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Case Report

Methotrexate-Induced Subacute Combined Degeneration in Acute Lymphoblastic Leukemia with CNS Relapse May Be Reversible

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Abstract: We describe a case of a female patient with acute lymphoblastic leukemia treated with high-dose systemic methotrexate and intrathecal methotrexate for leukemic relapse of the central nervous system. She developed complete bilateral lower-limb paralysis that was not attributable to any other cause. She was treated with folic acid, vitamin B12, methionine, S-adenosylmethionine, leucovorin, and dextromethorphan. After a 3-month period of paraplegia, she began to slowly recover motor function. She can now ambulate with assistance and continues to improve. There is a paucity of literature on methotrexate-induced subacute combined degeneration, which is typically described as irreversible. In addition to reporting our unique case, we review the published literature and call for more awareness and research in this area.

Keywords: ALL; leukemia; methotrexate; neurotoxicity; myelopathy; spinal cord; adverse effect; intrathecal chemotherapy

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1. Introduction

Methotrexate (MTX) is a chemotherapy used to treat hematological malignancies as well as chronic autoimmune diseases such as rheumatoid arthritis and psoriasis. MTX is a folate analog that competitively inhibits dihydrofolate reductase (DHFR), the enzyme that generates tetrahydrofolate (THF) from folic acid. THF is essential for both purine biosynthesis, as the methyl donor in its 10 formyl tetrahydrofolate form for one-carbon groups, as well as thymidine metabolism, through direct inhibition of thymidylate synthase [1–5]. Adverse effects of MTX caused by the disruption of the folate cycle and nucleotide biosynthesis include myelosuppression, pulmonary fibrosis, hepatoxicity, nephrotoxicity, gastrointestinal (GI) abnormalities, and neurotoxicity [6]. There are several possible mechanisms behind MTX-induced neurotoxicity. Primarily, MTX treatment reduces levels of essential metabolites in the cerebrospinal fluid (CSF), such as 5-methyl-tetrahydrofolate (THF), 5,10-methyl-THF, tetrahydrobiopterin (THB), and S-adenosylmethionine (SAM), which are required for neural function [7–10].

5,10-methyl-THF is vital for the conversion of uridine monophosphate (dUMP) to thymidine monophosphate (dTMP) and in the formation of 5-methyl-tetrahydrofolate (THF). 5-methyl-THF is essential in the homocysteine-methionine cycle, remethylating homocysteine to form methionine via methionine synthase and the cofactor methylcobalamin [11–13]. Homocysteine levels in the cerebrospinal fluid (CSF) are at baseline $\leq$ 0.5 nmol/mL in healthy individuals but can increase up to 1.0 nmol/mL with systemic MTX administration [14,15]. Methionine combines with adenosine triphosphate (ATP) to form SAM, the universal methyl donor vital for many biochemical pathways in humans,

including, myelin sheath formation and maintenance. The biochemical pathways of folate and methionine cycles are summarized in Figure 1 [16]. Conversely, homocysteine, adenosine, and S-adenosylhomocysteine (SAH) are sulfur-containing excitatory amino acids that have been implicated in neurological toxicity, and these metabolites are increased in the CSF post-MTX administration [4,15,17,18]. Secondarily, MTX directly interferes with astrocyte function, damaging the cells and resulting in axonal loss [4,19,20]. MTX-induced neurotoxicity due to MTX hypersensitivity or neuropraxia secondary to fluctuations in CSF osmolality have also been described [14,21,22]. Myelopathy secondary to MTX is likely multifactorial.

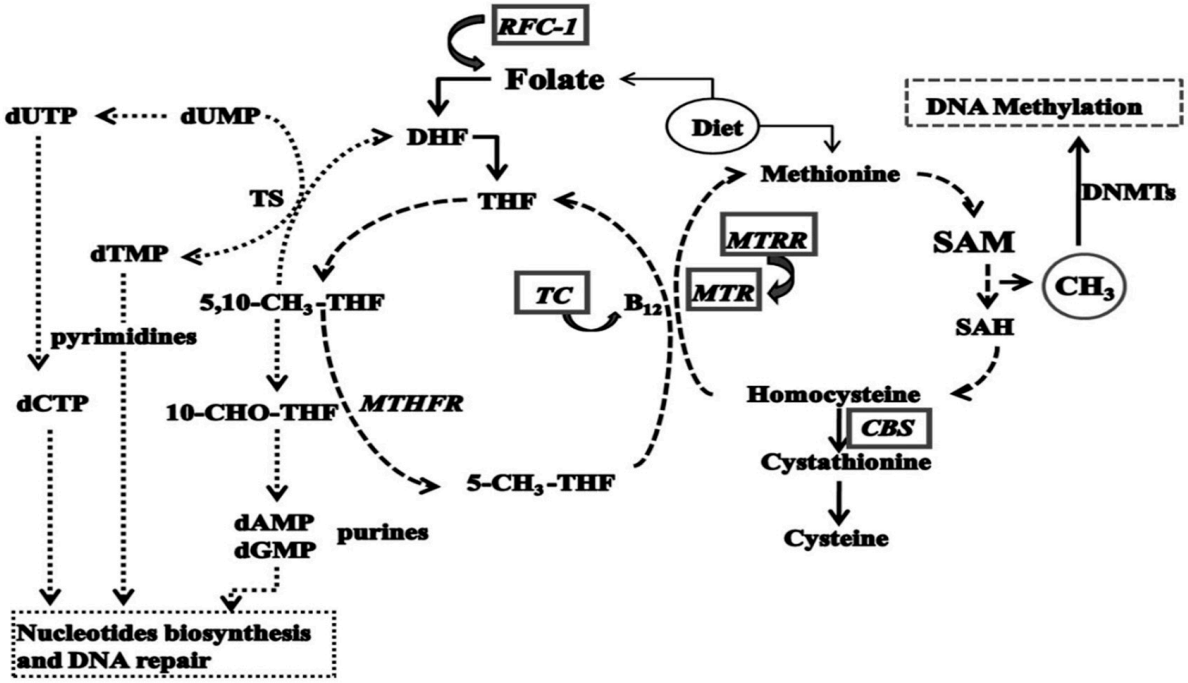


Figure 1. Folic acid biochemical pathways including the Methionine Cycle and folate's role in DNA synthesis.

There are three main types of toxicity described after MTX administration: Acute, subacute, and chronic toxicity. Patients with subacute toxicity may present with ataxia, speech difficulty, hemiparesis, seizures, and encephalopathy [4,23–25]. Researchers have proposed that adenosine partially mediates acute toxicity, whereas excitatory amino acids, homocysteine, S-adenosylmethionine (SAM)/S-adenosylhomocysteine (SAH) ratios, and biopterin are greater contributors to subacute and chronic toxicity [4]. In this case report, we describe a 36-year-old female with Philadelphia chromosome-positive (Ph+) B-cell acute lymphoblastic leukemia (Ph+ B-ALL) treated with pediatric-inspired multiagent systemic and intrathecal chemotherapy plus the tyrosine kinase inhibitor (TKI), who ultimately underwent matched unrelated donor allogeneic hematopoietic stem cell transplantation (MUD allo-HSCT) in first remission. After a prolonged first remission duration, the patient unfortunately relapsed with central nervous system (CNS) involvement of B-ALL. She subsequently received intrathecal chemotherapy, TKI, and high dose (HD) systemic infusional MTX, after which she became paraplegic due to subacute combined degeneration (SCD) of the spinal cord.

2. Detailed Case Description

A 36-year-old previously healthy woman presented five years ago with leukocytosis (white blood cell count 221.7 $\times$ 10^3/µL), hemoglobin (Hgb) 6.8 g/dL, platelets (PLT) 89 $\times$ 10^3/uL, and 77% circulating blasts. Bone marrow biopsy showed B-cell acute lymphoblastic leukemia (ALL) with 90% blasts by morphologic examination. Flow cytometry

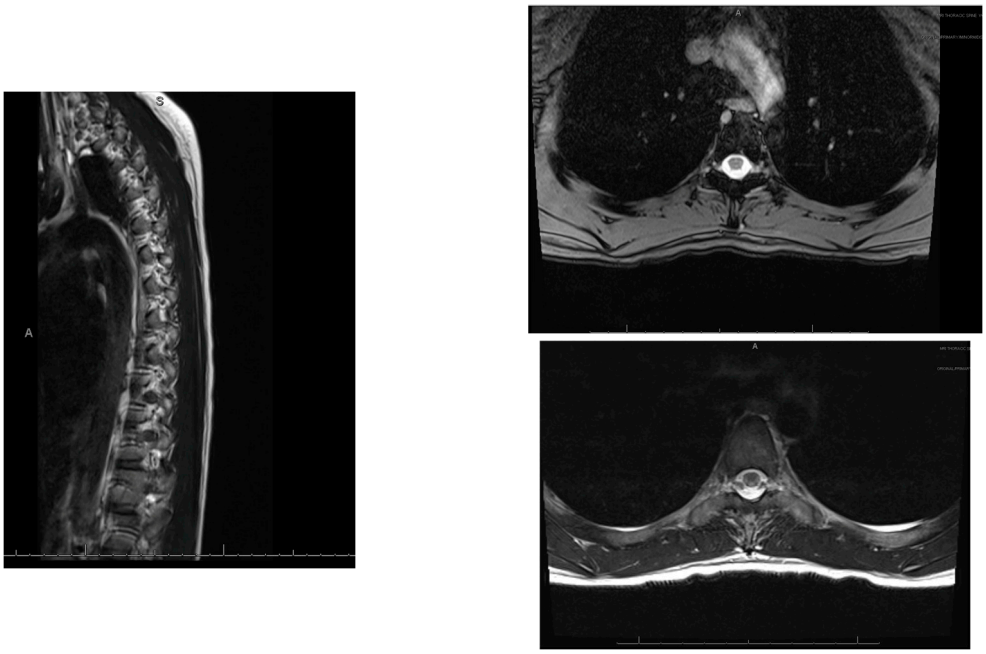
showed 70% blasts that were positive for CD34, HLA-DR, TdT, CD19, CD22, CD79a, and CD10, with aberrant expression of CD33 and CD13. MPO was negative. Chromosomal analysis revealed the Philadelphia chromosome t(9;22) in all metaphases and BCR-ABL was detected in 97% of cells by FISH. On her initial lumbar puncture (LP), she had evidence of CNS involvement with blasts representing 10% of events captured by flow cytometry. The patient underwent induction chemotherapy with a pediatric-inspired protocol (AALL1131) including daunorubicin, vincristine, PEG-asparaginase, prednisone, intrathecal (IT) MTX and cytarabine, and dasatinib 100 mg daily. She had received twice weekly alternating doses of IT MTX and IT cytarabine, with rapid clearance of the CSF by the third dose. A post-induction bone marrow biopsy showed complete remission and PCR for BCR-ABL was 0.0136%. She underwent consolidation therapy on AALL1131, with reduced-dose vincristine due to peripheral neuropathy (which eventually resolved). This was followed by interim maintenance with infusional HD MTX and leucovorin rescue. Pre-transplant bone marrow biopsy showed BCR-ABL at 0.009%. She subsequently underwent a successful MUD allo-HSCT with total body irritation (TBI), etoposide, and anti-thymocyte globulin (ATG) conditioning. She did not have significant graft-versus-host disease. She achieved complete molecular remission (CMR) post-transplant. She had received a total of 12 rounds of intrathecal chemotherapy (8 IT MTX, 4 IT cytarabine) prior to allo-HSCT. She received dasatinib 50–70 mg daily as maintenance after allo-HSCT, but it was stopped after ~1 year due to poor tolerance. She was monitored by peripheral blood BCR-ABL PCR testing and, after 2 years, had a molecular relapse at 0.3949%. She resumed dasatinib, and within two months had achieved a major molecular remission (MMR), which was maintained.

Approximately 4 years after allo-HSCT, she presented with severe headaches, photophobia, leukocytosis, and a diffuse rash. She was treated for presumed infectious meningitis. Diagnostic LP showed an abnormal immature B-cell population representing 8% of viable events by flow cytometry that were positive for CD45 (dim), CD19, CD38 (variable decreased), CD10, CD34, CD13/33, and CD58, consistent with CNS relapse of B-ALL. No infectious cause of meningitis was identified. Brain magnetic resonance imaging (MRI) showed no abnormalities. Bone marrow BCR-ABL PCR was 0.1676%. ABL kinase domain mutation was negative according to next-generation sequencing (NGS). Meningitis symptoms resolved with antibiotics before any IT chemotherapy was administered. After a discussion with the patient and considering her desire to receive outpatient therapy, she was then initiated on twice-weekly IT chemotherapy. She received a total of eight LPs performed twice weekly for four weeks with IT "triple-mix" chemotherapy consisting of MTX 12 mg, hydrocortisone 25 mg, and cytarabine 50 mg. During this time, dasatinib was changed to ponatinib 30 mg daily. She had delayed CSF clearance with persistent evidence of CNS leukemia up to the seventh LP, which showed residual abnormal immature B cells at 0.92% by flow cytometry. Due to delayed CSF clearance, she underwent an MRI of the thoracic, cervical, and lumbar spine with contrast to rule out masses, which revealed no abnormalities. Although she did not have an initial high burden of CNS involvement, given the resistance to IT chemotherapy, she was started on systemic HD MTX 3500 mg/m^2 given intravenously over 2 h with leucovorin rescue of 10 mg/m^2 given orally every six hours. After plasma MTX levels had cleared, she received a final IT (#8), which demonstrated that her CSF had cleared (no detectable leukemia). The next day, the patient reported bilateral lower extremity (BLE) paresthesias causing mild gait impairment, but she was ambulatory with full strength. No further IT administration of MTX or cytarabine was performed. Neurology evaluated the patient, noting probable nerve root irritation secondary to LP or neuropathic pain due to MTX. Two weeks later, she received, as planned, a second cycle of HD MTX 3500 mg/m^2 intravenously over 2 h with leucovorin rescue, and she was discharged with gabapentin for neuropathic pain. There was no delayed plasma clearance of HD MTX after either cycle. Systemically, she had achieved CMR with undetectable BCR-ABL by PCR.

The patient presented 4 days later with acute onset ascending bilateral lower extremity weakness. She denied bilateral upper extremity (BUE) symptoms, urinary or bowel

incontinence, or cognitive impairment. Upon neurological exam, the patient was noted to have normal mental status, cranial nerves intact, BUE strength 5/5, bilateral hip adduction 2/5, right-hip abduction 4/5, left-hip abduction 3/5, bilateral quadriceps 4/5, R-hamstring 3/5, L-hamstring 2/5, bilateral foot drop 2/5, bilateral inversion 3/5, R-eversion 3/5, L-eversion 0/5, and no thoracic sensory levels. Deep tendon reflexes were 2+ in the upper extremities, 3+ in the bilateral patellar, and 2+ in the bilateral ankle. There was no evidence of clonus. She had a positive R-Babinski, mute on the left. Sensation was decreased to pinprick but patchy in bilateral lower extremities up to her hips. Joint position sense was impaired in bilateral toes but normal at ankles. Vibration was 11–13 s distally in bilateral toes. She required moderate assistance to stand and could not ambulate without maximum assistance.

The patient underwent a repeat cervical, thoracic, and lumbar spine MRI, which revealed an increased T2 patchy signal in the posterior column of the thoracic spine, from T3 to the conus, shown in Figure 2. She was diagnosed with SCD of the spinal cord most likely due to MTX therapy. Laboratory testing was within normal limits, including folate level > 20 ng/mL, Vitamin B12 836 pg/mL, homocysteine 5.9 umol/L, and methylmalonic acid 106 nmol/L. Methylenetetrahydrofolate reductase (MTHFR) was the wild-type (normal) variant. Serologies for human immunodeficiency virus (HIV) 1/2, syphilis, cytomegalovirus (CMV), human T-lymphotropic virus (HTLV), West Nile Virus, and tuberculosis, as well as CSF analysis for bacterial/fungal cultures, Epstein–Barr virus (EBV), human herpesvirus 6 (HHV-6), varicella zoster virus (VZV), adenosine, JC, measles, West Nile Virus, herpes simplex virus 1 & 2 (HSV1/2), cryptococcus, enterovirus, *E. coli*, Hemophilus influenzae, parechovirus A, listeria monocytogenes, Neisseria, and streptococcus agalactiae, were all negative. She was initiated promptly on high-dose corticosteroids with methylprednisolone 1000 mg IV daily for 3 days, with supplementation of S-adenoslymethionine (SAM) 200 mg by mouth three times daily, leucovorin 20 mg IV every 6 h (eventually increased to 100 mg IV every 6 h), cyanocobalamin 500 mcg by mouth daily, cyanocobalamin 1000 mcg intramuscular injection weekly, and dextromethorphan 60 mg by mouth twice daily. Dextromethorphan is a non-competitive antagonist of the N-methyl-D-aspartate receptor (NMDA). TKI therapy with ponatinib was continued throughout the event. After transient improvement during the first 24–48 h of hospitalization, she progressively worsened and became paraplegic by the following week. She also developed a neurogenic bladder requiring catheterization, but bowel function was preserved. She was discharged on oral dextromethorphan, leucovorin, SAM, methionine 500 mg by mouth three times a day, and cyanocobalamin. She remained paraplegic for approximately 3 months. With intensive physical and occupational therapy, she began to recover motor function in her legs and improved bladder control ~3 months after the initial development of LE weakness. She continues to note steady improvement, and 6 months after the initial event, she is able to stand on her own and walk with assistance, and continues to have steady improvement in motor function, with the hope of a full recovery. Her B-ALL remains in CMR on ponatinib 30 mg daily. No further LPs have been performed (and none are planned), but there is no clinical or radiographic evidence of CNS relapse.



(**a**) (**b**)

Figure 2. (**a**) Sagittal view of T2-weighted MRI of the thoracic spinal cord showing hyperintensity from T3 to conus medullaris. (**b**) T2-weighted transverse view of the thoracic spinal cord.

3. Discussion

While MTX-induced leukoencephalopathy is well-reported, especially with HD MTX administration, subacute myelopathy is rare [4,26,27]. Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in folate metabolism, which catalyzes the conversion of 5-methyl-THF from THF for the re-methylation of homocysteine into methionine. The MTHFR allele variant 1298A>C (p.E429A) is associated with an increased risk of MTX-induced leukoencephalopathy [28–31]. Our patient was MTHFR wild-type, and the association between MTHFR polymorphisms and MTX-induced myelopathy is unclear [32–34]. Allele variants of dihydrofolate reductase (DHFR) may also be potential risk factors for MTX neurotoxicity [35–37].

The genetic profiles of children with ALL experiencing MTX toxicity were analyzed for trends in significant single nucleotide polymorphisms (SNPs). The majority of these SNPs were in genes involved in neural development and cell growth such as *TRIO*, *PRKG1*, *ANK1*, *COL4A2*, *NTN1*, *ASTN2*, *MBOAT-1*, *GIPC1*, *ZDHHC19*, *NXN*, and *PKN1* [14,31,38,39]. However, due to the limited sample size, these associations remain speculative. On targeted next-generation sequencing (128-gene panel), our patient did not have pathogenic mutations (other than BCR-ABL) or known SNPs that may be possibly associated with neurotoxicity. However, our patient did have what appeared to be germline polymorphisms in *PPM1D* and *ATM* genes based on variant allele frequencies (VAF) of ~50%.

Our patient's spinal MRI showed a diffuse abnormal T2 hyperintense signal within the dorsal columns from the level of T3 to the conus, indicative of white matter changes due to demyelination. There was no evidence of transverse myelitis. This finding is similar to other cases of MTX-myelopathy, which have also reported this T2 dorsal hyperintensity in a caudal to rostral pattern, usually along the posterior funiculi [14,27,40,41]. It is important to note that normal MRI imaging does not rule out MTX-induced myelopathy, and studies have shown that patients with overt clinical manifestations of myelopathy may have normal MRI scans [14,40,41]. This was evident in our case, as our patient had an initial negative MRI of the spinal cord after the development of lower extremity paresthesias. In our case, the patient was administered SAM, leucovorin, cyanocobalamin, methionine, and dextromethorphan, which is an NDMA antagonist. Though the exact

mechanism is still being investigated, dextromethorphan has been shown to block NDMA over-activation due to the elevated levels of homocysteine and other excitotoxic amino acids like glutamate that are present in the CSF of patients with MTX-myelopathy, reducing the duration and severity of neurological symptoms [42,43]. Leucovorin, a folate analog, has historically been the standard first-line treatment for MTX neurotoxicity. Recently, several cases have demonstrated the possible effectiveness of folate metabolites, such as SAM and methionine, in MTX-myelopathy, but the dosing and timing of administration are not standardized [29,44].

MTX-induced myelopathy has a reported incidence rate of 0.8% to 3% [27,45]. Our patient had several potential risk factors for developing MTX-myelopathy, including prior HSCT, relapsed disease, CNS relapse, suspected infectious or inflammatory meningitis, delayed CSF clearance with IT chemotherapy, and cumulative IT and intravenous MTX exposure within a short time period. After an initial prodrome of paresthesias, she developed acute onset ascending LE weakness shortly after a second cycle of high-dose infusional HD MTX, which worsened quickly to complete LE paralysis and loss of bladder control. Untreated SCD from nutritional deficiencies and drug-induced subacute combined degermation have been reported as irreversible. However, our patient recovered motor function after a 3-month period of paraplegia after receiving a combination of intensive drug therapy, supportive care, and physical therapy. Patients with myelopathy who recover some motor function within the first 3 months of developing paralysis have a more favorable prognosis and may achieve full recovery. In a literature review of patients with MTX-induced myelopathy, none had complete recovery of muscle strength to the level prior to MTX-induction [29], and three out of seven total reported patients (42%) partially recovered muscle strength, as in our case [29,46,47]. Previously described risk factors for symptomatic MTX neurotoxicity include IT MTX, systemic MTX, repeated IT chemotherapy injections within an interval of <1 week, history of exposure to other chemotherapy associated with neurotoxicity (cytarabine, cyclophosphamide, and nelarabine), radiation, elderly age, active CNS disease, and factors that affect the excretion of MTX such as acute kidney injury or dehydration [28,48,49]. A recent study reported that electromyography (EMG) testing of proximal and distal motor conduction showing F wave absence may be an early predictor of MTX-induced neurotoxicity including myelopathy [50]. There is currently no established therapy for MTX-induced myelopathy. Multiple studies have administered rescue leucovorin, with doses ranging from 10–50 mg/m^2 every 6 h, with the leucovorin dosage being administered 28–44 h after the first MTX dose [49,51–54]. In addition, prompt recognition of MTX-myelopathy is challenging as more than half of MRIs are normal at symptom onset, with the classical T2 dorsal cord hyperintensity only becoming evident on subsequent scans. Axial T2 MRI imaging is more sensitive for the detection of SCD [28]. Thus, careful evaluation of MRI scans and the recognition of symptoms is important to avoid further therapy, which may exacerbate the myelopathy, and to distinguish an evolving SCD from leukemic CNS involvement. Our patient with Ph+ B-ALL presented with symptomatic CNS relapse 4 years post-allo-HSCT and was initially treated with twice-weekly IT chemotherapy and ponatinib. Due to delayed CNS clearance, she received HD MTX for 2 cycles, both with leucovorin rescue. After the second HD MTX cycle, she developed SCD of the spinal cord resulting in complete lower-extremity paralysis. After receiving supportive medications and intensive physical therapy, she has regained motor function and can walk with assistance six months after the initial event, and her B-ALL remains in complete remission. A similar case of SCD-like imaging findings was reported in a 59-year-old male with diffuse large B-cell lymphoma who received five total doses of 14 mg IT MTX and no systemic HD MTX [14], and the authors summarized two other cases of lymphoma and three cases of ALL with similar findings reported in the literature [41]. Unfortunately, all of these patients died from either progression of their cancer or infectious complications. Our case is the first we are aware of to survive disease-free and with motor recovery.

4. Conclusions

MTX-induced SCD of the spinal cord is a rare but serious adverse effect of MTX treatment. Although the literature implicates multiple biochemical pathways behind the pathogenesis of MTX neurotoxicity, further studies are needed to examine the specific pathophysiology of MTX-induced SCD, such as genetic risk factors, including DHFR allele variant E429A c.1298 A>C and SNPs of genes important in the regulation of CNS myelination. Further guidance on standardizing treatment regimens for MTX myelopathy, including the use of folate metabolites, SAM, dextromethorphan, and corticosteroids, should be addressed by consensus. For ALL with CNS relapse, early administration of systemic HD MTX should be considered early after the initial failure of IT chemotherapy to avoid higher cumulative MTX doses and overlapping IT and systemic exposure. In Ph+ disease, TKIs with CNS penetration are needed, and ponatinib was initiated in this case because of reported blood–brain barrier penetration, though clear evidence is lacking [55]. Although clearance of the CNS is paramount for survival, we recommend considering a cap of four twice-weekly LPs with IT chemotherapy before systemic HD MTX is administered for persistent CNS disease; however, this is only based on our expert opinion, and empirical data are lacking. Alternating IT cytarabine and IT MTX and other treatment modalities such as Ommaya reservoir placement and changing TKI in Ph+ B-ALL should be considered. MTX should be held at the first sign of myelopathy, and MRI scans carefully read for T2 dorsal cord hyperintensity. Studies correlating MTX pharmacokinetics and the development of MTX-related toxicities, especially acute renal injury, have been described. However, the correlation between serum MTX levels and myelopathy is less well-established. As a result, frequent follow-ups and neurological exams are necessary to intervene earlier. In addition, prodromal symptoms, such as the new onset of mild peripheral neuropathy, should be considered as a potential harbinger for subsequent development of more serious myelopathy. CNS relapse of B-ALL is associated with a poor prognosis and the median OS is <1 year [56]. Our patient remains in CMR with no signs of CNS relapse >9 months after initial relapse in the CSF. Our case provides hope that recovery is possible for patients with severe MTX-induced myelopathy causing SCD. Prompt recognition of signs and symptoms, initiation of agents that may reverse MTX-induced neuronal damage, and intensive physical therapy are critical for successful recovery.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study and written informed consent has been obtained from the patient to publish this paper.

Data Availability Statement: Due to patient privacy and HIPPA, all data pertaining to the patient in this case report cannot be shared or distributed.

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Conflicts of Interest: The authors declare no conflict of interest.

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