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ARTICLE

Propsective evaluation of high-dose methotrexate pharmacokinetics in adult patients with lymphoma using novel determinants of kidney function

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Abstract

High-dose methotrexate (HDMTX) pharmacokinetics (PKs), including the best estimated glomerular filtration rate (eGFR) equation that reflects methotrexate (MTX) clearance, requires investigation. This prospective, observational, single-center study evaluated adult patients with lymphoma treated with HDMTX. Samples were collected at predefined time points up to 96 h postinfusion. MTX and 7-hydroxy-MTX PKs were estimated by standard noncompartmental analysis. Linear regression determined which serum creatinine- or cystatin C-based eGFR equation best predicted MTX clearance. The 80 included patients had a median (interquartile range [IQR]) age of 68.6 years (IQR 59.2–75.6), 54 (67.5%) were men, and 74 (92.5%) were White. The median (IQR) dose of MTX was 7.6 (IQR 4.8–11.3) grams. Median clearance was similar across three dosing levels at 4.5–5.6 L/h and was consistent with linear PKs. Liver function, weight, age, sex, concomitant chemotherapy, and number of previous MTX doses did not impact clearance. MTX area under the curve (AUC) values varied over a fourfold range and appeared to increase in proportion to the dose. The $eGFR_{cys}$ (ml/min) equation most closely correlated with MTX clearance in both the entire cohort and after excluding outlier MTX clearance values ($r = 0.31$ and 0.51, respectively). HDMTX as a 4-h infusion displays high interpatient pharmacokinetic variability. Population PK modeling to optimize MTX AUC attainment requires further evaluation. The cystatin C-based eGFR equation most closely estimated MTX clearance and should be investigated for dosing and monitoring in adults requiring MTX as part of lymphoma management.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Methotrexate (MTX) clearance has a relationship with glomerular filtration rate (GFR), which is often calculated using serum creatinine as a surrogate marker

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of renal clearance; however, kidney function estimation derived from serum creatinine-based GFR formulas has several known limitations, particularly in patients with cancer.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study attempts to answer the question of which estimated GFR (eGFR) equation has the strongest correlation with MTX clearance.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Results of this study suggest that, when high-dose MTX is administered, cystatin C based eGFR equations more strongly correlate with MTX clearance than eGFR equations based on serum creatinine alone.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Incorporating cystatin C into baseline evaluation when estimating kidney function has potential to improve MTX safety and optimize MTX exposure.

INTRODUCTION

High-dose methotrexate (≥1 gram/meter^2 [g/m^2]; HDMTX) is a cornerstone of treatment for central nervous system (CNS) lymphoma and an effective agent in the prophylaxis against CNS relapse of disease.[1,2] Optimizing methotrexate (MTX) exposure is necessary to maximize treatment efficacy and minimize dose-limiting toxicity.[3] MTX appears to readily cross the blood-brain barrier and achieve therapeutic levels in the CNS when more than 3 g/m^2 is administered.[4,5] MTX dosing strategies exist for primary CNS lymphoma ranging between 3.5 g/m^2 and 8 g/m^2; however, there is a lack of consensus for a preferred MTX strength, frequency, or number of doses.[2,6–8] Secondary prevention in patients with lymphoma at high-risk for CNS relapse is even less clear with doses ranging from 1 g/m^2 to 3.5 g/m^2.[2,9,10]

A thorough understanding of MTX pharmacokinetics (PKs) is important to maximize medication efficacy and safety. MTX area under the concentration-time curve (AUC) has been suggested as an important parameter for disease response with a proposed target range between 980 μmol·h/L (445 mg*h/L) and 1100 μmol·h/L (500 mg*h/L).[11,12] Additionally, an extremely elevated MTX AUC was associated with a decreased progression-free and overall survival in elderly patients.[13,14] Prolonged exposure after HDMTX administration places patients at increased risk for toxicities, including nephrotoxicity, dermatitis, hepatitis, debilitating mucositis, and life-threatening myelosuppression.[15] Historical studies have indicated that the best surrogate marker for HDMTX-associated toxicity is delayed MTX elimination, defined as a serum MTX concentration greater than or equal to 1 μmol/L (0.454 mg/L) at 48 h.[16–18] Whereas each organ-specific toxicity has a variable incidence depending on the study design and patient population observed, MTX-associated nephrotoxicity can occur in 5% to 40% of patients despite leucovorin administration.[19,20]

Renal clearance accounts for at least 90% of the MTX dose excretion as both an unchanged drug and its metabolite.[21] MTX clearance has a long-standing, well-known relationship with glomerular filtration rate (GFR), which is often calculated using serum creatinine as a surrogate marker of renal clearance.[22] Additionally, serum creatinine has been the solitary renal marker used to estimate GFR in population PK models for MTX.[23–25] The proven link between MTX and kidney function makes an accurate assessment of GFR paramount to appropriate dose selection and clearance estimation. Kidney function estimation derived from serum creatinine-based GFR formulas has several known limitations, particularly in patients with cancer, including advanced age, variable volume status, acute and chronic inflammation, reduced skeletal muscle mass, and deconditioning.[26–28]

Cystatin C is a serum marker of GFR that is less dependent on age, sex, race, or muscle mass than creatinine.[27] There is a recent increase in the use of cystatin C to inform drug dosing and monitor dynamic kidney function.[29,30] A vancomycin dosing algorithm based on cystatin C-inclusive estimated GFR (eGFR) equations improved target trough concentration achievement compared with creatinine-based methods.[31] Additionally, cystatin C has successfully been used in conjunction with, or as an alternative to, creatinine to dose antineoplastics, including carboplatin and topotecan.[32,33] Our study is a first attempt to characterize the dose-exposure relationship in patients prescribed HDMTX through noncompartmental analysis (NCA) that incorporates cystatin C into contemporary eGFR equations.

METHODS

Setting and participants

This prospective, single-center study included adult (≥18 years old) patients with histologically confirmed lymphoma admitted to Mayo Clinic in Rochester, Minnesota, for intravenous HDMTX between January 2018 and December 2019. Patients were eligible for inclusion with a new diagnosis or relapse of disease and could be enrolled with any number of previous MTX exposures. Exclusion criteria consisted of any patient receiving an MTX infusion scheduled to be administered longer than 4 h, as dictated by the treatment regimen, if they presented with any acute kidney injury (AKI) stage, or were currently receiving renal replacement therapy.[34] All patients provided written informed consent or had consent provided for them by their legal power of attorney before data collection and sampling. The protocol was approved by the Mayo Clinic Institutional Review Board and adhered to the ethical standards of the 1964 Declaration of Helsinki with adherence to all relevant regulations of the US health insurance portability and accountability act.

An HDMTX dose between 1.5 g/m^2 and 8 g/m^2 was prescribed per published treatment protocols. Selection of the HDMTX-inclusive regimen was at the discretion of the primary hematologist or the hospital-based care team. These protocols included HDMTX administered as monotherapy every 21–28 days, the combination of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone every 21 days with HDMTX administered on day 14 of the cycle (MR-CHOP), or HDMTX in combination with rituximab and temozolomide (MRT).[2,6,35] Details about the treatment regimens, MTX administration, and supportive management are available in the Supplementary Materials.

Study procedures

Clinical care was unaffected by study procedures. All data collected for the study were suppressed from the electronic health record. Upon admission for HDMTX, a trained study nurse from the Mayo Clinic Clinical Research Trials Unit obtained baseline study specimens. These were typically performed within 1–3 h of beginning pre-hydration with all baseline samples obtained before HDMTX administration. Blood samples for serum creatinine and cystatin C were measured at baseline, whereas blood samples for serum MTX concentration measurements were obtained at baseline, the end of infusion, 30-min after infusion completion, and at 12, 24, 48, and 96 h after the infusion. Participants could refuse any of the draws and, if discharged before the 96-h time point, no further specimens were obtained.

Serum creatinine and serum cystatin C were analyzed by the Mayo Clinic Central Chemistry and Renal Testing laboratories. All analyses were conducted by technicians masked to clinical data. Serum creatinine samples were assayed using the standardized (isotope dilution mass spectrometry traceable) Roche enzymatic creatinine assay. The interassay coefficients of variation for replicate samples using this method are 1.26% and 0.80%. Cystatin C was assayed on a Roche autoanalyzer using the standard clinically validated particle enhanced turbidimetric assay (PETIA) (Gentian AS, Moss, Norway). This assay is traceable to the same international certified cystatin C reference material (ERM-DA471/IFCC) used to develop the cystatin C-based Chronic Kidney Disease Epidemiology Collaborative (CKD-EPI) equations.[36] The eGFR was calculated from serum creatinine alone, serum cystatin C alone, or both biomarkers combined using one of four equations: the Cockcroft-Gault (C-G) estimated creatinine clearance (eCrCl) and the three CKD-EPI eGFR formulae (creatinine, cystatin C, and creatinine and cystatin C).[22,37] All CKD-EPI eGFR estimates were assessed as milliliters per minute (ml/min) and milliliters per minute per 1.73 m^2 (ml/min/1.73 m^2).[38]

In addition to prospectively collected patient specimens, data were abstracted from existing information available in the electronic health record. Patient demographics (age, sex, race, and ethnicity), height and weight, comorbid conditions, and laboratory data were collected. Other information gathered included date of cancer diagnosis, type of malignancy, indication for HDMTX (prophylaxis or treatment), chemotherapy regimen, protocol-defined dose, and delivered dose of MTX. Short-term outcome metrics, including incident AKI, need for intermittent hemodialysis, hospital length of stay, or death within 30 days were also collected.

Methotrexate assay

Reagents and materials

MTX, d3-MTX, and 7-Hydroxy-MTX (7-OH-MTX) were purchased from Toronto Research Chemicals Inc. Formic acid (95%) was purchased from Sigma Aldrich (St. Louis, MO). High-performance liquid chromatography grade methanol (MeOH) and acetonitrile (ACN) were purchased from Fisher Scientific. Drug-free human plasma was obtained from healthy volunteers under a protocol approved by the Mayo Clinic Institutional Review Board.

Instrumentation

The liquid chromatography-mass spectrometry (LC-MS) system consisted of a Waters Acquity H class ultra-performance liquid chromatography (UPLC) system, containing a quaternary solvent manager and sample manager-FTN coupled to a Xevo TQ-S mass spectrometer (Waters) equipped with an electrospray ionization (ESI) source. Data were acquired and analyzed by Waters MassLynx version 4.1 software.

Chromatographic conditions

The liquid chromatographic separation of MTX and its major circulating metabolite 7-OH-MTX was accomplished using a Waters Acquity UPLC HSS PFP precolumn (2.1 × 5 mm, 1.8 μ; Waters, Milford, MA) attached to a Waters Acquity UPLC HSS PFP analytical column (2.1 × 100 mm, 1.8 μ; Waters) at 40°C, eluted with a gradient mobile phase composed of water containing 0.1% formic acid (A) and ACN containing 0.1% formic acid (B) with a constant flow rate of 0.4 ml/min and a total run time of 11 min. The elution was initiated at 95% A and 5% B for 2.0 min, then B was linearly increased from 95 to 20% for 6 min, followed by 2% A 98% B from 8.2 to 9.8 min and returned to initial conditions over 1.2 min. Autosampler temperature was 10°C, and the sample injection volume was 2 μl.

Mass spectrometry conditions

Detection of MTX and its metabolite was accomplished using the mass spectrometer in positive ESI mode with multiple reaction monitoring (MRM) operating under the following settings: capillary voltage 1.0 kV, source temperature 150°C, desolvation temperature 600°C, cone gas flow 150 L/h, and desolvation gas flow 900 L/h. The cone voltages and collision energies were determined by MassLynx-Intellistart, version 4.1, software and were 10 and 19 for MTX, 34 and 10 for 7-OH-MTX, and 38 and 18 for d3-MTX. The MRM precursor and product ions were monitored at m/z 455.27>308.21 for MTX, 471.24>324.32 for 7-OH-MTX, and 458.3>311.32 for d3-MTX.

Sample preparation

Stock solutions of MTX, d3-MTX, and 7-OH-MTX were prepared at 1 mg/ml in DMSO and stored at −20°C. Working standard solutions were prepared in a methanol to water ratio of 1:1. Plasma standards (10–5000 ng/ml) containing MTX and 7-OH-MTX were prepared by adding 5 μl of a 20X working standard solution to plasma (95 μl) added to 1.5 ml microcentrifuge tubes. Samples were processed using an Orochem crash plate (ChromTech) with 450 μl of methanol containing internal standard and 50 μl of the plasma sample in each well. The plate was shaken at 1100 RPM on an Eppendorf mixer for 20 min. The samples were collected into a 96-well plate using positive pressure. All samples were diluted 1/5 with water and 2 μl injected on the LC-MS.

Pharmacokinetic and statistical analyses

The PKs of MTX and 7-OH-MTX were estimated by standard noncompartmental analysis using the program Phoenix WinNonlin version 8.1 (Certara Corporation). The apparent terminal elimination rate constant (k_z) was determined by linear least-squares regression and elimination half-life calculated as $0.693/k_z$. The AUC from time zero (AUC_{last}) to the time of the last detectable sample (C_{last}) was calculated using the linear trapezoidal approximation. The AUC through infinite time ($AUC_{0-\infty}$) were calculated by adding the value C_{last}/k_z to AUC_{last}. MTX plasma clearance (CL_p) was calculated as dose/$AUC_{0-\infty}$. Partial AUC values (AUC_{0-24h} and AUC_{0-48h}) were also estimated by trapezoidal approximation. The Spearman correlation coefficient was used to compare PK parameters and patient characteristics. We fit linear regression models to determine whether varying the approach to baseline kidney assessment (eGFR based on C-G or CKD-EPI equations using serum creatinine, cystatin C, or both biomarkers) improved the prediction of drug clearance or AUC. Continuous data were summarized using mean ± SD or median with the interquartile range (IQR) depending on the distribution. Frequencies (percentages) were used to describe discrete data. The Wilcoxon signed-rank test was used to detect intra-individual differences in kidney function estimates.

RESULTS

Participant characteristics

A total of 80 individuals met eligibility criteria and were enrolled in this study. A summary of baseline demographics, clinical characteristics, and laboratory values are displayed in Table 1. Patients had a median (IQR) age of 68.6 (IQR 59.3–75.9) years, 54 (68%) were men, and 74 (93%) were White. The median (IQR) body weight of the patient population was 80.5 kg (IQR 69.7–92.3) and the median (IQR) body surface area (BSA) was 1.97 (IQR 1.80–2.14)

TABLE 1 Baseline characteristics, laboratory values, and clearance estimations of adult patients with lymphoma treated with HDMTX

Characteristic	Patients ($N = 80$)
Age (years), median (IQR)	68.6 (59.3–75.9)
Patients aged 65 years or older, *N* (%)	51 (64)
Male, *N* (%)	54 (68)
White, *N* (%)	74 (93)
Weight (kg), median (IQR)	80.5 (69.7–92.3)
BSA (m^2), median (IQR)	1.97 (1.80–2.14)
Male BSA	2.04 (1.93–2.15)
Female BSA	1.77 (1.68–1.93)
Diagnosis, *N* (%)	
DLBCL	44 (57)
Primary DLBCL of the CNS	23 (30)
EBV positive DLBCL of the elderly	6 (8)
Other	7 (9)
Bone marrow involvement, *N* (%)	10 (12.5)
Percent involvement, median (IQR)	15 (9–35)
Kidney parameters at baseline	
History of chronic kidney disease, *N* (%)	5 (6)
Serum creatinine (mg/dl)[a]	0.8 ± 0.4
Cystatin C (mg/dl)[a]	1.1 ± 0.5
Estimated kidney function (ml/min), mean ±SD	
Cockcroft-Gault eCrCl	99 ± 46
CKD-EPI $eGFR_{creatinine}$	93 ± 27
CKD-EPI $eGFR_{cystatin\ C}$	83 ± 26
CKD-EPI $eGFR_{creatinine\text{-}cystatin\ C}$	88 ± 24

Abbreviations: BSA, body surface area; CNS, central nervous system; CKD-EPI, chronic kidney disease epidemiology collaboration; DLBCL, diffuse large B cell lymphoma; EBV, Epstein-Barr virus; eCrCl, estimated creatinine clearance; eGFR, estimated glomerular filtration rate; HDMTX, high-dose methotrexate; IQR, interquartile range; m, meters.

[a]Values expressed as means ± SDs unless noted.

TABLE 2 Summary of MTX-based chemotherapy treatment information

Characteristic	Patients ($n = 80$)
Chemotherapy regimen distribution, *N* (%)	
HDMTX 8 g/m^2 with rituximab and temozolomide	37 (46)
HDMTX 3.5 g/m^2 in combination with R-CHOP	29 (36)
HDMTX monotherapy	14 (18%)
Protocol-defined dose, *N* (%)	
8 g/m^2	36 (45)
3.5 g/m^2	37 (46)
1.5 g/m^2	7 (9)
Delivered dose (g), median (IQR)	7.55 (4.83, 11.25)
Prevention of CNS involvement	4.85 (3.4, 6.85)
Treatment of active CNS disease	10.85 (7.8, 13.5)
MTX dose history	
First MTX dose, *N* (%)	38 (48)
Second MTX dose, *N* (%)	26 (32)
Beyond second MTX dose, *N* (%)	16 (20)
Number of doses beyond second dose, median (IQR)	4 (3, 9)

Abbreviations: CNS, central nervous system; g/m^2, grams per meter squared; HDMTX, high-dose methotrexate; IQR, interquartile range; MTX, methotrexate; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.

as calculated by the Du Bois method. Baseline estimated kidney function differed according to equation utilized and ranged between a mean clearance of 83 ml/min when calculated with CKD-EPI eGFR-CysC and a mean clearance of 99 ml/min when calculated with C-G eGFR. There was a mean difference of −15 ± 42 ml/min between CKD-EPI eGFR-CysC (mean 83 ml/min) and C-G eGFR (mean 99 ml/min). There were five patients (6%) with a baseline diagnosis of CKD.

The primary indication for HDMTX, according to the World Health Organization (WHO) classification criteria, was diffuse large B cell lymphoma ($n = 73$). The majority of patients (67; 84%) were newly diagnosed, with 13 (16%) patients enrolled with relapsed disease. Ten patients had bone marrow involvement. A summary of chemotherapy, according to the treatment regimen, is provided in Table 2. MTX was prescribed at a median (IQR) dose of 10.85 g (IQR 7.8 g–13.5 g) as treatment for active CNS involvement for 44 (55%) patients and a median (IQR) dose of 4.85 g (IQR 3.4 g–6.85 g) as CNS prophylaxis against relapse for 36 (45%) patients. At study inclusion, 38 (47.5%) participants were receiving their first HDMTX dose. Chemotherapy regimen distribution included 37 (46%) participants prescribed MRT, 29 (36%) prescribed MR-CHOP, and 14 (18%) prescribed HDMTX monotherapy. There were seven (9%) patients who received MTX that was dose-reduced to 1.5 g/m^2 from the 3.5 g/m^2 protocols (HDMTX monotherapy $n = 4$, MR-CHOP $n = 3$) at provider discretion. Those patients had a median (IQR) age of 78 (IQR 76–81) years.

Pharmacokinetics of MTX

The PKs of MTX and its primary oxidative metabolite 7-OH-MTX were characterized for all 80 patients who participated in this study. Representative plasma profiles

are illustrated in Figure 1 (A: MTX; B: 7-OH-MTX) for patients who received full protocol-defined and protocol-adjusted doses of 1.5 g/m^2, 3.5 g/m^2, or 8 g/m^2. PK data for MTX and 7-OH-MTX are summarized in Table 3. The AUC values varied over a fourfold range at each dose level and appeared to increase in proportion to dose over the range of 1.5 g/m^2 to 8 g/m^2 investigated in this study (Figure 2a). The percent AUC (%AUC) extrapolated was minimal, indicating that there was sufficient characterization of the terminal phase for most patients. MTX had a median of 0.04% (IQR 0.02–0.08) whereas 7-OH-MTX had a median of 4.49% (IQR 1.76–7.70). There were not any MTX AUC that had an extrapolation of greater than 20%; however, there were three 7-OH MTX AUC had an extrapolation of greater than 20%. Clearance was similar across the dose range (Figure 2b) and there was no statistically significant difference between dosing groups observed ($p = 0.55$), consistent with linear PKs. BSA-normalized clearance was also similar across the dose range, and the MTX half-life found in this study was 9.5 ± 6.7 h.

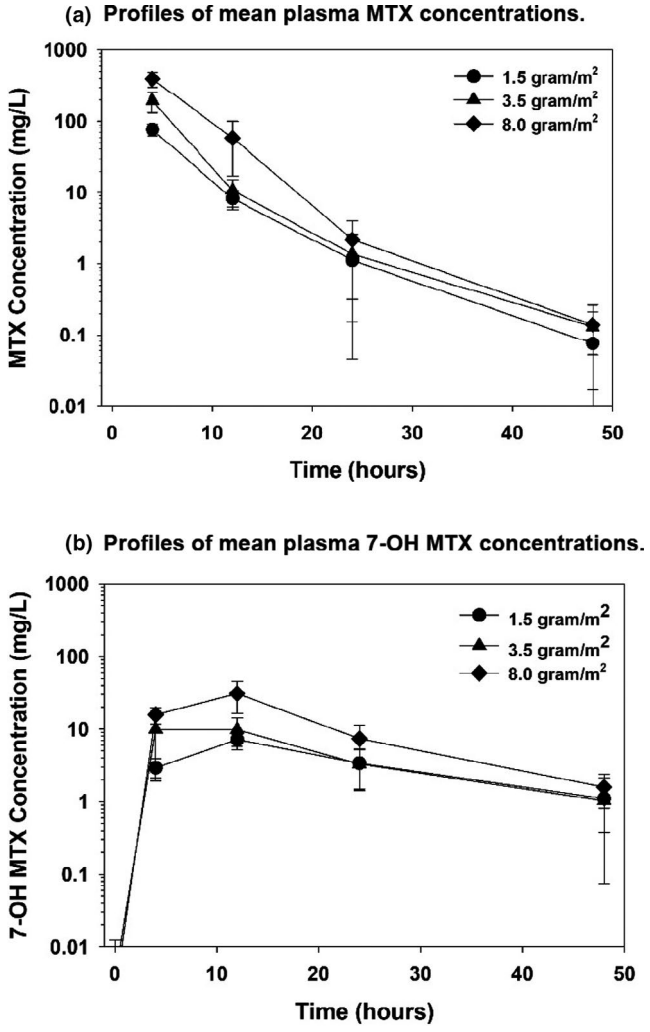

FIGURE 1 Representative plasma concentration profiles after receipt of HDMTX according to dosing group. The error bars represent the standard deviation for the mean value at each time point. (a) profiles of mean plasma MTX concentrations, (b) profiles of mean plasma 7-OH-MTX concentrations. 7-OH-MTX, 7-hydroxy-methotrexate; HDMTX, high-dose methotrexate; MTX, methotrexate

When comparing AUC and clearance for each MTX regimen, AUC values were highest (2515 ± 773 h*mg/L) for patients who receive 8 g/m^2 MTX in the MRT regimen and lowest (608 ± 204 h*mg/L) for patients who received 1.5 g/m^2 in the MR-CHOP regimen. Patients who received 3.5 g/m^2 MTX in the MR-CHOP or HD MTX regimens had intermediate AUC values (1055 ± 561 h*mg/L and 1264 ± 456 h*mg/L, respectively). The lower AUC values for the MR-CHOP regimen were consistent with a 39% higher cl value (3.37 ± 1.64 L/h/m^2 vs. 2.42 ± 0.67 L/h/m^2). Consistent with the dose-dependent AUC, the C_{24h} values and C_{48h} values for the 8 g/m^2 dose were higher than the 3.5 g/m^2. Negative correlations were observed between clearance and the 24-, 48-, and 72-h concentrations, as illustrated for the 24-h concentration in Figure 2c. The relationship between MTX clearance and age was determined by assessing age as both a continuous variable and by dividing patients into three age groups categorized as young adults (age <40 years $n = 4$), adults (ages 40–65 years, $n = 25$), and older adults (ages >65 years, $n = 51$). MTX clearance decreased with increasing age and according to the group with young adults (median 4.42 L/h/m^2) greater than adults (2.81 L/h/m^2) greater than older adults (2.22 L/h/m^2); however, the relationship was nonstatistically significant as a continuous ($p = 0.20$) or a categorical variable ($p = 0.62$). There were no relationships between clearance and baseline liver function (p values >0.20), weight ($p = 0.53$), sex ($p = 0.33$), or number of previous MTX doses ($p = 0.81$).

The PKs of the major circulating metabolite, 7-OH-MTX, appeared to parallel those of the parent molecule. AUC increased in proportion to dose over the range of 1.5 g/m^2 to 8 g/m^2 investigated in this study (Figure S1a). Negative correlations were observed for the 24-, 48-, and 72-h concentrations, as illustrated for the 24-h concentration in Figure S1b. There were no relationships between 7-OH-MTX concentrations and baseline liver function tests ($p > 0.33$), weight ($p = 0.62$), age ($p = 0.27$), or sex ($p = 0.57$); however, there was a statistically significant relationship between 7-OH-MTX concentrations and the number of previous MTX doses ($p = 0.021$).

MTX Clearance correlation with kidney function estimating equations

In the full cohort of 80 patients, the relationship between MTX clearance and baseline estimated kidney function based on creatinine, cystatin C, or both was modest

TABLE 3 Summary of MTX and 7-OH-MTX pharmacokinetics

Protocol-defined MTX dose (g/m^2)	MTX[a]			7-OH-MTX[a]		
	1.5 ($n = 9$)	**3.5 ($n = 34$)**	**8.0 ($n = 37$)**	**1.5 ($n = 9$)**	**3.5 ($n = 34$)**	**8.0 ($n = 37$)**
Half-life (h)	6.2 (5.9–6.8)	7.1 (5.6–14.8)	8.49 (5.9–9.8)	12.5 (10.7–15.3)	13.8 (11.3–20.7)	12.9 (10.6–17.8)
T_{max} (h)	3.7 (3.5–3.7)	3.7 (3.6–3.8)	3.9 (3.6–9.8)	11.7 (11.6–12.2)	11.7 (10.6–12.5)	12.0 (9.9–13.1)
C_{max} (mg/L)	72.0 (67.07–75.66)	142 (96.77–178.19)	316 (248.66–378.24)	6.5 (6.02–7.93)	12.80 (8.37–16.85)	28.3 (17.82–36.28)
C_{12h} (mg/L)	14.34 (5.51–19.22)	14.8 (11.43–23.20)	66.0 (35.77–85.75)	6.26 (5.91–6.81)	10.5 (7.79–14.11)	21.3 (15.86–23.33)
C_{24h} (mg/L)	1.12 (0.59–2.58)	1.3 (1.03–2.53)	5.5 (2.55–9.52)	3.08 (2.45–5.05)	4.9 (7.79–14.11)	12.2 (7.60–18.03)
C_{36h} (mg/L)	0.28 (0.08-.090)	0.4 (0.19–0.63)	0.98 (0.39–1.93)	1.98 (1.21–2.42)	2.6 (1.72–3.99)	5.6 (2.97–8.82)
C_{48h} (mg/L)	0.06 (0.01–0.26)	0.1 (0.05–0.22)	0.21 (0.12–0.45)	1.11 (0.58–1.28)	1.4 (0.81–2.09)	2.6 (1.18–5.02)
$AUC_{0-\infty}$ (h*mg/L)	567 (474–608)	1063 (824–1274)	2490 (1883–3077)	156 (148–222)	318 (240–482)	687 (399–905)
AUC_{0-24h} (h*mg/L)	522 (471–598)	1035 (810–1260)	2456 (1809–2937)	108 (97–117)	189 (149–265)	389 (270–565)
AUC_{0-48h} (h*mg/L)	565 (474–600)	1063 (823–1273)	2490 (1871–3058)	137 (133–210)	277 (198–387)	634 (356–721)
V_{ss} (L)	21.1 (14.11–23.57)	17.4 (14.3–25.7)	18.0 (13.2–23.0)	NC	NC	NC
CL_P ($L/h/m^2$)	2.84 (2.07–3.23)	2.68 (1.87–3.94)	2.46 (2.02–2.76)	NC	NC	NC
CL_P (L/h)	5.30 (4.77–5.64)	5.60 (3.83–8.03)	4.54 (3.96–5.46)	NC	NC	NC

Abbreviations: 7-OH-MTX, 7-hydroxymethotrexate; AUC, area under the concentration-time curve; C, serum concentration; CL_P, plasma clearance; C_{max}, maximum plasma concentration; g/m^2, grams per meter squared; $L/h/m^2$, liters per hour per meter squared; L/h, liters per hour; L, liter; MTX, methotrexate; NC, not calculated due to unknown fraction metabolized; T, time; T_{max}, time to maximum plasma concentration; V_{ss}, apparent volume of distribution at steady state.

[a]All data are represented in medians (Interquartile range) unless otherwise stated.

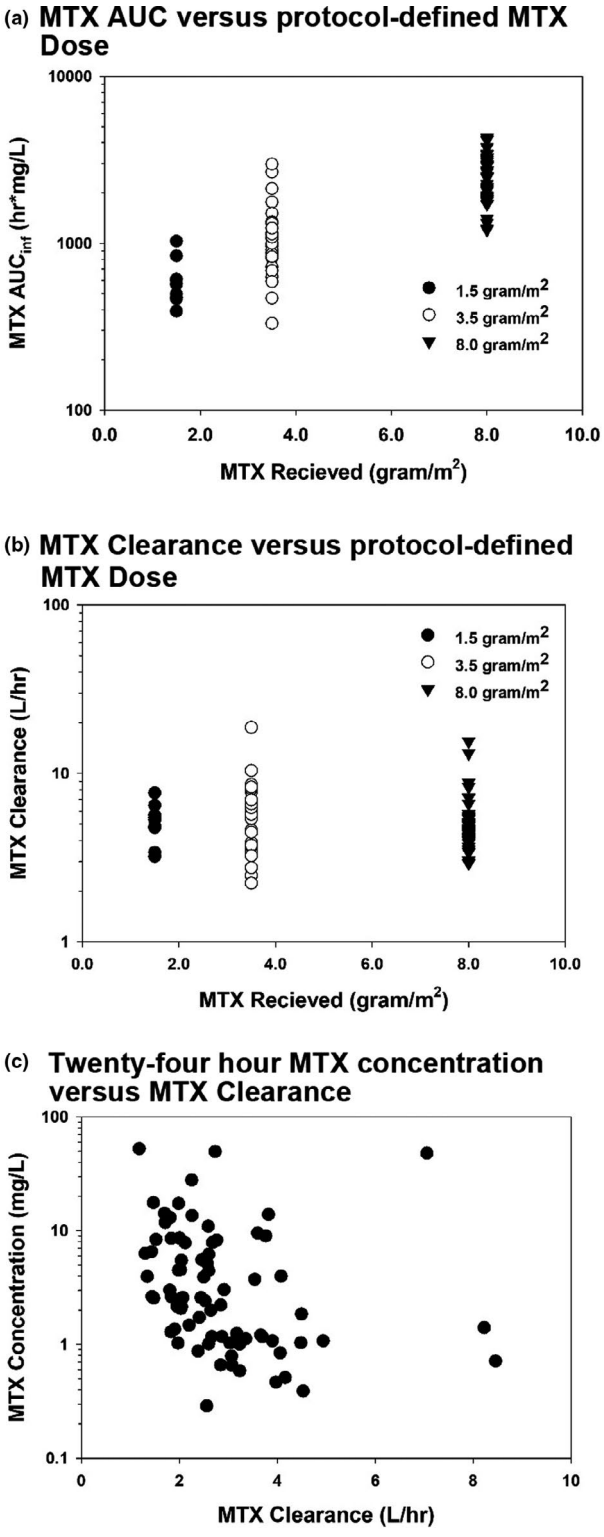

FIGURE 2 Area under the time-curve concentrations of methotrexate. AUC_{inf}, area under the time curve concentration through infinite time; MTX, methotrexate

(correlation coefficients between 0.11 and 0.31; Table 4). Among the seven kidney function estimates calculated, the $eGFR_{cys}$ (ml/min) most closely correlated with MTX clearance (L/h, r = 0.30). Seven patients had extreme values (top 10th percentile) for MTX clearance, which influenced these relationships (median [IQR] clearance 18.8 [IQR 13.2–38.1] L/h in these 7 patients vs. 4.8 [IQR 3.8–5.8] L/h in the remaining 73 patients). Analytical techniques were reviewed and a detailed chart review was performed. Neither revealed common features nor explanations for the extreme drug clearance values in these patients. In three of the seven patients, there was no postinfusion sample collected, resulting in the most extreme calculated values. The clearance of the other four patients was not explained by any obvious or identifiable features. These seven patients were all White, mostly men ($n = 6$, 85%), had a mean ±SD age of 53 ± 14 years, and a mean eCrCl 125 ± 55 ml/min. The protocol-defined MTX dose prescribed was 8 g/m^2 in two patients and 3.5 g/m^2 in the other five patients. A sensitivity analysis was performed after considering these seven patients to be outliers and excluding them, which strengthened the observed correlations between MTX clearance and kidney function estimates (Table 4). Patterns observed were overall similar, and the $eGFR_{cys}$ (ml/min) most closely correlated with MTX clearance ($r = 0.52$; Table 4, Figure 3).

DISCUSSION

This study of 80 patients receiving HDMTX as 4-h infusions for lymphoma management demonstrates a linear MTX clearance across dosing levels of 1.5 g/m^2, 3.5 g/m^2, and 8 g/m^2 with medians of 5.3 L/h, 5.6 L/h, and 4.5 L/h, respectively. Additionally, the AUC values exhibited marked variation at each dose level with a dose-proportional increase of MTX, as prescribed per protocol. Last, the relationship between MTX clearance and baseline kidney function estimated from equations, including serum creatinine, serum cystatin C, or both, was modest and most closely correlated with the $eGFR_{cys}$ (ml/min).

HDMTX PKs has been studied for over 50 years, with high inter- and intrapatient variability frequently described.[23,39,40] Our measured serum MTX concentrations and noncompartmental analysis determined that MTX clearance was linear and compared well to a previous report.[23] Two other studies demonstrated more rapid MTX clearance (>10 L/h); however, their patient populations had an age range of 4–51 years and a mean age of 35 ± 12 years, respectively, compared to our median age of 69 years.[24,41]

As expressed by AUC, absolute MTX exposure has been suggested as a surrogate marker for treatment outcomes.[11,12] However, there is controversy surrounding the optimal AUC target range in the management of CNS lymphoma.[11–13] An initial report identified longer survival for patients with primary CNS lymphoma when the MTX AUC exceeded 1100 μmol·h/L (454 mg*h/L).[12]

TABLE 4 Correlation and 95% confidence intervals for eGFR equations with MTX clearance

	Entire population ($n = 80$)		Population after excluding outliers ($n = 73$)	
	Correlation (95% CI) for MTX clearance (L/h)	Correlation (95% CI) for MTX clearance (L/h/BSA)	Correlation (95% CI) for MTX clearance (L/h)	Correlation (95% CI) for MTX clearance (L/h/BSA)
$eCrCl_{CG}$ (ml/min)	0.11 (−0.11 to 0.33)	0.13 (−0.09 to 0.34)	0.33 (0.10–0.52)	0.36 (0.14–0.55)
$eGFR_{cr}$ (ml/min)	0.17 (−0.05 to 0.38)	0.19 (−0.04 to 0.39)	0.38 (0.17–0.56)	0.39 (0.17–0.57)
$eGFR_{cys}$ (ml/min)	0.30 (0.09–0.49)	0.31 (0.09–0.49)	0.52 (0.33–0.67)	0.48 (0.28–0.64)
$eGFR_{cr\text{-}cys}$ (ml/min)	0.28 (0.06–0.47)	0.28 (0.07–0.47)	0.51 (0.31–0.66)	0.48 (0.29–0.64)
$eGFR_{cr}$ (ml/min/1.73 m^2)	0.14 (−0.08 to 0.35)	0.15 (−0.07 to 0.36)	0.24 (0.01–0.45)	0.27 (0.04–0.47)
$eGFR_{cys}$ (ml/min/1.73 m^2)	0.27 (0.05–0.46)	0.27 (0.06–0.46)	0.39 (0.18–0.57)	0.37 (0.16–0.56)
$eGFR_{cr\text{-}cys}$ (ml/min/1.73 m^2)	0.24 (0.02–0.44)	0.25 (0.03–0.44)	0.36 (0.15–0.55)	0.36 (0.15–0.55)

Abbreviations: BSA, body surface area; CI, confidence interval; MTX, methotrexate; $eCrCl_{CG}$, estimated creatinine clearance based on the Cockcroft-Gault formula; eGFR, estimated glomerular filtration rate based on the Chronic Kidney Disease Epidemiology Collaborative equation utilizing cr, serum creatinine, cys, cystatin C, or cr-cys, both serum creatinine and cystatin C; L/h, liters per hour, L/h/BSA, liters per hour per BSA; ml/min, milliliters per minute; ml/min/1.73 m^2, milliliters per minute per 1.73 m^2; MTX, methotrexate.

A subsequent investigation showed that an AUC greater than 980 μmol·h/L (445 mg*h/L) predicted event-free and overall survival.[11] Unfortunately, toxicity was observed at higher AUCs with an MTX AUC greater than 1047 μmol·h/L (476 mg*h/l) and greater than 1036 μmol·h/L (471 mg*h/L) associated with increased liver dysfunction and neutropenia, respectively.[11] In elderly patients, an inverse association was seen between escalating AUC and tumor response, where MTX AUC above 2126 μmol·h/L (966 mg*h/L) predicted worse progression-free and overall survival.[14]

We observed no association between MTX and nephrotoxicity in our population; however, based on previous literature, our median MTX AUC of 1739 μmol*h/L (790 mg*h/L) heightens the risk potential for nonrenal MTX-associated adverse events. Additionally, the median MTX AUC of 2543 μmol*h/L (1155 mg*h/L) in patients receiving HDMTX 8 g/m^2 is concerning for negative disease-related outcomes. Interestingly, the 3.5 g/m^2 administered primarily as prophylaxis for CNS relapse appeared to achieve the recommended AUC target for treatment with a median MTX AUC of 1063 μmol*h/L (483 mg*h/L). Dose reduction to 1.5 g/m^2 occurred in patients of advanced age or poor performance status eligible for CNS prophylaxis. Given the low AUCs observed in this group (median 567 μmol*h/L [258 mg*h/L]), the benefits and risks of this dosing modification should be revisited.

The methotrexate metabolite, 7-OH-MTX, has been implicated in crystalline formation in the kidneys of several animal species, particularly due to the limited solubility in acidic pH environments.[42,43] Additionally, two independent investigations demonstrated an association with 7-OH-MTX and nephrotoxicity in children with acute lymphoblastic leukemia and other hematologic malignancies treated with HDMTX.[44,45] Future studies in adult patients with lymphoma receiving HDMTX at 3.5 g/m^2 or 8 g/m^2 are needed to understand whether MTX-associated toxicity relies solely on serum MTX concentrations or should include the 7-OH-MTX detection to dictate our clinical supportive care measures. If a detectable 7-OH-MTX level at baseline is found to be a predictor of AKI in a patient scheduled to receive HDMTX, that detectable serum concentration could be an alert to the provider that MTX should be delayed until the 7-OH-MTX is completely cleared as a method to mitigate additional AKI risk.

One interesting finding in the present study is the relationship between biomarkers of kidney function and MTX clearance. We found that the eGFR based on cystatin C, whether expressed in ml/min or ml/min/1.73 m^2, predicted MTX clearance better than creatinine-based estimating equations. Additionally, the eGFR equation utilizing both serum creatinine and cystatin C also showed a stronger correlation with MTX clearance than the C-G eCrCl. This effect seems primarily driven by the cystatin C component, given that the correlation improved quite a bit when comparing the eGFR based on serum creatinine and the eGFR incorporating both serum creatinine and cystatin C. This differs considerably from the current approach to MTX dosing in clinical practice that relies solely on serum creatinine and eCrCl based on the C-G equation despite PK studies describing the poor performance of serum creatinine and creatinine clearance as a marker of MTX elimination.[24,40,41] Creatinine is heavily affected by nonrenal determinants of body habitus (i.e., age, sex, and muscle mass), whereas cystatin C is produced at a constant rate from all nucleated cells.[27,28,38,46,47] Additionally, cystatin C is freely filtered at the glomerulus and not

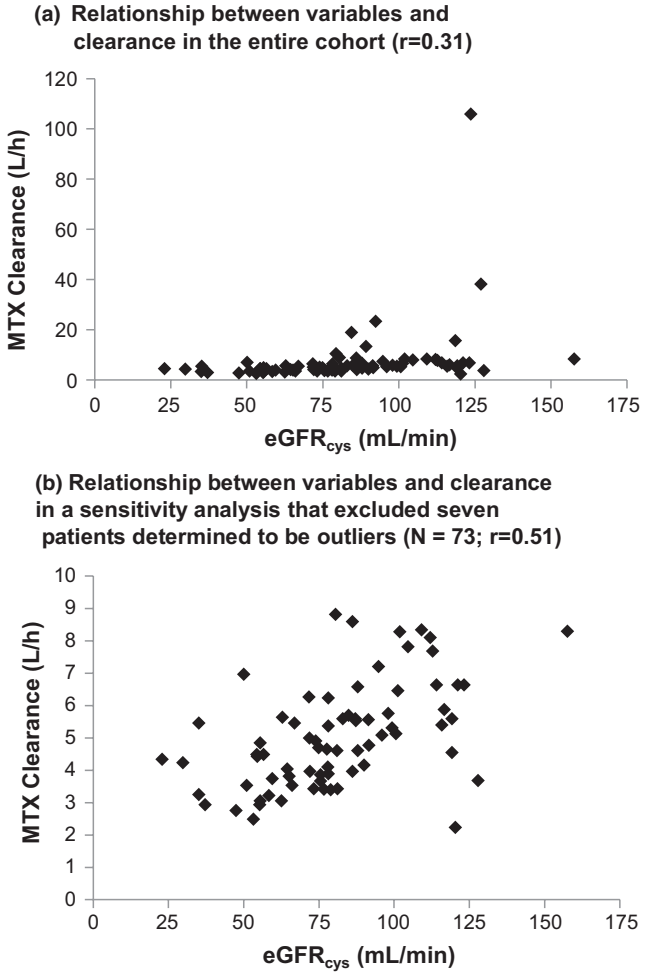

FIGURE 3 Correlation between cystatin C-based estimated glomerular filtration rate (eGFR) equation (ml/min) and clearance of methotrexate (MTX; L/h). (a) Relationship between variables and clearance in the entire cohort, (b) relationship between variables and clearance in a sensitivity analysis that excluded seven patients determine to be outliers

systemically reabsorbed or meaningfully secreted in the renal tubules.[48] In stable ambulatory patients, cystatin C and creatinine together predicted measured GFR better than either biomarker alone.[34,37] Concern has been raised about the viability of cystatin C for use in patients with cancer.[49] Nonrenal factors, including heightened cell turnover in rapidly proliferating malignancies, inflammation, and corticosteroids, may increase cystatin C concentrations independent of kidney function.[27] Notwithstanding these potential limitations, the incorporation of cystatin C measurements into kidney function estimates for dosing and monitoring medications with a narrow therapeutic index may be useful.[26,50] In a systematic review of data through 2017, 28 studies were identified that explored the relationship between cystatin C and drug pharmacokinetics in 3455 patients treated with renally eliminated medications, including carboplatin and topotecan.[51] Compared with estimates of kidney function based on creatinine, the use of cystatin C to predict kidney drug clearance was at least as accurate, if not superior, in most studies. No observational studies report the use of cystatin C to predict clearance of HDMTX in adults; however, two select cases published in the literature indicate the potential role.[26] Additionally, cystatin C has been suggested as a sensitive marker to monitor renal function during and after HDMTX prescribed to children with acute lymphoblastic leukemia.[52] Our data reinforce these observations and highlight the need for future study of the potential role for cystatin C to guide the dosing of HDMTX.

Cystatin C is not as universally accessible a biomarker as serum creatinine for the evaluation of kidney function.[53] However, a recent, multinational survey of nephrologists and critical care practitioners indicated that 19% of respondents reported use of cystatin C for evaluation of AKI, and 25% believed that alternative GFR biomarkers, such as cystatin C, should replace serum creatinine.[30] Additionally, a more focal evaluation of hospitals in Minnesota, USA, demonstrated that 79% of hospitals have access to cystatin C testing.[29] This makes cystatin C a reasonable addition to, or alternative for, serum creatinine.[37,50] Other strategies to estimate kidney function (i.e., measured creatinine clearance and measured GFR) can be cumbersome and technically complex to perform, with an expense that also limits availability or practicality of use. Additional study is needed to characterize successful application of these novel approaches to kidney assessment to guide drug dosing and monitoring, particularly in patients with cancer.

There are some limitations to our study beyond cystatin C accessibility that should be noted. First, our study is a single-center investigation with a limited sample size; however, our sample size is larger than most current available PK studies. Additionally, the consecutive enrollment over a condensed timeframe at our institution permitted consistent, supportive management strategies across our population. Second, there is some heterogeneity within the patient population given the intent of treatment versus prophylaxis, first MTX dose versus subsequent dose, and three MTX dosing levels. This population represents a real-world practice and facilitated comparisons among adult patients with lymphoma who received MTX as a 4-h infusion. Third, patients were permitted to refuse sampling at any point. Although patient refusal ended up restricting seven patients from parts of the analysis, over 90% of the requested samples were fulfilled, making investigators confident in the results provided. Last, the serum creatinine concentration is a lagging marker of kidney

function. It may take at least 48 h from the onset of kidney damage to observe a creatinine rise. Cystatin C may have a slightly more favorable kinetic profile than serum creatinine.[54] Whereas patients with overt AKI at the outset of therapy would have MTX administration deferred, it is possible that slight changes to kidney function occurred during the sampling period that may have affected observations.

In conclusion, our study presents a thorough analysis of MTX and 7-OH-MTX PKs in real-world patients with lymphoma receiving contemporary MTX doses over a 4-h infusion. The high median MTX AUC levels of 8 g/m^2 compared to 3.5 g/m^2 compels further, rigorous, prospective examination to determine the optimal dosing level. The relationship between cystatin C-based eGFR equations and MTX clearance also deserves intense, expanded investigation in similar patients.

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CONFLICT OF INTEREST

E.F.B. provides consultation for FAST Biomedical, unrelated to this work. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

J.N.B., J.M.R., C.A.T., K.C.M., A.D.R., K.B.K., N.L., T.R.L., R.M.M., T.E.W., and E.F.B. wrote the manuscript. J.N.B., J.M.R., C.A.T., K.C.M., A.D.R., K.B.K., N.L., T.E.W., and E.F.B. designed the research. J.N.B., K.C.M., and E.F.B. performed the research. J.N.B., J.M.R., K.C.M., A.D.R., K.B.K., T.R.L., R.M.M., T.E.W., and E.F.B. analyzed the data. J.M.R., T.R.L, and R.M.M. contributed new reagents.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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