Case Report

Myelopathy mimicking subacute combined degeneration in a Down syndrome patient with methotrexate treatment for B lymphoblastic leukemia: Report of an autopsy case

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We report clinicopathological features of a 23-year-old woman with Down syndrome (DS) presenting with subacute myelopathy treated with chemotherapy, including intravenous and intrathecal administration of methotrexate (MTX), and with allogenic bone-marrow transplantation for B lymphoblastic leukemia. Autopsy revealed severe demyelinating vacuolar myelopathy in the posterior and lateral columns of the spinal cord, associated with macrophage infiltration, marked axonal loss and some swollen axons. Pathological changes of posterior and lateral columns were observed from the medulla oblongata to lumbar cord. Proximal anterior and posterior roots were preserved. Cerebral white matter was relatively well preserved. There were no vascular lesions or meningeal dissemination of leukemia. Longitudinal extension of cord lesions was extensive, unlike typical cases of subacute combined degeneration (SACD), but distribution of lesions and histological findings were similar to that of SACD. DS patients show heightened sensitivity to MTX because of their genetic background. Risk factors for toxic myelopathy of DS are discussed, including delayed clearance of MTX despite normal renal function, alterations in MTX polyglutamation and enhanced folic acid depletion due to gene dosage effects of chromosome 21. Alteration of folate metabolism and/or vitamin B_{12} levels through intravenous or intrathecal administration of MTX might exist, although vitamin B_{12} and other essential nutrients were managed using intravenous hyperalimentation. To the best of our knowledge, this is the first report of an autopsy case that shows myelopathy mimicking SACD in a DS patient accompanied by B lymphoblastic leukemia. The case suggests a pathophysiological mechanism of MTX-related myelopathy in DS patients with B lymphoblastic leukemia mimicking SACD.

Key words: Down syndrome, methotrexate, myelopathy, precursor B-cell lymphoblastic leukemia-lymphoma, subacute combined degeneration.

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INTRODUCTION

Subacute combined degeneration (SACD) of the spinal cord is a rare neurological complication, which results from vitamin B_{12} deficiency induced by pernicious anemia, gastric resection, intestinal malabsorption, or maintaining strict veganism. Several risk factors are known to be associated with SACD, including deficiency of folic acid which affects methionine synthase activity, exposure to nitrous oxide during surgical procedures[1] or chronic recreational use of nitrous oxide, inherited defects of methylation, and abnormal plasma vitamin B_{12}-binding protein levels. Early manifestations are paresthesiae in the lower limbs, loss of fine touch, vibration and position sense. Further

progression leads to spastic paraparesis, ataxia and anesthesia of the lower limbs and trunk. Less common neurologic disturbances include visual impairment, depression, irritability, confusion and dementia. Abnormal morphology in the earliest lesions includes disappearance of the myelin sheath of the posterior and lateral columns and spongiform changes in the affected white matter of the spinal cord. In the chronic stages of SACD, the spinal cord shrinks and shows discolored posterior and lateral columns.

We report the clinicopathological features of a patient with Down syndrome (DS) with subacute myelopathy treated with chemotherapy, including intravenous and intrathecal administration of methotrexate (MTX), and with allogeneic bone-marrow transplantation for B lymphoblastic leukemia.

MTX is an inhibitor of folic acid metabolism and suppresses tumor cell proliferation by inhibition of dihydrofolate reductase, which reduces folic acid levels. Because this agent is soluble and has difficulty crossing the blood-brain barrier, the CNS is an appropriate target to treat or prevent the expansion of various neoplasms. This case suggests a pathophysiological mechanism of MTX-related myelopathy mimicking SACD in a DS patient with B lymphoblastic leukemia.

CLINICAL SUMMARY

The patient was a 23-year-old woman with DS. She had been on anti-epileptic medication since she was 8 years old, and this had been well controlled. At 21 years old, she was diagnosed with B lymphoblastic leukemia. She was treated according to the protocol for acute lymphoblastic leukemia in children (Tokyo Children's Cancer Study Group (TCCSG) ALL L04-16, for high-risk patients). The patient achieved remission after the first remission-induction therapy. The protocol contains intravenous and intrathecal administration of MTX as follows: intravenous administration, total 7 g/m^2 (1 g/m^2, 3 g/m^2, 3 g/m^2); and intrathecal administration, total 87.5 mg (12.5 mg, seven times each). Six weeks from finishing remission-induction therapy, leukemia had relapsed in the bone marrow. Although additional remission-induction chemotherapy was performed with intravenous or intrathecal administration (prednisolone, vincristine, cyclophosphamide, daunorubicin, and L-asparaginase, or prednisolone, cytrabine, and MTX (12.5 mg)), the patient failed to achieve complete remission. Two courses of chemotherapy were added, each containing intrathecal administration of MTX (total 24 mg, 12 mg each). Five months after bone-marrow relapse, an unrelated allogeneic bone-marrow transplantation was performed and proceeded to engraftment 1 month later. Bone-marrow transplantation was performed using busulfan, etoposide,

melphalan and systemic rardiotherapy (total 12 Gy, 2 Gy/day for 6 days). To prevent graft versus host disease (GVHD), MTX was administered by the parenteral route as follows: a total of 45 mg/m^2 four times at the early phase of bone-marrow transplantation (15 mg/m^2 and 10 mg/m^2 three times), and a total of 42 mg/m^2 at the late phase (15 mg/m^2 and 9 mg/m^2 three times). After bone-marrow transplantation, the patient gradually became more ill because of hematochezia, steroid diabetes, diastolic ventricular failure, hyperlipidemia, *Candida* esophagitis, diarrhea, and GVHD in the skin and digestive system. Respiratory impairment and disturbance of consciousness appeared 7 weeks before death. GVHD control was not enough even though tacrolimus, cyclosporine-A, MTX and prednisolone were used.
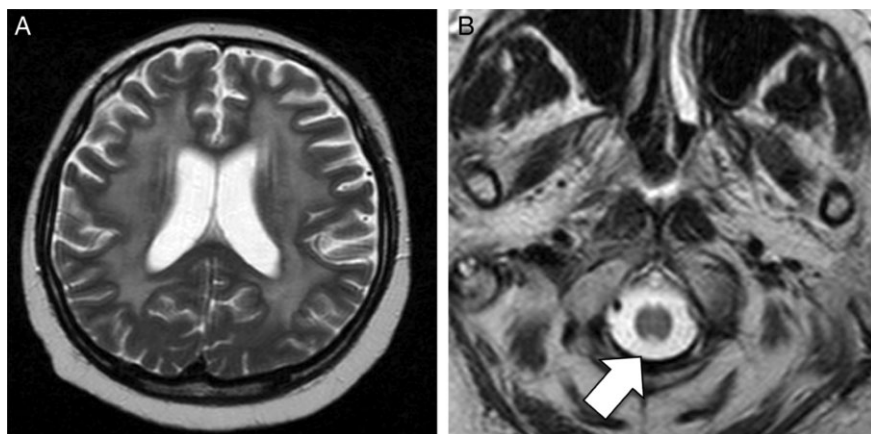
The patient had been able to eat meals independently and to do detailed tasks before the bone-marrow transplantation, but afterwards was bed-ridden. It was difficult to clinically identify whether muscle weakness of the lower limbs was caused by disuse atrophy or steroid atrophy. The presence of bladder and rectal disturbances was unclear as the patient's general condition deteriorated. Urethral catheterization was not needed.

Disturbances of consciousness became more evident at 18 weeks after bone-marrow transplantation. Around the same time, MRI showed high-signal intensity lesions on T2-weighted images (WI) in cerebrum white matter, and a T2-WI high signal was identified in the posterior column of the cervico-medulary junction (Fig. 1). These findings were not observed prior to the transplant. A spinal cord MRI was not obtained. The mild enlargement of the lateral ventricles have been observed as an accompanying finding of DS in a previous hospital. Immune suppression status was carefully controlled, but the patient became bed-ridden and needed ventilator support for severe pulmonary hemorrhage. The patient died because of pulmonary hemorrhaging 25 weeks (177 days) after the bone-marrow transplantation.

Neurological findings were difficult to assess near the end of the clinical treatment because of the patient's general deterioration in health and difficulty communicating. Vitamin B$_{12}$ and other essential nutrients were managed using intravenous hyperalimentation throughout the treatment; therefore, circulating levels of vitamin B$_{12}$ or folic acid were not examined throughout the antemortem course.

PATHOLOGICAL FINDINGS

A general autopsy was performed 15 h after death. Bilateral lungs showed diffuse alveolar hemorrhage and hemorrhagic infarction. Gram-positive cocci were also seen in alveoli from a few infiltrated inflammatory cells. Relapse of B lymphoblastic leukemia was not verified.

Microscopic examination revealed bone marrow cellularity under 10% due to marrow suppression by chemotherapy at the agonal stage. Leukemic cells were not observed in the bone marrow. Myocardium showed moderate fibrosis due to chemotherapy. The direct cause of death was respiratory failure resulting from diffuse alveolar hemorrhage.

The unfixed brain weighed 1150 g. Macroscopic examination revealed mild frontal lobe atrophy, although no atrophy was observed in the spinal cord. There was no atherosclerotic change, hemorrhage or infarction in the brain. Brain tissue samples were fixed with 10% formalin and paraffin embedded. We performed HE, KB and Bodian staining. Immunohistochemistry was performed with the following primary antibodies: anti-GFAP (6FC, anti-mouse monoclonal; Dako, Glostrup, Denmark), anti-myelin basic protein (anti-rabbit polyclonal; Dako), anti-CD68 (PG-M1, anti-mouse monoclonal IgG1; Dako), anti-phosphorylated neurofilament protein (2F11, anti-mouse monoclonal; Dako) and anti-PHF-tau (AT8, anti-mouse monoclonal IgG1; Immunogenetics, Gent, Belgium). Diamino benzidine was used for visualization.

The most prominent feature was seen in the spinal cord. The myelin sheath had disappeared in the posterior and lateral columns from the thoracic (Th5) to the lumbar spinal cord in KB staining (Fig. 2B,C). The myelin sheath disappearance in the cervical spinal cord showed only in the posterior column and not the lateral column (Fig. 2A). Lesions were nearly symmetrical and the posterior column and corticospinal and spinocerebellar tracts were severely involved. The pyramidal tract was maintained at the pons and medulla oblongata but was degenerated from the thoracic segments to the lumbar segments. Pathological change of the posterior column was observed from the cervicomedullary junction to the lumbar cord. The pathological change of dorsal column nuclei of the cervico-medullary junction was consistent with MRI findings (Fig. 1).

Microscopic examination revealed spongy vacuolation with foamy macrophage infiltration of the lateral and posterior columns from the cervical to lumbar levels associated with GFAP-positive hypertrophic astrocytes (Fig. 3A–D). Pathological change of the lateral column involved the pyramidal tract and the posterior spinocerebellar tract. Proximal anterior and posterior roots were preserved. Gray matter of the spinal cord was relatively well preserved. The same pattern of myelin sheath disappearance was confirmed by anti-myelin basic protein immunohistochemistry. Infiltration of foamy macrophages were observed in the posterior and lateral columns (Fig. 3B,C). Although some intact axons and swollen ones were remaining, marked axonal loss was observed in the posterior column of the cervical spinal cord and the pyramidal and the posterior spinocerebellar tract and the posterior column of the thoracic to lumber spinal cord (Fig. 3E,F). Among the posterior column of the cervical spinal cord, Wallerian degeneration was predominant in the fasciculus cuneatus rather than the fasciculus gracilis. Anti-tau positive NFTs and β-protein positive senile plaques were not found (data not shown). Histologically, other neurodegenerative diseases or neoplasm or metastasis of leukemia were not seen. Cerebral white matter which showed high-signal intensity lesions on T2-WI including around the area of the lateral ventricles was mildly edematous but there was no clarification with KB staining. Dorsal root ganglion was not obtained. No demyelination or fiber degeneration was observed in the cerebrum. No calcification or hyalinized vessels (which indicate radiological therapy) were observed in the entire CNS. Infiltration of leukemia cells was not found.

DISCUSSION

The pathological involvement of the posterior and lateral columns of the spinal cord in this patient mimics SACD. As the patient's general condition deteriorated, it was difficult to clearly assess clinical neurological symptoms. Pathological change of the lateral column involved the pyramidal tract and the posterior spinocerebellar tract. Longitudinal
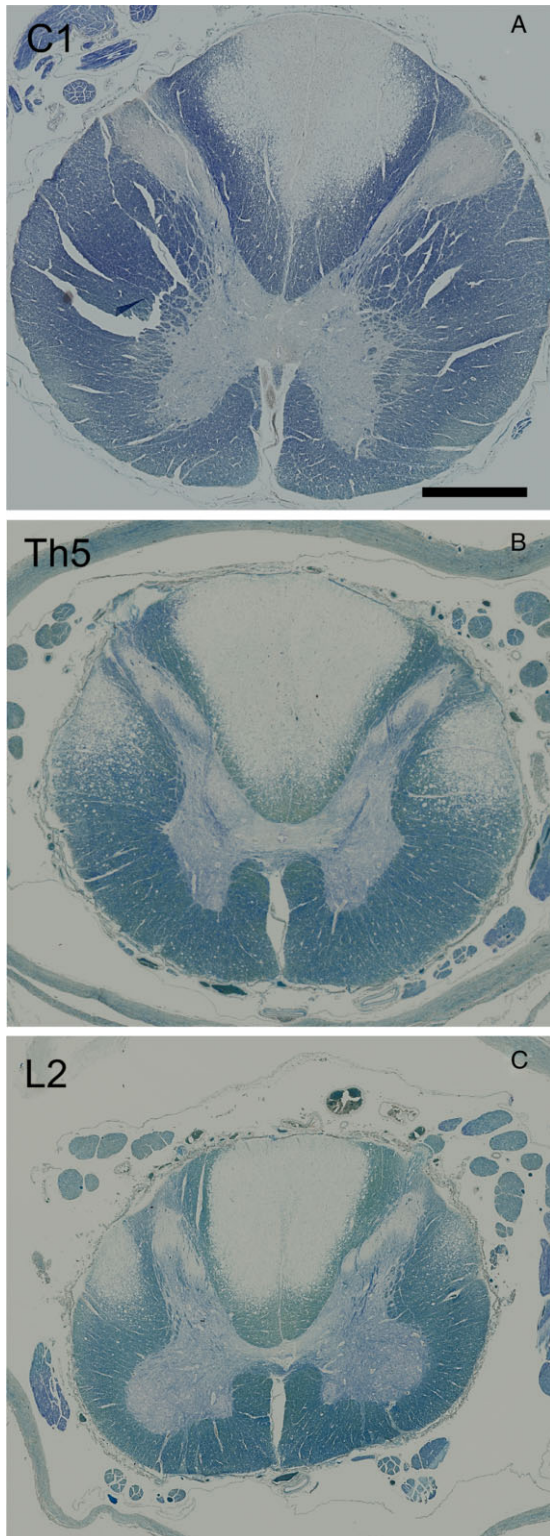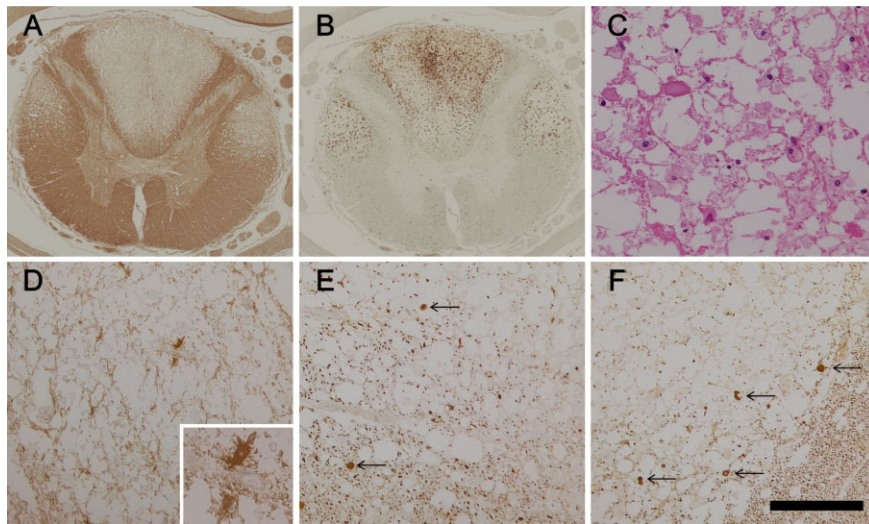
Fig. 2 Transverse sections of the spinal cord. The transverse sections of the spinal cord show edematous myelin pallor of the posterior column from the cervical segment to the lumbar segment, and the lateral column from thoracic segments to the lumbar segments symmetrically (KB stain). The cervical level (A: C1), the thoracic level (B: Th5), and the lumbar level (C: L2) of the spinal cord are shown. Bar = 2 mm.

extension of cord lesions was extensive, unlike typical cases of SACD,[2] in which posterior and lateral columns are involved, especially in the lower cervical and thoracic regions. The severity of the lesions in SACD usually diminishes toward the cervical and lumbar regions, although the severity of the lesions in this case is most prominent in the thoracic cord. Transverse distribution of lesions and histological findings were similar to that of SACD.

This finding corresponded to the high-intensity signal at the craniocervical junction in MRI. In addition, the posterior and lateral columns of the spinal cord showed edematous spongy vacuolation with active infiltration of numerous macrophage and axonal swelling, which suggested recent changes of the cord for some months.

Spinal venous stasis causes myelin loss of the posterior and lateral columns based on the anatomical venous drainage system. In this case, a multidisciplinary therapeutic approach was required to control the leukemia, including radiotherapy, various chemotherapy, bone-marrow transplantation and myelosuppressive therapy. In addition, the patient suffered from hematochezia, GVHD, pulmonary hemorrhage and heart failure. All of these circumstances certainly associated with edematous change of the posterior and lateral columns but only a few swollen axons in them indicate a pathophysiological mechanism of abnormal metabolic or toxic processes. MTX is known to injure cerebral white matter or the spinal cord by disturbing methionine synthesis, which is necessary for myelination in the CNS. The following types of encephalopathy associated with MTX therapy have previously been reported:[3] (i) disseminated necrotizing leukoencephalopathy;[4] (ii) diffuse parenchymatous degeneration with gliosis and axonal dystrophy;[5] (iii) diffuse and focal subpial necrosis of the gray matter;[6] and (iv) mineralizing microangiopathy and dystrophic calcification.[7] There are several reports of SACD accompanied with MTX therapy for leukemia; such patients are typically identified by radiological imaging.[8–10] A few autopsy cases have reported an association between spinal cord injury and intrathecal MTX administration. One case reported prominent necrotizing changes on the spinal cord surface as well as on the surface of the cerebrum, cerebellum and brain stem.[11] These histological findings are not identical to those observed in the present case. In addition, there was no apparent necrosis,[12] patchy myelin loss or perivascular foci of myelin loss in the cerebrum.[13]

The methylenetetrahydrofolate reductase (MTHFR) polymorphism C677T is one of the factors that causes subacute leukoencephalopathy.[14,15] In recent years, a patient with MTX-induced subacute neurotoxicity who was found to carry functional allelic variants in most of the genes involved in MTX and folate pathways, was reported.[16] The risk factors of spinal cord involvement in the present case

Fig. 3 Microscopic findings. The spinal cord shows myelin sheath disappearance (A: anti-myelin basic protein antibody immunohistochemistry). The same distribution of CD68-positive macrophages was recognized (B: anti-CD68 antibody immunohistochemistry). Spongy vacuolations and foamy macrophages infiltrate the myelin sheath disappearance area of the posterior column (C: HE stain, ×200). Hypertrophic astrocytes were recognized in the same area (D: anti-GFAP antibody immunohistochemistry ×100, inset is a high magnification). Some swollen axons were seen but marked axonal loss was observed in the pyramidal tract of chest spinal cord (E: anti-phosphorylated neurofilament antibody immunohistochemistry, ×100), and in the posterior column of chest spinal cord (F: anti-phosphorylated neurofilament antibody immunohistochemistry, ×100). Arrows are swollen axons. Bar = 2 mm (A, B); 100 μm (C); 200 μm (D, E, F).

are controversial. First, it is well known that DS patients have lower survival rates, and critical treatment-related toxicity, particularly with MTX was reported to be higher than that of B lymphoblastic leukemia with non-DS patients.[17,18] Children with B lymphoblastic leukemia-DS are very sensitive to MTX and frequently develop severe gastritis, myelosuppression and other complications following MTX administration.[19] The reasons why DS patients have unique clinical and biological features have been documented.[20] These reasons include delayed clearance of MTX in B lymphoblastic leukemia-DS patients despite normal renal function,[21,22] alterations in MTX polyglutamation[23] and enhanced folic acid depletion due to gene dosage effects of chromosome 21.[24] Gene dosage effects include genes involved in purine synthesis,[25] cystathionine β-synthase[26] and the folic acid carrier.[27]

There are no differences in the pharmacokinetic or pharmacodynamic effects of MTX between patients with and without DS. It is proposed that it may be safe in children with DS to start intermediate dosages of MTX (1–3 g/m²) and to carefully monitor these patients.[27]

Second, although Tomonaga et al. reported degeneration of white matter in the subpial zone of the brainstem and spinal cord of a patient on whom intrathecal MTX administration was performed,[28] direct spinal cord injury due to MTX did not seem likely in the present case.

Third, the possibility of vitamin B_{12} or folic acid deficiency is not high. This is because the patient had total parenteral nutrition, providing sufficient amounts of vitamin B_{12} and all other essential nutrients, although her circulating levels were not strictly observed. However, GVHD of the digestive system and worsening general health conditions could have caused leakage and/or inefficient utilization of folic acid or vitamin B_{12}. Furthermore, the alteration of folate metabolism and vitamin B_{12} levels

through intravenous or intrathecal administration of MTX is thought to exist.

To the best of our knowledge, this is the first report of toxic myelopathy mimicking SACD in a DS patient treated with MTX for B lymphoblastic leukemia. Since DS patients show heightened sensitivity to MTX, physicians take particular care and reduce the dose of MTX not to cause MTX-induced side effects. Even though physicians strictly performed chemotherapy acording to this consensus, this patient showed MTX-related myelopathy. All physicians should be reminded of the risk of MTX and perform a serial neurological assessment, appropriate nutritional management, and spinal MRI to detect an early stage of MTX-related myelopathy and to prevent a progression of it in the treatment of B lymphoblastic leukemia. Therefore, risk-adapted therapies adjusted for biological differences between the B lymphoblastic leukemia-DS and B lymphoblastic leukemia-non-DS populations are recommended. Additionally, in comming years, the progression of clinical medicine may lead to the achievement of a good prognosis for various diseases with MTX treatment. Substitution with multiple folate metabolites has been reported as a strategy for the treatment of MTX-induced neurotoxicity.[29] To maintain a patient's quality of life, it is important to prevent the occurrence of MTX-induced myelopathy.

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