A Systematic Review of Neuropsychological Studies Confirms that Adequate Folinic Acid Rescue Prevents Post Methotrexate Neurotoxicity

Michelle Sadeh[1], Helen Toledano[2], and Ian Cohen[3]

[1]Israel Cancer Association
[2]Schneider Children's Medical Center of Israel
[3]Schneider Children's Medical Center of Israel Department of Internal Pediatrics A

December 31, 2021

Abstract

A comprehensive literature search was performed of all databases of the Web of Science Citation Index, during 1990-2020, for the terms: neuropsychological, neurocognitive, cognitive, acute lymphoblastic (and lymphocytic) leukemia, and osteogenic sarcoma, to see if there was evidence of a correlation between folinic acid (FA) rescue inadequacy and long-term cognitive damage. All English language, peer-reviewed articles of neuropsychological assessments of children who had been treated with high-dose methotrexate without irradiation, and which included details of methotrexate and FA schedules, were selected. Four groups of studies were found and analyzed, Those with no evidence of cognitive deterioration, Those with evidence of cognitive deterioration, studies with more than one protocol grouped together, preventing separate analysis of any protocols, and those with significant serious methodical problems. In all studies, protocols without evidence of cognitive deterioration reported adequate FA rescue, and those with evidence of cognitive deterioration reported inadequate FA rescue.

A Systematic Review of Neuropsychological Studies Confirms that Adequate Folinic Acid Post Methotrexate Rescue Prevents Neurotoxicity

Sadeh Michelle,1 Toledano Helen,2,3 Cohen Ian Joseph,2,3

1.Israel Cancer Association, Tel Aviv, Israel.

2 . The Rina Zaizov Department of Pediatric Hematology-Oncology,

Schneider Children's Medical Center of Israel, Petach Tikva, Israel

3. Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Corresponding Author: Prof Ian Joseph Cohen, icohen@tauex.tau.ac.il

ORCID Numbers:

Sadeh M: 0000-0002-3312-6828

Toledano H: 0000-0002-1476-7937

Cohen IJ: 0000-0002-5364-3275

ABBREVIATIONS TABLE

FA Folinic acid

ALL Acute Lymphoblastic Leukaemia

MTX Methotrexate (MTX)

LD-IT-MTX Low-dose intrathecal methotrexate

HDMTX High-dose methotrexate

IT MTX Intrathecal MTX

CNS Central Nervous System

CSF Cerebrospinal fluid

WIPPSI Wechsler Preschool and Primary Scale of Intelligence

WISC-IV Wechsler Intelligence Scale for Children fourth edition

WAIS Wechsler Adult Intelligence Scale

IQ Intellectual Quotient

BFM Berlin Frankfurt Munster

POG Pediatric Oncology Group

GCI General cognitive index

WM Working memory

VIQ Verbal intelligence quotient

PIQ Performance intelligence quotient

PS Processing speed

WORD COUNT TEXT 5899 ABSTRACT 147

TABLES 4

RUNNING TITLE Adequate FA after MTX prevents cognitive damage .

Keywords:

Neuropsychological evaluation, high dose methotrexate, folinic acid rescue therapy, neurocognitive damage

ABSTRACT

A comprehensive literature search was performed of all databases of the Web of Science Citation Index, during 1990-2020, for the terms: neuropsychological, neurocognitive, cognitive, acute lymphoblastic (and lymphocytic) leukemia, and osteogenic sarcoma, to see if there was evidence of a correlation between folinic acid (FA) rescue inadequacy and long-term cognitive damage. All English language, peer-reviewed articles of neuropsychological assessments of children who had been treated with high-dose methotrexate without irradiation, and which included details of methotrexate and FA schedules, were selected. Four groups of studies were found and analyzed, Those with no evidence of cognitive deterioration, Those with evidence of cognitive deterioration, studies with more than one protocol grouped together, preventing separate analysis of any protocols, and those with significant serious methodical problems. In all studies, protocols without evidence of cognitive deterioration reported adequate FA rescue, and those with evidence of cognitive deterioration reported inadequate FA rescue.

.

INTRODUCTION

For over 50 years, our understanding of the pathophysiology of treatment-related side effects following high dose MTX has been based on several axioms, which require reassessment.

No evidence of the previous held belief of "the folinic acid methotrexate ratio" has been found. That concept suggested the effective dose of folinic acid (FA) needed to rescue patient after MTX is linearly correlated to the MTX dose [1]. Sirotnak [2] clearly demonstrated that in a mouse model one can "over rescue" and block the efficiency of MTX by giving too much leucovorin after the MTX dose and also that too little leucovorin or too prolonged starting of the rescue can result in death from neurotoxicity. The question remains whether is it possible to find a dose of FA that will prevent neurotoxicity without reducing the chemotherapy effect. The answer can be found in the same article showed that the appropriate, adequate, rescue dose of FA, after doubling the MTX dose, needs to be 3-4 times higher that used for the original MTX dose. "On certain schedules with methotrexate, toxicity can be virtually eliminated with no diminution in antitumor efficiency [2]. It follows that when the previous dose of FA was barely adequate for rescue, doubling both the MTX and FA doses will lead to neurotoxicity. The previously not understood " mysterious interaction" [3] that high-dose MTX caused brain damage (leukoencephalopathy) if given after, but not before, cranial irradiation, can now be explained, since both irradiation and intra-arterial mannitol [4] disrupt the blood brain barrier, and result in higher levels of both MTX and folinic acid. The level of FA, however, may now be inadequate since for the higher CSF level of MTX achieved, a much higher level of FA would be needed for effective rescue [5]. It is therefore not surprising that after low dose methotrexate, over rescue can occur after similar milligram doses of FA. Such a finding was reported by Bowman who noted that patients with head and neck cancer who received 40mg/m2 FA after 40mg/m2 MTX had a lower response rate than patients who received no FA [6]. It will be expected that following high dose methotrexate, over rescue will only be possible after "Mega doses" of FA. This has too the best of our knowledge only been reported to date, once, when by mistake, such a dose of FA (1275mg) was given after 12.5 grams of HDMTX[7].

The misleading advice that the FA rescue dose should be kept at a minimum to prevent a reduction in cure rates by "over rescue" has been accepted without any evidence, and no reports of reduced prognosis with increased FA rescue were found [8]. Reports that claimed increasing the FA dose reduces prognosis did not stand up to close scrutiny and have since been discredited [7], and many of the articles cited to support this concept were recently shown to be problematic [10]. These reports have resulted in the use of low-dose (inadequate) FA rescue after high dose MTX by certain groups, which was, as we predicted, associated with significant neurological damage [11].

Previous studies examining adequacy of FA doses used and occurrence of neurotoxicity were able to show correlations between neurotoxicity and inadequate FA rescue [11], but were less helpful in defining a critical FA dose, since they were hindered by the lack of a consensus definition of what constitutes neurological damage. One group initially limited reported neurotoxicity to the occurrence of strokes and seizures [12] (Later this specific group published a neuropsychological analysis of the same patients [13].) Current neuropsychological studies can now demonstrate subtle brain damage that result in long-term brain damage that should be assessed in order to prevent such damage. We predict that the dose of FA needed to prevent subtle cognitive damage found on neuropsychological assessment will be even higher than what is found to be adequate for the prevention of gross neurological damage.

Peterson [14] performed a meta-analysis of neuropsychological sequelae in children with ALL treated by different protocols without cranial irradiation. The meta-analysis did not find any consistency in the results with regard to visual-motor skills, visual memory, or verbal fluency. But, in multiple domains of intelligence and academic achievement (math and reading), processing speed, verbal memory, working memory perceptual reasoning skills and some aspects of executive functioning, worse functioning was found in ALL survivors. These findings strengthen the understanding that chemotherapy as the sole CNS relapse prevention protocol can cause cognitive and academic problems in children.

Since the meaning of some of the terms used have changed over the years, we define them as used in this manuscript.

1. High dose MTX refers to an intravenous infusion dose of > 1gm/m2 MTX [15].

2. Adequate FA rescue is the term used to describe a dose of FA which, when given after high-dose MTX, was not followed by any long-term toxicity. It can be given intravenously or orally. An inadequate rescue dose associated with toxicity could be due to an insufficient dose and/or too long an interval between initiation of MTX and start of FA rescue "Too little too late". The FA rescue after high dose MTX may be delayed safely up to 36 hours after initiating MTX without neurotoxicity [16]. However, the higher the dose of MTX and the longer the time to rescue, the higher the rescue dose required. Thus, the doses of MTX and FA, and time to rescue, both determine if a rescue protocol is adequate or inadequate.

3. Comprehensive Neuropsychological studies refer to assessments that include standard tests which address intelligence, learning and memory, working memory, processing speed, linguistic abilities, executive functions, problem solving – all differentiating between verbal and non-verbal domains, attention, academic achievements, visual abilities (perceptual, spatial) and fine motor functions. There are many tests that examine each and every one of these cognitive abilities, but in different research centres and countries researchers use different tests, which make it harder to compare between the diverse studies done in the world addressing cognitive abilities and impairments.

A sensitive issue when assessing any cognitive ability is related to age. Depending on the childrens' age, different assessment tools are needed. For example, the WIPPSI vs. WISC-IV vs. WAIS, all standard tests addressing the Intellectual Quotient (IQ) of the examinee, but are appropriate for different age brackets.

Another issue important to consider when asking about any type of cognitive ability affected by any intervention is the time of assessment. Most neuropsychological tests allow repeating them after a year, but if one asks about late effects of chemotherapy, one must state what is considered late effect. If the question is chemotherapies' detrimental effects on the childs' ability to become a well-functioning adult, then it is imperative that the effects of the oncological treatment be assessed at least after being in school. The effect of the oncological treatment on his or her reading, writing, comprehension and math is crucial in answering such a question.

Furthermore, in an ideal world, it is best to do the 1st neuropsychological testing before any intervention occurred. But that is very difficult to achieve, thus it is best done as soon as possible. When addressing the long-term effects of giving adequate or inadequate FA, the assessments should be at least 2 or 3 years after the 1st assessment, preferably using the same tests, asking about the same cognitive abilities one studied to begin with.

METHODS

A comprehensive search of the literature during 1990-2020 was performed in December 2020, of all the databases in the Web of Science Citation Index using the terms neuropsychological, neurocognitive, and cognitive, together with acute lymphoblastic (and lymphocytic) leukaemia and osteogenic sarcoma. In addition, a personal data base of over 500 reprints of articles from over 130 journals on the subject of methotrexate and FA and side effects was reviewed.

INCLUSION CRITERIA

English language peer-reviewed articles of neuropsychological assessments of children who had received treatment with high-dose methotrexate without irradiation, which included details of methotrexate and FA schedules, were selected. All articles found were examined and reported irrespective of difficulties in separating subgroups who received different therapy or those with methodological problems. Not all of these studies had full details of the ITMTX (Intrathecal MTX) doses These reports that could not be analysed were included to prevent any suggestion of bias and to prevent challenge of the results of this review in the future by citation of these problematic reports.

RESULTS

The selected literature was separated into four groups: (1) studies with no evidence of cognitive deterioration, (2) studies with evidence of cognitive deterioration, (3) studies of more than one protocol grouped together, preventing analysis of each protocols, and (4) studies with significant serious methodical problems. The results are presented below, and include the treatment protocols, neuropsychological assessments, and significance of the FA rescue regime used. It was found that the studies without evidence of cognitive deterioration were those in which the treatment protocol contained adequate FA rescue, while those with evidence of cognitive deterioration had inadequate FA rescue.

1. Studies with no evidence of cognitive deterioration (Table 1)

Protocols used at the Hospital for Sick Children (Sick Kids Toronto Canada) from1983-1996

Children aged 1-5 years were treated with three different Central Nervous System (CNS) prophylaxis protocols with either cranial irradiation, 3 doses of 8gm/m2 or 3 doses of 33.6 gm/m2 MTX. FA rescue was started after 36 hours. Those who received MTX were initially given 100mg/m2 of FA followed by 12mg/m2 of FA every 3 hours x 6 then every 6 hours until the plasma levels were < 0.08 micromol /L. The total dose was at least 172mg/m2. Patients who received 33.6gm/m2 MTX were given 200mg/m2 FA followed by 12mg/m2 FA every 3 hours x6 then every 6 hours until the serum levels were < 0.08 micromol/L. The total FA dose was at least 272mg/m2 [17].

Spiegler et al. tested 79 of the 156 pediatric patients treated in these studies: 22 after very high dose MTX, 32 after high dose MTX, and 25 after they received cranial irradiation. The patients were tested 10.5+2.7 years after diagnosis. Children in both the MTX groups had 17 of 18 measures of neurocognitive function, including intelligence, attention, memory, and academics compatible with population norms. Irradiated children had significantly lower results in 12 measures.[17]

This is an important group of studies that shows that adequate FA rescue, even after very high dose MTX, prevents cognitive damage.

The Dutch (DCLSG) Protocol ALL-7

Four 2 weekly 24-hour infusions of 5g/m2 MTX were followed by 75mg/m2 FA rescue at 36 hours from start of MTX, followed by twelve doses of 15 mg/m2 FA every 3 hours, for a total of 255mg/m2 (personal communication with Dr. Annette Kingma).

Kingma et al. performed two studies of these patients. In the first [18] they assessed 20 children < 7 years old at diagnosis, after approximately 2 and 7 years follow up (median 7 years), using14 intelligence tests evaluating learning and memory, attention and speed, visual motor integration, and fine motor function. No major cognitive impairment was found, 12/14 tests showed no significantly lower test score, and there were no differences in school achievement as compared to siblings ($p<0.004$). In the second study, 17 children were assessed 8 years after diagnosis with 12 psychometric measures [19]. Although they showed 16 defects on various test measures, all the patients and their healthy siblings attained the mean level of education for the current Dutch population of children 15 to 19 years of age. Excluding fine motor measures, 10/17 lacked measures of poor function.

Although this is a protocol based on the ALL Berlin- Frankfurt – Munster (BFM) 86 protocol without cranial irradiation, a significant major difference is that they received a FA rescue protocol of 225mg/m2 initiated 36 hours after the start of the MTX, instead of an FA dose of 45mg/m2 initiated at 42 hours as in the BFM study. Groups that adopted the rescue used by the BFM group have reported neurotoxicity [20].

Israel Osteosarcoma Group protocols

Three paediatric oncology units in Israel treated patients with osteosarcoma with protocols including HDMTX and FA. All patients received repeated doses of intravenous MTX (12-20 g/m2); however, different doses of FA were used according to institutional protocols.[21]

Bonda-Shkedi et al. examined long-term survivors without central nervous system involvement who agreed

to participate in the study, had not received any other potentially neurotoxic therapy, and had no previous neurological or psychiatric history. Twelve osteosarcoma patients aged 17-31 years were examined at least 4 years after completing therapy (average time from completion of therapy to examination was 10-17+5.57 years (personal communication Bonda-Skedi E.) and had received 300-600 mg/m2 of FA after each treatment. They had statistically significant ($p<0.025$) better results on 11 of 18 subtests (e.g., attention, visual perception, analysis and synthesis, verbal memory, visual memory, executive functioning, and comprehension) than those who received inadequate FA rescue (see below) [21].

The lack of cognitive damage after adequate FA rescue supports the concept that adequate rescue prevents cognitive damage.

The CCG-107 study

This study treated babies aged 1-12 months diagnosed with ALL with a protocol that included a CNS - directed therapy consisting of four 24-hour infusions of 33.6 gm/m2 MTX. Thirty six hours after the start of the MTX they received 200mg/m2 FA then six 12mg/m2 doses of FA every 3 hours, and 12mg/m2 FA every 6 hours until serum MTX level was < 8x10-8M. The total dose was at least 284 mg/m2.[22]

Thomas A. Kaleita assessed the neurodevelopmental effects of the intense chemotherapy treatment given to these children by testing them using the McCarthy Scales of Children's Abilities (27 children received this test, 3 received IQ tests). This assessment tool has 18 cognitive and motor subtests which provide six scales: general cognitive (GCI), verbal, perceptual-performance, quantitative, memory, and motor. The results on all scales were close to the normal population norm; though the range was wide (one child had a GCI more than two standard deviations above the mean, and two children a GCI between one and two standard deviations below the mean). They were tested aged 62.1+ 17.2 months [23].

Although this study was performed more than 2 years after the treatment with HDMTX, most of the 30 children participating in this study were assessed prior to entering school, which may have been too early to pick up many of the late deleterious cognitive and scholastic effects. The lack of cognitive damage at this very young age is certainly encouraging.

2. Studies with evidence of cognitive deterioration (table 2)

The ACL0131 STUDY [24] examined children who were treated in the POG 9201 and POG 9605 studies without cranial irradiation. Those on the POG 2901 protocol received six courses of 200mg/m2 MTX over an hour, followed by 800mg/m2 MTX over 23 hours together with (intrathecal methotrexate) ITMTX, every three weeks. FA rescue was started at 42 hours after the start of MTX. 5 doses of 10 mg/m2 FA was given every 6 hours, (total 50 mg/m2). Children on the POG9605 protocol received additional MTX at 2 of 3 weeks, either intravenous or intrathecal.[24]

Duffner et al. reported the results of neurocognitive studies in 52 patients aged 1-10 years at diagnosis who were tested more than 2.6 years after completion of therapy with Paediatric Oncology Group (POG) 9605 and POG 9201 protocols. Twenty subgroups of tests were assessed in seven functional areas: global intellectual function, verbal abilities, perceptual and spatial abilities, spatial planning, attention and concentration, processing speed, and memory. Forty percent of patients scored < 85 on either verbal IQ (VIQ) or performance IQ (PIQ). Note the average IQ is 100 with a standard score of +/- 15. Children in both studies had significant attention problems, but P9605 children scored below average on more neurocognitive measures than P9201 children (14/17 (82%) vs. 4/17 (24%) measures, respectively).[24]

The reason why the children on the P9605 did worse than those on the P9605 is unclear from the discussion by Duffner[24] who presents three different possible suggestions. We would suggest a more convincing reason is that the patients on both these protocols received only 50mg/m2 FA initiated after 42 hours, although 60mg/m2 initiated after 36 hours was the dose of FA shown to prevent neurological damage when this dose of MTX was given without ITMTX [25]. This dose was clearly inadequate to prevent cognitive damage especially since both protocols also included ITMTX. This inadequacy was more marked in the POG 9605 study patients who received more, inadequately rescued, MTX.

The POG AALL0232 protocol study

Children received four courses of 5gm/m2 MTX and IT MTX followed by FA rescue of 15mg/m2 at 42,48, and 54 hours. When MTX levels were higher than 1micromol/L at 42 hours or 0.4 micromole at 48 hours, FA rescue was continued. Another group received five doses of escalating dose MTX with PEG asparaginase but without FA rescue [13].

The COG AALL06N1 study assessed children who were treated with the COG AALL0232 protocol. Hardy reported the neurocognitive impact of this treatment in 192 children, aged 1-18 years, at diagnosis 8-24 months after completion of chemotherapy (average age 14.4+4.0 months). They were evaluated by a > 4-hour comprehensive neuropsychological battery or a screening battery (approximately 1 hour) by a psychometrist for IQ, working memory (WM), and processing speed (PS). Patients in both groups together demonstrated impairment in IQ of 21.4% and 28.6% had impaired processing speed as defined by scores of equal or less than 1 standard deviation below the mean-versus 15.9% of individuals in the normative samples for each Wechsler measure (two-sided P=2.04 and <.01 respectively). Cognitive outcome correlated with age and insurance status which in the US is a proxy for socioeconomic status. They found that children < 10 years old at diagnosis were at risk of deficits in IQ and PS. [13]

Thus the folinic acid dose that was given to the children after IVMTX was inadequate to prevent the damage seen to the children who received IV MTX (with L asparaginase) without any FA. Others who used this protocol with these rescue doses have reported considerable neurotoxicity [26].

The St Jude X protocol

This protocol compared the efficacy of two CNS prophylaxis protocols in ALL. One group received 1gm/m2 MTX over 24 hours (together with ITMTX 12mg/m2). FA rescue was given at 36 and 42 hours from the start of the HDMTX by 2 doses of 30 mg/m2 FA then 3mg/m2 FA at hours 54, 66 and 78 (total 69 mg/m2). This element of therapy was given once a week for 3 weeks then every 6 weeks for up to 75 weeks. The other group received cranial irradiation and ITMTX. (12mg/m2 x5 over 2.5 weeks then every 12 weeks for 30 months) [27].

Ochs et al. examined 26 children who received HDMTX and 23 who received cranial radiation. The aim of the study was to determine if different protocols resulted in differences in cognition at 6 years post diagnosis. One of the main findings, when comparing abilities at the beginning of the treatment vs. 6 years later, was that both treatment paradigms caused a decrease in the children's full-scale IQ, VIQ, and arithmetic and reading abilities. In addition, they found that 20% of those receiving parenteral MTX had white matter hypodensity and 58% abnormal electroencephalogram. The repeated inadequate FA rescue caused neuropsychological damage similar to that caused by cranial irradiation [3].

St Jude protocol Total Therapy XV

Patients in the low risk group received four courses of (approximately) 2.5gm/m2 MTX followed by a FA dose of 10mg/m2 at 44 hours, repeated four times every 6 hours (total = 50mg/m2), and the standard risk/high risk groups received (approximately) 5 gm/m2 MTX over 24 hours followed by a FA dose of 15mg/m2 initiated at 42 hours and repeated four times every 6 hours (total = 75mg/m2). Age-dependant intrathecal triple therapy of MTX, hydrocortisone and cytarabine was given with each infusion [28].

Three analyses [29, 30, 31] have been published showing the neuropsychological outcome of patients treated with the St Jude protocol Total Therapy XV.

The study reported by Krull reported 218 children treated with low-risk and standard/high risk arms. Executive functions were assessed, among them cognitive flexibility, verbal fluency, WM, organization, and problem-solving abilities. Additional cognitive abilities included IQ, processing speed, attention, memory, and fine motor dexterity. These children who were at least 5-years post diagnosis and older than 8 years of age, showed their full-scale intelligence was within normal limits, but scored significantly lower than the population mean on six measures of executive function (flexibility, fluency, WM, organization/planning, and

abstract reasoning), as well on measures of perceptual reasoning, attention, memory, and processing speed. Younger age at diagnosis and higher MTX dose were associated with lower activation in areas related to response inhibition, shifting of attention, language, executive functions and decision-related processes. The study concluded that "this study demonstrates that, at more than 5-years post diagnosis, a substantial proportion of survivors experience neurocognitive dysfunction" [29].

The 2012 study by Conklin tested 243 children with ALL, between the ages of 1-18 years old, who underwent a very extensive neuropsychological evaluation 120 weeks after the end of therapy, stratified by age, which also included academic achievement tests. Their conclusion was that "treatment with chemotherapy alone is not without risks". The negative cognitive effects seen in these children were: (1) when given more chemotherapy, there were more detrimental cognitive outcomes in processing speed and academic achievement, with parents reporting more learning problems; and (2) younger age at the beginning of treatment caused worse results in sustained attention and memory. The fact that these children were assessed around 2 years old, after cessation of treatment, in a group of children aged 1.02-18.73 years, raises significant concerns. An important subgroup of children who, when assessed, had not yet entered school, could potentially conceal cognitive, learning, and real-life future problems [30].

Sherief et al. in their study compared 50 patients treated with this St. Jude protocol to 50 who received a low-dose intrathecal methotrexate protocol (LD-IT-MTX ONLY CCG1991 PROTOCOL). The children were cognitively assessed using the Arabic version of Wechsler Intelligence Scale test (WISC-III), looking at the full-scale IQ as well as all subtests within the verbal and performance subtests. In the study each subject was tested once in a cross sectional design at one to seven years after completing therapy and the statistical design does not allow any attempt to control for age at diagnosis or any other known risk factors. Although both groups were negatively affected compared to controls, the total XV protocol treated children were negatively affected more than those receiving the LD-IT-MTX ONLY CCG1991 PROTOCOL. Furthermore, there was a negative correlation between duration from end of therapy to the verbal and performance abilities of these children. Thus, the more time that passed from end of therapy to the assessment, the worse the VIQ and PIQ became. In the first year after cessation of treatment the average VIQ was 90, while seven years later it decreased to 70 (the same trend was seen in PIQ, from 100 to 90). In terms of age and gender, the younger the patients were at diagnosis and treatment, the worse they performed specifically on the full scale IQ and PIQ. Female patients seemed to be more effected than males with regard to full-scale IQ, PIQ, and VIQ. It should be noted that this type of study cannot demonstrate a deterioration in neurocognitive function over time [31]

Not only was the FA rescue clearly inadequate, but it is relevant to mention the study by Fellah et al. [32] who tested 165 of the children reported by Krull [29] 6.7 years after diagnosis, and reported high rate of leukoencephalopathy with both the low-risk and high-risk protocols (67% and 86%, respectively).

THE UKALL X1 protocol [31]

This protocol compared different CNS prophylaxis protocols. Patients received HDMTX and ITMTX (6 gm/m2 for children at least 4 years of age, and 8 gm/m2 for infants) followed by FA rescue at hour 36; 15 mg/m2 FA was given every 3 hours until 48 hours, then every 6 hours until the MTX level was $<$ 10-7 micromol/L (since we would expect the MTX level to reach 10-7 micromol/L by 72 hours, the total FA dose would be expected to be 120-135 mg/m2) [33].

Halsey [34] reported the full-scale VIQ and PIQ scores in 555 children on this protocol compared with 311 controls; 202 low-risk children who received HDMTX and ITMTX were compared with 197 similar patients treated with ITMTX; 79 high-risk patients who received HDMTX and ITMTX were compared with 77 who received cranial irradiation. They were tested at 5 months, 3 years, and 5 years from start of therapy. At 5 months, no significant difference was seen in IQ scores between the randomised groups, but at 3 and 5 years all treatment groups showed a significant reduction ($p<0.002$) in all three IQ scores of 3.6 and 7.3 points, respectively, compared with controls [34].

This contrasts with the report by Rodgers on a small number of patients on this protocol tested $>$2 years after

completing therapy. They reported that patients did not exhibit the deficits witnessed in previous cohorts and were performing at comparable levels on all measures of attention [35]. However, when this study was included in the mega-analysis by Iyer, who found diminished function in working memory, attention /concentration, and information processing speed [36].

The rescue dose we calculated to be between 120-135mg/m2 were much less than the 225mg/m2 dose used after similar doses of MTX in a study with no reported major toxicity [37].

Treatment protocol Israel Osteosarcoma Group – also see above

Bonda-Shkedi found that 11 osteosarcoma patients, aged 17-31 years, who were examined at least 6 months after completing therapy (average time from completion of therapy to examination was 6.18+ 4.07 years, personal communication Bonda-Skedi) and had received 120-250 mg/m2 FA after each treatment with 12-20gm/m2 MTX, had statistically significant (p=0.025) worse results on 11 of 18 subtests of attention, visual perception, verbal memory, VM, executive functioning, and comprehension compared with the 12 who received adequate FA rescue (300-600mg/m2 FA).[21]

3. Studies of more than one protocol grouped together preventing analysis of each protocols (Table 3)

In the NOPHO-ALL- 86 Protocol, standard-risk patients received 1gm/m2 MTX rescued at 24 and 36 hours by 30mg/m2 FA (total 60 mg/m2) as given in the BFM-86 protocol. The high-risk NORPHO 1990 protocol patients received 8 gm/m2 MTX rescued at 36 hours by 50mg/m2, followed by 6 doses of 12mg/m2FA every 3 hours (total 112mg/m2) [39-41].

The NOPHO-ALL-1992 SR and intermediate-risk (IR) patients received 5gm/m2 MTX followed by 15mg/m2 FA at 42,48, and 52 hours, than every 6 hours until plasma MTX level was < 0.08 micromole/L (the total dose was at least 45mg/m2) (personal communication Dr Arja Harila –Saari). High-risk patients were given 8gm/m2 MTX rescued with 140mg/m2 FA, started at 36 hours.

Harila et al. [38] reported on neuropsychological outcomes seen in 64 survivors treated on several of these different protocols during 1972-1992, 20 of them without radiotherapy.

Nofstad et al. [39,40] and Reinfjell [41] looked at the NOPHO-ALL-1992 protocol survivors. Some received 5gm/m2 and others 8 gm/m2 MTX, nevertheless, they were analysed together. The FA rescue protocol was also different, preventing analysis of the significance of the FA protocols.

The dose of FA rescue 45mg/m 2 given 42 hours after the start of MTX was clearly inadequate, while 140 mg/m2 FA given after 8 gm/m2 HDMTX seems to be a more realistic dose. Analysis of the cognitive damage with such a mix of FA rescue doses was not possible.

4. Studies with significant serious methodical problems (Table 4)

ALL BFM 95 protocol [40-42]

Children $<$ 18years received 4 courses of HD-MTX 5gm/m2 over 24 hours followed by 15mg/m2 FA at 42,48, and 54 hours (total 45mg/m2) [42-44].

COALL 06-09 protocol

Children received eight courses of 1gm/m2 MTX followed by two doses of 15mg/m2 FA rescue starting at 48 hours (total dose 30mg/m2) (personal communication Dr Gritta Janke).

Krapmann et al. [45] examined 57 children on protocol ALL BFM 95 and nine children on protocol COALL-0609. The study did not differentiate between patients treated with two very different protocols.

The dose of MTX given in the COALL 06-09 protocol has not been reported to cause neurotoxicity, even without any FA rescue, and the rescue regime would not be expected to have any effect when started after 48 hours [16], while the 57 children on protocol ALL BFM 95 received inadequate FA rescue [16].

The findings as reported in the study by Krappmann et al. [45] of "almost normal cognitive function during therapy" have several serious methodical problems. The title of the article suggests that the authors presented what they intended to show and not what they found. The report does not differentiate between the 57 children who received protocol ALL BFM 95 and the nine children on protocol COALL 06-09. Although "Implications for follow up" appears in the title, the study examined performance at diagnosis and after 21-31 weeks of chemotherapy. Furthermore, the IQ measured at this early stage of follow up used the German version of the K-ABC for kids (2.5 – 12 years) and adolescent and adult version (the K-Tim in German), which are not standard tests used to measure intelligence. Nonetheless, they showed a significant reduction (p=0.035) especially in girls (p=0.004) and younger children (p=0.001). Other neuropsychological parameters were not addressed except for visual–motor function, known not to be affected in ALL patients, and concentration [14], which was not affected. An examination utilizing standard IQ tests with a verbal and nonverbal component was not performed. The analysis does not report the cognitive damage by comparison with a control group, but only compares the results at diagnosis (T1) with end of reinduction therapy (T2), 21-31 weeks after diagnosis when the children were 10 months older, which does not entitle it to be called a longitudinal study.

While 2- and 5-years post treatment would have been an acceptable time frame to look for cognitive difficulties, no report or publication of the promised results of re-examination after maintenance therapy were found. It would seem that following the publication of this article the BFM group accepted the premise that since this protocol resulted in "almost normal cognitive function during therapy", and thus they stopped doing any evaluations of neurotoxicity in subsequent studies. Other groups such as the Italian AIEO group with a similar protocol noted 5.8% acute neurotoxicity when 5gm/m2 MTX and intrathecal MTX was rescued by a FA dose higher than the 45mg/m2 used by the BFM group. They used the equivalent of 75mg/m2 FA (37.5mg/m2 l-folinic acid) [20]. Unfortunately, since this group combined with the BFM group study in the AIEOP-BFM ALL 2000 study, their neurotoxicity data is no longer reported. Not only has the BFM group decided that cognitive damage does not exist after treatment with high dose MTX whatever the FA rescue dose used, but they also convinced others to accept this fallacy. Some have used "the same dosing and leucovorin rescue as reported by the BFM for thousands of patients, without an increase in neurotoxicity or neurocognitive dysfunction" [46]. Even more worrying is the fact that the NOPHO 2008 protocol for all risk groups are "rescued" with a 15 mg/m2 FA dose started 42 hours after 5gm/m2 MTX and repeated every 6 hours until MTX is less than 0.2 micromoles/l (personal communication Dr. Arja Harila Saari). Thus, some may only receive a rescue dose of 30mg/m2 FA [9].

The Berlin-Frankfurt-Munster (BFM) Group has treated 6609 children $<$ 18 years of age in 82 centers in Germany, Austria, and Switzerland in five consecutive trials during 1981-2000. Since the ALL-BFM 86 study, all patients have received 4 courses of HD-MTX 5gm/m2 over24 hours. In the ALL-BFM 86 study, the FA rescue dose was originally 75mg/m2 at 36 hours and 15mg/m2 5 times every 3 hours then 4 doses every 6 hours (total 210mg/m2). This was reduced in 1988 to six 15mg/m2 doses of FA from 36 hours every 6 hours (total 90mg/m2). Later the ALL-BFM- 90 study started FA 30mg/m2 at 42 hours, and 15mg at 48 and 54 hours (total 75mg/m2). The ALL-BFM 95 subsequently have used 15mg/m2 at 42, 48, and 54 hours (total 45mg/M2) [42-44].

Studies comparing different FA rescue protocols

The above study by Bonda-Shkedi et al. [21] seems to be the only report, to date, to examine the cognitive function according to the adequacy of the FA rescue dose.

Eleven of 18 subtests of full neuropsychological assessment showed significantly favourable results (p=0.025) in the group who received the higher dose of FA, compared to the low FA group in attention, WM, verbal and visual memory, and in executive functions. It was however a retrospective report of osteogenic survivors who were treated in three centres who used different treatment protocols.

DISCUSSION

Different diseases will require different dose regimes of MTX. There is clear evidence to support the use of

a 24 hour infusion of least 6 g/m2 IV MTX [47] or 5g/ m2 with 12mg/m IT MTX [48] in the treatment of acute lymphoblastic leukemia to achieve a 24 hour serum MTX level of 1 molar. This level was achieved in only some of the children when 5g/m2 IV MTX was given alone [48,49]. A study that attempted CNS intensification /consolidation of four weekly courses of MIX(25mg/m q6hrs x4 and triple intrathecal therapy weekly x6 and oral folinic acid rescue) resulted in 21/239 patients developing a CNS relapse. In Pediatric Brain Tumors patients a 24 hour CSF MTX of 1 molar was achieved with 10g/m2 IV MTX or 5gm/m2 MTX following radiotherapy and in Osteogenic Sarcoma patients adjusting MTX levels to 2x10-5 M at 24 hours and 1x10-6 M at 48 hours followed by high dose FA rescue led to some of the best results reported to date (high cure rate and absence of neurotoxicity on neuropsychological testing) [50].

The appropriate dose of folinic acid for rescue will also depend on the time interval from the start of the MTX to the start of the FA as well as the dose of MTX being used. This can be critical. One study in which 2.5g/m2 MTX given over 24 hours had 16 cases of severe toxicity and 5 deaths when FA rescue was started after 48 hours reported no toxicity at all, when the rescue was started after 36 hours [51]. We have shown that it is safe to postpone the start of FA rescue until 36 hours from the start of the MTX [16].

Based on the data presented in this review we can support the use of FA rescue started 36 hours after the start of MTX

5g/m2 MTX FA 75mg/m2 +12mg/m2 q3hrs x12 (total 255mg/M2) [18,19]

8 g/m2 MTX FA 100 mg +12mg/m2 q3hs x6, then q6hr to 0.8x10-8 micromol /L [17]

12-20g/m2 MTX FA 50mg/m2 x8 then 45mg/m2 x8q6hrs (total 760mg/m2) [21,50]

33.3 g/m2 MTX FA 200 mg/2 +12mg/m2 q3hrs x6, then q6hrs to 0.8 x10-8 micromol/L[17] 33.6 g/m2 MTX FA 200 mg/2 +12mg/m2 q3hrs x6, then q6hrs to 0.8 x10-8 micromol/L[23]

The neuropsychological studies reviewed here show a clear correlation between inadequate FA rescue and cognitive damage. This analysis and the results being generated by ongoing studies such as the Ponte di Legno toxicity working group neurotoxicity analysis of the NOPHO-ALL- 2008 protocol and the neurotoxicity sub-study of the UKALL2011 protocol will, we hope, result in an improvement in the way FA rescue is used after high dose MTX.

DECLARATIONS

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest/Competing interests: The authors have no conflicts of interest relevant to this article to disclose.

Acknowledgements: We would like to thank Ms. Debby Mir for expert editorial help.

References

1. Bernard S, Etienne MC, Fischel JL, Formento P, Milano G (1991) Critical factors for the reversal of methotrexate cytotoxicity by folinic acid. Brit J Cancer 63;303-307. doi: 10.1038/bjc.1991.70

2. Sirotnak FM, Moccio DM, Dorick DM (1978) Optimization of high-dose methotrexate with leucovorin rescue therapy in the L1210 leukemia and sarcoma 180 murine tumor models. Cancer Res. 38:345-353.

3. Ochs J, Mulhern R, Fairclogh D, et al. (1991) Comparison of neuropsychologic functioning and clinical indicators of neurotoxicity in long-term survivors of childhood leukemia given cranial radiation or parenteral methotrexate: a prospective study. J Clin Oncol. 9:145-151. doi: 10.1200/JCO.1991.9.1.145

4. McAllister LD, Doolittle ND, Guastandisegni PE, et al. (2000) Cognitive outcome and long term follow - up after enhanced chemotherapy delivery for primary central nervous system lymphoma. Neurosurgery 46;51-60.

5. Cohen IJ (2007) Prevention of high-dose-methotrexate neurotoxicity by adequate folinic acid rescue is possible even after central nervous system irradiation. Med Hypotheses. 68:1147-1153. doi: 10.1016/j.mehy.2006.04.074

6. Bowman GP, Goodyear MDE, Levine MN, Russell R, Archibald SD, Young JEM.(1990) Modulation of the antitumor effect of methotrexate by low doseleucovorin in squamous cell head and neck cancer:a randomized placebo controlled clinical trial J Clin Oncol 8:203-8.

7. Cohen IJ (2003) Progression of osteosarcoma after high dose methotrexate:Over rescue by folinic acid Ped Hematol and Oncol 20:579-581.

8. Cohen IJ (2008) The importance of adequate methotrexate and adequate folinic acid rescue in the treatment of primary brain lymphoma. J Neurooncol. 140: 757. doi: 10.1007/s11060-018-2999-x

9. Cohen IJ (2013) Challenging the relevance of folinic acid over rescue after high dose methotrexate (HDMTX). Medical Hypothesis. 81;942-947.

10. Cohen IJ (2021) Folinic acid over rescue of high dose Methotrexate: how problematic citations conserve discredited concepts. Med Hypotheses. 146;110467.

11. Cohen IJ (2017) Neurotoxicity after high -dose methotrexate (MTX) is adequately explained by insufficient folinic acid rescue. Cancer Chemother Pharmacol. 79:1057-1065. doi: 10.1007/s00280-017-3304-y

12. Larsen EC, Devidas M, Chen S, et al. (2016) Dexamethasone and High-Dose Methotrexate Improve Outcome for Children and Young Adults With High-Risk B-Acute Lymphoblastic Leukemia: A Report From Children's Oncology Group Study AALL0232. J Clin Oncol. 34:3280-2388. doi: 10.1200/JCO.2015.62.4544

13. Hardy KA, Embry L Kairalla JA, et al. (2017) Neurocognitive Functioning of Children Treated for High-Risk B-Acute Lymphoblastic Leukemia Randomly Assigned to Different Methotrexate and Corticosteroid Treatment Strategies: A Report From the Children's Oncology Group. J Clin Oncol. 35:2700-2707. doi: 10.1200/JCO.2016.71.7587

14. Peterson CC, Johnson CE, Ramirez LY, et al. (2008) A meta-analysis of the neuropsychological sequelae of chemotherapy-only treatment for pediatric acute lymphoblastic leukemia. Pediatr Blood Cancer. 51:99-104. doi: 10.1002/pbc.21544

15. Ackland SP, Schilky RL (1987) High-dose methotrexate: a critical reappraisal. J Clin Oncol. 5:2017-2031. doi: 10.1200/JCO.1987.5.12.2017

16. Cohen IJ, Wolff JE (2014) How long can folinic acid rescue be delayed after high-dose methotrexate without toxicity? Pediatr Blood Cancer. 61:7-10. doi: 10.1002/pbc.24770.

17. Spiegler BJ, Kennedy K, Maze R, et al. (2006) Comparison of long-term neurocognitive outcomes in young children with acute lymphoblastic leukemia treated with cranial radiation or high-dose or very high-dose intravenous methotrexate. J Clin Oncol. 24;3858-3864. doi: 10.1200/JCO.2006.05.9055. Erratum in: J Clin Oncol. 2006 Nov 10;24(32):5181.

18. Kingma A, Van Dommelen RI, Mooyart EL, et al. (2002) No major cognitive impairment in young children with acute lymphoblastic leukemia using chemotherapy only: a prospective longitudinal study. J Pediatr Hematol Oncol. 24;106-114. doi: 10.1097/00043426-200202000-00010

19. Kingma A,Van Dommelen RI,Mooyart EL, et al. (2001) Slight cognitive impairment and magnetic resonance imaging abnormalities but normal school levels in children treated with acute lymphoblastic leukemia with chemotherapy only. J Paediatr. 139:413-420. doi: 10.1067/mpd.2001.117066

20. LoNigro L, DiCatalgo A, Schiliro G (2000) Acute neurotoxicity in children with B-lineage acute lymphoblastic leukemia (B-ALL) treated with intermediate risk protocols. Med Pediatr Oncol. 35;449-455. doi: 10.1002/1096-911x(20001101)35:5<449::aid-mpo2>3.0.co;2-x

21. Bonda-Skedi E, Weyl-Ben Harush M, Kaplinsky C, et al (2013) The correlation between dose of folinic acid neurotoxicity in children and adolescents treated for osteosarcoma with high dose methotrexate (HDMTX):a neuropsychological and psychosocial study. J Pediatr Hematol Oncol. 35;271-275. doi: 10.1097/MPH.0b013e31828c2da1

22. Reaman GH, Sposto R,Sensel MG, Lange B et.al. (1999) Treatment outcome and prognostic factors for infants with acute lymphoblastic leukemia treated on two consecutive trials of the Children's Cancer Group. J Clin Oncol. 17:445-455. doi: 10.1200/JCO.1999.17.2.445

23. Kaleita T A, Reaman GH, MacLean WE, Sather HN, Whitt JK (1999) Neurodevelopmental outcome of infants with acute lymphoblastic leukemia, A Children's Cancer Group Report. Cancer. 85:1859-6521. doi: 10.1002/(sici)1097-0142(19990415)85:8<1859::aid-cncr28>3.0.co;2-2

24. Duffner PK, Armstrong FD, Chen L, et al. (2014) Neurocognitive and neuroradiologic central nervous system late effects in children treated on Pediatric Oncology Group (POG) P9605 (standard risk) and P9201 (lesser risk) acute lymphoblastic leukemia protocols (ACCL0131): a methotrexate consequence? A report from the Children's Oncology Group. J Pediatr Hematol Oncol. 36;8-15. doi: 10.1097/MPH.0000000000000000. PMID: 24345882

25. Cohen I J (2014) Inadequate folinic acid rescue after methotrexate causing neurocognitive and neuroradiological central nervous system late effects in children with acute lymphatic leukemia. J Pediatr Hematol Oncol. 36;501. doi: 10.1097/MPH.0000000000000139

26. Cohen IJ (2017) Neurotoxicity after high-dose methotrexate (MTX) is adequately explained by insufficient folinic acid rescue. Cancer Chemother Pharmacol. 79:1057-1065. doi: 10.1007/s00280-017-3304-y

27. Abramowitch M, Ochs J, Pui C-H, et al. (1988) High-dose methotrexate improves clinical outcome in children with acute lymphoblastic leukemia: St. Jude Total Therapy Study X. Med Pediatr Oncol.16:297-303. doi: 10.1002/mpo.2950160502

28. Pui C-H, Campanad, Pei D et.al. (2009) Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Eng J Med 360:2370-2741

29. Krull KR, Cheung YT, Liu W, et al. (2016) Chemotherapy Pharmacodynamics and Neuroimaging and Neurocognitive Outcomes in Long-Term Survivors of Childhood Acute Lymphoblastic Leukemia. J Clin Oncol. 34: 2644-2653. doi: 10.1200/JCO.2015.65.4574

30. Conklin HM, Krull KR, Reddick WE, Pei D, Cheng C, Pui CH (2012) Cognitive outcomes following contemporary treatment without cranial irradiation for childhood acute lymphoblastic leukemia. J Natl Cancer Inst. 104:1386-1395. doi: 10.1093/jnci/djs344

31. Sherief LM, Sanad R, El Hadad A, et al. (2018) A Cross-sectional Study of Two Chemotherapy Protocols on Long Term Neurocognitive Functions in Egyptian Children Surviving Acute Lymphoblastic Leukemia. Curr Pediatr Rev. 14:253-260. doi: 10.2174/1573396314666181031134919

32. Fellah S, Cheug YT, Scoggins MA, et al. (2019) Brain Activity Associated With Attention Deficits Following Chemotherapy for Childhood Acute Lymphoblastic Leukemia. J Natl Cancer Inst. 111:201-209. doi: 10.1093/jnci/djy089

33. Hann I, Vora A, Harrison G, Harrison C, Eden O, Hill F, Gibson B, Richards S; UK Medical Research Council's Working Party on Childhood Leukaemia (2001) Determinants of outcome after intensified therapy of childhood lymphoblastic leukaemia: results from Medical Research Council United Kingdom acute lymphoblastic leukaemia XI protocol. Br J Haematol. 113:103-114. doi: 10.1046/j.1365-2141.2001.02668.x. Erratum in: Br J Haematol 2001 Jun;113(3):844. Erratum in: Br J Haematol 2001 Sep;114(3):738.

34. Halsey C, Buck G, Richards S, Vargha-Khadem F, Hill F, Gibson B (2011 The impact of therapy for childhood acute lymphoblastic leukaemia on intelligence quotients; results of the risk-stratified randomized

central nervous system treatment trial MRC UKALL XI. J Hematol Oncol. 4:42. doi: 10.1186/1756-8722-4-42

35. Rodgers J, Marckus R, Kearns P, Windebank K (2003) Attentional ability among survivors of leukaemia treated without cranial irradiation. Arch Dis Child. 88:147-150. doi: 10.1136/adc.88.2.147

36. Iyer NS, Balsamo LM, Bracken MB, Kadan-Lottick NS (2015) Chemotherapy-only treatment effects on long-term neurocognitive functioning in childhood ALL survivors: a review and meta-analysis. Blood. 126:346-353. doi: 10.1182/blood-2015-02-627414

37. Borsi JD, Wesenberg F, Stockland T, Moe PJ (1991) How much is too much? Folinic acid rescue dose in children with acute lymphoblastic leukaemia. Eur J Cancer. 27:1006-9. doi: 10.1016/0277-5379(91)90269-j

38. Harila MJ, Winqvist S, Lanning M, Bloigu R, Harila-Saari AH (2009) Progressive neurocognitive impairment in young adult survivors of childhood acute lymphoblastic leukemia. Pediatr Blood Cancer. 53;156-161. doi: 10.1002/pbc.21992

39. Lofstad GE, Reinfjell T, Hestad K, Desth TH (2009) Cognitive outcome in children and adolescents treated for acute lymphoblastic leukaemia with chemotherapy only. Acta Paediatr. 98:180-186. doi: 10.1111/j.1651-2227.2008.01055.x

40. Lofstad GE, Reinfjell T, Weider S, Diseth TH, Hestad K (2019) Neurocognitive Outcome and Compensating Possibilities in Children and Adolescents Treated for Acute Lymphoblastic Leukemia With Chemotherapy Only. Front Psychol. 10:1027. doi:10.3389/fpsyg 2019.01027

41. Reinfjell T, Lofstad GE, Veenstra M, Vikan A, Diseth TH (2007) Health-related quality of life and intellectual functioning in children in remission from acute lymphoblastic leukaemia. Acta Paediatr. 96:1280-5. doi: 10.1111/j.1651-2227.2007.00383.x

42. Moricke A, Zimmermann M, Reiter A, et al. (2010) Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia. 24:265-284. doi: 10.1038/leu.2009.257

43. Reiter A,Schrae S, Ludwig W-D, et al. (1994) Chemotherapy in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of the multicenter trial ALL-BFM 86. Blood. 84:3122-33

44. Reiter A, Schrappe M, Tiemann M, et al. (1999) Improved treatment results in childhood B-cell neoplasms with tailored intensification of therapy: A report of the Berlin-Frankfurt-Münster Group Trial NHL-BFM 90. Blood. 94:3294-306.

45. Krappmann P, Paulides M, Stöhr W, et al. (2007) Almost normal cognitive function in patients during therapy for childhood acute lymphoblastic leukemia without cranial irradiation according to ALL-BFM 95 and COALL 06-97 protocols: results of an Austrian-German multicenter longitudinal study and implications for follow-up. Pediatr Hematol Oncol. 24:101-109. doi: 10.1080/08880010601123281

46. Hardy KK, Embry LM, Kairalla JA, et.al. (2017) Reply to I.J. Cohen. J Clin Oncol. 35:3989-3990. doi: 10.1200/JCO.2017.75.7252

47. Borsi JD, Moe PJ. (1987) A comparative study of the pharmacokinetics of methotrexate in the dose range of 0-5g/m2 to 33.6g/m2 in children with acute lymphoblastic leukemia. Cancer 6 0:5-13.

48. Milano G, Thyss A, Serre Debeauvais F,et.al.(1990) CSF levels for children with acute lymphoblastic leukemia treated with 5g/m2 methotrexate .A study from the EORTC Childrens Leukemia Cooperative group Eur J Cancer 4:492-495.

49.Millot F, Rubie H, Mazingue F, Mechinaud F, and Thyss A.(1994)Cerebrospinal fluid drug levels of leukemic children receiving intravenous 5g/m2 methotrexate. Leukemia and Lymphoma 14:141-144.

50 Shkalim-Zemer V,Toladano H,Ash S et al.(2005) Highly effective reduced toxicity dose-intensive pilot protocol for non- metastatic limb osteosarcoma (SCOS 89)Cancer Chemotherapy Pharmocology 76:909-916.

51. Buendia MTA, Lozano JM, Saurez VGE , Saavedra G,Guevera G.(2008) The impact of acute lymphatic leuhkemia treatment on the central nervous system results in Bogata Colombia. J Peadiatr Hematol Oncol 30:643-50.

Table headings

Table 1 Studies with adequate folinic acid rescue without cognitive deterioration.

Table 2. Studies with inadequate folinic acid rescue and cognitive deterioration.

Table 3 Studies with several protocols with differences in folinic acid adequacy analyzed

together preventing analysis of results.

Table 4 Studies with significant serious methodical problems.

Hosted file

`table 1 pbc.docx` available at https://authorea.com/users/453314/articles/551194-a-systematic-review-of-neuropsychological-studies-confirms-that-adequate-folinic-acid-rescue-prevents-post-methotrexate-neurotoxicity

Hosted file

`table2 pbc.docx` available at https://authorea.com/users/453314/articles/551194-a-systematic-review-of-neuropsychological-studies-confirms-that-adequate-folinic-acid-rescue-prevents-post-methotrexate-neurotoxicity

Hosted file

`table 3 pbc.docx` available at https://authorea.com/users/453314/articles/551194-a-systematic-review-of-neuropsychological-studies-confirms-that-adequate-folinic-acid-rescue-prevents-post-methotrexate-neurotoxicity

Hosted file

`table 4pbc.docx` available at https://authorea.com/users/453314/articles/551194-a-systematic-review-of-neuropsychological-studies-confirms-that-adequate-folinic-acid-rescue-prevents-post-methotrexate-neurotoxicity