate-associated lymphoproliferative disorders (MTX-LPDs) are associated with Epstein-Barr virus (EBV) infection and occur in the trunk, including the lymph nodes or extranodal areas such as the epipharynx, lungs, thyroid glands, and skin. Intracranial MTX-LPD can also occur infrequently. Patients with MTX-LPD typically show remission merely upon withdrawal of MTX treatment. As a result, the first step in clinical management of MTX-LPD is close monitoring following MTX discontinuation.

However, even after discontinuing MTX, some MTX-LPD
cases were unable to achieve regression.\textsuperscript{14,15} These patients require further treatment. Unfortunately, there are no standard treatment options for MTX-LPD beyond MTX cessation. We present a case of EBV-negative MTX-LPD in the central nervous system (CNS) that was successfully treated with rituximab, methotrexate, procarbazine, and vincristine (R-MPV) chemotherapy, followed by low-dose whole-brain radiotherapy (rdWBRT).

**Case Report**

This case report was prepared after informed consent was obtained from the patient. A 59-year-old woman presented with a 6-week history of gradually developing staggering gait and a 4-week history of slowly progressive memory deficit. She was diagnosed with RA 9 years prior and had been receiving MTX (8 mg/week) therapy for 7 years. Neurological examination revealed impaired orientation (Glasgow Coma Scale 14/15; E4V4M6) and staggering gait with bilateral lower extremity weakness.

In gadolinium-enhanced T1-weighted images (Gd-T1WI), brain magnetic resonance imaging (MRI) revealed multiple lesions with heterogeneous enhancement (Fig. 1A). On fluid-attenuated inversion recovery (FLAIR), perifocal edema was also observed (Fig. 1B). Spinal MRI revealed no abnormalities, and contrast-enhanced whole-body computed tomography revealed no apparent lymphadenopathies or other lesions suspected of malignancy. Laboratory results showed elevated serum soluble interleukin-2 receptor levels (926 U/mL). Serum antigen tests for EBV were as follows: virus capsid antigen (VCA) IgG, 40 IU/mL; VCA IgM, <10 IU/mL; EBV nuclear antigen, 20 IU/mL; and early antigen-diffuse-type and restricted-type IgG, <10 IU/mL, which discredits the possibility of a previous infection. EBV-DNA was undetectable in her whole blood. Because CNS MTX-LPD was suspected, MTX administration was stopped based on her drug history and brain MRI findings. However, neurological symptoms did not improve, and brain MRI did not reveal any regression of these lesions.

One week after MTX discontinuation (Fig. 1C), fluorine-18-fluorodeoxyglucose-positron emission tomography (FDG-PET) revealed uptake in brain lesions without evidence for systemic lesions (Fig. 1D). The patient underwent biopsy on the left parietal lesion. Histopathological examination of the tumor specimen showed proliferation of large atypical lymphoid cells (Fig. 2A-B). These cells ex-
Successful Treatment of Intracranial MTX-LPD Using R-MPV

A: Hematoxylin and eosin (H&E) staining (original magnification ×40) showing lymphocytic cells, some of which show perivascular cuffing.
B: H&E staining (original magnification ×400) showing diffuse and perivascular infiltration of large atypical lymphoid cells.
C: Immunostaining for CD20 (original magnification ×400) demonstrating a positive result.
D: In situ hybridization (original magnification ×400) showing no evidence of EBER.

Fig. 2 The pathological findings of the left parietal lesion.

Expressed CD 20 (Fig. 2C) and CD 10, were partially positive for BCL6 and MUM1, and were negative for CD 5 and CD 3. When these findings were combined, they led to the diagnosis of diffuse large B-cell lymphoma, germinal center B-cell like subtype. In situ hybridization of EBV-encoded small RNA (EBER) was negative (Fig. 2D). Thus, on the basis of drug history and pathology results, she was diagnosed with EBV-negative CNS MTX-LPD.

Considering her disease progression despite MTX cessation, we started chemotherapy with R-MPV. Her neurological symptoms gradually improved after she started chemotherapy, and her intracranial lesion decreased in size. After 7 cycles of R-MPV chemotherapy, she achieved a complete response (CR). She received rdWBRT [23.40 Gy in 1.8-Gy fractions × 13] as consolidation treatment and was discharged from the hospital without disease (Fig. 3). She has remained in remission for 16 months after completion of chemo-radiotherapy.

Fig. 3 Brain MRI findings after 7 cycles of chemotherapy with R-MPV and rdWBRT.
A and B: Gd-T1WI (A) and FLAIR (B) showing no evidence of lesions.
Discussion

CNS MTX-LPD is exceptionally rare. To the best of our knowledge, only 10 cases have been reported.4-13 Out of 11 cases, including the case presented here, 4 were negative for EBV infection and all of the required additional treatment (Table 1).4,5,9

LPDs are caused by several types of immunodeficiency or drugs. The World Health Organization classified immunodeficiency-related LPDs into four categories: LPD associated with primary immune disorders, lymphomas associated with human immunodeficiency virus infection, post-transplant LPD, and other iatrogenic immunodeficiency-associated lymphoproliferative disorders (OIIA-LPD).16 OIIA-LPD includes lymphoid proliferations or lymphomas that develop in patients treated with anti-RA drugs, including MTX, tacrolimus, and anti-tumor necrosis factor α drugs.10 Most OIIA-LPD cases develop in patients with RA who are treated with MTX, and the median duration of MTX administration is 54 months.10 On the other hand, the OIIA-LPD category remains contentious due to the difficulty in distinguishing the direct effect of immunosuppressive drugs from the effect of autoimmune diseases themselves.17 Some studies have shown that patients with RA are more likely to develop LPD, regardless of the history of MTX use.10,17,18 As a result, MTX-LPD could be divided into two pathogeneses. One is truly caused by MTX, whereas the other is caused by RA or other factors other than MTX. Withdrawal from MTX may be ineffective in cases of MTX-LPD caused by RA.

MTX-LPD is typically associated with EBV infection. The EBV positivity rate in patients with MTX-LPD is approximately 60% in Japan, which is higher than that reported in Western studies.14,20 Previous research on all-site MTX-LPD has shown that spontaneous regression after MTX withdrawal occurs more frequently in EBV-positive patients than in EBV-negative patients.15,21 EBV infection is related to the pathogenetic mechanism of MTX-LPD through DNA methylation that can interrupt the expression of tumor suppressor genes.22 EBV infection has been linked to a lower incidence of CpG island methylator phenotype, apoptosis-related gene hypermethylation, and BCL2 expression in patients with RA with MTX-LPD, implying spontaneous regression after MTX withdrawal and better prognosis in patients with EBV-positive MTX-LPD. As a result, EBV-negative MTX-LPD cases are more likely to demonstrate partial or no regression after MTX withdrawal, requiring further intervention.22

Of the many reported approaches, no standard chemotherapy regimen has been established for MTX-LPD cases, including CNS.3-5 Cyclophosphamide, vincristine, doxorubicin, prednisolone, MTX, bleomycin, mitomycin C, mercaptopurine, melphalan, and rituximab are all chemotherapeutic agents that have been tried.3,14 Each chemotherapy regimen was essentially selected according to the histopathological appearance of tumor cells. For example, for non-Hodgkin lymphoma cases, the most commonly used chemotherapy regimens were rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone and rituximab, cyclophosphamide, pirarubicin, vincristine, and prednisolone.14,26 However, these regimens may be ineffective in the treatment of CNS MTX-LPD due to low penetration of the CNS.23

R-MPV followed by rdWBRT is an effective treatment for primary CNS lymphoma (PCNSL).24 Those who achieved CR after 5-7 cycles of induction chemotherapy with R-MPV

### Abbreviations

Ara-C = cytarabine; CR = complete response; DLBCL = diffuse large B-cell lymphoma; IVLBCL = intravascular large B-cell lymphoma; MTX = methotrexate; PSL = prednisolone; RA = rheumatoid arthritis; R-CHOP = rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone; R-MPV = rituximab, methotrexate, procarbazine, and vincristine

### Table 1 Summary of reported EBV-negative CNS MTX-LPD cases

| Study            | Age (years)/sex | Underlying disease | LPD site     | Histopathology | Spontaneous regression | Additional treatment                                      | Outcome | Follow-up duration |
|------------------|-----------------|--------------------|--------------|----------------|------------------------|----------------------------------------------------------|---------|-------------------|
| Liu et al., 2015 | 58/M            | RA                 | Cerebrum     | DLBCL          | Not available          | Radiotherapy                                             | Good    | 1 year            |
| Kikuchi et al., 2016 | 50/F         | RA                 | Dura mater   | IVLBCL         | Not available          | R-CHOP + intrathecal chemotherapy (PSL+ MTX + Ara-C)    | CR      | 2 years           |
| Uchida et al., 2018 | 52/F         | RA                 | Cerebrum     | DLBCL          | No                     | Rituximab + high-dose MTX + Ara-C, radiotherapy         | Died    | 17 months         |
| This study      | 59/F            | RA                 | Cerebrum     | DLBCL          | No                     | R-MPV, radiotherapy                                      | CR      | 10 months         |
receive rdWBRT (23.4 Gy). Aside from that, standard WBRT (45 Gy) is available. We used R-MPV in this case, as we did in the case of ordinary PCNSL, and achieved CR. Although the use of MTX is controversial for patients with MTX-LPD, regimens that included MTX have been effective in some cases.\(^{1,2}\) Our findings suggest that an R-MPV regimen could be a viable treatment option for patients with CNS MTX-LPD when MTX withdrawal is ineffective, particularly in EBV-negative cases.

**Conclusion**

R-MPV followed by rdWBRT may be considered for patients with CNS MTX-LPD without EBV infection.

**Acknowledgments**

The authors would like to thank Enago (www.enago.jp) for the English language review.

**Abbreviations in this paper**

MTX = methotrexate; RA = rheumatoid arthritis; LPD = lymphoproliferative disorder; MTX-LPD = methotrexate-associated lymphoproliferative disorder; CNS = central nervous system; R-MPV = rituximab, methotrexate, procarbazine, and vincristine; rdWBRT = reduced-dose whole-brain radiotherapy; MRI = magnetic resonance imaging; Gd-T1WI = gadolinium-enhanced T1-weighted images; FLAIR = fluid-attenuated inversion recovery; EBV = Epstein-Barr virus; VCA = virus capsid antigen; FDG-PET = fluorine-18-fluorodeoxyglucose-positron emission tomography; EBER = Epstein-Barr virus-encoded small RNA; CR = complete response; OIIA-LPD = other iatrogenic immunodeficiency-associated lymphoproliferative disorders; PCNSL = primary central nervous system lymphoma; H&E = hematoxylin and eosin

**Conflicts of Interest Disclosure**

All authors have no conflict of interest.

**References**

1) Shiroky JB, Frost A, Skelton JD, Haegert DG, Newkirk MM, Neville C: Complications of immunosuppression associated with weekly low dose methotrexate. *J Rheumatol* 18: 1172-1175, 1991

2) Wolfe F, Michaud K: The effect of methotrexate and anti-tumor necrosis factor therapy on the risk of lymphoma in rheumatoid arthritis in 19,562 patients during 89,710 person-years of observation. *Arthritis Rheum* 56: 1433-1439, 2007

3) Hoshida Y, Xu JX, Fujita S, et al.: Lymphoproliferative disorders in rheumatoid arthritis: Clinicopathological analysis of 76 cases in relation to methotrexate medication. *J Rheumatol* 34: 322-331, 2007

4) Kikuchi J, Kaneko Y, Kasahara H, et al.: Methotrexate-associated intravascular large B-cell lymphoma in a patient with rheumatoid arthritis. *Intern Med* 55: 1661-1665, 2016

5) Uchida Y, Hokkoku K, Hatanaka Y, Kikuchi Y, Tashiro H, Sonoo M: Primary central nervous system methotrexate associated lymphoproliferative disorders in a patient with rheumatoid arthritis. *Rinsho Shinkeigaku* 58: 485-491, 2018

6) Kleinschmidt-DeMasters BK, Damek DM, Lillehei KO, Dogan A, Giannini C: Epstein Barr virus-associated primary CNS lymphomas in elderly patients on immunosuppressive medications. *J Neuropathol Exp Neurol* 67: 1103-1111, 2008

7) Fukushima M, Katayama Y, Yokose N, et al.: Primary central nervous system malignant lymphoma in a patient with rheumatoid arthritis receiving low-dose methotrexate treatment. *Br J Neurosurg* 27: 824-826, 2013

8) Migita K, Miyashita T, Mijn T, et al.: Epstein-Barr virus and methotrexate-associated CNS lymphoma in a patient with rheumatoid arthritis. *Mod Rheumatol* 23: 832-836, 2013

9) Liu W, Xue J, Yu S, Chen Q, Li X, Yu R: Primary central nervous system lymphoma mimicking recurrent depressive disorder: A case report. *Oncol Lett* 9: 1819-1821, 2015

10) Shimada H, Dobashi H, Morimoto H, et al.: Primary central nervous system lymphoma in a rheumatoid arthritis patient treated with methotrexate: A case report. *BMC Res Notes* 8: 88, 2015

11) Matsuda I, Hirota S: Methotrexate-associated lymphoproliferative disorder masquerading as multiple cerebral metastases. *Br J Haematol* 180: 628, 2018

12) Miyaza S, Matsuda R, Nakamura M, Nakagawa I, Motoyama Y, Nakase H: Intracranial methotrexate-associated lymphoproliferative disorder in rheumatoid arthritis. *World Neurosurg* 130: 138-141, 2019

13) Uneda A, Hirashita K, Kanda T, et al.: Primary central nervous system methotrexate-associated lymphoproliferative disorder in a patient with rheumatoid arthritis. *Case report and review of literature. NMC Case Rep J* 7: 121-127, 2020

14) Ichikawa A, Arakawa F, Kiyasu J, et al.: Methotrexate/iatrogenic lymphoproliferative disorders in rheumatoid arthritis: Histology, Epstein-Barr virus, and clonality are important predictors of disease progression and regression. *Eur J Haematol* 91: 20-28, 2013

15) Rizzi R, Curci P, Delia M, et al.: Spontaneous remission of "methotrexate-associated lymphoproliferative disorders" after discontinuation of immunosuppressive treatment for autoimmune disease. Review of the literature. *Mod Oncol* 26: 1-9, 2009

16) Swerdlow S, Campo E, Harris N, et al.: Immunodeficiency-Associated Lymphoproliferative Disorders, WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues, ed 4. IARC Publications, 2017

17) Tokuhiro M, Tamaru JI, Kizaki M: Clinical management for other iatrogenic immunodeficiency-associated lymphoproliferative disorders. *J Clin Exp Hematop* 59: 72-92, 2019

18) Baecklund E, Ilidou A, Askling J, et al.: Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. *Arthritis Rheum* 54: 692-701, 2006

19) Simon TA, Thompson A, Gandhi KK, Hochberg MC, Sussa S: Incidence of malignancy in adult patients with rheumatoid arthritis: A meta-analysis. *Arthritis Res Ther* 17: 212, 2015

20) Kameda T, Dobashi H, Miyatake N, et al.: Association of higher methotrexate dose with lymphoproliferative disease onset in rheumatoid arthritis patients. *Arthritis Care Res* 66: 1302-1309, 2014

21) Gion Y, Iwaki N, Takata K, et al.: Clinicopathological analysis of methotrexate-associated lymphoproliferative disorders: Comparison of diffuse large B-cell lymphoma and classical Hodgkin lymphoma.
phoma types. Cancer Sci 108: 1271-1280, 2017

22) Ejima-Yamada K, Oshiro Y, Okamura S, et al.: Epstein-Barr virus infection and gene promoter hypermethylation in rheumatoid arthritis patients with methotrexate-associated B cell lymphoproliferative disorders. Virchows Arch 470: 205-215, 2017

23) O’Neill BP, Wang CH, O’Fallon JR, et al.: Primary central nervous system non-Hodgkin’s lymphoma (PCNSL): Survival advantages with combined initial therapy? A final report of the North Central Cancer Treatment Group (NCCTG) study 86-72-52. Int J Radiat Oncol Biol Phys 43: 559-563, 1999

24) Morris PG, Correa DD, Yahalom J, et al.: Rituximab, methotrexate, procarbazine, and vincristine followed by consolidation reduced-dose whole-brain radiotherapy and cytarabine in newly diagnosed primary CNS lymphoma: Final results and long-term outcome. J Clin Oncol 31: 3971-3979, 2013

25) Kameda H, Fujii T, Nakajima A, et al.: Japan College of Rheumatology guideline for the use of methotrexate in patients with rheumatoid arthritis. Mod Rheumatol 29: 31-40, 2019

Corresponding author: Shigeru Yamaguchi, MD, PhD
Department of Neurosurgery, Hokkaido University Graduate School of Medicine, North 15 West 7, Kita-ku, Sapporo, Hokkaido 060-8638, Japan.

e-mail: yama-shu@med.hokudai.ac.jp