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# Methotrexate-induced central nervous system toxicity in children treated for acute lymphoblastic leukemia: analysis of seven consecutive cases

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par

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# Introduction

Methotrexate (MTX), a folate analogue, is widely used in the treatment of patients with autoimmune disease or certain types of cancer such as acute lymphoblastic leukaemia (ALL), lymphoma and osteosarcoma. Depending on disease type, MTX can be administered intravenously (iv), by intrathecal (it) injection or as a high dose (HD) perfusion. One of the major MTX toxicities is neurotoxicity that can be classified in three groups depending on the timing: acute, subacute and late neurotoxicity. In acute neurotoxicity, symptoms like seizures, confusion, somnolence and chemical arachnoïditis with headache, nausea, vomiting and fever arise within hours of MTX administration. In the subacute form, stroke-like symptoms like hemiparesis, ataxia, speech disorder, or myelopathy like sensory changes, leg pain and paraplegia can appear after days or weeks of MTX administration. The recovery is often spontaneous after 48 to 72h (1). Finally, patients can develop a chronic form of neurotoxicity after months or years, characterized by learning disabilities, neurocognitive impairment and other leukoencephalopathic symptoms such as quadriparesia, dementia, coma, even death (2).

The pathophysiology of MTX-induced neurotoxicity is not clearly understood. It has been postulated that MTX causes direct damage to the central nervous system (CNS), with induction of early alterations in astrocytes, axonal loss and demyelination. Secondly, it also induces alterations of the metabolic pathway of folates, excitatory amino acids, homocysteine, adenosine and biopterins resulting in biochemical alterations potentially related to neurological signs (2). Biochemical pathways related to MTX activity may explain why neurotoxicity develops at high doses. DNA and RNA synthesis is altered because MTX interferes with the synthesis of thymidine and purines. MTX inhibits the enzyme dihydrofolate reductase (DHFR). As a result, dihydrofolate levels increase in the cell and tetrahydrofolates (THF), important for purine and thymidine synthesis, decrease. MTX also interferes with homocysteine metabolism. It reduces the level of 5-methyltetrahydrofolate (5-methyl-THF) which is used as a group methyl donor to methylate homocysteine to Methionine. This reaction needs vitamin B12 as a cofactor and is catalyzed by methionine synthetase. Then, if methionine reacts with adenosine triphosphate (ATP), the most important methyl donor in cellular metabolism, S-adenosylmethionine (SAM), is yielded. It can be used to methyl and help other metabolic pathways, always catalyzed by an enzyme methyltransferase, which

converts SAM in S-adenosylhomocysteine (SAH) which can be degraded to homocysteine and adenosine. Furthermore, excess homocysteine can be metabolized to sulfur-containing excitatory amino acid (SEAA) neuro-transmitters. These are important because of their agonist effect on N-methyl-D-aspartate (NMDA) receptors, which can lead to seizures, if it is an intensive stimulation, and a more severe phenomenon called excitotoxicity. The latter is mediated by high influx of calcium ions through NMDA receptors-linked calcium channel and may have great implication in the pathogenesis of acute and chronic neuronal degeneration (3).

These metabolic alterations induced by MTX may have possible clinical consequences. It was suggested that a decreased level of SAM was correlated with demyelination due to hypomethylation, and could be a factor to exacerbate and induce a leukoencephalopathy. High levels of homocysteine have a direct toxic effect on the vascular endothelium and may cause cerebrovascular ischemia associated with microangiopathy and focal neurological deficits. Elevated levels of adenosine can alter cerebral blood flow and neuronal excitability, which can lead to neurological symptoms like nausea, vomiting, headache and seizures (2). Bernini et al. (4) described in 1995 the use of aminophylline (AMP) in 6 patients with MTX induced acute neurotoxicity and high levels of adenosine in the CSF, unresponsive to leucovorin rescue. Leucovorin, a folinic acid rescue, is the standard measure to prevent systemic toxicity, however, no specific and validated approach is available. Bernini's report on use of AMP was based on the observation that MTX therapy in patients with rheumatoid arthritis led to the release of adenosine from fibroblasts and endothelial cells in vivo (5). In fact, elevated adenosine levels were found in the CSF of the 6 patients who developed MTX induced neurotoxicity. Thus, an adenosine antagonist, acting as a competitive analogue, could have the potential to counteract the toxic CNS effects of MTX. Aminophylline, a competitive adenosine antagonist, was used in the six patients and resulted in resolution of symptoms in four treated patients and in substantial improvement in the remaining two. The role of AMP as a potential reversal agent in this setting remains entirely unexplained. In rodents, however, under hypoxic conditions, AMP potentially induces brain damage and neuronal death. (6)

Based on Bernini's observation, we treated recently seven consecutive patients who presented with subacute MTX-related neurotoxicity with aminophylline. Although very similar, they presented differences in clinical behavior and imaging. The analysis of these patients is the objective of this report.

## Material and Methods

The medical charts of 7 patients on treatment for ALL were reviewed, all diagnosed at the Pediatric Hematology-Oncology Unit of CHUV between January 2014 and June 2017 and who presented with MTX-induced neurotoxicity. Clinical, radiological data, EEG results if available, management and outcome were included in the analysis.

## Results

Seven patients with ALL who were diagnosed with MTX-induced encephalopathy between January 2014 and June 2017 were studied. Their age at diagnosis of MTX neurotoxicity was < 5 years in 4 and > 12 years in 3. The median age at diagnosis was 4 years (interval 2 to 15 years). There were 2 girls and 5 boys. All patients presented a pre-B ALL, with CNS involvement only in one case (CNS2: blasts present at diagnostic lumbar punction). Three patients had a standard risk ALL and were treated with the protocol COG ALL0932 (COG = Children's Oncology Group); 4 patients were high risk or very high risk because of their age > 13 years at diagnosis or positive minimal residual disease (MRD) after induction and were treated with the protocol COG ALL1131. The cytogenetic analysis of their leukemia showed non-contributive results in 4, favorable prognostic features in 2 and no significance in one patient.

### Case reports

*Case 1.* F.B is a female patient born in 1999, who in 2014, at the age of 15 years, was diagnosed with a high risk ALL due to age > 13. Genetic characteristics were non-contributive. While on delayed intensification treatment (day 42), the patient developed neurological symptoms 6 days after the last it MTX with a left hemiparesis associated with left hand and foot paresthesia, which didn't allow her to hold a pen and provoked a limping while walking. The neurological examination showed no balance disorder, a Romberg positive on left side, no meningeal signs, no other sensitive disorder.

The symptoms were fluctuating and alternating between the left and right side, and temporarily associated with a dysarthria. A differential diagnosis between transient ischemic attack (TIA), focal epileptic seizures, functional disorder and MTX neurotoxicity was considered and investigated with laboratory, EEG and cerebral MRI. The patient was treated

with hyperhydration with 3000ml/m2 of glucosalin, iv leucovorin at 30mg/m2 every 3h, and oral vitamine B complex (Becozyme Forte) PO. As symptoms persisted, the patient received one dose of iv Euphyllin of 2mg/kg in 1 hour the night of the first day of hospitalization. The neurological symptoms and neurological status resolved two hours after Euphyllin perfusion. Leucovorin was decreased to 30 mg/m2 every 6 hours from the second day on and stopped after the fourth day of hospitalization when she went back home. She received 16 doses of Leucovorin at a dose of 30mg/m2 in total.

*Case 2.* P.B is a male patient born in 2002, who was diagnosed with very high risk ALL in 2015 at the age of 13 years. Genetic characteristics were non-contributive. While on interim maintenance 1 treatment, he developed fever at 38.2°C 6 days after the last it MTX associated with headache for 24h and painful throat for 72h without inflammatory syndrome (leucocyte and CRP normal). An MRI was performed although the neurological status remained normal, because of persisting frontal headache without phono-photophobia or vertigo. MRI showed diffuse white matter abnormalities and MTX neurotoxicity was suspected. He was hospitalized and received iv hyperhydration, iv leucovorin rescue at 30 mg/m2 every 3 hours for 24 hours and then every 6 hours until the end of hospitalization. He received 2 dose of iv Euphyllin at 2mg/kg/dose in 1 hour day 1 and 2 of hospitalization. During the hospitalization, there were no symptoms and the neurological examination remained normal. He was released after 3 days. He received 10 doses of leucovorin 30mg/m2 in total.

*Case 3.* P.E is a male patient born in 2010, who was diagnosed with a high risk ALL in 2015 at the age of 5 years because of hyperleucocytosis, and treated further on as a very high risk patient because of positive minimal residual disease after induction. The cytogenetic characteristics showed a deletion of the short arm of chromosome 12 containing the gene ETV6, without impact on prognosis. During consolidation, on day 28, 5 days after the last it MTX, he consulted because of left otalgia that had been present for 2-3 weeks. There was no fever, but important fatigue, episodes of vomiting almost every day, associated with rhinitis and intermittent loss of balance. As there was no satisfactory explanation for the non-specific general symptoms, an MRI and blood tests were performed. There was no inflammatory syndrome in the blood, but white matter lesions were found on MRI compatible with ischemic or embolic lesion. However, a MTX induced encephalopathy could not be excluded and the

patient was hospitalized for observation. No neurological symptoms occurred, the patient didn't receive any treatment and left the hospital after 3 days.

*Case 4.* P.N. is a male patient born in 2003, who was diagnosed with a high risk ALL in 2016, at the age of 13 years. Genetic characteristics were non-contributive. While on delayed intensification, the patient developed neurological symptoms on day 40, 1 week after the last it MTX, with paresthesia of the right hand alternating with right shoulder, then the right cheek and, right part of the tongue, associated with loss of strength of the right upper extremity. Later the same paresthesia was experienced in the right lower extremity. The episodes lasted about 15 min and resolved spontaneously at home. As the same type of episode recurred, the patient was transferred to the hospital. At admittance, the neurological examination was normal, but a pancytopenia was found without an inflammatory syndrome. EEG and MRI showed abnormalities that were in line with neurotoxicity. After a third episode occurring during hospitalization characterized by dyspraxia of the right hand and paresthesia of the right hemibody lasting 10-15 minutes, the patient received 2 doses of iv Euphyllin 2mg/kg/dose in 24 hours 1 hour per dose and iv leucovorin 75 mg/d in 3 doses. Although no more symptoms appeared. The evolution was favorable and the patient left hospital after a total of 2 days.

*Case 5.* D.M. is a male patient born in 2011, who is diagnosed with a standard risk ALL in 2015, at the age of 4 years. His cytogenetic characteristics were favorable with an hyperdiploïdy and trisomy 4/10/17. While on delayed intensification, neurological symptoms appeared on day 13, 17 days after the last it MTX with abnormal seizure-like movements. The child was examined at a pediatric emergency ward which didn't reveal any special clinical findings. Because of the reported movements, an MRI was performed revealing bilateral white matter lesions. As the patient remained somnolent after sedation for the MRI, he was transferred to our unit. Because of suspected intermittent hypotonia of the upper limbs, and taking into account the MRI lesions, it was decided to treat the patient. He was put on iv Euphyllin 2 mg/kg/dose 2 doses in 48 hours 1 hour per dose and iv leucovorin at 30 mg/m2 every 3h. He had tremor of upper limbs with a slight ataxia during 3 days. The neurological exam was normal afterwards, but dysautonomic episode were present for 2 days with sweating and tremor. This last episode was not objectified by nurses and evolved favorably. In the

meantime, the child had an EEG and a MRI was repeated the day before his last day of hospitalization of 7 days, which confirmed the most likely diagnosis of MTX neurotoxicity.

*Case 6.* R.M. is a male patient born in 2012, who was diagnosed with a standard risk ALL in 2014, at the age of 2 years without contributive genetic characteristics. While on interim maintenance 2, the child presented neurological symptoms on day 10, 9 days after the last it MTX injection, by an epileptic seizure with loss of contact, ocular revulsion, head deviation to the left side and clonic movements of the left arm, associated with a febrile neutropenia and a skin rash. He was put on meropenem and acyclovir. The neurological examination showed a slight left hemisyndrome which disappeared within one hour. Because of differential diagnosis including cerebral hemorrhage, cerebral venous thrombosis, neurotoxicity by MTX and viral or bacterial meningoencephalitis, CT Scan and MRI were performed. The MRI result was in favor of either herpetic encephalitis or toxic encephalopathy. Because herpetic encephalitis could not be excluded, the patient was treated with acyclovir for 3 weeks, although the infection was not documented (PCR negative). The evolution was favorable, without any new episode during the hospitalization of 11 days. Levetiracetam peros at a dose of 110 mg 2x/day for minimum 3 months was introduced and intranasal Midazolam of 2.5mg prescribed as emergency treatment for seizures. Oncological treatment was then resumed with iv Vincristine, iv MTX and it MTX. One month later, on day 40 of interim maintenance 2, 7 days after the last it MTX, the patient presented an episode of vomiting, confusion and walking instability, hypotonia and loss of contact with eye deviation for 2h, associated with one episode of fever at 38°C. The neurological examination showed a somnolent and apathic patient with bilateral mydriases and no ocular pursuit. The neurological symptoms and confusional state resolved spontaneously within 30 minutes. MRI and EEG were repeated to differentiate between infectious or toxic cause. Acyclovir iv 500 mg/m2/kg was given again while waiting for the microbiological and viral results. The patient was also put on iv hyperhydration and received as well iv leucovorin at 30mg/m2 every 3h and iv Euphyllin 2mg/kg/dose 3 doses in 72h 1 hour per dose to cover for MTX-induced neurotoxicity. Acyclovir was stopped during hospitalization one day before his departure when microbiological tests showed to be negative. The patient recovered well within the first day of treatment by Euphyllin and leucovorin, without any new episode of seizures. He went back home after 5

days of hospitalization with an adjustment of anti-epileptic treatment with Levetiracetam at a dose of 150 mg 2x/day.

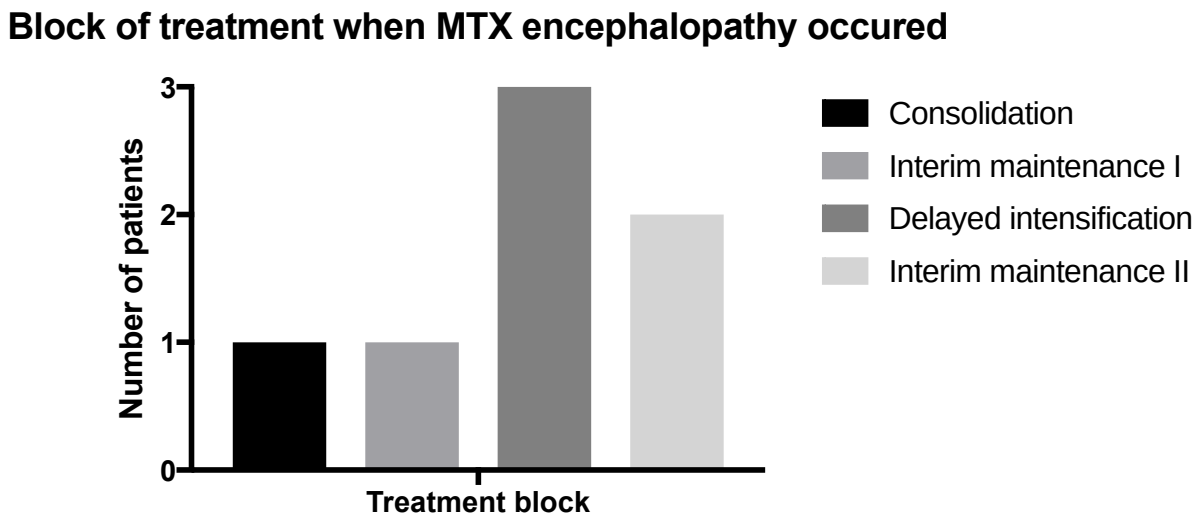
*Case 7.* B-D. S is a female patient born in 2013, who was diagnosed with a standard risk ALL in 2016, at the age of 3 years, with favorable cytogenetic characteristics by a trisomy 4, 10 and 17. While on interim maintenance 2, 13 days after the last it MTX, the patient presented with fatigue and an episode of loss of consciousness with tremor and extra-pyramidal syndrome, with posture and action tremor, fluctuating rigidity, automatic gait and a whispered voice. The neurological examination showed a pathological Barré and Mingazini with postural tremor, a clonus of the right foot, a fluctuating tonus with moments of rigidity of upper extremities. The child could not execute repeated fast movements. The clinical examination resembled the clinical picture of Wernicke encephalopathy (thiamine deficit). An EEG, MRI and blood tests were made to differentiate between MTX toxicity and Wernicke encephalopathy. The child was put on hyperhydratation and received iv Euphyllin at 2mg/kg/dose 2 doses in 48 hours 1 hour per dose and iv leucovorin at 30mg/m2 every 6 hours during 48 hours while waiting for MRI and metabolic results. A treatment of biotine, thiamine and riboflavin was introduced after MRI result showed signs in favor of Wernicke encephalopathy. After 48 hours of vitamin supplementation, the neurological examination improved without new episodes of loss of contact, with still a slight rigidity and slight tremor only when she is tired or upset. The patient went back home after 7 days of hospitalization.

The general schema of ALL protocol is summarized in figure 1. Standard risk protocol and very high risk/high risk protocol have the same schema, but treatment and timing are different.

*Figure 1. General treatment scheme of lymphoblastic leukemia*


The time of appearance of MTX-induced encephalopathy in the 7 patients is presented in Figure 2. No MTX toxicity occurred during the induction phase.

*Figure 2. Timing of encephalopathy*




Intrathecal MTX injections were given at different time points, alone or in combination with other medication. When neurological signs appeared, the protocol was put on hold during the time of hospitalization. Intrathecal MTX was stopped until the end of symptoms and the complete neurological recovery after hospitalization, or it MTX was completely removed from protocol.

The description of treatment blocks when MTX neurotoxicity occurred is summarized in table 1. Clinical data of the seven patients are summarized in table 2. An overview concomitantly on clinical symptoms, MRI and EEG findings are summarized on table 3. Blood test results and the outcome over time are summarized in table 4.

*Table 1. Description of treatments blocks and occurrence of MTX neurotoxicity.*

| Agent | Very high risk (patient 3) Consolidation | Very high risk (patient 2) Interim maintenance I | Very high risk/High risk (patient 1 2 and 4) Delayed intensification | Standard risk (patient 5) Delayed intensification | Standard risk (patient 6 and 7) Interim maintenance II |
|---|---|---|---|---|---|
| Cyclophosphamide iv | Day 1, 1000mg/m2/dose | | Day 29, 1000mg/m2/dose | | |
| Cytarabine iv | Day 1-4 and 8-11, 75mg/m2/dose | | Day 29-32 and 36-39, 75mg/m2/dose | Day 29-32 and 36-39, 75mg/m2/dose | |
| Mercatopurine po | Day 1-14, 60mg/m2/dose | Days 1-56, 25mg/m2/dose | | | |
| Vincristine iv | Day 15 and 22, 1.5/mg/m2/dose | Day 1, 15, 29 and 43, 1.5mg/m2/dose | Day 1, 8, 15, 43 and 50, 1.5mg/m2/dose | Day 1, 8 and 15, 1.5mg/m2/dose | Day 1, 11, 21, 31 and 41, 1.5mg/m2/dose |
| Dexamethasone po | | | Day 1-7 and 15-21, 5mg/m2/dose | Day 1-7 and 15-21, 5mg/m2/dose | |
| Pegaspargase iv | Day 15, 2500 UI/m2/dose | | Day 4 and 43, 2500 UI/m2/dose | Day 4, 2500 UI/m2/dose | |
| Prednisone po | | Days 1-5, 29-33 and 57-61, 20mg/m2/dose | | | |
| Doxorubicin iv | | | Day 1, 8 and 15, 25mg/m2/dose | Day 1, 8 and 15, 25mg/m2/dose | |
| Thioguanine po | | | Day 29-42, 60mg/m2/dose | Day 29-42, 60mg/m2/dose | |
| Methotrexate iv high dose | | Day 1, 15, 29 and 43, 5g/m2/dose | | | |
| Methotrexate iv (Capizzi) | | | | | Day 1, 11, 21, 31, and 41 |
| Methotrexate it | Day 1, 8, 15 and 22, 12mg/dose | Day 1 and 29, 15mg/dose | Day 1, 29 and 36, 15mg/dose | Day 1 and 29, 12mg/dose | Day 10 and 31, 10mg/dose and 12mg/dose respectively |

Legend: MTX Capizzi, iv doses escalation started from 100 mg/m2; iv, intravenous; po, peros; it, intrathecal

*Table 2. Clinical features of seven patients with acute MTX-induced encephalopathy*

| Patient no. | Age (years) | Type of Leukemia | Risk classification | Sex | Onset (days after last IT MTX) | Days to resolution | Treatment before Hospitalization | Treatment during Hospitalization |
|---|---|---|---|---|---|---|---|---|
| 1 | 15 | ALL pre-B CNS 1 | very high risk | F | 6 | 4 | Cotrimoxazole, Picosulfate of sodium, Paracetamol | Euphyllin IV, Leucovorin IV, Becozyme Forte PO, hyperhydratation |
| 2 | 12 | ALL pre-B CNS 1 | very high risk | M | 6 | 3 | Keppra, Xyzal 20gttes, Temesta, Clexane, Co-trimoxazole, Purinethol | Euphyllin IV, Leucovorin IV, hyperhydratation |
| 3 | 4 | ALL pre-B CNS 1 | very high risk | M | 5 | 3 | Co-trimoxazole, Ondansétron, Nutrini standard with NGS | None |
| 4 | 13 | ALL pre-B CNS 1 | high risk | M | 7 | 2 | Nopil (triméthoprime-sulfaméthazole) sirop | Euphyllin IV, Leucovorin IV, hyperhydratation |
| 5 | 3 | ALL pre-B CNS 1 | standard risk | M | 20 | 7 | Dexamethasone, Co-trimoxazole, Ondansetron | Euphyllin IV, Leucovorin IV, hyperhydratation |
| 6 | 2 | ALL pre-B CNS 2b | standard risk | M | 9 | 5 | Levetiracetam, midazolam if necessary, Co-trimoxazol | Euphyllin IV, Leucovorin IV, Acyclovir IV, hyperhydratation |
| 7 | 3 | ALL pre-B CNS 1 | standard risk | F | 13 | 7 | None | Euphyllin IV, Leucovorin IV, hyperhydration, Biotine, thiamine and Riboflavin PO |

Legend: IT, intrathecal; MTX, methotrexate; ALL, acute lymphoblastic leukemia; F, female; M, male; NGS, nasogastrictube feeding; CNS1: no meningeal disease, CNS2b: presence of blasts in cytospin of cerebrospinal fluid at diagnosis, without pleiocytosis, but with limited number of erythrocytes

*Table 3. Clinical symptoms and findings on brain MRI and EEG*

| Patient no. | Headache | Hemiparesis | Bilateral weakness | Dysphasia | Confusion, disorder of consciousness | Balance disorder | Movement disorder | Fatigue | Anatomic locations of MRI abnormality (pattern) | Restriced diffusion on DWI/Increased T2 signal +/- FLAIR (days after onset of symptoms) | Follow-up MRI findings (time after initial MRI) | Characteristics of EEG (days after onset of symptoms) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | yes | yes | yes | yes | no | no | no | yes | Centrum semiovale bilateral (R > L) (focal) | Yes/Yes (1 day) | 1) Decrease of Restricted diffusion and T2 hyperisgnal in centrum semiovale bilateral (always R>L) (11 days) 2) disappearance of restriced diffusion and stability of T2 minor lesions of white matter bihemispheric (5 months) | Normal (0 day) |
| 2 | yes | no | no | yes | no | no | no | yes | Supratentorial bilateral white matter prevailing at the right fronto-temporal and left parietal level (diffuse) | No/Yes (0 day) | 1) Same lesion similar to last MRI (10 days) 2) Progression of toxic lesions of white matter supratentoriel bilateral (20 days) 3) regression of toxic lesion (5 months) | None |
| 3 | yes | no | no | no | no | yes | no | yes | Frontal bilateral punctiform (focal) | Yes/no (1 day) | None | None |
| 4 | no | yes | yes | no | no | no | no | no | Periventricular bihemisperic white mattter (diffuse) | No/Yes (0 Day) | 1) regression of T2 hypersignal (2 months) 2) Still lesion as sequelae (5 months) | Slight bifrontal and left temporal deceleration and irregular basal activity, without epileptic element (0 day) |
| 5 | no | no | no | no | yes | no | yes | yes | Subcortical parietal bilateral and frontal right white matter (diffuse) | No/Yes (0 Day) | 1) significant decrease of abnormalities of frontal white matter and parietal bilateral (1 month) 2) Still slight abnormalities parietal bilateral in T2 signal(3 month) | Deceleration more in posterior bilateral, no epilieptic and no irritative foci (day 0) |
| 6 | no | no | no | no | yes | yes | no | no | Putamen, hippocamp bilateral and R centrum semiovale (diffuse) | Yes/Yes (1 day) | 1) Stability of hippocamp lesions with resolution of DWI, chronic aspect of putamen lesions, regression of T2 signal in centrum semiovale (1month) 2) Stability of putamen atrophy, centrum semiovale with sequelar lesion (9 month) | Diffuse deceleration to fronto-temporal lobe bilateral, more accentuated to the left (day 1) |
| 7 | no | no | no | yes | yes | no | yes | yes | Caudate nucleus, putmanen, pallidums and centrum semiovale anterior (diffuse) | No/Yes (1 day) | 1) Decrease of T2 signal of putamen, pallidum, caudate nucelus and centrum semiovale (11 days) 2) Still periventricual leucopathy signs with T2 hypersignal of white matter but decrease of tumefaction of basal ganglia | Slight diffuse deceleration which goes with encephalophaty, without irritative activity (day 0) |

Legend: MRI, magnetic resonance imaging; EEG, electroencephalography; DWI, diffusion-weighted imaging; FLAIR, fluid-attenuated inversion recovery.

*Table 4. Blood test abnormalities at the moment of clinical symptoms*

| Patient no. | Anemia (Hb g/l) | Leucopenia (G/L) | Leucocytosis (G/L) | Thrombopenia (G/L) | Neutrophil absolut number (G/L) | Lymphocyte absolut number (G/L) | Liver enzyme elevation | Renal function (creatinine µmol/l and urea mmol/l) |
|---|---|---|---|---|---|---|---|---|
| 1 | yes (77) | yes (1.3) | no | yes (52) | 1.03 | 0.1 | yes (both elevated with ASAT 64, ALAT 133) | normal |
| 2 | yes (94) | no | yes (15.0) | no | 12.9 (too elevated) | 1.2 | yes (both elevated with ASAT 50, ALAT 294) | anormal with creatinine 43 and urea 1.7 |
| 3 | yes (84) | yes (1.7) | no | yes (52) | 0.58 | 1 | no | normal |
| 4 | yes (83) | yes (1.7) | no | yes (87) | 1.12 | 0.39 | none | normal |
| 5 | yes (110) | yes (2.2) | no | no | 1.76 | 0.44 | no | normal |
| 6 | no | no | yes (12.6) | no | 11.3 (too elevated) | 0.9 | yes (both elevated with ASAT 180, ALAT 264) | normal |
| 7 | no | yes (4.0) | no | no | 1.08 | 1.8 | yes (both elevated with ASAT 52, ALAT 274) | normal |

Legend: Liver enzyme elevation = ASAT and ALAT only; number of neutrophil and lymphocyte in red are abnormal.

Looking at the clinical features of the seven patients with ALL pre-B and neurotoxicity (Table 1), one can distinguish two groups of patients by risk group, 3 being in the standard risk (patients number 5 to 7) 4 in the very high or high risk group (patients number 1 to 4). The median age at diagnosis was 12.5 years (range 4-15) for very high/high risk group, and 3 years for standard risk (range 2-3). Patient number 2 had a past history of venous sinus thrombosis and patient number 6 had a past history of herpetic encephalitis with inaugural epileptic seizure. Encephalopathy occurred after a total of 13 courses of it MTX in patient 1, from the beginning of treatment, after 8 it MTX in patient 2 and 3 had, and after 10 it MTX courses in patient 4. In this very high/high risk group the median number of MTX it injections before neurological symptoms was 9 (range 8-13). In the standard risk group, patient 5 had 6 it MTX courses and patient 6 and 7 had 8 it MTX courses since beginning of treatment. The median number for this group was 8 it MTX injections (range 6-8). Thus, there was no difference between the median number of it MTX injections received and the occurrence of encephalopathy between the 2 patient groups.

Considering the treatment blocks of the patients at the time when neurotoxicity occurred, there was one patient with neurotoxicity on Consolidation, one during Interim maintenance 1, two during Interim maintenance 2 and 3 patients during Delayed Intensification phase. Encephalopathy occurred after 2 courses of it MTX for patient number 1 and 4 during delayed intensification, after 4 courses of it MTX for patient number 3 during consolidation, after 1 course of it MTX and 1 course of HD MTX for patient number 2 during interim maintenance 1, in the high risk group. For the standard risk patients, neurological symptoms appeared after one course of it MTX for patient number 5 on delayed intensification and after 4 courses iv MTX and 2 courses of it MTX for patient number 6 and 7 on interim maintenance 2. The 7 patients developed signs of neurotoxicity after a median of 7 days after the last it MTX (range 5-20). In the high risk group the median interval was 6 days (range 5-7), and in the standard risk group 13 days (range 9-20). In the high risk group, the average number of days after it MTX when encephalopathy occurred is 6 days and for standard risk 13 days. Meopa (a mix equimolar of oxygen and azote protoxide) was used for sedation for the intrathecal injection for 6 patients during the treatment, one had general anesthesia for the injection.

The total number of it MTX injection before the neurotoxicity occurred is summarized in table 5. This table compares the number of days since last it MTX injection when neurotoxicity occurred and shows the treatment block in which every child was during neurotoxicity.

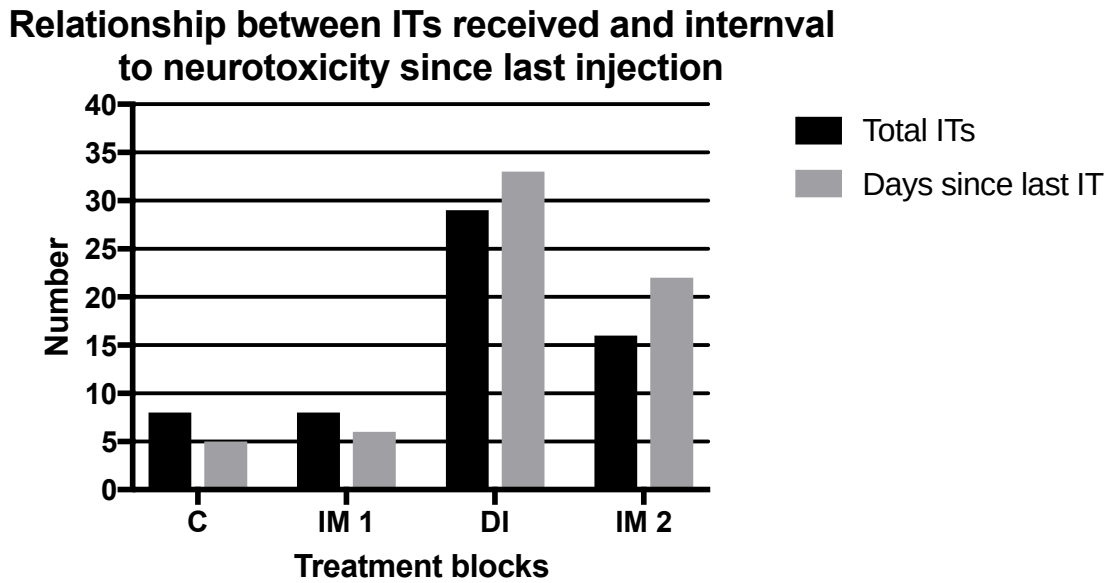
*Table 5. Number of IT MTX injections and interval to neurotoxicity*

| Patient No | Total IT before NT | Days since last IT | Treatment block |
|---|---|---|---|
| 1 | 13 | 6 | DI |
| 2 | 8 | 6 | IM1 |
| 3 | 8 | 5 | C |
| 4 | 10 | 7 | DI |
| 5 | 6 | 20 | DI |
| 6 | 8 | 9 | IM2 |
| 7 | 8 | 13 | IM2 |
| Median (range) | 8 (6-13) | 7 (5-20) | |

Legend: IT, Intrathecal; NT, neurotoxicity; DI, delayed intensification; IM2, interim maintenance 2; C, consolidation; IM2, interim maintenance 2.

Figure 3 depicts the relationship between the intensity of intrathecal treatment before occurrence of neurotoxicity and the interval observed.

*Figure 3. IT Interval between intrathecal MTX and neurotoxicity*




Legend: IT, Intrethecal; C, consolidation; IM1, interim maintenance 1; DI, delayed intensification; IM2, interim maintenance 2

## Neurological symptoms

The table 2 shows and compares neurological symptoms and signs which were present among patients with MTX induced encephalopathy: All patients were also examined by a senior neuropediatrician. The described signs and symptoms appeared after an it MTX with or without Capizzi MTX (moderate dose iv MTX starting at 100mg/m2 with dose escalation every 10 days based on blood results) or with or without HD MTX (5g/m2/dose). They were not specific and allowed a large differential diagnosis. The most frequent symptom was fatigue (4/7 patients), combined sometimes with unusual asthenia.

Three of the seven patients had headache (frontal in 2/3) without other signs of intracranial hypertension, except for patient 3 with vomiting episodes. This symptom quickly resolved during the hospitalization.

Three patients had general and nonspecific neurological symptoms like loss of contact and confusion. This confusional state was associated with other neurological symptoms like hypotonia which was present in 3 patients, or tremor which was present in 2 patients. Because of the suspicion of epileptic seizure, an EEG was made in these three patients, and pathological decelerations were found in all of them.

Two patients (patient 1 and 4) had a loss of strength and an objectified paresthesia before and during hospitalization. This was found either anamnestically, or at neurological examination. Both sensitive and motor deficits were found. The hemisyndrome was fluctuant and transient for both. For patient 1 the same symptoms appeared on the other body side during hospitalization. In all patients, the neurological symptoms disappeared during hospitalization. The follow-up was favorable. The treatment measures may have helped the recovery, but this is not definite and clear. There was no recurrence of neurotoxicity. This also includes the patient without treatment during hospitalization.

## Management of encephalopathy

Six of seven patients received iv Euhpyllin, iv leucovorin with iv hyperhydration. Euphyllin was given in 1 hour with NaCl 0.9% at 2 mg/kg. Patient 1 received a vitamin B complement by Becozyme Forte PO. Patient 2 was already on Levetiracetam (Keppra) because of his risk of epileptic seizures and on therapeutic Enoxaparin because of his recent history of venous sinus thrombosis. Patient 6 was already on Levetiracetam because of recent history of suspected herpetic encephalitis with one epileptic seizure and he received Acyclovir during his second

hospitalization to cover for potential viral encephalitis. Patient 7 received in addition a vitamin supplementation of biotine, thiamine and riboflavin, because of the suspicion of metabolic disease. Patient 3 didn't receive any treatment during his hospitalization because of the favorable evolution directly at the beginning of hospitalization.

## Imaging

Two patients had a computed tomography (CT) scan shortly after the onset of neurological symptoms. CT scan of patient 1 was normal, and the scan of patient 6 showed right frontal and right and left occipital hyperdensity evoking calcifications. All seven patients had an MRI the day of or one day after the beginning of the neurological symptoms. They all had conventional MRI with DWI and gadolinium contrasts studies. Two Patients had angio-MRI and patient 3 didn't have FLAIR 3D MRI. The angio-MRI didn't not show any abnormalities.

a) Four patients (1, 2, 4 and 5) had MRI with clear signs of MTX neurotoxicity with diffuse and confluent T2 hyper signal of white matter of both sides. Patient 1 had also restricted diffusion on the same location of T2 lesion. All the patients had follow-up MRIs at one month after the hospitalization and 3 to 5 months later to check the evolution of brain lesions. The first follow-up MRI showed a stability or a decrease of lesions with T2 hyper signal and for patient 1 an associated decrease of restricted diffusion. The second follow-up on MRI showed in 3/4 patients a stability of T2 hyper signal corresponding sequelae.

b) MTX neurotoxicity on MRI was less clear for patient 6 and 7. They both had T2 hyper signal of white matter in centrum semiovale, but it was associated with basal ganglia lesions as well, which is atypical for MTX-induced toxicity. The differential diagnosis was then enlarged to infectious encephalitis for patient 6, because of the hippocampal lesions suggesting more herpetic encephalitis, and to metabolic disease for patient 7, because of significant lesions of the basal ganglia associated with biotin-thiamine responsive basal ganglia disease. The follow-up MRI showed a decrease of T2 hyper signal of the basal ganglia and the centrum semiovale within the first month of hospitalization for both patients. Patient 6 had a particularity in follow-up images with persisting stable hippocampal lesions on both sides. Later MRIs in both patients continued to show white matter lesions, atrophy of basal ganglia, especially putamen of both sides, compatible with chronic sequelae of MTX toxicity.

c) Patient 3 had an MRI during his hospitalization that didn't show specific signs of leukoencephalopathy. There were two little frontal lesions with slight restricted diffusion

without T2 abnormalities, and without clinical repercussion. Because of the fast favorable recovery, within the first day, and because of lack of clear MRI signs of MTX neurotoxicity, he didn't have any more MRI follow-up.

*Figure 4. MRI image in DTI with restricted diffusion of white matter in centrum semiovale bilateral*

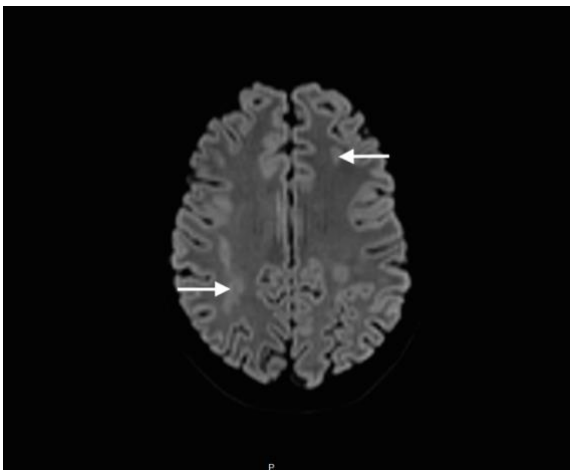
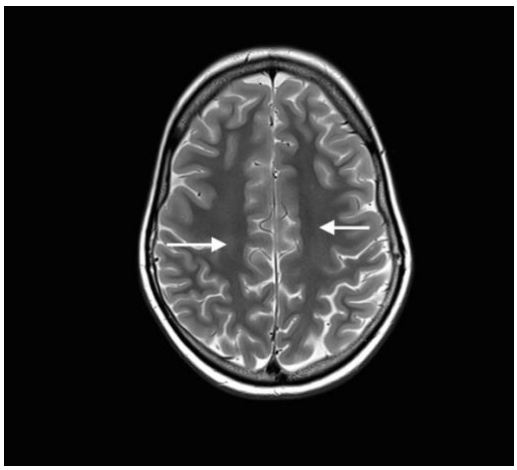


*Figure 5. MRI image in T2 with white matter hypersignal in centrum semiovale bilateral*



## Electroencephalography (EEG)

Comparison of EEGs showed similarities between patients. Five out of seven patients had one or several EEGs during their hospitalization. One was normal, and the rest had abnormalities. Two patients had diffuse deceleration without irritative foci, and the rest had more focal deceleration without epileptic or irritative foci. These EEGs were compatible with an encephalopathy.

## Blood tests

All patients had basic blood tests performed during their hospitalization, which included complete blood count (CBC) with leucocyte differentiation, creatinine, urea, electrolytes with sodium and potassium, and additional analysis if needed. Three patients presented a pancytopenia on arrival. One needed to be transfused with red cells and platelets. Two patients had a leukocytosis just below 10 G/l with 15 G/L for patient 2 associated with neutrophilia without left deviation and with one episode of fever at 38°C but without CRP elevation. The leucocytes for patient 6 were at 12.6 G/L without fever and inflammatory syndrome. It resolved for the two patients at the end of hospitalization. None of the patients had an acute renal failure which could have exacerbate the MTX toxicity. Four patients had an

elevation of liver enzyme, that resolved after hospitalization. Patient 5 had at arrival a neutropenia with an absolute number of neutrophil at 400. Because of suspected infectious disease provoking the encephalopathy, patient 6 had a serology and microbiology done without positive results. Enlarged metabolic analysis were run for patient 7, as a metabolic disease was suspected provoking the encephalopathy. This analysis included blood lactates, pyruvate, ketone bodies, electrolytes, liver and renal analysis, cholesterol, Vit B12, amine acid in the blood, urine and CSF, acylcarnitine profil, total carnitine, purine and pyrimidine in the urine. Everything revealed to be normal. In this child, a genetic sequencing of 7 genes associated with acute encephalopathy was performed without revealing any abnormalities.

## Discussion

The aim of our study was to review in detail the clinical chart of 7 patients who presented neurological symptoms related to MTX treatment for their ALL. The described neurological events occurred after it MTX only, after Capizzi MTX (100mg/$m^2$) combined with it MTX or HD MTX (5g/$m^2$) combined with it MTX. No neurotoxicity occurred during induction and none during maintenance. Thus, no toxicity was observed in the very low-dose MTX (20-40mg/$m^2$) setting. This is in concordance with Mahoney et al who observed significantly more neurotoxicity after intermediate-dose iv MTX +/- iv 6-mercaptopurin, compared to repetitive low dose oral MTX (7). The neurological events in our patients were of subacute nature, as they occurred after a median number of 7 days (range 5-20) after the last it MTX injection. However, the occurrence of MTX neurotoxicity is not so much based on the last it MTX, but there is a relationship with the amount of MTX received. Every patient had a median of 8 it MTX it injections before any neurological events happened (range 6-13). One patient received concomitantly iv MTX at 100mg/m2 with a dose escalation every 10 days up to a maximum of 300mg/m2 which is considered a low dose. There were two patients (1 and 2) receiving iv high dose MTX at a dose of 5g/m2. Patient 1 had received the full block of 4 courses of HD-MTX at 5g/m2/dose before occurrence of neurotoxicity in the subsequent treatment block. Patient 2 developed signs of neurotoxicty after the first course of HD-MTX at 5g/m2/dose. After recovery, he completed the lacking three courses of HD-MTX without any further toxicity. The patients who were on consolidation or delayed intensification received iv Arac at a low dose of 75mg/m2/dose. AraC is the only medication with potential neurotoxicty in this treatment

schedule, but given only intravenously. Thus, in contrast to Reddick et al (8) who showed in a study of 45 patients treated for low to high risk ALL a clear relationship between dose of iv MTX received and risk of leucoencephalopathy, our patients presented neurotoxicity, but mainly in the setting of it MTX, except for patient 2, who had HD MTX associated with it MTX.

All our patients underwent imaging and showed abnormalities, in concordance with a large prospective study conducted by Bhojwani et al (1) on 369 patients with ALL. They showed that all patients with clinical symptoms of MTX neurotoxicity (3.8%) presented leukoencephalopathy on MRI, but that leukoencephalopathy could also be found in 20.6% of asymptomatic patients. Fifty-eight percent of symptomatic patients and 78% of asymptomatic patients kept signs of leukoencephalopathy until the end of treatment. All had received 5 courses of HD-MTX and 13-25 triple intrathecal injections (MTX, hydrocortisone and cytarabine). Among various variables analyzed, only the ratio of 42h MTX level concentration to leucovorin dose was a significant risk factor. Among our patients, only 1 had 4 courses of HD-MTX and another patient 1 course before occurrence of neurotoxicity whereas the other 5 patients received either it MTX alone or combined with low doses. We could therefore not evaluate this ratio as potential risk factor. Our patients continued the protocol treatment after complete neurological recovery. The protocol treatment was resumed after a median number of 9 days (range 2-17). Four of seven (1,2,3 and 5) patients were rechallenged with it MTX again without further neurologic events. This is very much in line with the observation made by Badke et al (9) who showed that rechallenging with IT MTX in maintenance period of protocol was safe. They described 21 patients with ALL or lymphoblastic lymphoma who developed MTX neurotoxicity, 17 after it MTX only and 4 after combined HD-MTX and it MTX. Thirteen could be rechallenged with it MTX, without further symptoms in 13/17, while 4 developed second neurotoxicity. Two of them were switched to it AraC and the other 2 were successfully rechallenged during maintenance treatment. The authors observed, as in our patients, MTX neurotoxicty only during consolidation, induction, interim maintenance or delayed intensification, but no neurotoxicity during maintenance treatment. Patient 4, 6 and 7 didn't receive any more it MTX. A follow-up MRI in July 2018 will be done for patient 4 and it MTX recovery can be reconsidered. Patient 6 had already finished all it treatments in his treatment block and continued according to the maintenance treatment. In patient 7, who presented with Wernicke-like symptoms the decision was made to stop further it treatments, as no clear origin of this particular clinical presentation could be found and as an underlying

metabolic disease could not be definitely discarded. Also, the patient present second neurological symptoms when rechallenged with only oral low-dose MTX. A NGS analysis didn't provide any genomic clue for predisposition to toxicity. Further oral and iv chemotherapy other than MTX could be continued with only reduced doses, indicating that a predisposing metabolic disorder remained the best explanation for all the observed toxicities.

Dufourg and coll (10) found a significant association between MTX-related neurotoxicity and age > 10 years. In our small patient cohort three of our patients were more than 10 years old, with a mean age of 13 years, and were in the high risk protocol with it MTX and Capizzi MTX or HD MTX, and 4 patients were under the age of 5 years, with a mean age of 3 years. They had a wide range of neurological symptoms, corresponding mainly to described manifestations of subacute MTX neurotoxicity including general symptoms such as headache, fatigue, confusion, seizure and more specific and focal neurologic deficits like dysarthria and hemiparesis (1, 2, 4). Our observation was that the patients in the standard risk group presented more general neurological symptoms such as confusion, loss of contact and balance disorder (walking instability), whereas the high risk group had more focal and progressing symptoms, like paresthesia and dysarthria. Headache appeared only in the high risk group.

Genetic variations of genes that are included in MTX metabolism may give a clue to explain neurotoxicity induced by MTX. In 2013, 499 pediatric patients with ALL were investigated for pharmacogenetics in MTX (11). A strong association was found between a germline variant of SLCO1B1 SNP and MTX pharmacokinetic parameters such as peak MTX level at the end of 24h infusion, $AUC_{0\text{-}48h}$ and clearance, with $AUC_{0\text{-}48h}$ being the predictor of high overall toxicity scores. We couldn't analyze pharmacokinetic parameters, as only 2 patients had received high-dose MTX (patient 1 and 2). Gene sequencing was performed only in patient 7 because of the severity and unusual clinical presentation, without identifying any of the known candidate genes for MTX toxicity. A molecular analysis for MTHRF mutation was done, and revealed to be negative, only in patient 1 because of delayed MTX excretion during the first HD-MTX course, although there were no signs of organ toxicity. As demonstrated by Lopez-Lopez and coll. in a systematic review and meta-analysis of the two most frequent MTHFR polymorphisms (12), mutations of the MTHFR gene doesn't seem to be a good marker of MTX-related toxicity.

Interaction with concomitant medication could potentially interfere with MTX metabolism and contribute to toxicity. As summarized in Table 2, none of our patients was concomitantly on proton pump inhibitors or fluoroquinolone antibiotics, known to possibly increase the plasma or CNS level of MTX. However, among drugs that use the same metabolic pathway as MTX there is nitrous oxide (NO) which decreases the methionin levels necessary for myelin production, by depletion of functional Vit B12 (13). Nitrous oxide is used as sedative for it MTX treatments. Six of our patients (patient number 1,2,3,4,5 and 7) had it MTX under sedation with Meopa and one (patient 6) under general anaesthesia. Nitrous oxide inactivates methionine synthetase and prevents conversion of methyltetrahydrofolate to tetrahydrofolate and reduces the production of methylenetetrahydrofolate necessary for DNA synthesis and myelin production, while increasing the level of homocystein. Koblin (14) and al showed in mice that short exposure (15 minutes to 4 hours) at high pressure (0.8atm) markedly inhibited methionine synthetase activity in mouse liver and brain, whereas short exposure to low partial pressure (<0.05atm) did not provoke any laceration. Only small decrease in enzyme activity was found after prolonged continuous exposure to NO over 8-22 days. MTX neurotoxicity associated with Meopa was also described in humans. Lobel et al (15) reported on a case of neurotoxicity induced by MTX in a 7 year old child who developed 4 days after the 3$^{rd}$ it MTX injections during induction treatment, performed under NO sedation, dysarthria and carpopedal spasms followed by somnolence and decrease of muscle control, then a coma, not responding to leucovorin recue and later aminophylline administration. Recovery was slow over 12 months. Infectious and metabolic causes were excluded, and the authors concluded because of the unusual severity of the clinical presentation and follow-up that NO could be a possible additional risk factor, acknowledging that the MRI didn't show modifications that can be observed with chronic use of NO. Forster et al (13) described another case of a 12-year-old patient treated for ALL with regular it MTX injections. She always received propofol for induction followed by nitrous oxide before her injection and was currently on a PPI treatment. Four days after the 5$^{th}$ it MTX injection, she was hospitalized for neurological symptoms such as focal seizure and weakness of left upper limb. MRI showed signs of leukoencephalopathy (T2-weighted hyperintensity white matter lesions and restricted diffusion of subcortical white matter). Serum vitamin B12 level was measured and was under the normal range. Both cases show similarities with our patients with regard to the clinical pattern of neurotoxicity and the timing after it MTX. In the Forster's case report neurotoxicity

appeared earlier after 5 it MTX which may be explained by the presence of several other drugs the patient was receiving and that could have interacted with MTX, inducing methionine depletion and accumulation of homocysteine, and depletion of functional vitamin B12. In our patients, NO only without any other premedication was administered for it injections at a pressure of 9 litre/min (usual flow for patients under age of 7) or 12 litre/min (usual flow for patients above the age of 7) according to age, and limited to 30 minutes. The six patients who had NO for it MTX underwent a usual procedure without any complications observed. As highlighted by Forster et al (13), NO as any other concomitant medication during MTX treatment in patients with ALL should be used cautiously and limited in time.

The use of imaging for diagnosis of MTX-induced encephalopathy is not recent. However, conventional CT Scans, regular T1 and T2 MRI and angiography have failed to show consistent abnormalities that characterize MTX neurotoxicity. The hallmark of MTX-induced leukoencephalopathy on MRI are hyperintensities on T2-weighted images located in the periventricular white matter, especially the centrum semiovale. In spite of these changes, patients can recover as did our patients. Diffusion-weighted MRI (DWI) has shown to be a sensitive way of detecting cytotoxic edema on early MRI. The restricted diffusion of water in the brain of patients who presented stroke-like neurological complications after either HD-MTX, it MTX only or a combination of two produces this image contrast (16) . Restricted diffusion is supposed to correspond to motion reduction of water along axons as result of cytotoxic edema which resolves after resolution of symptoms. DWI may be a tool to evaluate and monitor acute neurotoxicity and detect early MTX white matter injury (16). This cellular cytotoxic swelling is not necessarily irreversible explaining the neurological recovery observed in our patients. Patients 1 had bilateral restricted diffusion that decreased and then cleared after the resolution of the clinical symptoms. The authors agree that diffusion abnormalities in acute/subacute MTX toxicity indicate a brain dysfunction, but not necessarily structural brain injury. Clinical recovery and disappearance of diffusion abnormalities allow to consider to rechallenge with it MTX and continue anti-leukemic treatment. This happened in 2 of our patients (1 and 6) who presented neurological symptoms and restricted diffusion abnormalities on MRI performed shortly after the occurrence of neurological events. Follow-up MRIs can show a regression or disappearance of restricted diffusion, and the occurrence, concomitantly or later, of T2-weighted hyperintensity changes of white matter. We found these abnormalities in 6/7 patients during hospitalization, 2 of whom also had signs of

restricted diffusion on DWI. Whereas restricted diffusion decreased or disappeared, the T2-weighted white matter hyperintensities persisted, even after resolution of neurological symptoms. Other case reports made similar observations (17, 18). However, interpretation of the MRI changes remains challenging with regard to neurological prognosis.

Another presentation of MTX-related neurotoxicity is the PRES syndrome, defined by neurological symptoms like seizure, vomiting episode, abnormalities of mental status associated with a neuroradiological pattern of symmetrical parietooccipital vasogenic edema (19). It can be caused by arterial hypertension, pre-eclampsia and immunosuppressive drugs. IT MTX is a very rare cause of PRES syndrome but it has been described (20). This was the case for patient 5, who had presented with seizure-like symptoms and then somnolence. His MRI didn't show diffusion restriction, but concomitantly T2-weighted white matter changes and different neuroradiological features associated with different clinical symptoms and allowed to conclude to a PRES syndrome.

Follow-up MRIs were done until the patients had no more radiological signs of neurotoxicity or presented a stability of the lesions on MRI. Five out of 6 patients still had T2-weighted hyperintensity lesions of white matter at 3-9 months follow-up MRI , that is signs of leukoencephalopathy, but without clinical impact. In patients with diffusion restriction, an early MRI within 1 month of the events has been done to document disappearance of the cytotoxic edema.

We described and analyzed 7 patients with MTX related subacute neurotoxicity, 6 of whom were treated with iv hyperhydration, iv leucovorin and iv aminophylline. All had pathological MRIs, but all recovered, and it MTX was continued in 4 out of 7 patients. The pathogenesis of MTX neurotoxicity is multifactorial, the elevation of adenosine in CSF is one of the mechanisms discussed which gives the rational for administration of AMP which may inhibit the indirect effect of increased adenosine by MTX (4). We can of course not be sure that recovery was due to AMP, as spontaneous recovery can occur. A prospective study in a larger patient cohort with well settled guidelines is needed to confirm the hypothesis. Among the risk factors that could explain the observed neurotoxicity, only the use of NO as sedative for intrathecal injections can be considered. Six out of 7 patients who presented MTX-related neurotoxicity had their it MTX performed under NO sedation. Although no particular observation could be made during the procedure and the recommended timing was respected, we cannot exclude a potential interaction between NO and MTX.

MTX neurotoxicity is complex and it touches various metabolic pathways. Because MTX is essential in the treatment of ALL, further studies must be conducted to elucidate the possible risk factors depending on the treatment schemes used in order to prevent potentially severe complications.

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# References


1. Bhojwani D, Sabin ND, Pei D, Yang JJ, Khan RB, Panetta JC, et al. Methotrexate-induced neurotoxicity and leukoencephalopathy in childhood acute lymphoblastic leukemia. J Clin Oncol. 2014;32(9):949-59.
2. Vezmar S, Becker A, Bode U, Jaehde U. Biochemical and clinical aspects of methotrexate neurotoxicity. Chemotherapy. 2003;49(1-2):92-104.
3. Quinn CT, Griener JC, Bottiglieri T, Hyland K, Farrow A, Kamen BA. Elevation of homocysteine and excitatory amino acid neurotransmitters in the CSF of children who receive methotrexate for the treatment of cancer. J Clin Oncol. 1997;15(8):2800-6.
4. Bernini JC, Fort DW, Griener JC, Kane BJ, Chappell WB, Kamen BA. Aminophylline for methotrexate-induced neurotoxicity. Lancet. 1995;345(8949):544-7.
5. Cronstein BN, Naime D, Ostad E. The antiinflammatory mechanism of methotrexate. Increased adenosine release at inflamed sites diminishes leukocyte accumulation in an in vivo model of inflammation. The Journal of clinical investigation. 1993;92(6):2675-82.
6. Somekawa-Kondo T, Yamaguchi K, Ishitsuka Y, Ito S, Tanaka K, Irikura M, et al. Aminophylline, administered at usual doses for rodents in pharmacological studies, induces hippocampal neuronal cell injury under low tidal volume hypoxic conditions in guinea-pigs. The Journal of pharmacy and pharmacology. 2013;65(1):102-14.
7. Mahoney DH, Jr., Shuster JJ, Nitschke R, Lauer SJ, Steuber CP, Winick N, et al. Acute neurotoxicity in children with B-precursor acute lymphoid leukemia: an association with intermediate-dose intravenous methotrexate and intrathecal triple therapy--a Pediatric Oncology Group study. J Clin Oncol. 1998;16(5):1712-22.
8. Reddick WE, Glass JO, Helton KJ, Langston JW, Xiong X, Wu S, et al. Prevalence of leukoencephalopathy in children treated for acute lymphoblastic leukemia with high-dose methotrexate. AJNR Am J Neuroradiol. 2005;26(5):1263-9.
9. Badke C, Fleming A, Iqbal A, Khilji O, Parhas S, Weinstein J, et al. Rechallenging With Intrathecal Methotrexate After Developing Subacute Neurotoxicity in Children With Hematologic Malignancies. Pediatr Blood Cancer. 2016;63(4):723-6.
10. Dufourg MN, Landman-Parker J, Auclerc MF, Schmitt C, Perel Y, Michel G, et al. Age and high-dose methotrexate are associated to clinical acute encephalopathy in FRALLE 93 trial for acute lymphoblastic leukemia in children. Leukemia. 2007;21(2):238-47.
11. Radtke S, Zolk O, Renner B, Paulides M, Zimmermann M, Moricke A, et al. Germline genetic variations in methotrexate candidate genes are associated with pharmacokinetics, toxicity, and outcome in childhood acute lymphoblastic leukemia. Blood. 2013;121(26):5145-53.
12. Lopez-Lopez E, Martin-Guerrero I, Ballesteros J, Garcia-Orad A. A systematic review and meta-analysis of MTHFR polymorphisms in methotrexate toxicity prediction in pediatric acute lymphoblastic leukemia. The pharmacogenomics journal. 2013;13(6):498-506.
13. Forster VJ, van Delft FW, Baird SF, Mair S, Skinner R, Halsey C. Drug interactions may be important risk factors for methotrexate neurotoxicity, particularly in pediatric leukemia patients. Cancer Chemother Pharmacol. 2016;78(5):1093-6.
14. Koblin DD, Watson JE, Deady JE, Stokstad EL, Eger EI, 2nd. Inactivation of methionine synthetase by nitrous oxide in mice. Anesthesiology. 1981;54(4):318-24.

15. Lobel U, Trah J, Escherich G. Severe neurotoxicity following intrathecal methotrexate with nitrous oxide sedation in a child with acute lymphoblastic leukemia. Pediatr Blood Cancer. 2015;62(3):539-41.
16. Fisher MJ, Khademian ZP, Simon EM, Zimmerman RA, Bilaniuk LT. Diffusion-weighted MR imaging of early methotrexate-related neurotoxicity in children. AJNR Am J Neuroradiol. 2005;26(7):1686-9.
17. Sandoval C, Kutscher M, Jayabose S, Tenner M. Neurotoxicity of intrathecal methotrexate: MR imaging findings. AJNR Am J Neuroradiol. 2003;24(9):1887-90.
18. Eichler AF, Batchelor TT, Henson JW. Diffusion and perfusion imaging in subacute neurotoxicity following high-dose intravenous methotrexate. Neuro Oncol. 2007;9(3):373-7.
19. Hinchey J, Chaves C, Appignani B, Breen J, Pao L, Wang A, et al. A reversible posterior leukoencephalopathy syndrome. The New England journal of medicine. 1996;334(8):494-500.
20. Guler T, Cakmak OY, Toprak SK, Kibaroglu S, Can U. Intrathecal Methotrexate-Induced Posterior Reversible Encephalopathy Syndrome (PRES). Turk J Haematol. 2014;31(1):109-10.