

## Methotrexate-Related Neurotoxicity in the Treatment of Childhood Acute Lymphoblastic Leukemia

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### Abstract

The addition of methotrexate to treatment protocols in children with acute lymphoblastic leukemia has been found beneficial in preventing central nervous system relapse. However, MTX itself may be associated with neurologic morbidities, the most significant of which is leukoencephalopathy. The present study describes the clinical spectrum of leukoencephalopathy, which ranges from a subclinical disease manifested only radiologically to a progressive, devastating encephalopathy. The interaction of MTX with other components of the treatment protocol is discussed, as is the effect of leucovorin. A summary is presented of the metabolic pathways that may be involved in the development of MTX toxicity. Researchers are still seeking a biochemical marker to aid in the determination of the amount of MTX that may be safely administered.

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The central nervous system is recognized as a sanctuary for tumor cells in patients with lymphoreticular malignant diseases. CNS radiation therapy is useful for preventing disease recurrence in this area but is associated with a wide range of untoward effects, from vasculopathies to tumorigenesis. Methotrexate, a major antineoplastic agent, can sometimes serve as an effective alternative to XRT. The addition of intrathecal MTX or high dose intravenous MTX to leukemia treatment protocols has been associated with an increased survival rate in children with acute lymphoblastic leukemia [1,2]. However, the drug also has a significant toxic effect on the CNS and can potentially lead to severe neurologic morbidity [3-8]. The toxic effect can appear acutely, soon after treatment, or as a long-lasting, progressive neurologic and cognitive deterioration [4]. The overall incidence of acute MTX neurotoxicity may be estimated at 3-11% of treated children, depending on the amounts of MTX and leucovorin in the treatment protocol [3].

Two frequent clinical manifestations of MTX neurotoxicity are seizures and occlusive vascular-like events. The seizures are mostly sporadic, with a minority of patients progressing to chronic epilepsy [9]. The occlusive vascular-like events mimic transient ischemic attacks, sometimes even with persistent paresis or paralysis; the diagnosis is confirmed by the presence of white matter infarcts on imaging scans [10,11]. Other manifestations have also been reported, such as ataxia, tremor, somnolence, headaches, and

cognitive and psychiatric disturbances [12]. More peripherally, radiculopathy following intrathecal treatment has been observed [13]. Studies with evoked potentials have demonstrated peripheral nervous system damage during the treatment of ALL, with an additive effect of methotrexate [14].

The most frequent neurologic manifestation of MTX toxicity is leukoencephalopathy. This entity is defined by neurologists as abnormalities in the white matter of the brain, and by oncologists as abnormalities of both the gray ("encephalopathy") and white ("leuco-") matter [15,16]. Leukoencephalopathy may be subclinical, notable only by magnetic resonance imaging, with neither subjective nor objective changes in the patient's health, and it may improve or even disappear over a period of weeks to months. The frequency of subclinical leukoencephalopathy varies with the amount of MTX included in the treatment protocol [15]. It can also be slowly progressive and neurologically devastating, manifested by dementia, seizures, pyramidal signs, visual deficits and coma [4,16]. Pathologic examination reveals diffuse bilateral necrotizing lesions, demyelination, reactive astrocytosis and calcium deposition [16].

Children with leukemia are known to be at high risk of neuropsychological abnormalities owing to the traumatic impact of the disease itself and its treatment, the dependence on oncology services, missed school days, missed social contacts, and so on. Thus, treating these susceptible children with an agent that can cause neurocognitive deficits can be very deleterious to their future development.

Other drugs used today in treatment protocols for leukemia may cause toxicity themselves, and their combination with MTX could have an additive effect. Cytosine arabinoside, for instance, which is being given intrathecally together with MTX (triple therapy), has also been reported to cause leukoencephalopathy [17], cerebellar damage, and neuropsychological deterioration [18]. Apparently, the most harmful treatment protocol is XRT in conjunction with MTX, probably because of the disruption of the blood-brain barrier by XRT, which allows a markedly increased penetration of MTX to the brain, thereby increasing tissue levels [19]. The combination of the tissue damage from the XRT and the intensified MTX can lead to extremely devastating leukoencephalopathy.

The amount of leucovorin (5-formyl-tetrahydrofolate) administered to rescue cells from MTX damage is also an important factor. Leucovorin rescues normal cells by a mechanism involving 1-carbon

MTX = methotrexate  
CNS = central nervous system  
XRT = radiation therapy

ALL = acute lymphoblastic leukemia

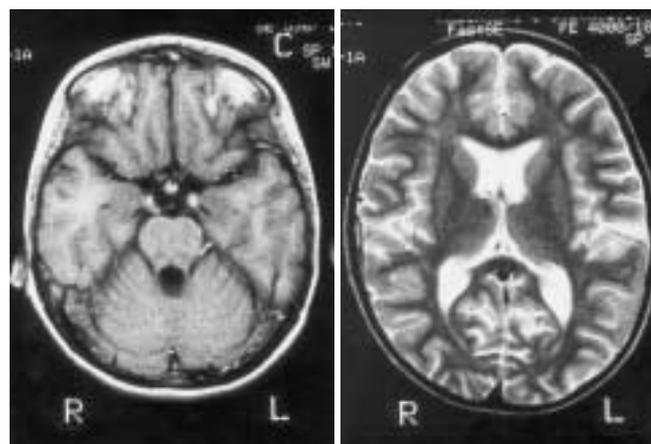
transfer reactions in the biosynthesis of purines and pyrimidines of nucleic acids [20]. Mahoney et al. [3] reported that increased cumulative exposure to repeated high dose MTX or an increased MTX/leucovorin ratio was associated with an increased incidence of neurotoxicity. The frequency of MTX-related neurotoxicity in patients with solid tumors is much lower than in those with ALL, probably owing to the use of higher doses of leucovorin. The clinical effect of an increase in leucovorin dosage in ALL protocols and its ability to decrease neurotoxicity at the expense of decreasing the response to treatment are not yet fully delineated [21].

Both the degree and sites of MTX toxicity are difficult to determine. There is no clear correlation either between findings on ancillary tests (MRI study, radionuclide scan of the brain, electroencephalography, neuropsychological testing) and the clinical manifestations that prompted their performance, or among the findings themselves on the different modalities. Functional changes can be observed after MTX treatment in SPECT (single photon emission computed tomography) studies of the brain. These changes probably represent perfusion abnormalities and resultant cerebral dysfunction in different brain regions. However, the clinical significance of these findings and their implication for the treatment protocols are not yet established [4,22]. Recently, motor-evoked potentials were suggested as a tool for assessing treatment-related neurotoxicity in both the CNS and the peripheral nerves. As with other measures, the specific aspects of the whole spectrum of neurotoxicity covered by the test and its short- and long-term clinical significance remain unclear [23].

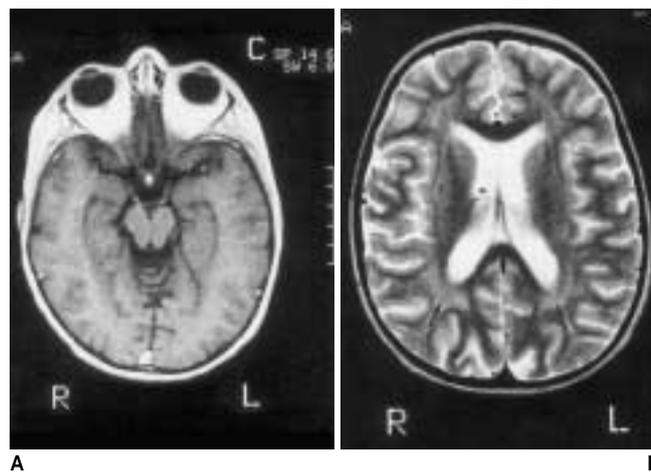
Figures 1 and 2 show the MRI scan of a 12 year old girl with relapse of ALL in the central nervous system. Leukemic infiltration in the pituitary stalk as well as the third cranial nerve is demonstrated. The child was treated with an intensified dosage of MTX, intrathecally and intravenously. Two months later, the infiltrates disappeared, but leukoencephalopathy was apparent, leading to discontinuation of the treatment.

The mechanism whereby MTX causes neurotoxicity is not fully understood, and more than one mechanism may be involved. It is noteworthy that the effect of MTX on nervous tissue is immediate. Shuper et al. [4] described a child who moved forcefully in the midst of an intrathecal injection of MTX. The cord, which was directly exposed to the MTX, was immediately damaged, leading to persistent monoparesis. Packer and co-workers [24] reported focal brain tissue damage around the tip of a misplaced intraventricular catheter used to deliver MTX.

The main action of MTX is inhibition of the enzyme dihydrofolate reductase, which is necessary for the reduction of dihydrofolate to THF [Figure 3], a key intracellular compound. THF deficiency leads to depletion of intracellular folates, and thereby, to decreased synthesis of both purines and pyrimidines [25–27]. It also markedly interferes with transmethylation reactions, which are crucial for the formation of proteins, lipids and myelin, presumably leading to demyelination [14]. Cerebrospinal fluid levels of methionine and S-adenosyl methionine decrease and levels of S-adenosyl homo-

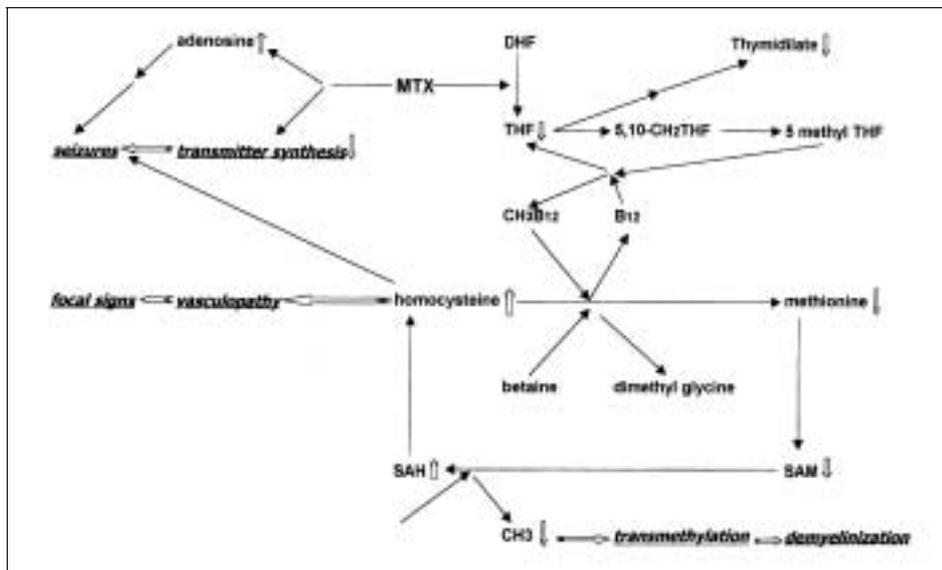


**Figure 1.** At relapse. **[A]** T1-weighted axial image after gadolinium injection. A thickened pituitary stalk is visualized in the suprasellar cistern (arrow). Note also the bilateral thickening and enhancement of the oculomotor nerves (arrows). These findings are secondary to leukemic infiltrates. **[B]** T2-weighted axial image. Mild parenchymal loss may be noted. The periventricular white matter is normal.



**Figure 2.** Scan done 2 months after that in Figure 1, following chemotherapy. **[A]** T1-weighted axial image after gadolinium injection. The appearance of the pituitary stalk and the oculomotor nerves has reverted to normal. **[B]** T2-weighted axial image. Hyperintensity of the periventricular white matter is notable (arrows), indicative of leukomalacia.

cysteine and homocysteine increase. Elevated blood homocysteine levels are known to be associated with endothelial cell injury and infarcts, and these may account for the vascular phenomena of MTX neurotoxicity [28]. They may also be associated with seizures. However, the significance of hyperhomocystinemia in neuronal injury has not yet been fully elucidated [29], and the association of changes in the levels of the specific components of the transmethylation process with the different presentations of MTX neurotoxicity is not known. Interference with normal transmethylation reactions can also lead to more remote effects, for instance on catecholamine synthesis. Changes in pteridines and monoamines



**Figure 3.** Schematic representation of the metabolic pathways involved in MTX activity and their possible association with different forms of neurotoxicity. DHF = dihydrofolate, THF = tetrahydrofolate, SAH = S adenosyl homocysteine, SAM = S adenosyl methionone.

have been found to be associated with neurologic damage [30]. In addition, other effects of MTX usage, such as increased adenosine levels and decreased biogenic amine neurotransmitter synthesis, may be important in the pathogenesis of MTX toxicity. In this regard, the reversal of MTX neurotoxicity by aminophylline, presumably by displacement of adenosine from its receptor in the CNS by aminophylline, has been suggested and should be studied further [31,32].

The approach to the child with MTX neurotoxicity is not well established. For instance, what should be done when the MRI shows significant leukoencephalopathy but the child is clinically asymptomatic? Should further doses be withheld? Decreased? Postponed? Or continued unchanged, given that MTX is the hallmark of childhood ALL treatment? Currently, the finding of an asymptomatic leukoencephalopathy on brain MRI study is not itself considered a clear indication for a decrease in MTX dose, and clinical judgment is also applied. The same is true for other modalities, such as motor-evoked potentials. In cases of transient MTX neurotoxicity, if the patient fully recovers MTX may be repeated. But, can neurologic damage recur in the next treatment? Can it recur in a more severe form? Furthermore, does a finding of subclinical leukoencephalopathy have any impact on the future neurologic or neuropsychological state of the patients? All of these questions point to the need for a biologic marker or markers for the quantification of MTX neurotoxicity and its correlation with acute, subacute, and long-term morbidity. Once this is accomplished, physicians will be better able to decide when to intensify treatment and when to stop it.

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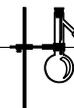
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## Research Project



### **Bcl-2 expression correlates positively with serum basic fibroblast growth factor (bFGF) and negatively with cellular vascular endothelial growth factor (VEGF) in patients with chronic lymphocytic leukemia (CLL)**

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**Background:** A large proportion of B-CLL cells express high levels of the anti-apoptotic protein Bcl-2. bFGF has been shown to up-regulate the expression of Bcl-2 in B-CLL cell lines and the elevated levels of Bcl-2 were associated with enhanced survival of CLL lymphocytes upon treatment with fludarabine. VEGF was shown to enhance the survival of endothelial cells by up-regulating the expression of Bcl-2.

**Objectives:** To measure the serum and cellular levels of bFGF and VEGF in 85 CLL patients, and correlate them with Bcl-2 levels.

**Methods:** bFGF and VEGF levels were determined using a commercial quantitative sandwich enzyme immunoassay technique. Bcl-2 levels were assayed by Western blot analysis.

**Results:** The mean serum bFGF level was 53.4 pg/ml (range 0–589) and that of VEGF was 459.2 pg/ml (range 33–1,798). The mean cellular bFGF level was 158.3 pg/2x10<sup>5</sup> cells and that of

cellular VEGF was 42.4 pg/2x10<sup>5</sup> cells. A high correlation was found between serum and cellular bFGF levels ( $P < 0.001$ ). Of the 69 evaluated patients 29 expressed Bcl-2, whose level of positively correlated with that of serum bFGF ( $P = 0.007$ ). Surprisingly, a negative correlation was found between Bcl-2 expression and intracellular VEGF level ( $P = 0.003$ ). A correlation was also found between serum bFGF and disease follow-up time.

**Conclusions:** In CLL there seems to be a correlation between angiogenesis-related factors and apoptosis-related protein expression. Elevated bFGF levels may account for the elevated Bcl-2 levels.

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