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Original research

# Eight-year national multicenter experience on the use of glucarpidase as effective rescue therapy for delayed methotrexate elimination after high-dose methotrexate cycles administered in children with hemato-oncological diseases

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## ABSTRACT

*Background:* High-dose methotrexate (HDMTX) for cancer treatment can be complicated by delayed methotrexate elimination (DME) and associated acute kidney injury (AKI). This study evaluated glucarpidase for the treatment of DME and HDMTX-AKI in pediatric hemato-oncology patients.

*Methods:* This multicenter, retrospective study reviewed the medical records of pediatric patients (1–18 years) with ALL or NHL who were given glucarpidase as rescue therapy for DME or HDMTX-AKI at 13 Italian AIEOP centers between January 1, 2015, and June 30, 2023. Patients also received uniform supportive therapy, per study protocols and guidelines, to prevent and treat AKI.

*Results:* Data were available for 42/44 patients given glucarpidase, following i.v. HDMTX as monotherapy (non-high risk ALL [non-HR ALL], n=24), or within combined intensive chemotherapy blocks (HR-ALL, n=13; NHL, n=5). Median time to glucarpidase infusion was 53 hours (range 32–72). Most patients required glucarpidase during the first cycle of HDMTX. Glucarpidase led to a rapid decrease in plasma MTX levels (median 72.53 %; range 12.62–94.57 %). Median time for complete elimination of MTX and its metabolites was 216 hours (range 120–672). Recovery of renal function (return of serum creatinine to ≤1.5 times baseline value) took a median 18 days (range 4–72). Of the 22/42 patients (52 %) re-challenged with HDMTX (at 40–100 % of recommended dose), none experienced DME and all completed per-protocol number of HDMTX cycles.

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*Conclusions:* Our experience shows that glucarpidase is effective and well tolerated for the treatment of DME and HDMTX-AKI in pediatric patients with hemato-oncologic diseases.

## 1. Introduction

Methotrexate (MTX) is essential in the treatment of childhood cancers, including acute lymphoblastic leukemia (ALL), non-Hodgkin lymphoma (NHL), and osteosarcoma [1]. High-dose methotrexate (HDMTX; $\geq$500 mg/m$^2$) is associated with significant toxicity, which manifests predominantly as acute kidney injury (AKI; an increase in serum creatinine and decrease in glomerular filtration rate), myelosuppression, and hepatotoxicity. HDMTX-induced AKI (HDMTX-AKI) is associated with a multifactorial pathogenesis mainly resulting from pH-dependent crystallization of MTX in the renal tubular lumen, but also from direct MTX tubular toxicity and reduced renal perfusion [2,3].

Modern HDMTX treatment protocols include standardized, rigorous, supportive preventive measures to reduce the risk of severe toxicity, including aggressive hydration, urine alkalinization, and rescue therapy with folinic acid [4]. Nevertheless, HDMTX-AKI still represents a significant toxicity issue, occurring in around 2–13 % of patients receiving HDMTX [5–7]. A recent Delphi epidemiological study estimated incidence of delayed methotrexate elimination (DME) and HDMTX-AKI, respectively, as 20.0 % and 11.8 % in adults, and 13.6 % and 7.5 % in children [8]. Of note, for pediatric patients with ALL treated with HDMTX courses, HDMTX-AKI was reported to occur with a lower incidence, ranging from 1.3 % to 3.6 % of the patients receiving HDMTX [9,10], with a relative risk for DME per HDMTX course of 0.5 % [10].

The toxic effects exerted by HDMTX on tissues mainly depend on the duration of exposure rather than the peak level during infusion [2]. Therefore, when DME occurs, prolonged toxic plasma MTX concentrations can lead to severe toxicity, including myelosuppression, mucositis, hepatotoxicity, and further worsening of renal function. Timely and effective treatment for DME is essential to counteract these potentially life-threatening complications. The management of DME usually consists of increased hyper-hydration, optimized urine alkalinization, administration of higher doses of folinic acid proportionally to the MTX levels, and, in rare cases, extracorporeal techniques to remove MTX [4,11].

Glucarpidase (carboxypeptidase G2) is a recombinant bacterial enzyme that cleaves MTX into two non-toxic metabolites, 4-deoxy-4-amino-N10-methylpteroic acid (DAMPA) and glutamate, which are metabolized by the liver, providing an effective strategy to inactivate circulating MTX in patients with AKI [12].

Glucarpidase was approved by the US Food and Drug Administration in 2012 for the treatment of toxic plasma concentrations of MTX in patients with DME due to impaired renal function [13]. US consensus guidelines on the use of glucarpidase for HDMTX-AKI and DME have been published [2]. Glucarpidase has also recently been approved by the European Medicines Agency to reduce toxic plasma MTX concentrations in patients with DME or at risk of MTX toxicity [14]. In the absence of European-based guidelines, and with few specific indications for use in the pediatric setting, the decision to administer glucarpidase often rests with the treating physician.

Herein, we report a case series of children diagnosed with ALL or NHL, treated in the centers of "*Associazione Italiana di Ematologia e Oncologia Pediatrica*" (AIEOP), who received glucarpidase due to DME and/or HDMTX-AKI from 2015 to 2023.

## 2. Patients and methods

This multicenter, national, retrospective, observational study reviewed the medical records of pediatric patients (aged >365 days and <18 years) diagnosed with ALL or NHL who received glucarpidase as rescue therapy for DME or HDMTX-AKI at AIEOP centers between January 1, 2015, and June 30, 2023.

Patients with ALL were treated according to the AIEOP-BFM ALL 2009 (NCT01117441) or AIEOP-BFM ALL 2017 (NCT03643276) protocols. Patients with NHL were treated according to the AIEOP LNH-97 protocol [15]. Patients with Philadelphia-chromosome positive ALL (Ph+ ALL) were treated according to the EsPhALL 2010 (NCT00287105) or EsPhALL2017/COGAALL1631 protocols (NCT03007147). HDMTX dosages are shown in Table 1. These protocols include uniform

**Table 1**
HDMTX courses per protocol by risk classification and recommended supportive measures.

| Diagnosis | Protocol | Risk group | N | HDMTX #numbers of courses and dosages | Time of MTX plasma level measured | Supportive measures | Folinic acid (i.v.) rescue | Recommended procedures in case of DME |
|---|---|---|---|---|---|---|---|---|
| **ALL** | AIEOP-BFM ALL 2009 (NCT01117441) or AIEOP-BFM ALL 2017 (NCT03643276) or EsPhALL 2010 (NCT00287105) or EsPhALL2017/ COGAALL1631 (NCT03007147) | Non-HR | 24 | 4 ×5 g/m$^2$/24 hours every 2 weeks (Protocol M) | 24, 36[a], 42, 48 hours after the start of HDMTX and, in case of DME, until MTX level <0.25 µmol/L | - Hyper-hydration (3000 mL/m$^2$/24 hours) at least 6 hours before and for 48 hours after the start of HDMTX<br>- Urinary pH stabilization >7 by sodium bicarbonate administration, during the 48 hours following the start of HDMTX or, in case of DME, until MTX level <0.25 µmol/L | **Timing**<br>Every 6 hours since 42 hours after start of HDMTX, until MTX level <0.25 µmol/L<br>**Dosage**<br>- 15 mg/m$^2$ i.v. (racemic product)<br>- 7.5 mg/m$^2$ i.v. (levoproduct) | - Hyper-hydration 4500 mL/m$^2$/24 hours with careful fluid balancing and frequent electrolyte tests<br>- Administration of folinic acid 30 mg/m$^2$ if MTX level >3 µmol/L at 36 hours<br>- 6-hourly folinic acid rescue continued until MTX level <0.25 µmol/L with dose adjustment according to MTX level determined 6 hours prior, as specified by the protocol nomogram<br>- Daily monitoring of serum creatinine and urine output until complete excretion of MTX was confirmed or until AKI was resolved |
| | | HR | 13 | 2 ×5 g/m$^2$/24 hours (HR1, HR2) | | | | |
| **NHL** | NHL−97 (15) | R1 | - | 2 ×1 g/m$^2$/24 hours (Courses A, B) | | | | |
| | | R2 | - | 4 ×1 g/m$^2$/24 hours (Courses A, B) | | | | |
| | | R3/R4 | 5 | 4 ×5 g/m$^2$/24 hours (Courses AA, BB) | | | | |

**Abbreviations**: AKI, acute kidney injury; ALL, acute lymphoblastic leukemia; DME, delayed methotrexate elimination; HDMTX, high-dose methotrexate; HR, high risk; i.v., intravenous; MTX, methotrexate; NHL, non-Hodgkin lymphoma

[a] Only in cases when DME was suspected based on clinical and hematological data obtained at the 24-hour timepoint (such as onset of diarrhea or severe vomiting during MTX infusion, or significant increase in serum creatinine compared with baseline).

**Table 2**
Recommended folinic acid doses (given every 6 hours) for elevated MTX levels, applicable to levels from 42 hours onwards.

| Plasma MTX level (μmol/L) | Folinic acid rescue dose (mg/m$^2$)[a] |
|---|---|
| **0.25–1.0** | 15 |
| **>1.0–2.0** | 30 |
| **>2.0–3.0** | 45 |
| **>3.0–4.0** | 60 |
| **>4.–5.0** | 75 |
| **>5.0** | mg = plasma MTX level [μmol/L] x KG [kg][b] |

**Abbreviations**: ALL, acute lymphoblastic leukemia; MTX, methotrexate
[a] Doses listed are for the racemic product and must be halved for levoproduct.
[b] In October 2023, a risk of calcium overdose warning for this formula was issued by the AIEOP-BFM ALL 2017 study coordinating unit. It was recommended to not administer more than 625 mg/m$^2$ of calcium levofolinate in a single dose, to prevent any potentially dangerous side effects secondary to the high calcium content of calcium folinate products.

indications for monitoring plasma MTX and supportive care guidelines for managing HDMTX toxicity (Table 1), including hyper-hydration, urine alkalinization, and folinic acid administration (Table 2). We also retrospectively evaluated our cohort to determine in which patients glucarpidase would have been indicated based on the consensus recommendations published by Ramsey et al. [2].

Plasma MTX levels were determined using fluorescence polarization immunoassay (FPIA). AKI was defined as a rise in serum creatinine >1.5 times the patient's baseline value occurring at any time after HDMTX infusion start. The definition of DME according to the protocols is reported in Supplementary Table 1; however, this was not used to guide glucarpidase administration.

In the absence of indications for glucarpidase use in the protocols, glucarpidase was given as rescue therapy in case of severe DME or HDMTX-AKI at the discretion of the treating physician. Glucarpidase was administered intravenously at the recommended dose of 50 IU/kg over 5 minutes [13,14]. Folinic acid was not administered within 2 hours before or after glucarpidase administration. Thereafter folinic acid was continued at least for the subsequent 48 hours, with dosage calculated according to plasma MTX level (measured by FPIA and as specified by the study protocols). Folinic acid rescue was stopped when the plasma MTX level was below the threshold according to the treatment protocols (<0.25 μmol/L). Recovery of renal function was defined as the return of serum creatinine to ≤1.5 times the baseline value.

Data were extracted from the AIEOP database and completed using a specific clinical report form sent to each of the AIEOP centers (n=13) that administered glucarpidase to at least one patient during the study period. Data included demographics, MTX doses and plasma levels, serum creatinine values, glucarpidase dose, and follow-up information. Toxicity data were collected and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

All guardians of included patients gave informed consent to data collection related to each single study, including toxicity patterns. The study was performed in accordance with the Declaration of Helsinki and approved by each AIEOP center's ethics committee.

Descriptive statistics were used. Quantitative variables were expressed as median and range and qualitative variables as percentage.

## 3. Results

Of 44 patients who received glucarpidase, 42 had available data. Patient characteristics are summarized in Table 3. The incidence of glucarpidase administration in the overall cohort of patients with ALL treated in the 13 AIEOP centers who received HDMTX in the study period was 2.69 % (37/1375).

Considering risk factors associated with DME, two patients were affected by syndromic conditions: one ataxia-telangiectasia (A-T) syndrome and one microdeletion on chromosome 9. The latter patient also

**Table 3**
Demographic, hematological, and clinical characteristics of 42 patients who received glucarpidase.

| Characteristics | n (%) |
|---|---|
| **Age (years)** | |
| Median | 13 |
| 1–2 | 1 (2) |
| 3–10 | 12 (29) |
| 11–18 | 29 (69) |
| **Sex** | |
| Male | 32 (76) |
| Female | 8 (19) |
| Unknown | 2 (5) |
| **Diagnosis** | |
| ALL | 37 (88) |
| BCP-ALL | 29 (78) |
| T-ALL | 4 (11) |
| Ph+ ALL | 4 (11) |
| NHL | 5 (12) |
| Primary mediastinal lymphoma | 2 (5) |
| DLBCL | 2 (5) |
| Gray zone lymphoma | 1 (2) |
| **ALL risk group** | |
| Standard/intermediate risk | 24 (65) |
| High risk | 13 (35) |
| **Additional therapy for AKI**[a] | |
| Furosemide | 22 (52) |
| Analgesic morphine | 9 (21) |
| Anti-hypertensive drugs | 6 (14) |
| Hemodialysis (after glucarpidase) | 3 (7) |
| Plasma exchange (before glucarpidase) | 1 (2) |

**Abbreviations**: AKI, acute kidney injury; ALL, acute lymphoblastic leukemia; BCP-ALL, B-cell precursor ALL; DLBCL, diffuse large B-cell lymphoma; NHL, non-Hodgkin lymphoma; Ph+ ALL, Philadelphia-chromosome positive ALL; T-ALL, T-cell ALL
[a] Refer to the Patients and Methods section for AKI definition.

presented a body mass index >97th percentile. No additional patients presented with obesity. All patients treated with glucarpidase received supportive therapy to prevent and treat AKI, according to protocol recommendations (Table 1). Additional treatment for AKI most commonly comprised furosemide (22/42 [52 %] patients]) (Table 3).

In all but one patient, HDMTX was infused at 5 g/m$^2$ over 24 hours. In the patient affected by A-T syndrome, HDMTX was administered at 3 g/m$^2$.

The median time between start of HDMTX infusion and glucarpidase infusion was 53 hours (range 32–72). Glucarpidase was given intravenously over 5 minutes at 50 IU/kg in all patients except one who received half the recommended dosage (25 IU/kg) due to availability. Most patients received a single dose of glucarpidase, with only two receiving an additional dose due to persistently high MTX levels after the first dose. These patients received their first/second doses of glucarpidase at 48/75 hours and 32/60 hours, and had MTX levels prior to first/second glucarpidase dose of 10.7/7.4 μmol/L and 95.0/40.4 μmol/L, respectively (measured by FPIA). No patient experienced glucarpidase-related adverse effects.

Among the 24 patients with non-HR ALL who developed DME during Protocol M, 16 (67 %) required glucarpidase during the first cycle of HDMTX, two (8 %) during the second, five (21 %) during the third, and one (4 %) during the fourth cycle. Among the 13 patients classified as high risk, 10 (77 %) developed DME during HR1 course and three (23 %) during HR2 course. Among the five patients with NHL, one developed DME during the first AA course, two during the second AA course, and two during the second BB course.

Table 4 presents the median (range) plasma MTX level at 24, 42, and 48 hours from the start of MTX infusion, and plasma MTX level before and after glucarpidase administration. Glucarpidase administration resulted in a rapid decrease in plasma MTX (median 72.53 %; range 12.62–94.57 %), measured by comparing the last plasma MTX level

**Table 4**
Plasma MTX levels, serum creatinine levels, and glucarpidase administration time for 42 patients who received glucarpidase.

| | Median | Range |
|---|---|---|
| **Glucarpidase administration time from HDMTX start (hours)** | 53 | 32–72 |
| **24-hour plasma MTX level (μmol/L)** | 104.7 | 19–327 |
| **42-hour plasma MTX level (μmol/L)** | 18.35 | 4.2–127 |
| **48-hour plasma MTX level (μmol/L)** | 11.75 | 3.02–110 |
| **Pre-glucarpidase plasma MTX level (μmol/L)** | 10.76 | 2–104 |
| **Post-glucarpidase plasma MTX level (μmol/L)** | 4.2 | 0.45–83 |
| **Reduction in MTX plasma level after glucarpidase as measured by FPIA (%)** | 72.53 | 12.62–94.57 |
| **Serum creatinine level before HDMTX courses (mg/dL)** | 0.48 | 0.2–0.8 |
| **Serum creatinine level measured immediately before glucarpidase administration (mg/dL)** | 1.97 | 0.54–4.53 |
| **Maximum serum creatinine level (mg/dL)** | 2.3 | 0.7–7 |
| **Time to renal recovery[a] (days)** | 18 | 4–72 |

**Abbreviations**: FPIA, fluorescence polarization immunoassay; HD, high-dose; MTX, methotrexate

[a] Refer to the Patients and Methods section for renal recovery definition.

before glucarpidase and the first plasma MTX level after glucarpidase. In the patient given a reduced dose of glucarpidase (25 IU/kg), plasma MTX decreased by 58 %, with an MTX clearance time of 9 days. Overall, the median time for MTX elimination, defined according to treatment protocols as MTX level below 0.25 μM/L, was 216 hours (range 120–672). It is important to underline that all plasma MTX levels after the administration of glucarpidase reported here were measured with FPIA; therefore, these levels also comprised metabolites deriving from the MTX cleavage ensuing from the administration of glucarpidase.

The median serum creatinine level immediately prior to glucarpidase administration was 1.97 mg/dL (range 0.54–4.53), and the median peak serum creatinine level was 2.3 mg/dL (range 0.7–7). All patients who received glucarpidase experienced AKI, with maximum serum creatinine concentration increased to ≥1.5 times the baseline value. All but one patient (who died) recovered renal function (return of serum creatinine to ≤1.5 times baseline value), within a median 18 days (range 4–72).

We retrospectively applied to our cohort of patients the criteria for the use of glucarpidase in patients with HDMTX-AKI and DME recommended by the consensus guidelines published by Ramsey et al. [2]. In our cohort, glucarpidase was administered in accordance with these guidelines in 38/42 patients, as shown in Table 5. In two patients, glucarpidase was administered 60 hours after the start of infusion due to HDMTX-AKI and 48-hour MTX levels of 3.7 and 3.75 μmol/L. In the other two patients, early administration of glucarpidase was initiated

**Table 5**
Patients who received glucarpidase in accordance with current guidelines.

| | Glucarpidase administration in accordance with Ramsey et al. guidelines [2] | | | Glucarpidase administration not in accordance with Ramsey et al. guidelines [2] |
|---|---|---|---|---|
| | 36-hour plasma MTX >30 μM and AKI | 42-hour plasma MTX >10 μM and AKI | 48-hour plasma MTX >5 μM and AKI | |
| **Number of patients who received glucarpidase (n=42)** | 5 | 11 | 22 | 4 |

**Abbreviations:** AKI, acute kidney injury; MTX, methotrexate

(32 hours after start of infusion) due to AKI and high 24-hour MTX levels of 184 and 140 μmol/L.

Considering non-nephrological HDMTX-related grade ≥3 adverse events, 13 patients (31 %) experienced hematologic toxicity, eight (19 %) mucocutaneous toxicity, eight (19 %) gastrointestinal toxicity, three (7 %) hepatic toxicity (increase in alanine aminotransferase/aspartate aminotransferase levels), and three (7 %) neurological toxicity, all of which resolved. One treatment-related death occurred: this patient had Ph+ ALL, chromosome 9 microdeletion, and obesity, and developed severe mucositis, hepatic toxicity, renal failure, and sepsis during severe and prolonged neutropenia.

## 4. Re-challenge with HDMTX

After receiving glucarpidase, 22/42 patients (52 %) were re-challenged with HDMTX, all of whom safely completed the per-protocol number of HDMTX cycles.

No patient was immediately re-challenged with full-dose HDMTX; doses were increased and ranged from 40–100 % of the recommended dose, with four patients (18 %) reaching the full dosage (5 g/m$^2$). No patient experienced further DME. Among the 20 patients who were not re-exposed to HDMTX (48 %), 11 should have received per-protocol further HDMTX but parents declined owing to concerns over repeat DME; of these, seven (16 %) received a course with high-dose cytarabine in place of HDMTX and four omitted other high-dose therapies. Of the remaining nine patients, five had already completed the per-protocol cycles of HDMTX, three patients with high-risk ALL continued without HDMTX (per-protocol indication), and one patient died (Fig. 1).

When comparing the groups of patients who were/were not re-challenged with HDMTX, no specific characteristics were identified as specific indicators for re-challenge; all re-challenged patients had fully normalized renal function at the time of re-challenge.

Of the 42 patients, 38 (90 %) are currently in complete remission (CR), three (7 %) subsequently underwent stem cell transplantation due to relapse and are currently in second CR, and two (5 %) died (one patient from septic shock during HDMTX treatment and one patient after disease relapse).

## 5. Discussion

This multicenter, national, retrospective study describes the characteristics and outcomes of a series of pediatric patients uniformly given HDMTX as part of ALL or NHL treatment and requiring glucarpidase due to DME and/or AKI, along with the feasibility of re-challenging patients with HDMTX.

The incidence of glucarpidase administration in our cohort was consistent with that in previous pediatric studies [9,10]. There was a median 72.53 % decrease of plasma MTX after glucarpidase administration, also consistent with previous pediatric studies that report a 75–80 % decrease in plasma MTX following glucarpidase, when measured using immunoassay [9,10].

Immunoassay techniques are known to overestimate plasma MTX after glucarpidase due to cross-reactivity of MTX with its non-cytotoxic metabolite DAMPA; however, the results of tests performed with these methods likely approximate intracellular MTX concentrations, which allows for an appropriate folinic acid rescue even after glucarpidase administration [2]. In a cohort of 20 pediatric oncology patients given glucarpidase for DME and HDMTX-AKI, the median (range) reported decrease in plasma MTX was 80 % (61.9–95.9 %) by FPIA and 99.6 % (99.2–99.9 %) using high-performance liquid chromatography (HPLC) [9]. Similar reductions in plasma MTX (median 93–100 %) following glucarpidase have been reported in other studies of pediatric patients when using HPLC [10,16,17]. The most accurate method for measuring plasma MTX after glucarpidase is HPLC–mass spectrometry (HPLC-MS); however, this method has very limited availability and does not measure intracellular MTX levels. Therefore, measuring MTX plasma levels by

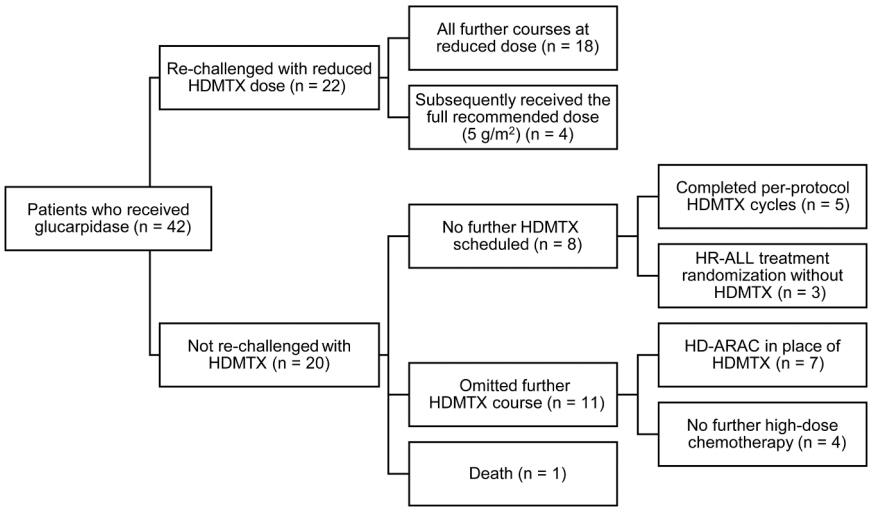


**Fig. 1.** Re-challenge with HDMTX. **Abbreviations**: HDMTX, high-dose methotrexate; HR-ALL, high-risk acute lymphoblastic leukemia; HD-ARAC, high-dose cytarabine

HPLC only leads to an underestimation of the actual intracellular MTX content and might result in lower than needed folinic acid doses [2]. Immunoassay is a more widely available tool that is usually sufficient to show the progressive decrease in plasma MTX concentration following glucarpidase; its reliability increases 48 hours after glucarpidase administration given that the half-life of DAMPA is approximately 10 hours [12].

Clinical studies in adult and pediatric patients show a rapid onset of action by glucarpidase, leading to a >95 % median decrease of MTX concentration (measured by HPLC) within 15 minutes [17,18]. Nevertheless, the timing of glucarpidase is crucial for preventing potentially life-threatening toxicities in the setting of DME: Widemann et al. found that delaying glucarpidase beyond 96 hours from the start of HDMTX infusion increased the risk of severe toxicity and mortality [19], and consensus guidelines state that administration within 48–60 hours is optimal [2]. Real-world data further support earlier intervention with glucarpidase in patients with HDMTX-related toxicities for reducing length of hospital stay [20]. Our patients received glucarpidase within 96 hours (range 32–72) from the start of HDMTX infusion. Therefore, it seems advisable for institutions in which patients are treated with HDMTX to implement all necessary measures to ensure rapid access to glucarpidase.

Although the biochemical efficacy of glucarpidase has been widely demonstrated, there are scarce data about the impact on clinical outcome, and no randomized trials have been conducted. The results of a first study comparing clinical outcomes of adult patients receiving glucarpidase for HDMTX-AKI with those of untreated controls have recently been published [21]. This study reported higher odds of renal recovery after glucarpidase compared with no glucarpidase, particularly when administered within 60 hours from the start of HDMTX. In our study of pediatric patients who received glucarpidase, all but one patient (who died) recovered renal function.

In our cohort, glucarpidase was administered at the recommended dose in all but one patient. In this case, a half-dose was given due to difficulties in procurement, and a decrease in plasma MTX level of 52 % was documented. Small retrospective studies of glucarpidase used at lower doses in children and adults report clinically significant reductions in plasma MTX levels [22,23], and a similar time to serum creatinine recovery compared with full-dose glucarpidase [22], but more data are needed to determine the safety and efficacy of lower-dose glucarpidase.

Age is a reported predictor of DME and HDMTX toxicity in pediatric patients [24], and consistent with those findings, 69 % of patients in our study were >11 years old, while only 2 % were <3 years old. This phenomenon is well explained by the different pharmacokinetics observed in different-aged children [25].

In our cohort, 64 % of patients experienced DME and HDMTX-AKI after the first planned HDMTX course. This finding correlates well with literature reports that the vast majority of HDMTX toxicity occurs during the first treatment cycles [10,19].

There are few published data about re-challenging with HDMTX after glucarpidase in pediatric oncology patients. Christensen et al. reported on 13 pediatric patients in whom HDMTX after glucarpidase was very well tolerated, with only one case of DME upon re-challenge of HDMTX and no further requirement for glucarpidase. Of note, nine patients received a reduced HDMTX dose (50–75 % of the recommended dose) and four received the full recommended dose [9]. Furthermore, a study conducted by the Nordic Organization for Pediatric Hematology and Oncology in pediatric patients with ALL receiving glucarpidase for DME demonstrated that subsequent HDMTX courses can be administered without severe adverse events in most patients. In this prospective study, 45/47 patients received a full dose in the subsequent HDMTX course (5 g/m$^2$) without signs of DME recurrence [10]. In our experience, 22/42 patients were re-challenged with HDMTX with no further episodes of DME. Although none were immediately re-exposed to the full per-protocol dose of HDMTX, four patients who tolerated a reduced dose subsequently received the full dose, showing good MTX clearance. In our study, we were not able to identify specific parameters, such as patient characteristics, HDMTX dosing regimens, or clinical, pharmacological, and laboratory findings, that allowed the formulation of solid assumptions regarding conditions for re-challenge.

However, in our opinion, and also based on the information available in the literature, re-challenge with HDMTX may be considered a viable option in patients previously treated with glucarpidase because of severe DME and AKI.

Notably, the present case series is one of the largest cohorts of pediatric patients treated with glucarpidase reported to date. However, it has some limitations. Given its impact on MTX clearance, it is important

to underline the value of screening for hypoalbuminemia before administration of HDMTX [26]. Unfortunately, this information was not available for this case series, meaning we cannot speculate on any potential impact of hypoalbuminemia. Other limitations are that glucarpidase administration was solely based on the decision of local treating physicians (i.e., without formal indication or defined threshold MTX plasma concentration or serum creatinine elevation) and that the retrospective nature and long timespan of the study could, over the years, have influenced the attitude of physicians in adopting the countermeasures reported in this paper. For the above-mentioned reasons, we do not make specific recommendations on the optimal indications and timing of glucarpidase administration based on our retrospective data. However, based on our knowledge of the literature, the US guidelines published in 2018 [2] represent a valid and reliable decision-making tool.

## 6. Conclusions

In conclusion, our experience shows that glucarpidase is effective and well tolerated for the treatment of DME and HDMTX-AKI in pediatric patients with hemato-oncologic diseases and may contribute to a safe continuation of the treatment journey of these patients.

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## CRediT authorship contribution statement

**Nicolò Peccatori**: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Marta Coppola**: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Antonella Colombini**: Writing - review & editing. **Daniela Silvestri**: Statistical analysis, Writing - review & editing. **Nicoletta Bertorello**: Data collection, Writing - review & editing. **Valentina Kiren**: Data collection, Writing - review & editing. **Fraia Melchionda**: Data collection, Writing - review & editing. **Rosamaria Mura**: Data collection, Writing - review & editing. **Daniela Onofrillo**: Data collection, Writing - review & editing. **Simona Gobbi**: Data collection, Writing - review & editing. **Raffaele Mattera**: Data collection, Writing - review & editing. **Luciana Vinti**: Data collection, Writing - review & editing. **Tommaso Casini**: Data collection, Writing - review & editing. **Nicola Santoro**: Data collection, Writing - review & editing. **Domenico Sperlì**: Data collection, Writing - review & editing. **Carmelita D'Ippolito**: Data collection, Writing - review & editing. **Valentino Conter**: Writing - review & editing. **Andrea Biondi**: Writing - review & editing. **Carmelo Rizzari**: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing, supervision.

All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

## Declaration of Competing Interest

The author, Carmelo Rizzari, is a Section Editor for *EJC Paediatric Oncology* and was not involved in the editorial review or the decision to publish this article. The authors declare the following financial interests/personal relationships, which may be considered as potential competing interests: Carmelo Rizzari and Rosamaria Mura received fees from SERB Pharmaceuticals to attend advisory boards and to give speeches at sponsored symposia, as well as support for travel and meeting registration. The remaining authors have nothing to declare.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejcped.2024.100202.

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