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Italian expert consensus recommendations for the safe administration of high-dose methotrexate in children with cancer and for the use of glucarpidase to manage delayed methotrexate elimination and/or high-dose methotrexate-induced acute kidney injury

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ABSTRACT

Background: High-dose methotrexate (HD-MTX) is used to treat various malignancies in adults and children. However, HD-MTX can lead to severe toxicity, which can require the use of glucarpidase, a recombinant enzyme that rapidly reduces MTX concentrations. Current guidelines, approximated from both adult and pediatric studies and established prescribing indications, do not provide detailed recommendations tailored to children to guide glucarpidase use in clinical practice.

Methods: The current study used modified Delphi methodology to develop expert consensus recommendations for the safe administration of HD-MTX, and management of delayed MTX elimination (DME) and/or HD-MTX–induced acute kidney injury (AKI) in children with cancer. Consensus statements were developed by a multidisciplinary Scientific Board of eight Italian physicians, then circulated to an expert panel of 20 pediatric hemato-oncologists for voting. Expert panel members rated their level of agreement using a 5-point Likert scale; consensus was reached if ≥66.6 % of respondents indicated that they "agreed" or "strongly agreed" with each statement.

Results: Of the 46 statements, 40 achieved consensus. Clinicians agreed that harmonized glucarpidase treatment guidelines are needed. Careful risk assessment performed by a multidisciplinary team should be established to ensure correct management of pediatric patients on relevant factors such as concomitant medications, monitoring of plasma MTX levels for DME, collection and processing of blood samples, monitoring of renal function for AKI prevention or management, and prompt availability of glucarpidase.

Conclusions: These expert consensus recommendations will help pediatric hemato-oncologists to better deal with the various aspects associated with managing HD-MTX in general and with DME or AKI in particular.

1. Introduction

Methotrexate (MTX) is a key chemotherapy agent used to treat various malignancies in adults and children, including acute lymphoblastic leukemia (ALL), osteosarcoma, and non-Hodgkin lymphomas. It is generally well tolerated, although tolerability can vary depending on

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dose, schedule of high-dose MTX (HD-MTX) and patient profile [1–4]. However, HD-MTX (>500 mg/m^2) can cause HD-MTX–associated acute kidney injury (HD-MTX–AKI) and delayed MTX elimination (DME), in turn leading to severe toxicity, and eventually representing a challenge in clinical practice [1,3–7]. Strategies such as folinic acid (FA)/leucovorin (i.e., calcium levofolinate), hyperhydration, and urine alkalinization may be insufficient to prevent or manage DME [4].

Glucarpidase is a recombinant enzyme that converts circulating MTX into the inactive metabolites 2,4-diamino-N(10)-methylpteroic acid (DAMPA) and glutamate. It is able to reduce MTX concentrations by up to 98 % within 15 min and represents an effective antidote for counteracting HD-MTX–associated toxicity [8–13]. Although previous expert consensus papers provide recommendations for the management of DME and HD-MTX toxicity, including the use of glucarpidase [14–16], they are based on both adult and pediatric data, as well as data from solid and hematological malignancies. A guidance document comprehensively covering the safe administration of HD-MTX, the management of related toxicities with or without glucarpidase, and specifically tailored to the pediatric setting is lacking.

Thus, the current study aimed to establish practical recommendations for managing HD-MTX–courses and related toxicity in pediatric patients, based on evidence from the literature and expert opinion. Since protocols and clinician choices may vary between countries, it is important to note that these recommendations reflect the management attitudes applied in pediatric patients with cancer who are treated with HD-MTX in Italy.

2. Methods

The study used modified Delphi methodology to develop Italian expert consensus recommendations for the management of HD-MTX–associated toxicities in pediatric patients with cancer. The development and finalization of these consensus recommendations comprised four stages (Supplementary Fig. S1).

2.1. Development of the expert consensus statements

In stages 1 and 2, a multidisciplinary Scientific Board of eight clinical experts (i.e., the authors) was convened to identify key topics, review relevant literature, and develop draft consensus statements. The Scientific Board included six pediatric hemato-oncologists, one pediatric nephrologist and one pharmacologist, who were selected by the senior author (CR). The pediatric hemato-oncologists were selected based on the size of their centers, their expertise in using glucarpidase in routine clinical practice, their level of expertise in the management of toxic HD-MTX courses (as testified by several clinical interactions and high-level professional relationships), and their publication history in this therapeutic area. The nephrologist and pharmacologist were selected based on their level of pediatric expertise and in-depth knowledge of renal pathology and the effects of drugs, respectively, in cases of DME and HD-MTX–AKI. The Scientific Board was also balanced in terms of the pathologies managed by the experts (i.e., leukemia vs sarcomas).

On May 20, 2024, the Scientific Board met in-person to identify key clinical topics to be covered by the consensus recommendations. The lead author (NP) conducted a PubMed database search (May 25, 2024) to collect relevant literature on the topics raised during the in-person meeting. The literature search used the keywords “methotrexate (Methotrexate)”, and “glucarpidase” OR “carboxypeptidase G2” AND “children”, and was filtered to English-language papers. From this search 21 publications were selected based on their relevance and were shared with the Scientific Board, who were divided into two working groups of four experts each. Working Group A reviewed the literature with respect to clinical management of HD-MTX in children; specific topics included risk factors, prevention of DME, sample management, support strategies, and timing and tools used to monitor MTX concentrations. Working Group B reviewed the literature with respect to the

Table 1
Italian expert consensus statements related to risk assessment.

No.	Risk assessment statement	Consensus achieved (level of agreement)
1	Before starting treatment with HD-MTX, it is necessary to carefully check the medications taken by the patient and to consider suspending any drugs with potential interactions with MTX clearance; in particular: • Hepatotoxic medications (e.g., leflunomide, azathioprine, sulfasalazine, retinoids) • Anticonvulsants or 5-fluorouracil • Salicylates, phenylbutazone, diphenylhydantoin (phenytoin), barbiturates, tranquilizers, oral contraceptives, tetracyclines, amidopyrine derivatives, sulfonamides, thiazide diuretics, oral hypoglycemics, doxorubicin, p-aminobenzoic acid • Probenecid • Proton pump inhibitors (e.g., esomeprazole, lansoprazole, omeprazole, rabeprazole, pantoprazole) • Antibiotics (e.g., penicillin, glycopeptides, sulfonamides, ciprofloxacin, cephalothin) • Concomitant use of medications that can cause folic acid deficiency (e.g., sulfonamides, trimethoprim/sulfathiazole) • Ciclosporin • Cholestyramine • Tyrosine kinase inhibitors	Yes (80 %)
2	Patients with hypoalbuminemia have lower MTX clearance compared with those with normal albumin levels. In cases of low albumin, fluid retention in the third space (e.g., pleural effusion, ascites, edema) may occur due to reduced oncotic pressure, leading to prolonged MTX exposure and potential renal injury. Physicians should check plasma[a] albumin levels before each cycle of HD-MTX chemotherapy and assess fluid retention via CT, X-ray, or ultrasound before the first HD-MTX dose in patients with low albumin. Hypoalbuminemia should be corrected when identified.	Yes (90 %)
3	It is essential to assess the patient’s hydration status before initiating HD-MTX infusion and to correct any dehydration with appropriate infusion support.	Yes (100 %)
4	Children with Down syndrome are at increased risk of poor tolerance to HD-MTX due to heightened cellular sensitivity in affected tissues, such as the mucosa and bone marrow. This increased sensitivity often necessitates dose reductions of MTX in this population.	Yes (100 %)
5	Studies indicate that delayed MTX excretion becomes more pronounced with increasing age, even within the pediatric population. The incidence of severe delayed MTX excretion is significantly higher in adolescents and young adults compared with younger children.	Yes (85 %)
6	Obesity/overweight[a] are recognized as risk factors for delayed elimination of MTX. In obese patients, the MTX dose should be calculated based on ideal body weight rather than actual weight.	Yes (85 %)
7	Excessive consumption of caffeine- or theophylline-containing beverages (e.g., coffee, caffeinated drinks, black tea) should be avoided during MTX therapy. These may reduce the effect of MTX due to possible interactions between MTX and methylxanthines at adenosine receptors.	No (45 %)

[a] For accuracy, the word ‘plasma’ has replaced ‘serum’, which was used in the original statements prior to voting.
[b]The phrase ‘large body size’ that was used in the original statements prior to voting was replaced by ‘Overweight’, which is a more accurate description of this patient characteristic.

CT, computed tomography; HD-MTX, high-dose methotrexate (Methotrexate); MTX, methotrexate (Methotrexate).

management of patients with DME or HD-MTX–associated toxicities; specific topics included folinic acid versus glucarpidase, DME and emergency criteria for initiating glucarpidase, AKI parameters, the impact of glucarpidase on subsequent HD-MTX treatment, and the role of the nephrologist.

Between June 17–24, 2024, Working Group members attended online meetings to discuss the available evidence and generate an initial list of expert consensus statements. Draft statements were further refined, with the final survey comprising 46 expert consensus statements. These statements covered five topics: risk assessment (Table 1); supportive therapy (Table 2); monitoring of plasma MTX levels (Table 3); treatment of DME and HD-MTX toxicity (Table 4); and sample management (Table 5).

2.2. Finalization of the expert consensus statements

On September 11, 2024, the online survey was circulated to a representative panel of clinical experts from major pediatric oncology centers across Italy (stage 3; Supplementary Fig. S1). These clinicians, due to their high patient volumes, have encountered and managed numerous episodes of HD-MTX toxicity with glucarpidase.

Expert panel members were invited to participate by the study sponsor (with a target sample size of 20 clinicians), based on their experience in managing episodes of HD-MTX toxicity with glucarpidase in clinical practice. Those who agreed to participate received the most relevant literature on the topic before completing the survey. Participants rated their level of agreement with each consensus statement using a 5-point Likert scale (1, strongly disagree; 2, disagree; 3, neither agree nor disagree; 4, agree; 5, strongly agree).

A supplementary questionnaire (developed by the Scientific Board following a literature review and based on their own expertise in clinical practice) was circulated to the 20-member expert panel on September 18, 2024, aiming to provide context regarding the use of glucarpidase in Italy. On January 24, 2025, a subsequent questionnaire was addressed to the expert panel to ascertain their demographic and additional clinical information.

Expert panel responses to the surveys were analyzed, with consensus considered reached when ≥66.6 % of respondents agreed on each statement indicating that they “agreed” or “strongly agreed” with the statement. The survey results were presented to the Scientific Board and expert panel at an online plenary meeting on September 27, 2024, during which practical recommendations related to the expert consensus statements were discussed (stage 4).

3. Results

3.1. Surveyed experts

Of the 23 pediatric hemato-oncologists invited to join the expert panel, 20 agreed and subsequently evaluated the consensus statements. Of these, 17 experts returned supplementary survey data describing demographic and clinical characteristics (Supplementary Table S1), and provide insights into HD-MTX–related toxicities, the management of DME, and the use of glucarpidase in Italy (Table 6).

3.2. Expert consensus recommendations

Of the 46 statements, 40 achieved consensus among the expert panel (Supplementary Table S2). The authors believe lack of consensus was mostly due to insufficient evidence in the literature causing uncertainty among the clinicians, or the highly technical nature of the statement. Consensus statements (grouped by topic) are presented in Tables 1–5 and contextualized with relevant literature in the following subsections.

Table 2
Italian expert consensus statements related to supportive therapy.

No.	Supportive therapy statement	Consensus achieved (level of agreement)
8	A preliminary assessment of the patient’s hydration status and renal function is essential to minimize the risk of acute renal toxicity secondary to HD-MTX therapy (i.e., doses >500 mg/m^2).	Yes (100 %)
9	Accurate assessment of renal risk (including pre-existing kidney disease, past acute events, or chronic damage from previous MTX exposure) and early identification of signs of renal damage allow for the implementation of therapeutic strategies aimed at minimizing both acute consequences (further accumulation and damage) and long-term outcomes. This is particularly important due to the progressive nature of chronic kidney disease and the long-life expectancy of pediatric patients.	Yes (100 %)
10	Before initiating a HD-MTX cycle, comprehensive evaluation of renal function is mandatory, including a medical history review for previous episodes of AKI; repeated blood pressure measurements (adjusted for age, sex, and height); and renal function tests such as plasma[a] creatinine (within age-appropriate ranges), cystatin C (whenever available), urine analysis, and measurements of urinary protein, creatinine, sodium, and potassium (normal urinary Na/K ratio >1).	Yes (85 %)
11	Cystatin C is more reliable to estimate renal function than creatinine, particularly in patients with reduced physical activity, reduced muscular mass, or hypoalbuminemia.	Yes (75 %)
12	Since >90 % of MTX is eliminated by the kidneys, a hyper-hydration strategy is crucial to maintain high urinary flow rates. It is recommended to start hyper-hydration ≥12–18 h before HD-MTX infusion (at 100–150 mL/m^2 per hour) and to continue for 48 h. This is particularly important for patients who are not in a state of euvolemia at baseline.	Yes (94 %)
13	The hyper-hydration infusion should include ≥50 mEq/L of NaCl to favor volume expansion, along with $NaHCO_3$ (50–60 mEq/L or 2–4 mEq/kg) as a continuous infusion before and after HD-MTX administration.	Yes (100 %)
14	Close monitoring of fluid input and output balance is required, with daily weighing of the patient. In cases of negative fluid balance, an increase in IV fluid infusion is indicated, while in cases of highly positive fluid balance, diuretics (e.g., furosemide) should be administered.	Yes (90 %)
15	A low urine pH is associated with delayed MTX elimination and potential nephrotoxicity due to decreased MTX solubility and increased intratubular precipitation. Intensive hydration and sodium bicarbonate administration are recommended to maintain urine pH ≥7.	Yes (100 %)
16	Urine pH should be measured before starting HD-MTX infusion and at each voiding. Additional boluses of $NaHCO_3$ (2 mEq/kg) should be administered if urine pH is <7, with repeats as necessary based on urine pH results.	Yes (100 %)
17	Antiemetics (e.g., ondansetron) are recommended for pediatric patients undergoing HD-MTX therapy to prevent a reduction in blood volume due to vomiting.	Yes (100 %)
18	For long infusions of MTX (i.e., 1–5 g/m^2 over 24 h continuous infusion), folinic acid rescue should be administered at doses of 15 mg/m^2 of the racemic product or 7.5 mg/m^2 of the levo product at 42, 48, and 54 h after the start of HD-MTX infusion. The folinic acid dose should be adjusted based on the MTX plasma level determined 6 h prior to each dose.	Yes (100 %)
19	For short MTX infusions (i.e., 8–12 g/m^2), folinic acid rescue should begin 24 hours after the start of MTX infusion. Folinic acid doses (15 mg/m^2 of the racemic product or 7.5 mg/m^2 of the levo product every 6 h until 90 h post-MTX infusion) should be adjusted according to plasma MTX concentrations following the Standard Folinic acid Nomogram (Bleyer), and continued until adequate MTX clearance is achieved.	Yes (95 %)

(*continued on next page*)

Table 2 (*continued*)

No.	Supportive therapy statement	Consensus achieved (level of agreement)
20	Strict monitoring of plasma[a] calcium levels after prolonged administration of high doses of calcium levulinate is highly recommended.	Yes (100 %)
21	Prolonged use of excessive doses of folinic acid may pose a risk of interfering with the antitumor activity of HD-MTX.	No (60 %)

[a] For accuracy, the word 'plasma' has replaced 'serum', which was used in the original statements prior to voting.

AKI, acute kidney insufficiency; HD-MTX, high-dose methotrexate (Methotrexate); IV, intravenous; K, potassium; MTX, methotrexate (Methotrexate); Na, sodium; NaCl, sodium chloride; $NaHCO_3$, sodium bicarbonate.

Table 3
Italian expert consensus statements related to the monitoring of plasma MTX levels.

No.	Monitoring of plasma MTX levels statement	Consensus achieved (level of agreement)
22	Serial monitoring of plasma[a] MTX levels following the initiation of HD-MTX therapy is essential for the early detection of DME, allowing for timely interventions to mitigate HD-MTX–induced toxicity.	Yes (100 %)
23	Immunoassay is the most widely used and accessible method for determining plasma[a] MTX levels. However, following glucarpidase administration, immunoassay captures both MTX and DAMPA; therefore, overestimating the real MTX plasma[a] concentration. HPLC can be used instead, especially in the first 48 h after glucarpidase administration since it differentiates MTX from DAMPA. However, since HPLC does not measure intracellular MTX content, it might result, in the long run, in a folinic acid dose insufficient for restarting the intracellular folic acid cycle. For this reason, in the calculation of the folinic acid rescue dose, the plasma[a] MTX concentration measured by immunoassay seems to be more appropriate.	Yes (80 %)
24	For long HD-MTX infusions (i.e., 5 g/m^2 over 24 h), plasma MTX levels should be systematically monitored at 24, 42, and 48 h from the start of infusion. If plasma[a] MTX levels exceed 0.5 μmol/L at 42 h, monitoring should continue every 6–12 h until plasma[a] MTX levels are reduced to <0.25 μmol/L.	Yes (90 %)
25	For short HD-MTX infusions (i.e., 8–12 g/m^2 over 4 h), plasma[a] MTX levels should be systematically monitored at 24 h (with a normal value of ≤10 μmol/L) and at 48 h (with a normal value of ≤1 μmol/L) from the start of infusion.	Yes (80 %)
26	Additional MTX level monitoring at 36 h from the start of HD-MTX infusion is recommended if the 24-hour MTX level exceeds 120 μmol/L for long infusions or 50 μmol/L for short infusions.	Yes (90 %)
27	The MTXPK.org tool is useful for clinicians since it takes into account the major variables useful to predict the MTX elimination curve.	Yes (90 %)

[a] For accuracy, the word 'plasma' has replaced 'serum', which was used in the original statements prior to voting.

DAMPA, 4-deoxy-4-amino-N10-methylpteroic acid; DME, delayed methotrexate (Methotrexate) elimination; HD-MTX, high-dose methotrexate (Methotrexate); HPLC, high-performance liquid chromatography; MTX, methotrexate (Methotrexate).

3.2.1. Patient risk assessment

Risk assessment of pediatric patients prior to initiating HD-MTX is critical and should consider medical profile (i.e., concomitant medications [Statement 1], renal function and presence of hypoalbuminemia [Statement 2], hydration status [Statement 3], comorbidities [Statement 4]), and demographics (i.e., patient age [Statement 5] and body weight [Statement 6]; Table 1). It is important to assess patient risks

Table 4
Italian expert consensus statements related to the treatment of DME and HD-MTX toxicity.

No.	Treatment of DME and HD-MTX toxicity statement	Consensus achieved (level of agreement)
28	Urine alkalinization and folinic acid administration are recommended as first-line treatments for the prevention of acute renal toxicity associated with HD-MTX.	Yes (100 %)
29	The involvement of a nephrologist at the onset of HD-MTX toxicity (pre-AKI phase) is beneficial for optimizing supportive and therapeutic interventions.	Yes (80 %)
30	HD-MTX toxicity can occur even in the absence of identifiable risk factors.	Yes (100 %)
31	Body weight and creatinine clearance are significant covariates in the distribution of MTX.	Yes (90 %)
32	Glucarpidase administration is recommended for patients with significant DME or DME associated with signs of acute renal failure, defined as a 50 % increase in creatinine versus baseline within 24–48 h after HD-MTX administration. Specific DME thresholds are >120 μM at 24 h, >30 μM at 36 h, >10 μM at 42 h, and >5 μM at 48 h[a].	Yes (95 %)
33	When glucarpidase is indicated, early administration is critical, ideally within 48–60 h after HD-MTX administration start. To ensure the timely use of glucarpidase, it is crucial to have the drug readily available to allow effective therapeutic intervention.	Yes (100 %)
34	There is no consolidated evidence supporting dose capping in the administration of glucarpidase.	No (65 %)
35	DME is a risk factor for inappropriate continuation of treatment, possible delays in subsequent cycles, subacute and chronic renal damage, systemic toxicity, and in some cases, treatment-related mortality.	Yes (95 %)
36	In general, DME indicates kidney damage unless it is due to reduced nephron endowment, previous kidney damage, or underlying pathology.	Yes (85 %)
37	Patients are considered to be in a state of DME if MTX plasma[b] levels exceed two standard deviations above the mean MTX excretion curve. However, exceptions may occur in cases of reduced excretion due to drug interactions (e.g., treatments inhibiting renal transporters), where DME might develop without initial renal impairment, though renal damage could eventually occur.	Yes (90 %)
38	Patients who develop DME in the absence of AKI do not have optimal renal function.	No (50 %)
39	Delayed elimination in a patient with ALL receiving HD-MTX is defined by a 36-hour, 42-hour, or 48-hour plasma MTX level >30 μM, 10 μM, or 5 μM, respectively[c], or two standard deviations above the mean expected MTX plasma concentration (as determined using MTXPK.org), along with a 1.5-fold increase in plasma creatinine above baseline.	Yes (75 %)
40	No specific instrumental examinations are available to assess limited renal functional impairment (i.e., impairment <30 %).	Yes (75 %)
41	The use of glucarpidase facilitates the continuation of subsequent cycles of HD-MTX.	Yes (95 %)
42	In cases of functional recovery after DME with AKI, re-exposure to HD-MTX should be preceded by a thorough nephrological evaluation.	Yes (85 %)

[a] A typographical error was made when developing this statement, such that the units presented were 'μmol' rather than 'μM'. This has been corrected in the statement presented in this table.

[b] For accuracy, the word 'plasma' has replaced 'serum', which was used in the original statements prior to voting.

[c] A typographical error was made when developing this statement, such that the units presented were 'μM/L' rather than 'μM'. This has been corrected in the statement presented in this table.

AKI, acute kidney insufficiency; ALL, acute lymphoblastic leukemia; DME, delayed methotrexate (Methotrexate) elimination; HD-MTX, high-dose methotrexate (Methotrexate); MTX, methotrexate (Methotrexate).

Table 5
Italian expert consensus statements related to sample management.

No.	Sample management statement	Consensus achieved (level of agreement)
43	To ensure the integrity of MTX samples, they should ideally be taken from a site different from the infusion site to prevent contamination, protected from light, and kept at room temperature prior to centrifugation.	Yes (70 %)
44	Blood sampling for the assessment of MTX concentrations affects analytical performance; ideally, samples should be processed within 2 h of collection.	Yes (90 %)
45	MTX remains stable in untreated plasma samples for up to three freeze–thaw cycles, for 6 h at room temperature, 24 h at 4 °C, and 1 month at −80 °C.	No (45 %)
46	In treated plasma samples, MTX remains stable for 24 h in the autosampler, maintaining accuracy and RSD within ±15 %.	No (30 %)

MTX, methotrexate (Methotrexate); RSD, relative standard deviation.

individually and recognize that risk factors can vary between visits.

It should be standard practice to consider the potential for drug–drug interactions; however, reliable sources allowing a practical decision-making process are lacking. The consequences of temporary discontinuation of drugs potentially affecting MTX clearance should also be carefully evaluated. For instance, tyrosine kinase inhibitors (TKIs) can interfere with hepatic metabolism of drugs, as well as hepatobiliary, tubular, and glomerular transporters. Although some case reports have indicated the possibility of higher DME and AKI incidence in children with Philadelphia chromosome–positive ALL (pH+ALL) treated with concurrent TKIs during HD-MTX courses [17–20], TKI suspension is not currently indicated by international treatment protocols (i.e., European intergroup study of post-induction treatment of pH+ALL and Children's Oncology Group protocols) [21,22].

A preliminary assessment of the patient's hydration status and renal function is essential to minimize the risk of acute renal toxicity secondary to HD-MTX [Statement 8]. Accurate assessment of renal risk should include the recording of pre-existing kidney disease, past acute events, or chronic damage from previous MTX exposure [Statement 9]. It is recommended that physicians check plasma albumin levels before each HD-MTX dose and that patients with hypoalbuminemia (serum albumin level <35 g/L) be evaluated for possible fluid retention [Statement 2]. Of note, a serum albumin level of <30 g/L before starting HD-MTX was shown to have a clinically significant impact on HD-MTX elimination and risk of HD-MTX–related mucositis [23].

Severe hypoalbuminemia (<25 g/L), particularly when associated with edema, pleural effusion or ascites should be corrected by albumin replacement infusion when identified [Statement 2] and any dehydration should be corrected with appropriate infusion support [Statement 3].

Physicians also need to be aware that DME becomes more pronounced with increasing age, with the risk for severe DME significantly greater in adolescents and young adults than in younger children [Statement 5] [24]. The presence of comorbidities may also affect the risk of developing HD-MTX–associated toxicities, thus possibly necessitating MTX dose reduction. For example, children with Down syndrome are at increased risk of poor tolerance to HD-MTX due to heightened cellular sensitivity in affected tissues, such as the mucosa and bone marrow [25,26]. Also, overnutrition (obesity/overweight) is a recognized risk factor for DME [27], and is relevant to the metabolism of other drugs besides MTX [Statement 6]. Patients with obesity presented a 2-fold higher risk for delayed MTX elimination at 48 h post infusion in a study including children and adolescents with ALL [28]. In patients with obesity, it is recommended that the MTX dose be calculated based on ideal body weight (IBW) rather than actual weight [Statement 6]. Furthermore, it seems reasonable to also use IBW for glucarpidase dose calculation [29].

Table 6
Results of supplemental survey providing Italian context.

	$N = 17$
Annual number of oncohematologic patients treated with HD-MTX–containing chemotherapy, n (%)[a]	
0–30	12 (70.6)
31–60	4 (23.5)
61–90	1 (5.9)
Frequency of HD-MTX–related toxicity (any grade), n (%)[b]	
1–5 %	9 (52.9)
6–10 %	4 (23.5)
11–15 %	4 (23.5)
Most frequent HD-MTX–related toxicity encountered, n (%)	
Mucositis	10 (58.8)
Hepatic toxicity	6 (35.3)
AKI	1 (5.9)
Consequences of HD-MTX–related toxicity, n (%)[c]	
Discontinuation of therapy	11 (64.7)
Complete resolution	16 (94.1)
Resolution with consequences	1 (5.9)
Death	0
Frequency of discontinuation of HD-MTX–containing chemotherapy due to toxicity, n (%)	
Never	7 (41.2)
1–10 % of patients	10 (58.8)
Time to laboratory MTX result, n (%)	
≤1 h	4 (23.5)
2–3 h	12 (70.6)
>3 h	1 (5.9)
Patients treated with glucarpidase over the last 4 years, n (%)	
0	7 (41.2)
1–2	8 (47.1)
3–5	2 (11.8)
Re-exposure of glucarpidase-treated patients to HD-MTX, n (%)	
No, toxicity occurred during the last treatment cycle	1 (5.9)
No, due to fear of a new toxicity episode	3 (17.6)
Yes, at a reduced dose	8 (47.1)
Yes, without dose reduction	1 (5.9)
No answer given	4 (23.5)
Awareness of MTXPK.org tool, n (%)	
No	5 (29.4)
Yes, but do not use it	2 (11.8)
Use it occasionally	8 (47.1)
Use it regularly	2 (11.8)
Perceived unmet needs and critical issues, n (%)[c]	
Physician education	1 (5.9)
Timely/accurate assessment of MTX toxicity	4 (23.5)
Rapid access to glucarpidase	7 (41.2)
Cost of glucarpidase	2 (11.8)
Guidelines for glucarpidase use	1 (5.9)

Note: some percentages may not total 100.0 % due to rounding.
[a] At the respondent's center.
[b] Frequently observed toxicities were mucositis, liver toxicity, liver disease, transient hyperbilirubinemia, transient hepatitis, renal toxicity, pleural and pericardial serositis, and neurotoxicity.
[c] More than one answer permitted.
AKI, acute kidney insufficiency; HD-MTX, high-dose methotrexate (Methotrexate); MTX, methotrexate (Methotrexate).

No consensus was reached regarding the need to educate pediatric patients and their parents about avoiding the consumption of caffeine- or theophylline-containing beverages during MTX therapy [Statement 7], which may have been due to uncertainty because of insufficient published evidence.

3.2.2. *Supportive measures*

3.2.2.1. Renal function/hydration. Given the progressive nature of chronic kidney disease [30] and the long life expectancy of pediatric oncology survivors [31], early identification of signs of renal damage is particularly important to allow the implementation of therapeutic strategies that minimize both acute consequences and long-term

outcomes on renal function [Statement 9] (Table 2). Thus, prior to initiating each HD-MTX cycle, comprehensive evaluation of renal function is mandatory [Statement 10]. Clinicians considered the use of cystatin C to be a reliable indicator of renal function more than creatinine [32], particularly in patients with reduced physical activity, reduced muscular mass, or hypoalbuminemia [Statement 11] [33]. Despite this, cystatin C is not routinely used in all centers in Italy.

Since >90 % of MTX is eliminated by the kidneys [34], a hyper-hydration strategy is crucial for maintaining high urinary flow rates [Statements 12 and 13]. This is particularly important in patients who are not in a state of euvolemia at baseline. Close monitoring of fluid input and output is also required, and patients should be weighed daily [Statement 14]. In the case of negative fluid balance, an increase in intravenous fluid infusion is indicated; in cases of highly positive fluid balance, diuretics are indicated. A dosage of furosemide 0.5 mg/kg is useful for the recovery of diuresis (with the objective of increasing MTX elimination) without causing hypovolemia.

Implementation of a hyper-hydration strategy has implications for the timing of patient admission relative to HD-MTX, and this might vary due to organizational factors among centers or patient preference. A typical protocol involves patient admission in the evening, with 12-hour prehydration (and possible intensified alkalinization during the final 3 h) then HD-MTX administered the following day.

3.2.2.2. Maintenance of urine pH/alkalinization. Low urine pH is associated with DME and potential nephrotoxicity due to decreased MTX solubility and increased intratubular precipitation [Statement 15] (Table 2) [34]. Thus, in addition to intensive hydration, administration of sodium bicarbonate is recommended to maintain a urine pH ≥7. Measure urine pH prior to each HD-MTX infusion and at each voiding [Statement 16]. Additional boluses of sodium bicarbonate 2 mEq/kg should be administered if urine pH is <7.

3.2.2.3. Antiemetics. Current clinical guidelines recommend prophylactic treatment with antiemetics to manage chemotherapy-induced nausea and vomiting in patients with cancer [35]. The expert panel agreed that prophylactic antiemetics should also be considered for all pediatric patients receiving HD-MTX therapy, aiming to also prevent a reduction in blood volume due to vomiting [Statement 17] (Table 2).

3.2.2.4. Folinic acid. FA is routinely used to prevent HD-MTX–induced toxicities [3], with the dosage and the duration of its administration increased in case of DME and according to plasma MTX levels. Administration of FA must start after HD-MTX initiation because, if given too early, it would negatively affect the antitumor effects of MTX [36]. For recommended standard doses and timings of FA rescue after long and short infusions of MTX, see Statements 18 and 19, respectively (Table 2). We recommend after long MTX infusions (1–5 g/m^2 over 24 h continuous infusion) to stop FA rescue at 54 h (3 doses of FA in total – at 42, 48 and 54 h) in case of appropriate MTX clearance (MTX <0.25 μM at 54 h), while 6-hourly FA rescue should be continued until the MTX level has dropped below 0.25 μM in case of delayed MTX excretion. For short MTX infusions FA should be started 24 h after HD-MTX infusion and repeated every 6 h until 90 h, for a total of 11 doses or until the MTX level is <0.1 or <0.2 μM depending on the protocol. For both long and short infusions, FA doses should be adjusted according to plasma MTX concentrations following the Standard Folinic acid Nomogram [16].

Monitoring of plasma calcium levels after prolonged administration of high doses of FA is highly recommended [Statement 20]. Since the calcium content of some FA preparations could lead, in rare cases, to various degrees of hypercalcemia, the use of sodium-conjugated FA preparations is advised [37].

Consensus was not reached on whether prolonged use of high-dose FA may interfere with the antitumor activity of HD-MTX [Statement 21]. This lack of consensus may be due to a paucity of data on this issue in the pediatric oncology setting, thus resulting in an inconclusive judgment among physicians.

3.2.3. Monitoring of plasma MTX levels

Serial monitoring of plasma MTX following initiation of HD-MTX is essential for the early detection of DME, allowing for timely interventions to mitigate HD-MTX–induced toxicity [Statement 22] (Table 3). Timing of such monitoring depends on the duration of MTX infusion [Statements 24–26]. Immunoassay is the most widely used and accessible method for determining plasma MTX levels [Statement 23] [38], although high-performance liquid chromatography (HPLC)-based methods are more accurate [39]. Following glucarpidase administration, immunoassay captures both MTX and DAMPA, thus overestimating the actual MTX plasma concentration [38]. As such, HPLC is particularly useful in the first 48 h after glucarpidase administration since it differentiates MTX from DAMPA [Statement 23] [38]. However, HPLC is relatively time consuming [38,39], potentially limiting its use when urgent determination of MTX levels is required. Therefore, when calculating the FA rescue dose, measurement of plasma MTX concentrations by immunoassay may be more feasible.

Of the many PK software packages available, MTXPK.org, a free online tool designed specifically to evaluate HD-MTX pharmacokinetics in the context of DME (to predict MTX clearance), was chosen by the Italian experts since it was the most used in their routine practice aimed at managing MTX excretion. MTXPK.org is a widely-used, useful, practical clinical decision support tool for physicians who encounter DME and HD-MTX–AKI [40,41]. It considers the major variables useful to predict MTX elimination in pediatric patients [Statement 27] and provides an objective indication for glucarpidase administration, especially when MTX levels are borderline for glucarpidase treatment [16].

3.2.4. Treatment of DME and HD-MTX toxicity

3.2.4.1. Prevention of acute renal toxicity associated with HD-MTX. The clinicians agreed that urine alkalinization and FA administration are recommended as first-line treatments for the prevention of acute renal toxicity associated with HD-MTX [Statement 28] (Table 4). This recommendation is in line with available evidence and current standardized protocols [3,4]. Furthermore, the involvement of a nephrologist at the onset of HD-MTX (pre-AKI) was considered beneficial for optimizing supportive and therapeutic interventions [Statement 29]. Clinicians agreed that HD-MTX toxicity can also occur in the absence of identifiable risk factors [Statement 30], and that body weight and creatinine clearance are significant covariates in the distribution of MTX [Statement 31].

3.2.4.2. Use of glucarpidase. The clinicians agreed that glucarpidase administration is recommended in patients with significant DME, or DME associated with signs of AKI (i.e., 50 % increase in creatinine versus baseline within 24–48 h after HD-MTX administration) [Statement 32] (Table 4). Specific DME thresholds, chosen from the literature and based on the therapeutic indication of glucarpidase [42] and clinical judgement, are: ≥120 μM for long infusions of MTX or ≥50 μM for short infusions at 24 h; ≥30 μM at 36 h; ≥10 μM at 42 h; and ≥5 μM at 48 h. When glucarpidase is indicated, early administration is critical, ideally within 48–60 h after the initiation of HD-MTX administration [Statement 33]. The recommended dosing of glucarpidase is to administer a single dose of 50 units/kg by bolus intravenous injection over 5 min [43]. To ensure timely use of glucarpidase, it is crucial to have the drug readily available to allow effective therapeutic intervention. After glucarpidase administration, continuation of FA rescue (to be restarted ≥2 h post-glucarpidase) is recommended until MTX is eliminated (MTX <0.2 μM or <0.1 μM depending on protocols) [42].

Consensus was not reached on the statement that there is no consolidated evidence supporting glucarpidase dose-capping [Statement

34]. Nevertheless, it is important to note that, given the United States Food and Drug Administration- and European Medicines Agency (EMA)-approved dosage for glucarpidase and a lack of evidence, dose capping of glucarpidase is not advisable; the pharmacologist on the Scientific Board concurred.

3.2.4.3. HD-MTX treatment discontinuation. DME has been associated with inappropriate HD-MTX treatment continuation, delays in subsequent treatment cycles, subacute and chronic renal damage, systemic toxicity, and in some cases, treatment-related mortality [Statement 35] (Table 4). In patients with life-threatening diseases such as ALL, lymphomas, or osteosarcoma, recovery from severe HD-MTX–associated toxicities is important in order to maintain outcomes. In a recent large study, adults with cancer and HD-MTX–AKI had 2.7-fold higher adjusted odds of renal recovery and lower risk of grade ≥2 liver enzyme elevations or neutropenia when they received glucarpidase, compared with those who did not receive glucarpidase [44]. Such recovery would not only allow continuation of chemotherapy, but would also possibly permit rechallenge with HD-MTX.

3.2.4.4. Causes and definition of DME. Patients are considered to have DME if plasma MTX exceeds two standard deviations above the mean MTX excretion curve [Statement 37] [14] (Table 4). The presence of DME generally indicates the presence of AKI, except in cases of reduced nephron endowment, previous kidney damage, or underlying pathology [Statement 36]. It is important for physicians to be aware that DME might also develop without initial renal impairment (i.e., because of drug interactions), although renal damage could eventually occur in these patients.

Clinicians failed to reach agreement that patients who develop DME in the absence of AKI do not have optimal renal function [Statement 38]. However, the nephrologist deemed that this doubt among clinicians (35 % neither agreed nor disagreed with the statement) was reasonable, given that the great variability in nephron endowment (between 300,000–1800,000 per kidney) and its consequences are not completely well-known outside of the nephrology community.

DME may or may not be accompanied by a 1.5-fold increase in plasma creatinine above baseline [16]; however, the EMA-approved indications for glucarpidase do not necessarily require the presence of increased creatinine [42]. Slightly lower consensus (75 % agreement) was reached between clinicians [Statement 39], possibly because of suboptimal formulation of the statement regarding creatinine increase and due to the modified Delphi methodology constraints, where statements were not modified after the vote.

Clinicians agreed that there are no specific instrumental examinations available to assess limited renal functional impairment [Statement 40], highlighting that renal impairment is only detectable when it causes a 40 % decrease in glomerular filtration rate. The only exception is scintigraphy which is not an option in the setting of pediatric oncology due the difficulty in obtaining high-quality diagnostic images in children [45].

3.2.4.5. Re-challenge with HD-MTX. Clinicians agreed that the use of glucarpidase to treat HD-MTX–AKI facilitates the continuation of subsequent cycles of HD-MTX [Statement 41] (Table 4). Indeed, favorable (although still limited) data regarding the feasibility of rechallenging glucarpidase-treated pediatric patients with HD-MTX have been reported [46–49]. The majority of responding clinicians in the current study had re-exposed such patients to HD-MTX, either with (47.1 %) or without (5.9 %) dose reduction (Table 6). However, it is recommended that in cases of functional recovery after DME with AKI, re-exposure to HD-MTX is preceded by a thorough nephrological evaluation [Statement 42].

3.2.5. Sample management

Correct sample management is essential to avoid underestimating MTX levels. Clinicians agreed that, to ensure the integrity of MTX samples, they should be taken from a site other than the infusion site to prevent contamination [Statement 43] (Table 5). However, considering the age of patients and the presence of a central venous catheter, the ideal option is rarely pursued in clinical practice. Thus, it is recommended that if MTX levels in blood taken from a central venous catheter are unexpectedly high, re-evaluation using blood sampled from a peripheral vein should be considered.

Blood samples should ideally be processed within 2 h of collection [Statement 44], since longer processing times typically result in sample deterioration and unreliable data. Furthermore, blood samples should be protected from light and kept at room temperature prior to centrifugation. Among the responding clinicians, the majority (70.6 %) received plasma MTX results from the laboratory within 2–3 h (Table 6). There was uncertainty among clinicians regarding the stability of plasma samples, with no consensus reached [Statements 45 and 46] (Supplementary Table S2), although these were considered to be technically specialized statements.

4. Discussion

The interdisciplinary Scientific Board utilized in this study (including six pediatric oncologists, a pediatric nephrologist, and a pharmacologist) ensured that all relevant aspects of treating pediatric patients with HD-MTX were addressed, which distinguishes this study from others that sought to develop recommendations for managing HD-MTX–related toxicities [14–16]. The nephrologist and pharmacologist provided insightful input into several issues, some of which are discussed below. Further, all Scientific Board and expert panel members were from Italy, meaning that their clinical perspectives were similar and not impacted by differing international clinical practices.

Given that the consensus statements were developed specifically for pediatric patients, the importance of prioritizing continuation of chemotherapy despite toxicity was acknowledged to maximize life expectancy; however, there is minimal guidance regarding re-exposure to HD-MTX following renal toxicity. Nevertheless, various published studies have indicated that HD-MTX resumption, using different doses and modalities, is feasible and safe in pediatric patients [46–48].

Ideally, one should be able to identify patients at higher risk of renal insufficiency prior to re-exposure. The authors suggested that re-exposure to HD-MTX should depend on the grade of toxicity experienced. For example, if previous toxicity was limited and well-managed, with adequate clinical recovery, re-exposure to MTX at a full or reduced dosage may be considered, based on the judgement of the treating physician.

From a nephrology perspective, previous toxicity from HD-MTX could be related to nephron endowment [50], with a higher risk of toxicity if nephron endowment is low. The risk of toxicity is also higher in patients who have previously experienced HD-MTX toxicity, since HD-MTX toxicity entails nephron loss. Nephron endowment or previous renal damage should be considered along with modifiable risk factors and any underlying causes of toxicity (e.g., diarrhea, suboptimal hydration).

The feedback from the clinicians in this study indicates that rapid availability of glucarpidase is a major unmet need in pediatric oncology. Given the life-saving characteristics of the drug, physicians should be aware of the supply modalities in their local area ahead of the need to ensure timely use of glucarpidase.

The need to educate younger physicians regarding tools to aid clinical decision-making was identified as another unmet need. In particular, awareness of a published flowchart for correct and timely glucarpidase administration [14,16] and the MTXPK.org tool would be useful, as well as dedicated training during national and international scientific pediatric and/or hemato-oncology meetings. Finally, timely

measurement of MTX levels and rapid availability of data are critical since they affect treatment decisions and, ultimately, patient outcomes.

5. Conclusions

The harmonization of consensus recommendations regarding glucarpidase treatment for different neoplasms in pediatric oncology is desirable. Risk assessment by a multidisciplinary team is recommended to ensure the correct management of pediatric patients, specifically considering individual patient characteristics, concomitant medications, therapeutic relevance of HD-MTX, monitoring of plasma MTX levels, management of blood samples, impairment of liver and renal function, early detection of DME and AKI, and the prompt availability of glucarpidase. The recommendations in this consensus document will assist clinicians with the various aspects relevant for optimal management of pediatric patients in this specific clinical setting.

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Author contributions

All authors meet the authorship criteria of the International Committee of Medical Journal Editors, contributed to the development of the consensus statements and the drafting and revising of this manuscript, and share the role as lead authors.

CRediT authorship contribution statement

Nicolò Peccatori: Writing – review & editing, Methodology, Conceptualization. **Gianluigi Ardissino:** Writing – review & editing, Conceptualization. **Simone Cesaro:** Writing – review & editing, Conceptualization. **Romano Danesi:** Writing – review & editing, Conceptualization. **Stefania Gaspari:** Writing – review & editing, Conceptualization. **Cristina Meazza:** Writing – review & editing, Conceptualization. **Rossella Mura:** Writing – review & editing, Conceptualization. **Carmelo Rizzari:** Writing – review & editing, Conceptualization.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.retram.2025.103551.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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