

Toxicity of High-Dose Methotrexate and Its Correlation with Serum Levels in Adult Acute Lymphoblastic Leukaemia

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Abstract: ***Background:** High-dose methotrexate (HDMTX) is central to consolidation in acute lymphoblastic leukaemia (ALL) but causes substantial toxicity, and data on adults are limited. **Methods:** We retrospectively analysed 400 HDMTX cycles (5 g/m²) given to 106 adults with ALL on the BFM-95 protocol at Kidwai Memorial Institute of Oncology, Bengaluru (June 2023–November 2024). Toxicity was graded by CTCAE v5.0 and related to serum methotrexate levels at 48 and 72 hours (toxic: >1 and >0.1 µmol/L). **Results:** Toxicity occurred in 154 cycles (38.5%); the next dose was reduced by 15% in 94 (23.5%). Levels were toxic at 48 hours in 34 cycles and at 72 hours in 24. Toxic levels were associated with leucopenia, neutropenia, thrombocytopenia, raised SGOT and raised creatinine at both times, and with raised SGPT and bilirubin at 48 hours (p<0.05). Vomiting (18%), febrile neutropenia (5.5%) and mucositis (5.5%) were the commonest non-haematological events. No treatment-related deaths occurred. Toxicity prolonged admission (mean 11.48 days against about 5 days usually) and delayed the next cycle by 15–17 days on average. **Conclusion:** Serum methotrexate monitoring at 48 and 72 hours identifies cycles at risk of toxicity and supports timely leucovorin escalation.*

Keywords: High-dose methotrexate; Acute lymphoblastic leukaemia; Adults; Toxicity; Serum methotrexate level

1. Introduction

Acute lymphoblastic leukaemia (ALL) occurs mainly in childhood, with more than two-thirds of cases diagnosed before 20 years of age, and a second incidence peak appears in adult life [1]. Cure rates approach 80% in children, whereas adults fare considerably worse [2,3]. Treatment is multi-agent chemotherapy, with drugs of different mechanisms given across successive phases.

Methotrexate (MTX), a folate analogue, is active in several cancers. Doses above 500 mg/m² are classed as high-dose; they penetrate the central nervous system (CNS) and are used for CNS prophylaxis [4,5]. HDMTX is tolerated well by most patients, yet it can cause life-threatening toxicity [6,7]. In ALL it forms the consolidation phase of protocols such as BFM-95. Its main toxicities are myelosuppression, mucositis, and hepatic and renal dysfunction, which hydration, urinary alkalinisation and leucovorin rescue aim to prevent.

Serum MTX concentrations guide rescue therapy: levels above 1 µmol/L at 48 hours and above 0.1 µmol/L at 72 hours after the start of the infusion predict more toxicity [4]. This study describes the toxicity of HDMTX in adults with ALL treated at a tertiary cancer centre and examines how serum MTX levels relate to it.

2. Literature Survey

MTX is the one cytotoxic drug for which serum level monitoring is routine; the level is an important determinant of toxicity and allows early intervention when it is high [8]. Toxicity can be unpredictable because MTX inhibits normal proliferating tissue as well as tumour cells [8]. Most published HDMTX experience comes from paediatric cohorts, including reports from resource-limited settings [9–12], which have linked plasma MTX with neutropenia, hepatic dysfunction, vomiting and mucositis [11], although the link with mucositis is disputed [13]. Pharmacogenetic factors such as the MTHFR C677T polymorphism have also been studied as predictors [14–16]. Where in-house assays are unavailable, a single level at 36 hours has been proposed as a low-cost monitoring strategy [17]. Reports on adults with ALL receiving HDMTX are comparatively few.

3. Problem Definition and Objectives

It is unclear how well serum MTX levels at 48 and 72 hours track clinical and laboratory toxicity in adults, and what that toxicity costs in dose reductions, hospital stay and treatment delay. The objectives were (i) to describe the toxicities of HDMTX in adult ALL; (ii) to correlate them with serum MTX levels at 48 and 72 hours; (iii) to assess whether serum level estimation is needed at these time points; and (iv) to

measure inpatient burden and delay in subsequent treatment attributable to toxicity.

4. Methodology

4.1 Study design and setting

This retrospective observational study was conducted at Kidwai Memorial Institute of Oncology, Bengaluru, Karnataka, from June 2023 to November 2024. Demographic data, treatment details, toxicity profile, serum MTX values and subsequent management were extracted from treatment records. The unit of analysis was the HDMTX cycle.

4.2 Participants

Adults (>18 years) with ALL who had completed Induction phases A and B of the BFM-95 protocol, were in remission after Induction phase B, and received HDMTX at 5 g/m² were included. Patients were excluded if they had deranged liver function tests (direct bilirubin, SGOT or SGPT) or deranged renal function tests (creatinine), defined as CTCAE Grade 1 or worse, or any pleural or pericardial effusion, or if they were not in remission after Induction phase B.

4.3 Treatment protocol and supportive care

Patients were treated per the BFM-95 protocol, whose consolidation includes four HDMTX courses (Table 1). In each course 10% of the dose was given intravenously over 30 minutes and the remainder as an infusion over 23.5 hours. Drugs known to interact with MTX (proton pump inhibitors, aminoglycosides, NSAIDs, cephalosporins, fluoroquinolones and antifungals) were withheld until the 72-hour level was in the non-toxic range. Tyrosine kinase inhibitors were withheld during HDMTX and restarted after each cycle in Ph-positive patients. Patients were hydrated for 24 hours before MTX with 0.9% saline and Ringer lactate; the infusion began after two consecutive urine pH readings of 7 or more, six hours apart. Potassium chloride and sodium bicarbonate were added to maintain urine pH and electrolyte balance, and urine pH was checked six-hourly. Hydration continued to 72 hours, or until MTX levels were non-toxic when toxicity occurred, together with urine alkalinisation.

Leucovorin rescue began 12 hours after the end of the infusion (36 hours from its start): a 50 mg bolus, then 15 mg/m² every 6 hours for 8 doses per institutional protocol. When levels were toxic, leucovorin was increased to 30 mg/m² every 6 hours, with continued hydration and alkalinisation and MTX levels every 12 hours until non-toxic. Concurrent oral 6-mercaptopurine (6-MP) was given in the consolidation phase and was withheld in patients with baseline Grade 2 toxicity.

Table 1: Consolidation phase of the BFM-95 protocol as used in this study

Drug	Dose	Days
6-Mercaptopurine (oral)	25 mg/m ²	1–56
High-dose methotrexate (IV)	5 g/m ²	8, 22, 36, 50
Methotrexate (intrathecal)	12 mg/dose	8, 22, 36, 50

4.4 Blood sampling and MTX assay

Blood was collected for complete blood count, biochemistry and serum MTX at 48 and 72 hours after the start of the infusion. Serum was assayed in-house by the ARCHITECT chemiluminescent microparticle immunoassay (CMIA). Levels above 1 µmol/L at 48 hours and above 0.1 µmol/L at 72 hours were taken as toxic. When levels were toxic, monitoring continued until they fell below 0.1 µmol/L.

4.5 Assessment of toxicity

Toxicity was defined and graded with CTCAE v5.0 [18] and recorded from the start of the MTX infusion. Haematological and biochemical toxicity (haemoglobin, total leucocyte count, absolute neutrophil count [ANC], platelets, direct bilirubin, SGOT, SGPT and creatinine) was graded from the 48-hour and 72-hour samples. Gastrointestinal, respiratory, dermatological and other events were recorded from the records. Cycles with toxicity below Grade 2 proceeded with subsequent MTX doses. When toxicity warranted dose reduction, the next dose was reduced by 15% under institutional policy, so that at least 85% of the planned dose was delivered, because dose intensity below this level has been linked to poorer outcomes [19].

4.6 Statistical analysis

Data were analysed in SPSS version 23. Categorical variables are given as numbers and percentages and age as median (range). Cycles were classed by serum MTX level (toxic vs non-toxic) at 48 and at 72 hours. For each laboratory parameter, cycles were classed as normal (CTCAE Grade 0) or abnormal (Grade ≥1), and the proportions were compared between the two groups with Fisher's exact test, as many cells were small. Odds ratios (OR) with 95% confidence intervals (Woelf method) are reported, and are not shown when a cell was zero. The association between concurrent 6-MP use and toxic MTX levels was tested the same way. All tests were two-sided with p<0.05 taken as significant; because 16 comparisons were made for MTX level, a Bonferroni-adjusted threshold of 0.003 is also noted. Analyses were done per cycle and were not adjusted for repeated cycles in the same patient.

4.7 Ethics

This was a retrospective review of existing hospital records. There was no patient contact or intervention, and no patient identifiers are reported. Formal Institutional Ethics Committee approval was not obtained for this record-based analysis. The study followed the principles of the Declaration of Helsinki.

5. Results and Discussion

5.1 Characteristics of patients and cycles

Four hundred HDMTX cycles were given to 106 adults with ALL (median age 24 years; range 18–56). Males received 330 cycles (82.5%). B-lineage ALL accounted for 296 cycles (74%) and T-lineage for 104 (26%); 278 cycles (69.5%) were given to medium-risk and 122 (30.5%) to

high-risk patients. Diabetes mellitus was the commonest comorbidity (28 cycles, 7%), and anaemia the commonest baseline abnormality (264 cycles, 66%), mostly Grade 1 (Table 2). The male predominance and the excess of B-lineage disease agree with known ALL epidemiology [20,21].

Table 2: Baseline characteristics (400 cycles in 106 patients)

Variable	Category	Cycles (n)	%
Age, years (median, range)		24 (18–56)	
Sex	Female	70	17.5
	Male	330	82.5
Comorbidity	Diabetes mellitus	28	7
	Hypertension	8	2
	Deep vein thrombosis	8	2
	Pulmonary thromboembolism	8	2
	None	364	91
	Any comorbidity (≥ 1)	36	9
Haemoglobin	Normal	136	34
	<LLN–10 g/dL	246	61.5
	<10–8 g/dL	18	4.5
Total leucocyte count	Normal	384	96
	<LLN–3000/ μ L	14	3.5
	<3000–2000/ μ L	2	0.5
ANC	Normal	372	93
	<LLN–1500/ μ L	28	7
Platelets	Normal	392	98
	<LLN–75,000/ μ L	4	1
	<75,000–50,000/ μ L	4	1
Risk group	Medium risk	278	69.5
	High risk	122	30.5
ALL lineage	B-cell	296	74
	T-cell	104	26

Percentages are of 400 cycles. Comorbidity categories overlap (some cycles had more than one), so they sum to more than the 36

cycles with any comorbidity. LLN, lower limit of normal; ANC, absolute neutrophil count. Patients with deranged liver or renal function were excluded (Section 4.2).

5.2 Overall toxicity, dose modification and outcome

Toxicity occurred in 154 of 400 cycles (38.5%). As per institutional policy (Section 4.5) the next MTX dose was reduced by 15% in 94 cycles (23.5%), and the consolidation regimen was changed in 12 cycles. Thirteen of the 106 patients who had Grade 3 or higher toxicity in an earlier cycle had similar toxicity in later cycles, and received 85% of the dose for the rest of consolidation. Three cycles needed intensive care and one patient required parenteral nutrition for two days because of severe mucositis. There were no treatment-related deaths and no CNS toxicity. Most toxicity was Grade 1 or 2 and self-limiting; Grade 3 or higher events were managed as inpatients as required.

5.3 Serum MTX level and toxicity at 48 hours

The 48-hour level was toxic in 34 cycles (8.5%) and non-toxic in 366 (Table 3). Any-grade abnormality (Grade ≥ 1) was more frequent when the level was toxic for leucopenia, neutropenia, thrombocytopenia, raised direct bilirubin, SGOT, SGPT and creatinine, but not for anaemia (Table 5). Examples: neutropenia in 14/34 (41.2%) vs 66/366 (18.0%); OR 3.2 (1.5–6.6), $p=0.003$; thrombocytopenia in 12/34 (35.3%) vs 22/366 (6.0%); OR 8.5 (3.7–19.5), $p<0.001$; raised SGOT in 22/34 (64.7%) vs 86/366 (23.5%); OR 6.0 (2.8–12.6), $p<0.001$. Raised creatinine occurred only in cycles with a toxic level (2/34 vs 0/366, $p=0.007$). After Bonferroni adjustment (threshold 0.003), thrombocytopenia, neutropenia, raised SGOT and raised SGPT remained significant.

Table 3: Toxicity grade by serum MTX level at 48 hours

Parameter	Category	CTCAE grade	Total	Non-toxic level (n=366) n (%)	Toxic level (n=34) n (%)
Haemoglobin	Normal	0	108	102 (27.9)	6 (17.6)
	<LLN–10 g/dL	1	258	234 (63.9)	24 (70.6)
	<10–8 g/dL	2	30	26 (7.1)	4 (11.8)
	<8 g/dL	3	4	4 (1.1)	–
Total leucocyte count	Normal	0	268	252 (68.9)	16 (47.1)
	<LLN–3000	1	96	84 (23.0)	12 (35.3)
	<3000–2000	2	32	28 (7.7)	4 (11.8)
	<2000–1000	3	2	–	2 (5.9)
	<1000	4	2	2 (0.5)	–
ANC	Normal	0	320	300 (82.0)	20 (58.8)
	<LLN–1500	1	58	50 (13.7)	8 (23.5)
	<1500–1000	2	20	14 (3.8)	6 (17.6)
	<500	4	2	2 (0.5)	–
Platelets	Normal	0	366	344 (94.0)	22 (64.7)
	<LLN–75,000	1	28	18 (4.9)	10 (29.4)
	<75,000–50,000	2	4	2 (0.5)	2 (5.9)
	<50,000–25,000	3	2	2 (0.5)	–
Direct bilirubin	Normal	0	312	292 (79.8)	20 (58.8)
	>ULN–1.5 $\times$	1	46	44 (12.0)	2 (5.9)
	1.5 $\times$ –3 $\times$	2	30	18 (4.9)	12 (35.3)
	3 $\times$ –10 $\times$	3	12	12 (3.3)	–
SGOT	Normal	0	292	280 (76.5)	12 (35.3)
	>ULN–3 $\times$	1	96	76 (20.8)	20 (58.8)
	3 $\times$ –5 $\times$	2	10	8 (2.2)	2 (5.9)
	>5 $\times$ –20 $\times$	3	2	2 (0.5)	–
SGPT	Normal	0	304	292 (80.0)	12 (35.3)
	>ULN–3 $\times$	1	84	64 (17.5)	20 (58.8)
	3 $\times$ –5 $\times$	2	10	8 (2.2)	2 (5.9)
	>5 $\times$ –20 $\times$	3	1	1 (0.3)	–

Parameter	Category	CTCAE grade	Total	Non-toxic level (n=366) n (%)	Toxic level (n=34) n (%)
Creatinine	Normal	0	398	366 (100)	32 (94.1)
	>ULN-1.5×	1	2	–	2 (5.9)

Toxic level, serum MTX >1 µmol/L at 48 hours. Units: haemoglobin g/dL; counts per µL. Tests of association are in Table 5. SGPT was available for 399 cycles (non-toxic n=365); its percentages use these denominators. No cycle had Grade 3 ANC (<1000–500) at 48 hours.

Myelosuppression was common: 80 cycles (20%) had neutropenia of any grade at 48 hours, most of it Grade 1, and Grade 2 or worse neutropenia and thrombocytopenia were more frequent when the level was toxic. This matches earlier reports linking MTX exposure with myelotoxicity [9–11]. Transaminase elevation was seen in 108 cycles for SGOT (27%) and 95 for SGPT (23.8%), mostly Grade 1 and reversible, as others have found [9]; values normally return to baseline before the next cycle, and dose reduction is advised if they do not [4]. Holmboe et al. implicated the metabolite 7-OHMTX in hepatotoxicity [22].

Direct bilirubin was raised more often when the level was toxic (14 of 34 cycles, 41.2%, against 74 of 366, 20.2%), but all 12 cycles with Grade 3 elevation occurred in the non-toxic-level group. Viral markers were negative where available. Cumulative hepatotoxicity of concurrent 6-MP and MTX is one possible explanation and needs further study. Acute kidney injury (creatinine above ULN) occurred in 2 cycles (0.5%) at 48 hours. Reported incidence with HDMTX is 2–12% despite supportive care [23]. The mechanism is crystal nephropathy from MTX and its metabolites precipitating in the tubules; urinary alkalinisation helps prevent it [4]. Renal injury delays MTX

clearance and so increases other toxicities, and it is managed with higher-dose leucovorin, hydration and alkalinisation [24,25]. The low incidence here probably reflects patient selection (patients with deranged renal or liver function or effusions, or without remission, were excluded), strict hydration and alkalinisation, and the leucovorin bolus.

5.4 Serum MTX level and toxicity at 72 hours

The 72-hour level was toxic in 24 cycles (6.0%) (Table 4). Any-grade abnormality was more frequent with a toxic level for leucopenia, neutropenia, thrombocytopenia, raised SGOT and raised creatinine, but not for anaemia, bilirubin or SGPT (Table 5). Neutropenia showed the strongest association (16/24 (66.7%) vs 110/376 (29.3%); OR 4.8 (2.0–11.6), $p < 0.001$). Creatinine exceeded the ULN in 2 of 24 cycles (8.3%) with a toxic level, both Grade 2, against 4 of 376 (1.1%) otherwise; acute kidney injury (AKI) was seen in 6 cycles (1.5%) at 72 hours. Only neutropenia remained significant after Bonferroni adjustment. The 48-hour level was associated with a wider range of toxicities, including hepatic toxicity, whereas the 72-hour level mainly reflected myelosuppression and renal dysfunction.

Table 4: Toxicity grade by serum MTX level at 72 hours

Parameter	Category	CTCAE grade	Total	Non-toxic level (n=376) n (%)	Toxic level (n=24) n (%)
Haemoglobin	Normal	0	86	82 (21.8)	4 (16.7)
	<LLN-10 g/dL	1	276	262 (69.7)	14 (58.3)
	<10–8 g/dL	2	36	30 (8.0)	6 (25.0)
	<8 g/dL	3	2	2 (0.5)	–
Total leucocyte count	Normal	0	246	238 (63.3)	8 (33.3)
	<LLN-3000	1	94	84 (22.3)	10 (41.7)
	<3000–2000	2	42	36 (9.6)	6 (25.0)
	<2000–1000	3	16	16 (4.3)	–
	<1000	4	2	2 (0.5)	–
ANC	Normal	0	274	266 (70.7)	8 (33.3)
	<LLN-1500	1	88	74 (19.7)	14 (58.3)
	<1500–1000	2	22	20 (5.3)	2 (8.3)
	<1000–500	3	12	12 (3.2)	–
	<500	4	4	4 (1.1)	–
Platelets	Normal	0	360	342 (91.0)	18 (75.0)
	<LLN-75,000	1	30	24 (6.4)	6 (25.0)
	<75,000–50,000	2	4	4 (1.1)	–
	<50,000–25,000	3	4	4 (1.1)	–
	<25,000	4	2	2 (0.5)	–
Direct bilirubin	Normal	0	300	282 (75.0)	18 (75.0)
	>ULN-1.5×	1	56	52 (13.8)	4 (16.7)
	1.5×–3×	2	32	30 (8.0)	2 (8.3)
	3×–10×	3	12	12 (3.2)	–
SGOT	Normal	0	306	292 (77.7)	14 (58.3)
	>ULN-3×	1	78	70 (18.6)	8 (33.3)
	3×–5×	2	10	8 (2.1)	2 (8.3)
	5×–20×	3	6	6 (1.6)	–
SGPT	Normal	0	318	300 (79.8)	18 (75.0)
	>ULN-3×	1	70	64 (17.0)	6 (25.0)
	3×–5×	2	8	8 (2.1)	–
	5×–20×	3	4	4 (1.1)	–
Creatinine	Normal	0	394	372 (98.9)	22 (91.7)
	>ULN-1.5×	1	4	4 (1.1)	–
	1.5×–3×	2	2	–	2 (8.3)

Toxic level, serum MTX >0.1 µmol/L at 72 hours. Units as in Table 3. Tests of association are in Table 5.

Table 5: Association of toxic MTX level with any-grade toxicity (Grade ≥ 1)

Parameter	48 h: toxic level n/N (%)	48 h: non-toxic level n/N (%)	48 h: OR (95% CI)	48 h: p	72 h: toxic level n/N (%)	72 h: non-toxic level n/N (%)	72 h: OR (95% CI)	72 h: p
Haemoglobin	28/34 (82.4)	264/366 (72.1)	1.8 (0.7–4.5)	0.231	20/24 (83.3)	294/376 (78.2)	1.4 (0.5–4.2)	0.798
Total leucocyte count	18/34 (52.9)	114/366 (31.1)	2.5 (1.2–5.1)	0.013	16/24 (66.7)	138/376 (36.7)	3.4 (1.4–8.3)	0.005
ANC	14/34 (41.2)	66/366 (18.0)	3.2 (1.5–6.6)	0.003	16/24 (66.7)	110/376 (29.3)	4.8 (2.0–11.6)	<0.001
Platelets	12/34 (35.3)	22/366 (6.0)	8.5 (3.7–19.5)	<0.001	6/24 (25.0)	34/376 (9.0)	3.4 (1.2–9.0)	0.024
Direct bilirubin	14/34 (41.2)	74/366 (20.2)	2.8 (1.3–5.7)	0.008	6/24 (25.0)	94/376 (25.0)	1.0 (0.4–2.6)	1.000
SGOT	22/34 (64.7)	86/366 (23.5)	6.0 (2.8–12.6)	<0.001	10/24 (41.7)	84/376 (22.3)	2.5 (1.1–5.8)	0.044
SGPT	22/34 (64.7)	73/366 (20.0)	7.3 (3.5–15.5)	<0.001	6/24 (25.0)	76/376 (20.2)	1.3 (0.5–3.4)	0.602
Creatinine	2/34 (5.9)	0/366 (0.0)	–	0.007	2/24 (8.3)	4/376 (1.1)	8.5 (1.5–48.7)	0.045

OR, odds ratio of any-grade abnormality with a toxic versus non-toxic level; p from Fisher's exact test (bold, $p < 0.05$). OR not shown where a cell was zero. Cycle-level analysis, not adjusted for repeated cycles per patient. Bonferroni threshold 0.003.

5.5 Non-haematological adverse events

Vomiting of Grade 1 or 2 was the commonest non-haematological event (72 cycles, 18%) (Table 6). Febrile neutropenia occurred in 22 cycles (5.5%); cultures were positive in 17, with MRSA the most frequent blood isolate. Mucositis of any grade occurred in 22 cycles (5.5%): Grade 1–2 in 18, Grade 3 in 3 and Grade 4 in 1. Abnormal liver function after discharge (Grade 1–2) occurred in 6 cycles (1.5%). Less common events were epistaxis (5), bradycardia (4), Grade 2 acute gastroenteritis (4), congestive heart failure (3), erosive gastritis (3), rash (2) and ARDS (2).

Table 6: Non-haematological adverse events (400 cycles)

Adverse event	Cycles (n)	%
Vomiting, Grade 1–2	72	18.0
Febrile neutropenia	22	5.5
Mucositis, any grade	22	5.5
Grade 1–2	18	4.5
Grade 3	3	0.75
Grade 4	1	0.25
Abnormal LFT after discharge (Grade 1–2)	6	1.5
Epistaxis	5	1.25
Bradycardia	4	1.0
Acute gastroenteritis, Grade 2	4	1.0
Congestive heart failure	3	0.75
Erosive gastritis	3	0.75
Skin rash	2	0.5
ARDS	2	0.5

LFT, liver function test; ARDS, acute respiratory distress syndrome.

The predominance of vomiting is consistent with earlier work [14,26]. Vomiting is thought to follow serotonin release from enterochromaffin cells acting on the chemoreceptor trigger zone; the same injury to gut epithelium underlies mucositis, diarrhoea, erosive gastritis and acute gastroenteritis [26]. Maiguma et al. found no relationship between serum MTX and oral mucositis, and suggested that infusion duration, rather than level, drives cell injury [13]; MTX excretion in saliva may also contribute [9]. The relationship between mucositis and MTX level was not analysed in this study. Hydration, leucovorin and supportive measures such as glutamine or low-level laser therapy may help. Epistaxis in 5 cycles occurred with normal platelet counts, in keeping with reports that HDMTX alters coagulation only transiently, without clinically important bleeding or thrombosis [27,28]. Rash was Grade 1 and self-limiting, as in other series [4,12,14]. The two ARDS cases, in patients with normal cardiac function, recovered before the next cycle; pulmonary toxicity is reported in about 1–7% of MTX-treated patients, mostly on low-dose therapy, and

early recognition is important [29,30]. Bradycardia occurred in 4 cycles and no other conduction abnormality was seen. Three cycles of congestive heart failure were related to hydration and resolved when hydration intensity was reduced; MTX cardiotoxicity is rare and described mainly in case reports [31].

5.6 Concurrent 6-mercaptopurine

Concurrent 6-MP was given throughout 280 cycles (70%). It was omitted in 120 cycles (30%) because of baseline Grade 2 toxicity, to avoid cumulative toxicity; those patients restarted 6-MP at discharge if no Grade 2 or worse toxicity persisted. A toxic MTX level was recorded in 36 of 120 cycles (30.0%) without 6-MP, against 34 of 280 (12.1%) with it (OR 3.1, 95% CI 1.8–5.3; Fisher's exact $p < 0.001$; Table 7).

Table 7: Toxic MTX level at any sampling time point, by concurrent 6-MP

6-MP given	Non-toxic n (%)	Toxic n (%)	Total	p
No	84 (70.0)	36 (30.0)	120 (100)	<0.001
Yes	246 (87.9)	34 (12.1)	280 (100)	

Toxic level at any sampling time point; Fisher's exact test.

This finding is hard to interpret. Adding 6-MP is expected to increase, not reduce, toxicity, and omitting it is not known to raise MTX levels. The groups were not comparable: 6-MP was withheld in patients who already had Grade 2 toxicity, who may have had poorer MTX clearance or less marrow, hepatic or renal reserve, so confounding by indication is likely. The finding needs confirmation in a prospective study. Omitting 6-MP, together with MTX dose reduction, may also lower antileukaemic intensity and raise relapse risk, since the two drugs act synergistically and lower metabolite exposure has been linked to relapse [32]; interruptions or reductions in therapy may compromise cure [33]. Those data come from maintenance therapy in children, and relapse was not assessed here.

5.7 Leucovorin rescue, hospital stay and treatment delay

Eight leucovorin doses sufficed in 342 cycles (85.5%); 58 cycles (14.5%) needed more. Patients usually stayed in hospital for about 5 days; those with MTX-related toxicity stayed longer, with a mean stay of 11.48 days. After discharge, recovery from toxicity was delayed for ANC in 46 instances, for platelets in 15 and for liver tests in 2. Subsequent treatment was delayed by at least 2 weeks in these patients, by a mean of 17.39 days for ANC recovery

and 15.33 days for platelet recovery. MTX toxicity therefore disrupts the treatment schedule, as reported previously [16].

In-house serum MTX assay allowed early detection of toxic levels and prompt preventive measures. Many centres lack this and either outsource the test, at considerable cost, or rely on clinical and laboratory signs. A single level at 36 hours has been proposed as a cost-effective alternative where assays are unavailable, which is relevant to low- and middle-income countries such as India [17]. Whether a single level can replace the 48- and 72-hour levels in adults needs study.

6. Limitations and Future Scope

This was a single-centre retrospective study, and the 400 cycles came from 106 patients, so cycles were not independent, and the analysis was not adjusted for this, which may overstate significance; 16 comparisons were made, so some associations may be chance findings. The paediatric BFM-95 protocol was modified for adults according to institutional policy. Serum levels at 24 hours were not analysed. Glucarpidase was unavailable, and MTHFR polymorphism and MTX metabolite levels were not measured. Concurrent 6-MP use was confounded by baseline toxicity, and relapse and survival were not assessed. Prospective multicentre studies with pharmacogenetic profiling, patient-level modelling and outcome follow-up are needed, as is a test of limited-sampling strategies for adults. A prediction model combining the level with baseline organ function might help guide early leucovorin escalation.

7. Conclusion

HDMTX caused toxicity in 38.5% of cycles in adults with ALL, leading to dose reduction in 23.5%, longer admissions and delayed subsequent cycles, with no treatment-related deaths. At 48 hours, toxic MTX levels were associated with myelosuppression, raised transaminases and bilirubin, and renal dysfunction; at 72 hours, with myelosuppression, raised SGOT and renal dysfunction. Measuring serum MTX at these time points identifies cycles at risk and supports early leucovorin escalation.

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