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【 CASE REPORT 】

Prognosis Prediction Using Magnetic Resonance Spectroscopy and Oligoclonal Bands in Central Nervous System Methotrexate-associated Lymphoproliferative Disorder

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Abstract:

Central nervous system methotrexate-associated lymphoproliferative disorder (CNS-MTX-LPD) is rare, but its spontaneous regression has been observed in some patients after withdrawal of agents. We herein report three cases of primary CNS-MTX-LPD that received oral MTX for rheumatoid arthritis. Epstein-Barr virus and oligoclonal bands (OCBs) were positive, while proton magnetic resonance spectroscopy (^{1}H-MRS) showed an elevated lipid peak and slightly elevated choline/N-acetylaspartate ratio in common. After MTX withdrawal, brain lesions showed spontaneous regression in all cases. Our patient's ^{1}H-MRS findings and OCBs may reflect a non-monoclonal lymphoproliferative histology as benign-type lesions in CNS-MTX-LPD.

Key words: methotrexate-associated lymphoproliferative disorder, EBV, MRS, oligoclonal bands

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Introduction

Although primary central nervous system methotrexate-associated lymphoproliferative disorder (CNS-MTX-LPD) is exceptionally rare, several clinical case studies have recently been reported (1-10). Given that the spontaneous regression has been observed in some patients with MTX-LPD, uniform chemotherapy is not recommended (11). MTX-LPD includes a broad spectrum of presentations, from benign lesions to malignant lymphoma (12). Epstein-Barr virus (EBV)-positivity and non-diffuse large-B cell lymphoma (DLBCL) histology are implicated as essential factors for regression after MTX withdrawal. To avoid an unnecessary brain biopsy, we must identify noninvasive biomarkers that can help distinguish between benign and malignant histologies.

We herein report three cases of primary CNS-MTX-LPD in which the findings on proton magnetic resonance spectroscopy (^{1}H-MRS) and oligoclonal bands (OCBs) were associated with an uneventful course.

Case Reports

Case 1

A 64-year-old right-handed man who had received MTX for rheumatoid arthritis (RA) was admitted to our hospital, where he was referred to the Department of Neurosurgery with a suspected brain tumor. The RA had been diagnosed 4

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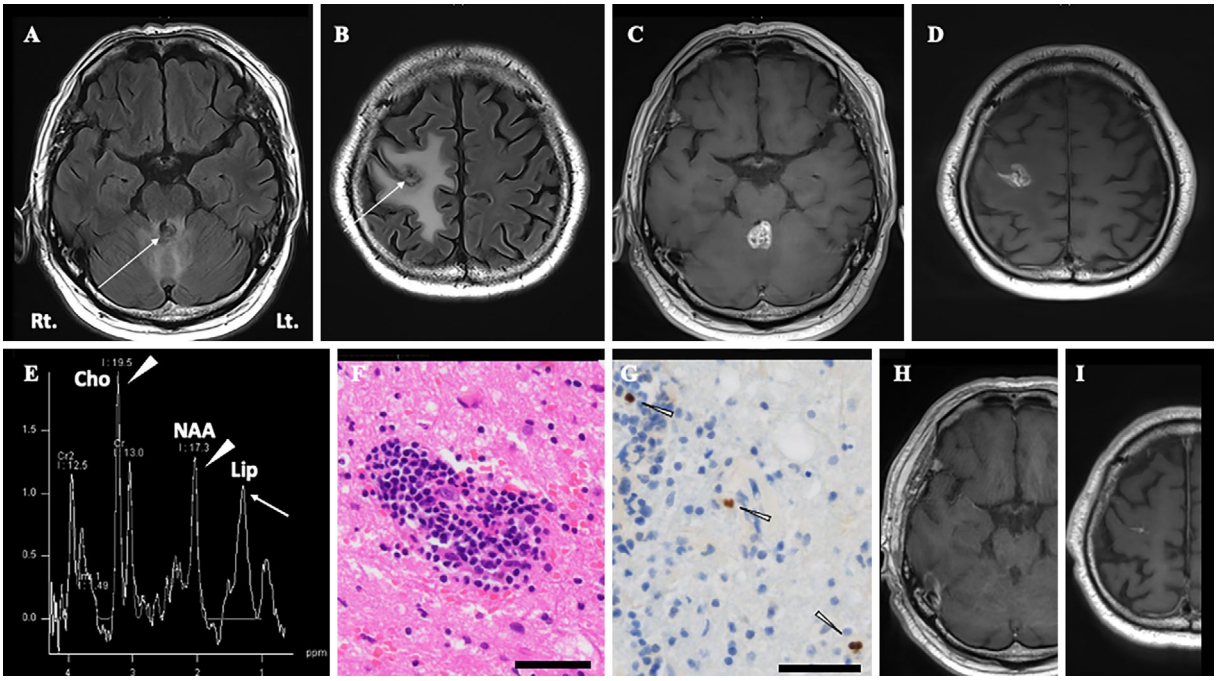

Figure 1. **MRI of the brain obtained on admission (A-E) shows iso-intense nodules (arrow) with surrounding edema in the cerebellar vermis (A) and the right frontoparietal lobe (B) on fluid attenuated inversion recovery (FLAIR) imaging. Post-contrast-enhanced T1-weighted imaging (CE-T1WI) shows enhancement (C, D). The ^{1}H-MR spectrum acquired from a localized voxel of interest in the enhancing lesion on T2-weighted imaging (T2WI): ^{1}H-MR spectroscopy shows a markedly elevated lipid (Lip) peak (arrow) and mildly elevated choline (Cho)/N-acetylaspartate (NAA) ratio (arrowheads, Cho/NAA=1.13) (E). Histological and immunohistochemical findings from the parietal lobe biopsy. Perivascular lymphocytic infiltration is present (Hematoxylin and Eosin staining, Bar=50 μm) (F). An EBV-encoded small RNA (EBER) *in situ* hybridization analysis shows positivity in scattered cells (arrowheads, bar=50 μm) (G). Follow-up brain MRI obtained 21 months after MTX withdrawal shows the almost complete absence of contrast enhancement on CE-T1WI (H, I).**

years previously, and he had been receiving oral MTX 16 mg once weekly for 3 years. He presented with progressive ataxia for five weeks.

On admission, the patient was alert and well-oriented. A neurological examination revealed slight weakness in the left extremities and truncal ataxia. Fluid attenuated inversion recovery (FLAIR) showed high-signal lesions in the cerebellar vermis and right frontoparietal lobe, with iso-signal small lesions located inward (Fig. 1A, B). Contrast-enhanced T1-weighted imaging (CE-T1WI) showed gadolinium enhancement in areas corresponding to isointense lesions on FLAIR (Fig. 1C, D). ^{1}H-MRS showed an elevated lipid (Lip) peak and mildly elevated choline (Cho)/N-acetylaspartate (NAA) ratio (Cho/NAA=1.13) (Fig. 1E). Whole-body computed tomography (CT) showed no apparent lymphadenopathies or other lesions suggestive of malignancy. The white blood cell count was 6.0×10^{3}/μL (lymphocytes, 1,320/μL). A cerebrospinal fluid (CSF) examination revealed 24 white blood cells/μL comprising 100% lymphocytes, and a total protein level of 46 mg/dL. CSF cytology revealed no evidence of malignant cells. OCBs were subsequently detected in the CSF.

The patient underwent an open biopsy of the right parietal lesion for a definitive diagnosis. The biopsy specimens showed mild gliosis and perivascular lymphocytic infiltration (Fig. 1F), composed of CD3+ T cells and scattered CD20+ B cells, with no definite evidence of malignancy. An EBV-encoded small RNA (EBER) *in situ* hybridization analysis was positive for scattered CD20+ B cells (Fig. 1G). Thus, he was diagnosed with CNS-MTX-LPD, and MTX was discontinued.

After MTX withdrawal, his symptoms improved, along with resolution of the lesions. Follow-up brain MRI at 21 months after admission showed no evidence of recurrence (Fig. 1H, I).

Case 2

An 86-year-old right-handed woman who had received MTX for RA was admitted to our hospital. RA had been diagnosed three years previously, and she had been receiving oral MTX 8 mg once weekly since then. She presented with progressive weakness developed in her left arm and leg for two weeks.

On admission, she was alert and well-oriented. A neurological examination revealed flaccid paralysis in the left upper and lower limbs. FLAIR showed high-signal mass le-

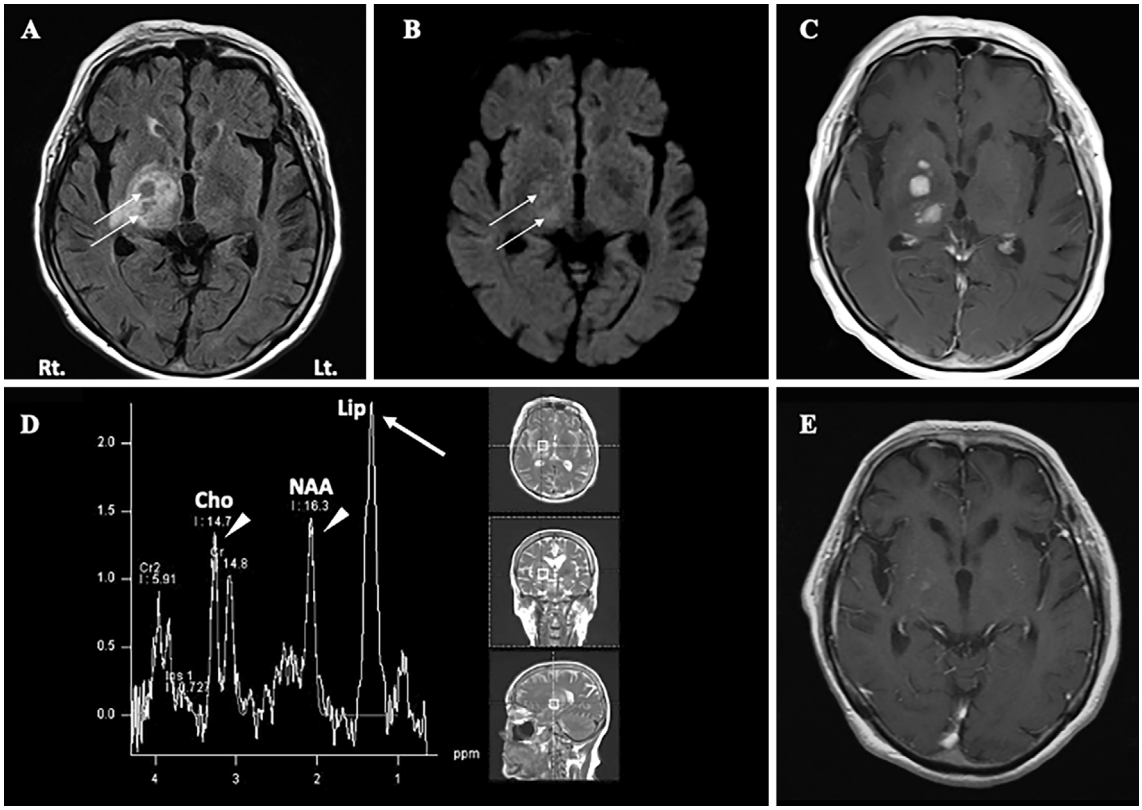

Figure 2. **MRI of the brain obtained on admission (A-D) shows iso-intense nodules (arrows) with surrounding edema in the right lentiform nucleus, internal capsule, and thalamus on FLAIR imaging (A). Diffusion-weighted imaging (DWI) (b-value, 1,000 s/mm^2) shows moderately hyperintense signals in part of the iso-intense lesions on FLAIR (B). Post CE-T1WI shows homogeneous enhancement (C). ^{1}H-MR spectrum acquired from a localized voxel of interest in the enhancing lesion on T2WI: ^{1}H-MR spectroscopy shows a markedly elevated Lip peak (arrow) and mildly elevated Cho/NAA ratio (arrowheads, Cho/NAA=0.90) (D). Follow-up brain MRI obtained 4 weeks after MTX withdrawal shows the almost complete absence of contrast enhancement on CE-T1WI (E).**

sions in the right internal capsule, lentiform nucleus and thalamus, with iso-signal small lesions located inward (Fig. 2A). Diffusion-weighted imaging (DWI) showed slightly high signals in part of the isointense lesions identified on FLAIR (Fig. 2B). CE-T1WI showed gadolinium enhancement in areas corresponding to the isointense lesions on FLAIR (Fig. 2C). ^{1}H-MRS showed an elevated Lip peak and mildly elevated Cho/NAA ratio (Cho/NAA=0.90) (Fig. 2D). Whole-body CT showed no apparent lymphadenopathies or other lesions suggestive of malignancy. The white blood cell count was 5.7×10^3/μL (CD4+ lymphocytes, 413/μL; CD8+ lymphocytes, 198/μL). The level of serum soluble interleukin-2 receptor was 881 U/mL. EBV-specific antibodies, including virus capsid antigen (VCA)-IgG, early antigen-DR-IgG, and EBV nuclear antigen (EBNA)-IgG, were positive, whereas VCA-IgM was negative. Polymerase chain reaction (PCR) for EBV DNA was positive (3.36 Log IU/mL). A CSF examination revealed 3 white blood cells/μL, comprising 100% lymphocytes, and a total protein level of 57 mg/dL. The CSF interleukin-10 level was elevated (134 pg/mL), and CSF cytology revealed no evidence of malignant cells. OCBs were subsequently detected in the CSF. Thus, she was given a provisional diagnosis of CNS-MTX-LPD, and MTX was discontinued.

After MTX withdrawal, her symptoms began to improve dramatically, along with the gradual resolution of the mass lesions. The findings of follow-up brain MRI four weeks after admission were unremarkable (Fig. 2E). Seven months after the onset, she was able to walk independently and has been free from recurrence.

Case 3

A 66-year-old right-handed woman who had received MTX for RA was admitted to our hospital. RA had been diagnosed 13 years previously, and she had been receiving oral MTX 8 mg once weekly since then. She presented with gait disturbance for three days.

On admission, she was alert and well-oriented. A neurological examination revealed slight weakness in the left lower limb. FLAIR showed high-signal lesions in the right frontoparietal lobe, with an iso-signal lesion located inward (Fig. 3A). DWI showed a low signal with a high-signal rim that corresponded to the isointense lesion identified on FLAIR (Fig. 3B). CE-T1WI was not performed due to her history of bronchial asthma. ^{1}H-MRS showed an elevated Lip peak and mildly elevated Cho/NAA ratio (Cho/NAA=

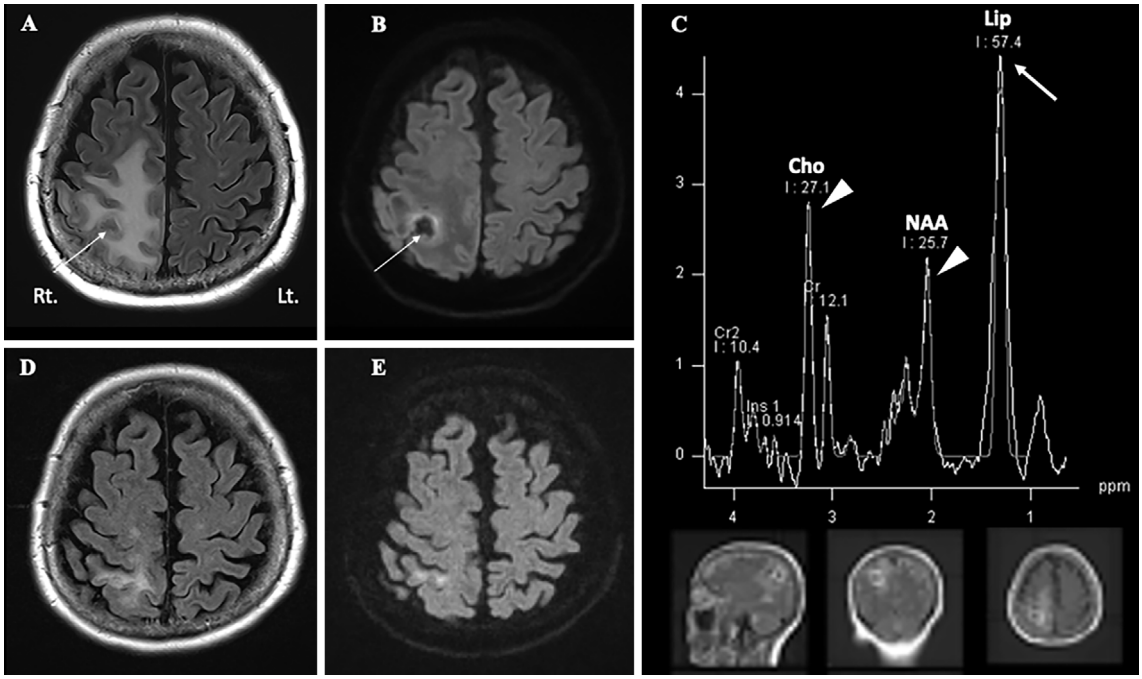

Figure 3. **MRI of the brain obtained on admission (A-C) shows iso/low-intensity nodules (arrow) with surrounding edema in the right parietal lobe on FLAIR imaging (A). DWI (b-value, 1,000 s/mm^2) shows hypointense with a hyperintense signal rim in part of the iso/low-intensity lesions on FLAIR (B). The ^{1}H-MR spectrum acquired from a localized voxel of interest in the parietal lesion on T2WI: ^{1}H-MR spectroscopy shows a markedly elevated Lip peak (arrow) and mildly elevated Cho/NAA ratio (arrowheads, Cho/NAA=1.05) (C). Follow-up brain MRI obtained 2 months after MTX withdrawal shows regression on FLAIR (D) and DWI (E).**

Table. **Case Series Characteristics.**

	Case 1	Case 2	Case 3
Age/Sex	64/M	86/F	66/F
RA disease duration	4 years	3 years	13 years
MTX dose (duration)	16mg/week (2 years)	8mg/week (2 years)	8mg/week (6 years)
EBV positivity	EBER expression in brain biopsy specimens	EBV DNA detection in peripheral blood (PCR, 3.36 Log IU/mL)	EBV DNA detection in peripheral blood (PCR, 4.70 Log IU/mL)
OCBs (No. of bands)	Positive (9)	Positive (5)	Positive (5)
Cho/NAA on MRS	1.13	0.90	1.05

1.05) (Fig. 3C). Whole-body CT showed no apparent lymphadenopathies or other lesions suggestive of malignancy. The white blood cell count was 9.8×10^3/μL (lymphocytes, 310/μL). The level of serum soluble interleukin-2 receptor was 500 U/mL. EBV-specific antibodies, including VCA-IgG, VCA-IgM, early antigen-DR-IgG, and EBNA-IgG, were positive. PCR for EBV DNA was positive (4.70 Log IU/mL). A CSF examination revealed 11 white blood cells/μL comprising 10 lymphocytes and 1 neutrophil, and a total protein level of 35 mg/dL. CSF cytology revealed no evidence of malignant cells. OCBs were subsequently detected in the CSF. Thus, she was given a provisional diagnosis of CNS-MTX-LPD, and MTX was discontinued.

After MTX withdrawal, her symptoms and brain lesions improved. Follow-up brain MRI two months after admission showed spontaneous regression (Fig. 3D, E).

The clinical characteristics of the cases are summarized in Table.

Discussion

MTX-LPD arises under host immunosuppression, with a significant pathophysiology of autonomous proliferation of EBV-infected lymphocytes. Tokuhira et al. found that most cases of EBV-positive DLBCL were classified into the subtype of regressive LPD without relapse/regrowth after immunosuppressive agent withdrawal (13). In the present cases, PCR for EBV or EBER was also positive, with a regressive and uneventful course. Cytotoxic T-lymphocytes respond to EBV-infected cells by recognizing EBNA or latent

membrane protein-derived peptides expressed on the human leukocyte antigen (HLA) complex, while those labeling viral proteins accelerate the access of T-lymphocytes to EBV-infected cells. In this regard, it is conceivable that withdrawal of MTX would inhibit signals for the proliferation and/or survival, leading to spontaneous regression without additional chemotherapy in MTX-LPD (11, 14).

Three clinical courses were observed in MTX-LPD after immunomodulator agent withdrawal: spontaneously regressive LPD without relapse/regrowth event, regressive LPD with relapse/regrowth event, and persistent LPD. Chemotherapy was required for patients with non-regressive LPD under watchful waiting after immunomodulator agent withdrawal (13). MTX-LPD includes both oligoclonal/polyclonal and monoclonal lymphoproliferation. The most frequent histological subtype of MTX-LPD is the large B-cell type, including DLBCL. Other common subtypes include reactive lymphoid hyperplasia (RH), classic Hodgkin lymphoma, polymorphic B-cell LPD (poly-LPD), and indolent lymphoma including follicular lymphoma. In the category of post-transplant LPD in the World Health Organization (WHO) classification, benign and borderline lesions are described as RH and poly-LPD, respectively. A subset of these benign-type lesions spontaneously regresses after withdrawal of the immunomodulator agent (13-15). Although a biopsy can presumably serve as a histological diagnostic approach for selecting an appropriate management for MTX-LPD, a brain biopsy is not indicated in benign-type with primary-CNS-MTX-LPD because spontaneous regression can occur. Accordingly, noninvasive examinations are required to provide extensive histological information in these brain lesions.

To our knowledge, no clinical studies have investigated the association between findings on ^{1}H-MRS and the prognosis in MTX-LPD. Large Lip peaks on ^{1}H-MRS images of tumors without central necrosis are characteristic of malignant lymphomas, whereas small or absent Lip peaks are suggestive of glioma (16). In the present case series, although the ^{1}H-MRS finding of elevated Lip was similar to that typically seen in primary CNS lymphoma (PCNSL), the Cho/NAA ratio was lower than that of typical PCNSL. EBV-infected-lymphoma cells release extracellular vesicles called exosomes, while exosomes alter the gene expression and convert the macrophages into "tumor-associated macrophages" (17). Ito et al. carried out an analysis of EBV-infected-lymphoma derived exosomes, suggesting that exosomes were rich in immunomodulatory proteins and phospholipids (18). In MTX-LPD with EBV-positive, an elevated Lip peak on ^{1}H-MRS may reflect macrophages containing lipid-rich EBV-infected cell-derived exosomes. Raizer et al. evaluated 16 pretreated patients with PCNSL using ^{1}H-MRS, where the mean NAA/Cho ratio was 0.39 (Cho/NAA ratio, 2.56) (19). Lu et al. enrolled 44 patients with PCNSL and 21 with tumefactive demyelinating lesions (TDLs), finding that PCNSL rather than TDLs was indicated when the Cho/NAA ratio was >1.73 (20). Markedly elevated Cho levels and a decreased NAA peak are obvious in PCNSL. This may be due to DLBCL-type LPD potentially having greater phosphocholine turnover due to membrane biosynthesis caused by proliferating cells and a larger loss of normal neurons than benign and borderline lesions, such as RH and poly-LPD in MTX-LPD. The slightly elevated Cho/NAA ratio on ^{1}H-MRS is more likely to be helpful for indicating non-DLBCL histology in MTX-LPD than lymphoma.

Although some case studies of primary CNS-MTX-LPD have been reported, few have investigated OCBs in the CSF in MTX-LPD. We suspect that the OCBs detected in the CSF indicated intrathecal polymorphous lymphoproliferation in the present patients. In this regard, the presence of OCBs may aid in identifying non-monoclonal lymphoproliferation in MTX-LPD with a spontaneous regression course following withdrawal of MTX. However, intrathecal synthesis of OCBs was occasionally found in patients with non-Hodgkin lymphoma and chronic lymphocytic leukemia (21). There thus remain unanswered questions concerning the clinical significance of OCBs in the CSF. Further studies will be required to examine the validity of these findings in a greater number of cases of CNS-MTX-LPD in order to avoid unnecessary brain biopsies.

Statement of Ethics: This study is a retrospective observational case series study and does not require the approval of the Institutional Review Boards in our Institute, but written consent was obtained from the patients and their families for the publication of this case series and any accompanying images.

The authors state that they have no Conflict of Interest (COI).

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