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REVIEW

Factors influencing methotrexate pharmacokinetics highlight the need for individualized dose adjustment: a systematic review

Yunyun Yang[1] · Zhengyue Liu[1] · Jingxia Chen[1] · Xuebin Wang[1] · Zheng Jiao[2] · Zhuo Wang[1]

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Abstract

Purpose To develop a population pharmacokinetic (PPK) model for methotrexate (MTX) dosage for all ages, assess the association between concentration and clearance, and determine covariates affecting MTX disposition.

Methods We compared MTX PK profiles among neonates, children, and adults by performing a systematic literature search for published population MTX models and conducted a Monte Carlo-based meta-analysis. Subsequently, we evaluated study quality and covariates significantly affecting dosage regimens and compared LDMTX and HDMTX PK profiles.

Results Of the total 40 studies included, 34 were HDMTX, and six were LDMTX studies. For HDMTX, three studies involving neonates reported estimated apparent clearances (median, range) of 0.53 (0.27–0.77) L/kg/h; for 14 studies involving children, 0.23 (0.07–0.23) L/kg/h; and for 13 involving adults, 0.11 (0.03–0.22) L/kg/h. Neonates had a higher volume of distribution than children and adults. For LDMTX studies, apparent clearance was 0.085 (0.05–1.68) L/kg/h, and volume of distribution was 0.25 (0.018–0.47) L/kg, lower than those of HDMTX studies, with large between-subject variability. Bodyweight significantly influenced apparent clearance and volume of distribution, whereas renal function mainly influenced clearance. Mutations in certain genes reduced MTX clearance by 8–35.3%, whereas those in others increased it by 15–48%. Body surface area (BSA) significantly influenced apparent clearance with a median reduction of 51% when BSA increased in pediatric patients.

Conclusions Methotrexate dosage regimens were primarily based on body surface area and renal function. Further studies are needed to evaluate MTX pharmacokinetics and pharmacodynamics in both children (especially infants) and adults.

Keywords Methotrexate · Population pharmacokinetics · Apparent clearance · Volume of distribution

Introduction

Methotrexate (MTX), a folate antimetabolite, can be administered over a broad range of doses via different routes for its antitumor and anti-inflammatory effects [1]. An MTX dosage < 50 mg/m^2 is defined as low-dose MTX (LDMTX) and is mainly used for the treatment of rheumatoid arthritis (RA), breast cancer, and prevention of acute graft-versus-host disease (aGVHD) [2]. Dosages of MTX > 500 mg/m^2 are defined as high-dose MTX (HDMTX) and are used effectively to treat infant, pediatric, or adult patients with acute lymphoblastic leukemia (ALL), osteosarcoma (OS), or lymphoma malignancies [3].

Methotrexate can be administered orally, subcutaneously, intramuscularly, or intravenously. Oral MTX absorption is highly variable, ranging from 23 to 95% in the dose range used to treat RA, with a dose-dependent response [4]; its absorption declines at higher doses, especially at > 40 mg/m^2 for pediatric patients and > 80 mg/m^2 for adult patients [5]. However, MTX absorption is complete after intramuscular injection. The time-to-peak for oral administration is 0.7–4 h for children and 0.75–6 h for adults; for intramuscular administration, it is 30–60 min for both children and adults. MTX

Yunyun Yang, Zhengyue Liu, and Jingxia Chen equally contributed to this study.

✉ Zheng Jiao
jiaozheng@online.sh.cn

✉ Zhuo Wang
wangzhuo088@163.com

[1] Department of Pharmacy, Shanghai Changhai Hospital, the First Affiliated Hospital of Navy Medical University, 168 Changhai Rd, Shanghai 200433, China

[2] Department of Pharmacy, Shanghai Chest Hospital, Shanghai Jiao Tong University, 241 Huaihai West Road, Shanghai 200030, China

penetrates slowly into third-space fluids (e.g., pleural effusions and ascites) and exits more slowly from these compartments than from plasma.

Major side effects of LDMTX include nausea, stomatitis, abnormal liver chemistry, and rash [6]. HDMTX is associated with toxicity, including acute kidney injury (AKI), oral mucositis, hepatotoxicity, and myelosuppression [7]. The activity and toxic effects of MTX are closely related to the dosage regime and the duration of exposure above the threshold concentration. There is also a higher probability of disease relapse among patients with rapid clearance [8]. These effects are not readily distinguishable between HD and LD indications. As the MTX dose affects both its PK and pharmacodynamics (PD), various pharmacogenetic associations may also have an impact. HDMTX in chemotherapy and LDMTX in immunosuppression therapy have different mechanisms of action. For LDMTX, the PK may be nonlinear and exhibit large heterogeneity, indicating that accurate LDMTX dosing is complicated [9]. To ensure the safety and effectiveness of MTX, therapeutic drug monitoring (TDM) can be employed to measure the MTX plasma concentration [10]. However, most TDM has been focused on HDMTX, which is guided by population means reflected in nomograms, and is not based on the individual characteristics of patients [11].

The population pharmacokinetic model (PPK) is used to analyze sparse TDM data for highly diverse populations, estimate intra- and inter-individual variability, and identify the impact of covariates. Bayesian estimation using PPK parameters has been effectively applied for dosage adjustment to avoid under or overestimating MTX concentrations [12, 13]. Although many PPK studies have been conducted to quantitatively describe the pharmacokinetic characteristics of MTX, these studies have mainly focused on identifying covariates with potentially significant effects on MTX PK.

The overarching aim of our analysis was to perform a Monte Carlo simulation of concentration-time profiles to assess the statistical and structural models within each MTX PPK model [14]. A robust PPK model with Monte Carlo simulation of various dosing regimens and patient populations can generate the expected range of MTX concentrations throughout the therapy and assist in predicting the therapeutic effect and risk of MTX toxicity in patients. Further, we aimed to compare the clearance (CL) of the included MTX models via formal meta-analysis, analyze the covariates significantly affecting MTX PK, and identify challenges that remain to be explored.

Methods

Information sources and search strategy

A systematic literature search for data published on PPK models for MTX until December 31, 2022, was conducted using the following electronic databases: PubMed, Embase, and MEDLINE. The language was limited to English. The PPK studies on MTX were searched using the following terms: "methotrexate," "population pharmacokinetic," "pharmacokinetic modeling," "nonlinear mixed effect model," "nonmem or P-metrics," "WINNONMIX," "ADAPT," "P-PHARM," "nlmixed," "NLME," "USC*PACK," "MONOLIX," or "Bayes," and "adult," "adolescent," "children," or "infant." We also checked the references of related studies. The literature search was performed by two authors, and a senior researcher was consulted to resolve discrepancies.

All relevant articles selected from the databases and reference lists were screened to evaluate their eligibility for inclusion, according to the following inclusion criteria: (1) patients receiving MTX; (2) MTX dosage, administration route, and sampling time provided; (3) use of the PPK method to analyze data; and (4) availability of essential PK parameters. The following studies were excluded: (1) the article was a review or external evaluation article; (2) the PK parameters were incomplete; (3) the articles were duplicated; (4) the full text could not be obtained; and (5) non-English articles.

Data extraction

We extracted the following information from the included articles: (1) basic demographics, including dosage regimens (e.g., MTX dosage, administration route), age, weight, diagnosis, study design (e.g., study type, number of patients and sample points, and sampling time), and (2) information on PPK-related parameters that contained the PPK formula, model evaluation methods, structural models, between-subject variability (BSV), and residual unexplained variability (RUV).

Study comparison

The concentration–time profiles of the virtual patients were generated based on the final PPK models. For HDMTX-based articles, we performed 2000 simulations in three age groups (infants, children, and adults) with three disease types (OS, ALL, or malignant lymphoma); for LDMTX used to treat RA or breast cancer or prevent aGVHD, we simulated 2000 virtual adult male patients each. They received intravenous or oral MTX at different dosage regimes, and we simulated the MTX concentration at the end of the administration and elimination phase. Patients were assumed to have received monotherapy if they reached a steady state for oral administration, with MTX plasma measured in μmol.

Standard HDMTX doses for ALL, lymphoma, and OS were determined according to the following relevant guidelines:

ALL: 500 mg/m^2 over 0.5 h, followed by 4500 mg/m^2 over 23.5 h, as per the ALL BFM95 [15].
Malignant lymphoma: 3000 mg/m^2 for 6 h infusion, according to the guidelines of lymphoma [16].
OS: 12,000 mg/m^2 for 6 h infusion [8].

Standard LDMTX doses for breast cancer, aGVHD, and RA were administered according to the following guidelines:

Breast cancer: 40 mg/m^2 intravenously for 6–12 cycles [17].
Hematopoietic stem cell transplant (HSCT):15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, and 11 after HSCT for aGVHD [18].
RA: 7.5 mg oral dose once weekly [19].

The simulation profiles were plotted according to the established PPK models, as follows: neonates (3 kg, post-menstrual age [PMA] 36 weeks), serum creatinine (SCr) set to 0.5 mg/dL; children (10 years, 30 kg), SCr set to 0.5 mg/dL, according to a multicenter study of children [20]; and adults (40 years, 70 kg), SCr set to 1 mg/dL, according to the mean estimated glomerular filtration rate of 120 mL/min for patients between 20 and 60 years [21].

We analyzed the effects of the potential covariates retained in the included PPK studies on MTX CL; the results are represented using a forest map. Continuous covariates (e.g., weight, age, creatinine clearance [CrCL], and body surface area [BSA]) were standardized to the same range for comparison. The weight ranges were 16–40 kg for children and 40–100 kg for adults. The CrCL range was 20–200 mL/min/1.73 m^2. For some continuous covariates that were retained in only one study (e.g., hematocrit), the minimum and maximum values were obtained from the study. For binary covariates, such as sex, 0 and 1 were used. We tested the range of covariates in each study and the calculated minimum and maximum apparent clearance (CL) values; CL was normalized to the median covariate values in each study. We defined effects on CL greater than 80–125% to have a significant clinical correlation [22].

We conducted simulations using NONMEM (version 7.4; ICON Development Solutions, Ellicott City, MD, USA). R (version 3.5.1; http://www.r-project.org/) was used to generate concentration–time profiles and forest plots.

Results

Study identification

We preliminarily screened 1085 articles identified using the search strategy from PubMed ($n=263$), Embase ($n=104$), and Medline ($n=718$). Twelve additional articles were identified using the reference lists of the selected articles. After removing the duplicates, 154 studies were screened, and 63 full-text articles were retrieved for a more detailed evaluation of eligibility. After literature retrieval and screening (Fig. 1 and Online Resource S1), we finally included 40 articles containing 34 studies on HDMTX and six studies on LDMTX for evaluation.

Study characteristics

The HDMTX studies all applied an infusion time of 1–24 h to treat ALL, OS, and lymphoma; four of these were for infants [23–26], 14 for adults [27–40], 11 for children [41–51]; the remaining five addressed both children and adults [52–56]. The LDMTX studies were all conducted using adults [57–62]. Of the HDMTX PPK studies, seven addressed only ALL [24, 41, 44, 47–50]; eight, only primary central nervous system lymphoma (PSCNL) [23, 25, 26, 34, 36, 39, 40, 53]; four, only OS [42, 46, 52, 54]; and one, only non-Hodgkin lymphoma (NHL) [35]. The remaining studies addressed two or more diagnoses. For LDMTX, three studies used oral MTX for patients with RA [57, 59, 62], two used intravenous MTX for breast cancer [58, 60], and one used intravenous MTX for preventing aGVHD [61]. Nine articles were prospective studies [24, 29, 31, 46, 48, 52, 53, 59, 61], and the rest were retrospective studies.

Only three articles were multicenter studies [25, 46, 53]; the remainder were single-center studies. Ten studies were conducted in China [26, 29, 34, 35, 40, 45, 47, 50, 51, 54], nine in the USA [23–25, 33, 36, 49, 55–57], five in France [27, 30, 31, 37, 46], four in Japan [28, 32, 39, 59], two in Spain [41, 42], two in the UK [52, 58], and one each in Slovenia [43], Switzerland [53], Germany [38], India [60], Korea [61], México [48], and Egypt [44].

Thirteen of the studies, all using HDMTX, had > 100 subjects [25, 26, 37, 38, 46, 47, 49–51, 53–56], and one [53] was a PK/PD study. The number of patients in each study ranged from 14 and 772, with MTX observations per individual ranging from 1 to 10. The daily dose of intravenous MTX ranged between 15 and 18,000 mg/m^2 and the oral dose between 7.5 and 15 mg weekly. Four articles did not mention the MTX bioassay method [33, 36, 44, 45]. Fluorescence polarization immunoassay (FPIA), enzyme multiplied immunoassay technique (EMIT), aldo–keto reductase (AKR), chemiluminescent immunoassay (CMIA), high-performance liquid chromatography(HPLC) with an ultraviolet detector, a special mode of HPLC called ultra-high performance liquid chromatography with tandem mass spectrometry (UPLC–MS/MS), and liquid chromatography with tandem mass spectrometry (LC–MS/MS) were employed as the MTX bioassay methods. The lowest limit for quantitative assays ranged between 0.238 and 5000 nM. The modeling

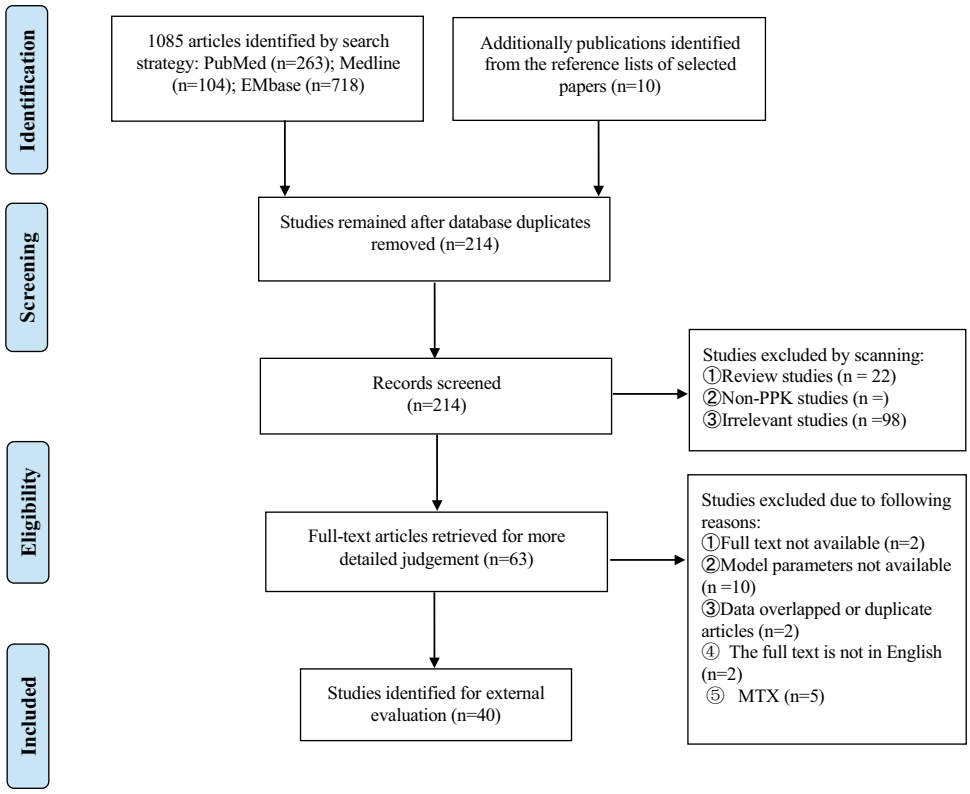

Fig. 1 PRISMA flow diagram for identification of Methotrexate PPK studies. (*n* number of articles returned by search)

strategies and final PPK parameters of each study are summarized in Tables 1 and 2.

In these studies, a two-compartment model with first-order elimination (FOCE) was used in most of the included studies; four used a three-compartment model [31, 38, 49, 50], and only one used a compartment model [26, 62].

Comparison of studies

For HDMTX, three studies were conducted in neonates, with an estimated CL (median, range) of 0.53 (0.27–0.77) L/kg/h; 14 articles addressed children with estimated CL (median, range) of 0.23 (0.07–0.23) L/kg/h; and 13 addressed adults with estimated CL (median, range) of 0.11 (0.03–0.22) L/kg/h. The median CL was higher in neonates than in children and adults. The volume of distribution (V_d) was determined as 1.51 (0.91–1.82) L/kg for neonates, 0.51 (0.19–1.22) L/kg for children, and 0.37 (0.03–1.13) L/kg for adults. V_d was also higher for neonates than for children and adults. For the six studies that applied LDMTX in adults, the estimated CL (median, range) was 0.085 (0.05–1.68) L/kg/h, V_d was 0.25 (0.018–0.47) L/kg, and the BSV was large. The median CL values were lower for LDMTX than for HDMTX studies. The concentration–time profiles of these six studies are presented in Fig. 2.

In all the included studies, BSV was described mainly using exponential models. For CL and V_d, the BSV median (range) was as follows: CL 22.9% (0.3–81.73%) [$n = 39$], V_d 25.9% (0.03–116%) [$n = 30$]. The RUV, commonly described using proportional models, ranged from 2 to 67.7%. In one study, the median RUV in patients receiving LDMTX was considerably lower than that in patients receiving HDMTX (18.6% vs. 33.94%, respectively).

Comparison of different dosing regimens for HDMTX

HDMTX over-exposure was defined as a serum MTX concentration > 1.0 μM at 48 h (MTX C_{48h}) and > 0.1 μM at 72 h (MTX C_{72h}) and was associated with increased MTX-related toxicity [39].

OS patients

Recent studies have reported that a serum MTX concentration of 700 μM after a 6 h (MTX C_{6h}) infusion can achieve good efficacy in treating OS, while a higher incidence of MTX-induced side effects was associated with MTX $C_{6h} > 1000$ μM [63]. Three studies, by Johansson et al. [52], Zhang et al. [54], and Kawakatsu et al. [55], included both children and adults, and their concentration–time profiles differed substantially. However, two studies, by Johansson et al. [52] and Watanabe et al. [32],

Table 1 Characteristics of population pharmacokinetic studies included in the systematic review

Study (publication year)	Type of study	Country (single/ multiple sites)	Number of subjects (samples)	MTX dosage regime	Administration time	Sampling design	Age Mean ± SD Median [range]	Weight Mean ± SD Median [range]	MTX assay [LOQ]	Diagnosis
HDMTX										
Faltaos et al. (2006) [27]	Retrospective	France (Single)	51 (496)	1–8 g/m^2 i.v	1–6-h infusion	24, 48, and 72 h	62 (39–72)	67 (55–76)	EMIT [0.0372ion	Malignant lymphoma and ALL
Aumente et al. (2006) [41]	Retrospective	Spain (Single)	37 (1514)	3 g/m^2	4-h or 24-h infusion	① 1, 2, 4, 7, 12, 19, 24 h (4 h infusion) ② 1, 6, 23, 26, 30, 36, 42 h (24 h infusion) every 24 h until < 0.2 µmol/L	6.97 (0.5–17)	30.56 (7.46–80)	TDX/FPIA [0.02µmol/L]	ALL
Fukuhara et al. (2008) [28]	Retrospective	Japan (Single)	51 (348)	0.5–16.2	3–6-h infusion	6, 12, 24, 48, and 72 h	38.0 ± 14.6	55.6 ± 9.18	TDx/FPIA [0.02µM/L]	Malignant tumors
Colom et al. (2009) [42]	Retrospective	Spain (Single)	14 (1304)	7.5–13.6 g/m^2	3.5-h or 4-h infusion	1, 2, 3.5, or 4 (end of infusion), 24, 48 h. Then, every 12 h until < 0.2 µmol/L	14.8 ± 2.1	50.1 ± 16.6	TDX/FPIA [0.02µmol/L]	OS
Min et al. (2009) [29]	Clinical trial	China (Single)	82 (400)	1.5–9 g	24-h infusion	6, 12, 18, 24, 30, 36, 44, 50, 56, 68, 74, 80, 92 h until < 0.2 µmol/L	40 ± 18.7	66 ± 14.1	TDX/FPIA [fixed 0.025µM/L]	Malignant lymphoma
Faganel et al. (2011) [43]	Retrospective	Slovenia (Single)	64 (252)	2.0–5.7 g/m^2 i.v	24-h infusion	24, 36, 42, and 48 h, every 6 h until MTX < 0.25 µmol/L	5.0 (1.6–16.8)	17.1 (10.3–78.3)	TDX/FPIA [0.2µmol/L]	Malignant lymphoma and ALL
Johansson et al. (2011) [52]	prospective	England (Single)	46 (943)	8–13 g/m^2	NA	Every 24 h until < MTX < 0.2 µmol/L	19.3 (4–51)	60.9 (17.4–107)	TDX/FPIA [0.02µmol/L]	OS
Joerger et al. (2012) [53]	prospective	Switzerland (Multiple)	131 (NA)	0.3–12 g/m^2	> 3 h	6, 8, and 12 h every 12 h until undetectable	NA	NA	HPLC [0.04µM/L]	PCNSL
Simon et al. (2013) [30]	Retrospective	France (Single)	50 (NA)	1–8 g/m2	1–6 h	End of infusion, 8–12, 24,48 h, then every 6 h until < 0.03 µmol/L	**62 (20–81)**	**67 (40–98)**	EMIT [0.015µM/L]	Malignant lymphoma and ALL
Desoky et al. (2013) [44]	Retrospective	Egypt (Single)	41 (NA)	2 g/m^2	2-h infusion	24 and 48 h	6.5 ± 3.4	**17.6 ± 9.9**	**NA**	**ALL**
Benz-de Bretagne et al. (2014) [31]	Clinical trial	France (Single)	81 (363)	0.96–8 g/m^2	3 or 24-h infusion	24, 48, and 72 h, and then every 24 h until < 0.2 µmol/L	60.6 (18.8–84.6)	**75 (43–110)**	TDX/FPIA [0.01µM/L]	Malignant lymphoma
Watanabe et al. (2014) [32]	Retrospective	Japan (Single)	57	3.5–18 g	3 h and 6 h	3, 6, 12, 24, 48, 72, 96, 120, 144, and 192 h	42.0 ± 24.0	**55.5** ± 12.2	TDX/FPIA [0.02µM/L]	Malignant lymphoma or OS
Wright and Stewart (2015) [23]	Retrospective	Memphis (Single)	75 (NA)	5 g/m^2 or 2.5g/ m^2	24-h infusion	42 and 66 h	1.6 (0.022–3.5)	**NA**	TDx/FPIA [0.03µM]	PCNSL
Zhang et al. (2015) [54]	Retrospective	China (Single)	148 (274)	10 g/m^2	6–8-h infusion	0, 6, 12, 24, 48,72 h every 24 h until < 0.05 µmol/L	17 (6–49)	**58 (20–97)**	TDX/FPIA [0.01µmol/L]	OS
Nader et al. (2017) [33]	Retrospective	USA (Single)	37 (530)	0.5–7 g/m^2	4–6 h or 24 h	every 12 or 24 h until < 0.05 µmol/L	35 ± 12	69 ± 15	NA [0.05µmol/L]	Lymphoma and ALL

Table 1 (continued)

Study (publication year)	Type of study	Country (single/ multiple sites)	Number of subjects (samples)	MTX dosage regime	Administration time	Sampling design	Age Mean ± SD Median [range]	Weight Mean ± SD Median [range]	MTX assay [LOQ]	Diagnosis
Hui et al. (2019) [45]	Retrospective	China (Single)	ALL: 36 (354); OS: 16 (585)	2–18 g/m^2	6 h for OS and 24 h for ALL	Every 24 h until < 0.1 μmol/L	ALL:7.3 ± 4.48 OS:12.17 ± 4.18	ALL: 23.4 ± 12.8 OS: 40.9 ± 16.5	NA	OS and ALL
Lui et al. (2018) [46]	Clinical trial	French (multicenter)	187 (7898)	12 g/m^2	> 4-h infusion	Every 24 h until < 0.15 μM/L	14.2 (5.8–24.5)	49 (19–88)	ARK [0.02μmol/L]	OS
Zang et al. (2019) [47]	Retrospective	China (Single)	190 (1659)	2–5 g/m^2	24-h infusion	45, 69, and 93h, then until < 0.1 μmol/L	4.92 (1.42–15.92)	18.5 (10–59)	TDx/FPIA [NA]	ALL
Mei et al. (2018) [34]	Retrospective	China (Single)	98 (701)	0.90–5.44 g/m^2	1.34–8.17-h infusion	5 to 6 am every day until < 0.05 μmol/L	52.14 ± 18.49	63.73 ± 11.99	UPLC-MS/MS [0.01μmol/L]	PCNSL
Kawakatsu et al. (2019) [55]	Retrospective	USA (Single)	320 (5116)	0.73–12.312 g/m^2	3–24-h infusion	0, 24, 48, 72, and 96 h	16.4 (0.6–78.6)	56.7 (7.8–163)	TDX/FPIA and ARK [NA]	ALL, OS and Lymphoma
Beechinor et al. (2019) [24]	Clinical trial	USA (Single)	71 (672)	4.0 g/m^2	24-h infusion	Every 12–24 h until < 0.18 μmol/L	① PK sub-study group:0.71 (0.24–1.08) ② Routine clinical group: 0.58(0.08–1.08)	① PK sub-study group: 8.6 (4.5–11.9) ② Routine clinical group: 7.6 (3.0–12.1)	HPLC [0.05μoml/L]	ALL
Panetta et al. (2020) [25]	Retrospective	USA (Multi)	162 (3916)	2.5 or 5 g/m^2	24-h infusion	Pre-infusion, 6, 23, 42, and 66 h, then < 0.1 μM/L	1.72 ± 0.97	11.4 ± 3.66	TDx/FPIA [0.03PIA	Malignant brain tumors
Medellin-Garibay et al. (2020) [48]	Prospective	Mexican (Single)	50 (109)	2.0–5.0 g/m^2	24-h infusion	24, 36, 42, and 48 h	5 (1–15)	21.2 (8–57.3)	CMIA [NA]	ALL
Yang et al. (2020) [35]	Retrospective	China (Single)	91 (64/27)	1–3 g/m^2	24-h infusion	24, 36, 42, 48, and 72 h until < 0.2 μmol/L	48 (18–73)	61 (44–90)	HPLC [0.09μmol/L]	malignant lymphoma
Pai et al. (2020) [36]	Retrospective	USA (Single)	73 (291)	1.11–10.99 g/m^2	NA	NA	Non obese: 64.1 ± 11.3 Obese: 64.9 ± 11.7	Non obese: 69.8 ± 11.6 Obese: 104 ± 14.9	NA	PCNSL
Gallais et al. (2021) [37]	Retrospective	France (Single)	328 (1179)	1 to 8 g/m^2	0.5–4 h or 22–24 h	36 and 48 h, then every 24 h until < 0.2 μmol/L	AKI patients: 53.4 ± 16.7 Non-AKI patients: 49.8 ± 17.1	AKI patients: 72.4 ± 13.4 Non-AKI patients: 70.2 ± 15.5	AKR and LC/MS/MS [0.05μ0.0]	ALL and NHL
Taylor et al. (2020) [49]	Retrospective	USA (multiple)	772 (31,672)	5 g/m^2 or 8 g/m^2	24-h infusion	24, 36, and every 6 h until < 0.2 μmol/L	4 (1–18.83)	17.8 (7.2–105)	EMIT and TDX/FPIA [NA]	ALL
Shi et al. (2020) [26]	Retrospective	China (Single)	105 ()	5 g/m^2	24-h infusion	Every 6 h until concentration < 0.1 μmol/L	3 ± 3.63	16 ± 11.52	CMIA [0.02μoml/L]	Medulloblastoma

Table 1 (continued)

Study (publication year)	Type of study	Country (single/ multiple sites)	Number of subjects (samples)	MTX dosage regime	Administration time	Sampling design	Age Mean ± SD Median [range]	Weight Mean ± SD Median [range]	MTX assay [LOQ]	Diagnosis
Arshad et al. (2021) [38]	Retrospective	Germany (Single)	229 (2182)	NA	4 h or 24 h	42, 48 h	58 (19–82)	78.4 (41.5–227)	Immunoassays [0.009assayse	Hematological malignancies and solid tumors
Gao et al. (2021) [50]	Retrospective	China (Single)	331 (4517)	1–5 g/m^2	24-h infusion	24, 42, and 48 h, every 6 h until concentration < 0.25 μmol/L	5.0 (0.75–15.2)	19 (4.5–113)	Fluorescence polarization immunoassay	ALL
Schulte et al. (2021) [56]	Retrospective	USA (Single)	106 (1990)	1–8 g/m^2	24 or 36-h infusion	24, 42, and 48 h until < 0.1 μmol/L	10.1 (0.6–27.6)	NA	ARK [0.0427.6)iv	Malignant lymphoma and ALL
Isono et al. (2021) [39]	Retrospective	Japan (Single)	16 (193)	3.5g/m^2	4-h infusion	24, 48, and 72 h	66 (49–85)	61.5 (48.4–77.4)	FPIA[a](ABBOTT ARCHITECT® analyzer i1000SR) [0.04TECT®	PCNSL
Mao et al. (2022) [40]	Retrospective	China (Single)	77 (1458)	1.1–10.2 g/m^2	NA	24, 42, and 48 h until < 0.2 μmol/L	54.6 ± 9.2	67.8 ± 10.7	EMIT (SYVA Viva-Emit 2000 Kit) [0.3 Viva-E	PCNSL
Zhan et al. (2022) [51]	Retrospective	China (Single)	205 (1658)	0.97–8.3 g/m^2	24-h infusion	24, 48, and 72 h. If 72 h > 0.2 μmol/L need further monitored	4.61 (1.07–14.24)	28.70 (8.3–68)	FPIA (Viva-E/VT)	Malignant lymphoma and ALL
LDMTX										
Godfrey et al. (1998) [57] USA	Retrospective	USA (single)	62 (3260)	7.5 mg po/im	Weekly	0.5, 1, 2, 3, 4, 6, 8, and 24 h	58 ± 12	75 ± 18	FPIA [0.01μM]	RA
Batey et al. (2002) [58] UK	Retrospective	UK (single)	46 (NA)	40 mg/m^2 iv	1–5 min	5, 15, 30, 60, 90, 120, 180, 240, 360, and 480 min	51 (34–79)	70 (52–102)	HPLC [NA]	Breast cancer
Yukawa et al. (2007) [59]	Prospective	Japan (single)	34 (153)	① volunteers:2 mg single dose ② patients:6–8 mg (3 doses/ week)	Not applicable	0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 12 h of the third MTX tablet on the second day for patients while on the first day for volunteers	① volunteers: 22.1 ± 2.2 ② patients: 56.9 ± 14.4	① volunteers: 60.5 ± 5; ② patients: 53.1 ± 11	LC–MS/ MS[NA]	RA
Nagulu et al. (2010) [60]	Retrospective	India (single)	45 (122)	45 (122) 60 mg/ m^2 iv	1–5 min	NA	51.11 ± 10.5	49.1 ± 11.5	HPLC–UV [5μM]	Breast cancer
Kim et al. (2012) [61]	Prospective	Korea (Single)	20 (NA)	15 mg/m^2 i.v (post d1),10 mg/m^2 on d3, d6, and d11	NA	D1: 5 min, 2, 3, 6, 12, and 24 h D11: 6, 12, and 24 h	28 (18–49)	55.6 (44.8–80.8)	LC–MS/MS [2ng/ml]	Post-HSCT patients

Table 1 (continued)

Study (publication year)	Type of study	Country (single/multiple sites)	Number of subjects (samples)	MTX dosage regime	Administration time	Sampling design	Age Mean ± SD Median [range]	Weight Mean ± SD Median [range]	MTX assay [LOQ]	Diagnosis
Wang et al. (2019) [62]	Retrospective	China (Single)	71 (85)	NA	Weekly	NA	48.01 ± 15.24	59.37 ± 10.74	LC–MS/MS [0.5ng/ml]	RA

TDX/FPIA fluorescence polarization immunoassay TDx® analysers, *ARK* homogeneous enzymatic immunoassay (ARK methotrexate assay), *EMIT* enzyme multiplied immunoassay technique, *LC–MS/MS* liquid chromatography tandem mass spectrometry, *HPLC* high-performance liquid chromatography, *UPLC-MS/MS* ultra-performance liquid chromatography tandem mass spectrometry, *CL* apparent clearance (L/h), *V1* apparent volume of distribution of central compartment (L), *V2* apparent volume of distribution peripheral compartment (L), *Q* apparent intercompartmental clearance (L/h), *CLnr* the non-renal clearances (L/h), *CLr* the renal clearances (L/h), *CLfilt* filtration part of clearance (L/h), *CLsec* secretion/metabolism part of clearance (L/h), K_{el} elimination rate constant, K_{10} elimination rate constant from the central compartment, K_{21} the rate constant from central compartment to peripheral compartment, K_{12} the rate constant from peripheral compartment to central compartment, *Q2 and Q3* inter-compartmental clearance, *V2 and V3* second and third volume of distribution, *CMT* compartment, *BSV* between subject variability, *OCCL OCCL* equal to 0 (first chemotherapy) or 1 (second chemotherapy), *WT* weight(kg), *SCR* serum creatinine (μmol/L), *HB* hemoglobin(g/L), *BSA* body surface area (m^2), *ALT* alanine aminotransferase (U/L), *AST* aspartate transferase (U/L), *HT* height(cm), *CCR* creatinine clearance (ml/min), *DOSE10G* 0 when MTX dose is < 10 g and 1 when MTX dose is ≥ 10 g, *CCR* Cockcroft-Gault equation, *GFR* expected glomerular filtration rate, for adults was calculated using Cockcroft–Gault and normalized to a BSA of 1.73 m^2 in mL/min/1.73 m^2, *PEN* concurrent penicillin, *HOMZ* HOMZ was 1 in patients with the homozygous ABCB1 3435 TT genotype, both were 0 otherwise, *DP3* the difference of the urinary coproporphyrin concentrations I/(I + III) ratio between P3 and P1 (DP3), SCO2 means the presence or absence of at least one score 2 drug, *NUM* number of MTX chemotherapy cycles, *HCT* hematocrit, *OST* OST = 1 in osteosarcoma patients and OST = 0 in all other patients, *ALB* albumin(g/dl), *VBH* vertebral body height(cm), *IOV* interoccasion variability

revealed that the target C_{max} values were not achieved in either adults or children.

For patients with OS, MTX concentrations were higher in adults than in pediatric patients. In six studies of OS in children, MTX C_{6h} (median, range) was 702 μM (475–1199 μM), with large BSV. Three studies of patients with OS defined MTX C6h < 700 μM as subtherapeutic, and one defined MTX C6h > 1000 μM as supratherapeutic. Four studies simulated adults with MTX C_{6h} (median, range) of 877 μM (566–1006 μM). MTX C_{72h} (median, range) was 0.17 μM (0.06–0.28 μM) in children and 0.21 μM (0.055–0.267 μM) in adults (Fig. 3).

ALL patients

For ALL patients receiving 24 h infusion, target MTX concentrations were defined based on previous literature, which suggested a serum MTX concentration after 24 h infusion (MTX C_{24h}) < 16 μM as subtherapeutic with risk of relapse and MTX C_{24h} > 100 μM as supratherapeutic and associated with increased toxicity [64].

Nader et al. [33] studied ALL in Middle Eastern populations, for CL in this article was higher than that estimated in other PPK studies of non-Middle Eastern populations (Fig. 4). Our simulation results revealed that dosing regimens of 5 g/m^2 provided sufficient MTX exposure based on the 24-h MTX target of 16 μM. However, eight of the ALL simulation studies [44–51] examined supratherapeutic MTX concentrations (> 100 μM) at 24 h. Our results revealed that only one study was conducted in infants with supratherapeutic MTX C_{24h} for the simulated treatment period, suggesting the need for further research in this special population. The simulated MTX concentrations at 24, 48, and 72 h were higher in children than in adults, indicating that children are more likely to experience MTX toxicity.

Lymphoma malignancy

There are no studies regarding the optimal effective target MTX concentration in patients with lymphoma malignancy (Fig. 5). For PSCNL patients, Joerger et al. [53] found that MTX C_{24h} of 4.0–5.0 μM could achieve a target area under the concentration–time curve (AUC) of 1000–1100 μM at an MTX dose ≥ 3000 mg/m^2 administered via 4- or 6-h infusions. Three studies were for adults with PCNSL; the MTX C_{24h} in Pai et al. [36] was supratherapeutic, whereas that in the others was subtherapeutic (Fig. 6).

Table 2 Characteristics of MTX population pharmacokinetic studies for the included studies

Study (publication year)	Estimation method	Structural model	PK parameters and formula		BSV (%)	Residual error	Internal validation	External validation (N = samples)
Faltaos et al. (2006) [27]	FOCE	2CMT	CL	$7.1*(62/AGE)^{-0.22}*(67/SCR)^{-0.43}$	22 (IOV:16.5)	46% 0.015 μmol/L (fixed)	GOF	NR
			V1	25.1	22.5			
			V2	2.7	64			
			Q	0.15	51			
Aumente et al. (2006) [41]	FOCE	2CMT (Age > 10 y)	CL	0.149*WT (For age > 10 year) $0.287*TBW^{0.876}$(For age ≤ 10 year)	41.71	25.2% 0.00157 μmol/L	GOF	$N = 12$
			V1	0.437*WT (For age > 10 year); 0.465*TBW (For age ≤ 10 year)	41.59			
			K12	0.0155	20.76			
			K21	0.0724	35.21			
Fukuhara et al. (2008) [28]	FOCE	AVANS6	CL_r	$5.57*(CCR^b/80.0)**0.112$	26.4	50.7% 0.00334 μmol/L	GOF	NR
			CL_{nr}	0.567*3.39**DOSE10G	32.1			
			V1	26.9	/			
			Q	0.0778	/			
			V2	2.27	41.9			
Colom et al. (2009) [42]	FOCE-I	2CMT	CL (L/d)	88.5*(AGE/15) + 27.4*(WT/50)	11.9 (IOV:8.2)	25.5%	GOF	$N = 10$
			V1	11.0 + 5.6*(WT/50)	8.9			
			V2	0.464	/			
			Q(L/d)	0.456	/			
Min et al. (2009) [29]	FOCE	2CMT	CL	7.45*[1 + (0.89-SCR)*0.224];	50.6	0.0376 μmol/L 49.3%	GOF	NR
			V1	25.9*[1 + (0.89-WT)*(− 0.00937)];	22.5			
			V2	9.23	97.8			
			Q	0.333	70.4			
Faganel et al. (2011) [43]	FOCE	2CMT	CL (L/h/kg)	7.87*(WT/25)**0.395*Theta(1) if MTHFR677C > T = 677TT, Theta (1) = 0.738 else Theta (1) = 0.938;	29.7 (IOV:16.6)	0.0867 μoml/L 67.6%	VPC	NR
			V1(L/kg)	11.1*(WT/25)	14.2			
			V2	2.52	50.6			
			Q	0.213	16.5			
Johansson.et al. (2011) [52]	FOCE-I	2CMT	CLfilt	$fu*GFR^c(fu = 0.4^3)$	/	29.3%	VPC	NR
			CLsec	10.9*(WT/70)**0.944;	12.9 (IOV:12.0)			

Table 2 (continued)

Study (publication year)	Estimation method	Structural model	PK parameters and formula		BSV (%)	Residual error	Internal validation	External validation (N = samples)
			CLtot	(1) (CLfilt + CLsec)*(SCR_{mean}/SCR)$^{0.0831}$ Age ≤ 15y: SCR_{mean} = −2.37330 −12.91367*ln(age) + 23.93581 *$\sqrt{age}$; (2) 15y < age ≤ 17y: for boys, SCR_{mean} = 9.5471*age-87.847; for girls, SCR_{mean} = 4.7137*age-15.347; (3) Age ≥ 18y: for men, SCR_{mean} = 84; for women,SCR_{mean} = 69.5	/			
			V1	74.3	8.02			
			V2	4.1	36.5			
			Q	0.110*(WT/70)**0.693;	29.2			
Joerger et al. (2012) [53]	FOCE	2CMT	CL	10.8*(CCR^b/95)$^{0.28}$*(BSA/1.75)$^{0.15}$;	33.9 (IOV:13.3)	31.8%	GOF	NR
			V1	34.0	/			
			V2	6.3	35.8			
			Q	0.35	/			
Simon et al. (2013) [30]		3CMT	CL	3.99*(ABCC2-24C > T) + 1.91*CCR/89 (ABCC2-24T > T = 1 if CC else = 1.63 if CT or TT)	28.7 V1, CL (block = 0.78	44.4% (exp)		
			V1	19*(ABCC2-24C > T)(ABCC2-24T > T = 1 if CC else = 1.63 if CT or TT);	36.7		GOF	NR
			Q2	0.10	/			
			V2	1.58	/			
			V3	1.99	/			
			Q3	0.021	/			
Desoky et al. (2013) [44]	FOCE-I	2CMT	CL	2.18*(BSA/0.81)$^{-0.407}$*1.18*$^{(1-SEX)}$	22.9	0.196 (proportional error model)	GOF,VPC, bootstrap	NR
			V1	5.74*(WT/20)$^{0.163}$	30.3			
			V2	11.2	/			
			Q	0.132 FIXED	/			
Benz-de Bretagne et al. (2014) [31]	FOCE	2CMT	CL	7.05*(CCR/91.6)$^{0.272}$ *(DP3/0.6)$^{0.163}$–0.929*SCO2 (SCO2 = 1 for patents receiving at least one score 2, SCO2 = 0 for patients not receiving any score 2 drug during MTX infusion)	23	41.7%	GOF,NPDE, bootstrap	NR
			V1	23.5	34			
			V2	3.01	32.1			
			Q	0.13	/			

Table 2 (continued)

Study (publication year)	Estimation method	Structural model	PK parameters and formula		BSV (%)	Residual error	Internal validation	External validation (N = samples)
Watanabe et al. (2014) [32]	using the maximum likelihood method	2CMT	K10	0.214	10.6	15%	NA	$N = 17$
			V1	0.379	23			
			V2	0.001	50.6			
			Q	0.036	44.5			
Wright and Stewart (2015) [23]	FOCE	2CMT	CL	74.5*EXP (0.154*AGE)	12.6	0.212	NA	NA
			V1	16.6*EXP (0.113*AGE)	10.6			
			V2	1.42	49.8			
			Q	0.0431*EXP[0.887*(IFC-IFC_{median}]	31.5			
Zhang et al. (2015) [54]	FOCE	2CMT	CL	6.20*(1–0.0183*NUM)*[(1–0.0416*($CRCL^{e}_{1}$-1.89)]	8.48	10%	GOF, Bootstrap	NR
			V1	19.6	/			
			V2	0.515*[1–0.874*(BSA-1.62)]	39.1			
			Q	0.0172*[1–0.880*(BSA-1.62)]	50.9			
Nader et al. (2017) [33]	FOCEI	2CMT	CL	$15.7*(HCT/32)^{0.85}$	34.9 (IOV2:47.4;IOV3:31.1)	33.4%	GOF,VPC, Bootstrap	NR
			V1	79.2*(WT/69)**1.29	63.2			
			V2	51.4	/			
			Q	0.97	/			
Hui et al. (2019) [45]	FOCE	2CMT	CL	ALL: $7.73*(BSA/0.735)^{0.721}*(CLCR/192)^{0.256}$	14.3 (IOV:14.9);	30.2% (ALL); 31.3% (OS)	GOF,VPC, Bootstrap	NR
				OS: $5.17*(HT/156)^{1.59}*(CLCR/179)^{0.357}[(Dose/BSA)]^{0.407}$	60 (IOV:16.9)			
			V1	ALL: $19.0*(BSA/0.735)^{0.985}$	0 FIXED			
				OS: $21.4*(HT/156)^{2.31}$	6.9			
			V2	ALL: 6.63	34.6			
				OS: $(AGE/146)^{0.652}$ age(unit,mo)	27.1			
			Q	ALL:$0.283*(AGE/63.5)^{0.278}$	0 FIXED			
				OS:$0.0315*(WT/40)^{0.744}$	18.8			
Lui et al. (2018) [46]	FOCE	2CMT	CL	rs13120400(ABCG2) = (AA/AG) = $15.4*(WT/70)^{0.75}$; ABCG2 gene = (GGs) = $18*(WT/70)^{0.75}$	17.6 (IOV: 14.8)	30.7%	GOF,VPC	NR
			V1	81.7*(WT/70)	20.1			
			V2	4.96*(WT/70)	IOV: 25.2			
			Q	$0.18*(WT/70)^{0.75}$	/			
Zang et al. (2019) [47]	FOCE ELS	2CMT	CL	$6.53*(BSA)^{0.34}$	7.4	0.40 (Pro)	GOF,VPC, Bootstrap	NR
			V1	67.88	$1.33*10^{-5}$			
			V2	37.99	7.3			
			Q2	0.77	1.5			

Table 2 (continued)

Study (publication year)	Estimation method	Structural model	PK parameters and formula		BSV (%)	Residual error	Internal validation	External validation (N = samples)
Mei et al. (2018) [34]	FOCE ELS	2CMT	CL	$6.67*(SCR/68.1)^{(-0.48)*}(BSA^f/1.75)^{1.17}$	39.99	0.67 mg/L	GOF,VPC, Bootstrap	NR
			V1	$24.46*(AGE/57.16)^{0.81}$;	42.72			
			V2	1.32	25.11			
			Q	0.047	63.04			
				OS: $5.17*(HT/156)^{1.59}*(CLCR/179)^{0.357}[(Dose/BSA)]^{0.407}$	60 (IOV:16.9)			
			V1	ALL: $19.0*(BSA/0.735)^{0.985}$	0 FIXED			
				OS: $21.4*(HT/156)^{2.31}$	6.9			
			V2	ALL: 6.63	34.6			
				OS: $(AGE/146)^{0.652}$	27.1			
			Q	ALL: $0.283*(AGE/63.5)^{0.278}$	0 FIXED			
				OS: $0.0315*(WT/40)^{0.744}$	18.8			
Kawakatsu et al. (2019) [55]	FOCE	2CMT	CL	$12.0*(WT/70)^{0.75}*(GFR/137)^{0.48}*$ (1.14 if NUM greater than 7)	37.3 (IOV:23.8) 34.9 (CL-V1) −20.7 (CL-V2)	43.4%;(PRO) 0.004 μg/mL(ADD)	GOF	NR
			V1	$52.1*(WT/70)*(AGE/15)^{-0.13}*(ALT/47)^{-0.06}$	41.4 26.8(V1-V2)			
			V2	$5.64*(WT/70)*(ALT/47)^{-0.05}*OST^{0.63}$	57.4			
			Q	0.13 *(WT/70)** 0.75	/			
Beechinor et al. (2019) [24]		2CMT	CL	11.0*(WT/70)**0.75	10.7(IOV:25.4)	37.5%	GOF,VPC	NR
			V1	63.4*(WT/70)	13.2			
			V2	13.6*(WT/70)	/			
			Q	0.13*(WT/70)**0.75	/			
Panetta et al. (2020) [25]	FOCE		CL (ml/min/m^2)	$89.81*(BSA/0.52)^{0.70}*(GFR/128.9)^{0.15}*(1.19)^{DEX}*(1.20)^{VANC}$	4.1% 1.4% (IOV on CL)	0.020 (Pro); 0.001 μM(Add)	GOF,VPC, Bootstrap	NR
			V(L/m)	$14.40*(BSA/0.52)^{0.68}**(1.27)^{DEX}*(1.22)^{VANC}$	4.5%; 1.9 (IOV on V1)			
			K12	0.0079	3.2%			
			K21	0.0753	1.5%			
Medellin-Garibay et al. (2020) [48]	FOCE-I	2-CMT	CL	$6.5*(BSA)^{0.62}$	8.2	20.1%	GOF,VPC, Bootstrap	N = 9
			V1	0.36*WT	25.9			
			V2	3.2	26.7			
			Q	0.41	/			
Yang et al. (2020) [35]	FOCE-I	2CMT	CL	$6.03*(CCR/115.1)^{0.414}$	51.6	0.139%	GOF,VPC, Bootstrap	N = 42
			V1	20.70	48.3			
			V2	3.76	/			
			Q	$0.074*(BSA/1.65)^{1.43}$	65.6			

Table 2 (continued)

Study (publication year)	Estimation method	Structural model	PK parameters and formula		BSV (%)	Residual error	Internal validation	External validation (*N* = samples)
Pai et al. (2020) [36]	SAEM	2CMT	CL	6.87–0.508*(AGE/66)+0.642*(ALB/4.0)–(0.234*SCR+0.322*(VBH/3)	16	0.24%; 0.0013 μmol/L;	GOF,VPC,NPDE	NR
			V1	42.4	11			
			V2	2.88	55			
			Q	0.0825	48			
Gallais et al. (2021) [37]	FOCE-I	2CMT	CL	$8.3*(AGE/50)^{-0.317}$	23 (IOV:22)	34%	GOF	NR
			V1	27.4	/			
			V2	$3.1*(WT/70)^{0.453}$	38			
			Q	0.15fixed	/			
Taylor et al. (2020) [49]	FOCE	3CMT	CL	$11*(BSA/1.73)*(SCR/29)^{(-0.247)}$	8	44%	GOF	NR
			V1	16.5*(BSA/1.73)	0 FIXED			
			Q2	0.602*(BSA/1.73)	0 FIXED			
			V2	4.55*(BSA/1.73)	12			
			Q3	0.111*(BSA/1.73)	13			
			V3	13.1*(BSA/1.73)	10			
Shi et al. (2020) [26]	FOCE-I	1CMT	CL	CLi = 9.23*(1 + 0.0005*(CLCR-105.78)*(1 + 0.0017*(WT-16)), if Co-Dex, CL = 1.19*CLi	3	30.6%; 0.023 μmol/L	GOF, Bootstrap	NR
			V	32.8FIXED	/			
Arshad et al. (2021) [38]	FOCE-I	3CMT	CL	$4.52*(SCR/0.74)^{-0.49}*(AGE/58)^{-0.18}*(BSA/1.73)^{-0.23}*(1+SEX*-0.16)*$ (SEX = 0 for male and 1 for female)	33 (IOV:30); COV (CL,V1)53.85;	51%; 0.02 μmol/L	GOF,NPC	NR
			V1	4.29	116			
			V2	2.51	/			
			V3	2.36	/			
			Q2	0.37	/			
			Q3	0.02	/			

Table 2 (continued)

Study (publication year)	Estimation method	Structural model	PK parameters and formula		BSV (%)	Residual error	Internal validation	External validation (N = samples)
Gao et al. (2021) [50]	FOCE-I	3CMT	CL	6.9*(SCR-26)*0.0097	17.5	35.4%;	GOF,VPS	NR
			V1	20.7	/			
			V2	41.0	/			
			V3	3.17	/			
			Q2	0.255	26.2			
			Q3	0.217	/			
			V(L/m)	$14.40*(BSA/0.52)^{0.68}$ $*(1.27)^{DEX}*(1.22)^{VANC}$	4.5%; 1.9 (IOV on V1)			
			K12	0.0079	3.2%			
			K21	0.0753	1.5%			
			V1	0.36*WT	25.9			
			V2	3.2	26.7			
			Q	0.41	/			
Schulte et al. (2021) [56]	FOCE-I	2-CMT	CL	$CL = 10.79*(WT/70)^{0.58}$ $*(0.84)^{SNP1}*1^{SNP2:AG}*0.92^{SNP2:GG}$ $1.15^{SNP1*SNP2:AG}*1.23^{SNP1*SNP2:GG}$	32.9; 20.3 (BOV1);11.0(BOV2); 13.2 (BOV3);10.4(BOV4)	31.6%	GOF,VPC	NR
			V1	$38.8*(WT/70)^{0.67}$	35.9			
			V2	$4.54*(WT/70)^{0.87}$	81.9			
			Q	$0.13(WT/70)^{0.75}$	72.8			
Isono et al. (2021) [39]	FOCE	2CMT	CL	$4.9*(CrCL/94.5)^{0.456}*(UV/HV)^{0.458}$	25.3	25.259%; 0.0509%	GOF, VPC,Bootstrap	NR
			V1	9.010	/			
			V2	5.730	16.1			
			Q	0.669	/			
Zhan et al. (2022) [51]	FOCE	2CMT	CL	$9.33*(BSA/1.73)^{0.75}*e^{0.13*rs17004785}$ $*e^{0.39*rs4148416}$	25.79	0.46(Pro)	GOF, VPC,Bootstrap	NR
			V1	24.98*(BSA/1.73)	50.82			
			V2	4.70*(BSA/1.73)	6.80			
			Q	$0.18*(BSA/1.73)^{0.75}$	1			
Mao et al. (2022) [40]	FOCE-ELS	2CMT	CL	$4.8*(CrCL/98)^{0.49}*(ALB/40)^{0.35}*(0.89,$ if age > 60)	20.9 24.7 (IOV on CL)	40.1%	GOF, VPC	NR
			V1	20.9	19.6			
			V2	0.09	30.4			
			Q	5.9	18.3			

Table 2 (continued)

Study (publication year)	Estimation method	Structural model	PK parameters and formula		BSV (%)	Residual error	Internal validation	External validation (*N* = samples)
Godfrey et al. (1998) [57]	FOCE	2CMT	CL(L/h)	(0.081*CCR*0.06–0.166*WT)*(1 + 0.312*GEN)	27.2	15%	GOF	NR
			V1	0.468*WT	27.9			
			V2	− 0.169*WT + 4.25*AGE	31.2			
			Q	− 0.354*(1 + 0.121*GEN)	40.5			
			Ka_{po}	(− 0.0416–0.469*DOSE)*(1 + 0.225*FED)	76.7			
			LAG_{po}	0.934	/			
Batey et al. (2002) [58]	FOCE	2CMT	CL	128 − (CFR-80)*1.05	20	17%	NR	NR
			V1	15.5*(1.5–3.33*OCCL) + 0.229*(WT-75)	37			
			V2	10.9	22			
			Q	206	28			
Yukawa et al. (2007) [59]	FOCE	2CMT	CL (L/kg/h)	$0.177*0.394^{MULT}$	25.7	17.8%	GOF	NR
			V1(L/kg)	0.0501	22.3			
			V2(L/kg)	0.368	/			
			Q(L/kg/h)	0.056	217.9			
			Ka	0.503	/			
Nagulu et al. (2010) [60]	FO	2CMT	CL	3.5	0.3	20%(PRO)	GOF	NR
			V1	1.25	0.03			
			V2	6.45	8.6			
			Q	8.43	20.7			
Kim et al. (2012) [61]	FOCE	2CMT	CL	$6.93*[1 + 0.376*(GFR/116)]*0.613^{PEN}*1.3^{HOMZ}$(ABCB1 3435TT = 1)	21.6	37.8%	GOF	NR
			V1	19.4	73.3			
			V2	2.57	/			
			Q	11.8	/			
Wang et al. (2019) [62]	FOCE	1CMT	CL	$7.75*e^{0.167}*0.805$(if RS2030 E.Q. 1) $7.75*e^{0.167}*0.647$(if RS2030 E.Q. 2) $7.75*e^{0.167}$(if RS2030 E.Q. 3)	0.167 FIX	0.713 (pro) 2.83 ng/ml	GOF, Bootstrap	NR
			V	32.8	0 FIX			
			Ka	1.69	0 FIX			
			F	0.704	0 FIX			

Table 2 (continued)

CL apparent clearance (L/h), *V1* apparent volume of distribution of central compartment (L), *V2* apparent volume of distribution peripheral compartment (L), *Q* apparent inter-compartmental clearance (L/h), *CLnr* the non-renal clearances (L/h), *CLr* the renal clearances (L/h), *CLfilt* filtration part of clearance (L/h), *CLsec* secretion/metabolism part of clearance (L/h), K_{el} elimination rate constant, K_{10} elimination rate constant from the central compartment, K_{21} the rate constant from central compartment to peripheral compartment, K_{12} the rate constant from peripheral compartment to central compartment, *Q2 and Q3* inter-compartmental clearance, *V2 and V3* second and third volume of distribution, *CMT* compartment, *BSV* between subject variability, *OCCL OCCL* equal to 0 (first chemotherapy) or 1 (second chemotherapy), *WT* weight(kg), *SCR* serum creatinine (μmol/L), *HB* hemoglobin(g/L), *BSA* body surface area (m^2), *ALT* alanine aminotransferase (U/L), *AST* aspartate transferase (U/L), *HT* height (cm), *CCR* creatinine clearance (ml/min), *DOSE10G* 0 when MTX dose is < 10 g and 1 when MTX dose is ≥ 10 g, *CCR* Cockcroft-Gault equation, *GFR* expected glomerular filtration rate, for adults was calculated using Cockcroftotherapy);Application/7.0.1.0227/resultu^2 in mL/min/1.73 m^2, *PEN* concurrent penicillin, *HOMZ* HOMZ was 1 in patients with the homozygous ABCB1 3435 TT genotype, both were 0 otherwise, *DP3* the difference of the Urinary coproporphyrin concentrations I/(I + III) ratio between P3 and P1 (DP3), SCO2 means the presence or absence of at least one score 2 drug, *NUM* number of MTX chemotherapy cycles, *HCT* hematocrit, *OST* OST = 1 in osteosarcoma patients and OST = 0 in all other patients, *ALB* albumin(g/dl), *VBH* vertebral body height (cm), *IOV* interoccasion variability

[a]GFR = (6230–32.8*age)*BSA*(1–0.23*Sex)/Scr

[b]CCR = [140-Age (years)]*Body weight (kg)/[0.818*SCR (μmol/L)] × (0.85, If the patient is female)

[c]GFR = 6.72*WT/70**0.632

[d]GFR was estimated using the Cockcroft-Gault formula

[e]$CRCL_1$ Creatinine clearance before administration

[f]BSA = 1.05 + (WT-30)*0.02(WT > 30 kg) or 0.035*WT + 0.1(WT ≤ 30 kg)

CRCL of Zhang et al. [54] used baseline Scr by Cockcroft-Gault equation

Cockcroft-Gault equation: CRCL = [140-Age (years)]*Body weight (kg)/[0.818*SCR (μmol/L)] × (0.85,If the patient is female);

Johansson et al. [52] calculated GFR as following:

GFR = 6.72*WT/70**0.632 [7]

[e]$CRCL_1$ Creatinine clearance before administration [13]

Male: BSA = 0.00607*height + 0.0127*weight-0.0698

Female: BSA = 0.00586*height + 0.0126*weight-0.0461

[f]BSA = 1.05 + (WT-30)*0.02(WT > 30 kg) or 0.035*WT + 0.1(WT ≤ 30 kg)

Yang et al. used (CKD-EPI2009Scr) equation:

For female: if SCR(μmol/L) ≤ 62, GFR = 144*(SCR/0.7)**(− 0.329)*0.993**Age and if SCR(μmol/L > 62, GFR = 144*(SCR/0.7)**(− 1.209)*0.993**Age

For male: if SCR(μmol/L) ≤ 80, GFR = 144*(SCR/0.9)**(− 0.411)*0.993**Age and if SCR(μmol/L > 80, GFR = 144*(SCR/0.9)**(− 1.209)*(0.993)**Age

SNP1, SLCO1B1 521CT, or 521CC (any variant); SNP2:AG, SLCO1B1 388AG; SNP2:GG, 388GG

BOV1, BOV2, BOV3, and BOV4, inter-occasion variance for CL from course 1 to course 4, respectively

Error variance

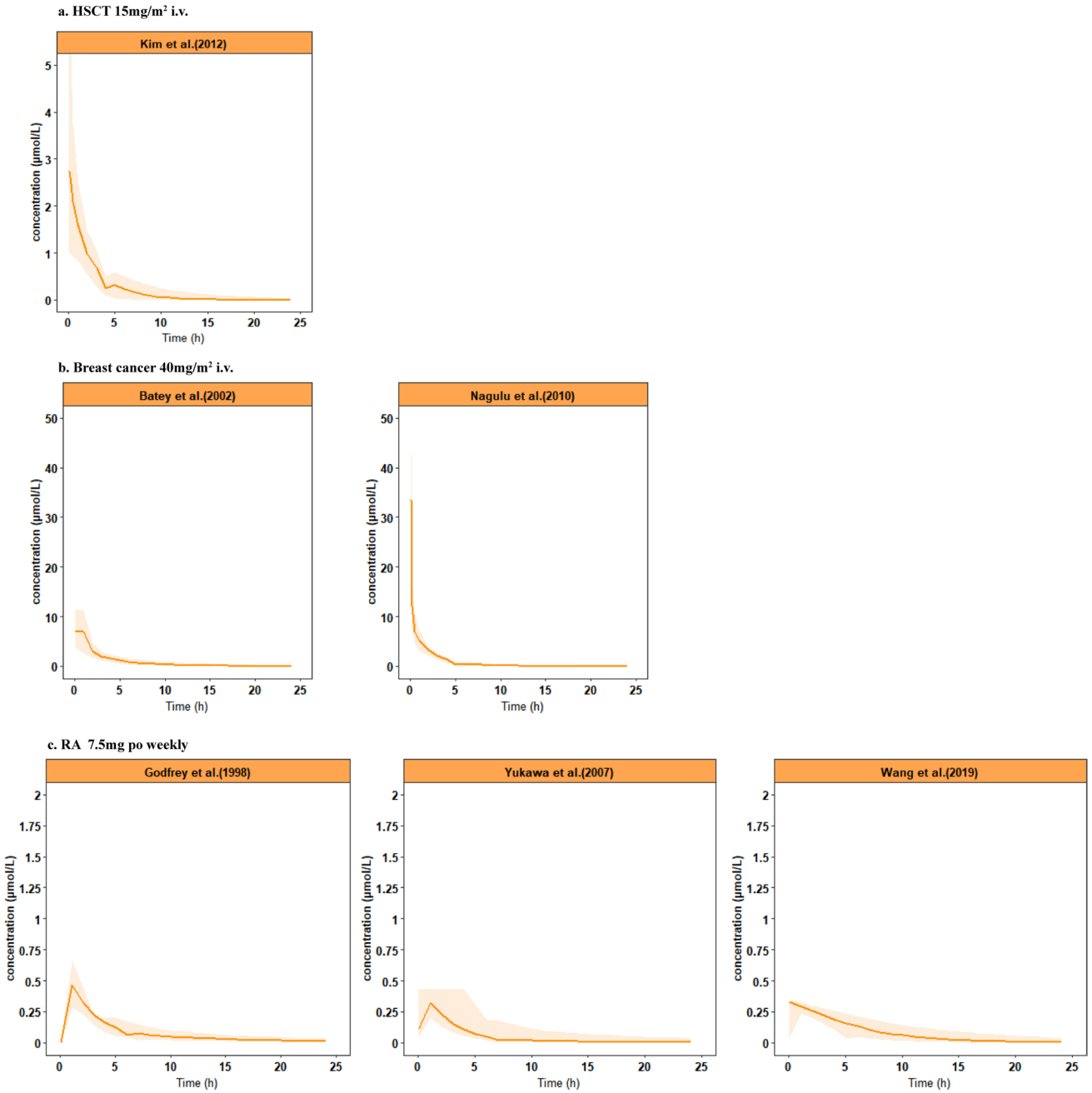

Fig. 2 Concentration–time profiles of LDMTX for HSCT patients (**a**), breast cancer patients (**b**), and RA patients (**c**), respectively. The solid line represents the median of the simulated concentration–time profile, and the light shadows represent the 10th–90th percentiles of the simulated concentration–time profiles

Comparison of studies for covariates

All of the included PPK studies were used to identify potential covariates to describe the BSV of MTX PK. Three studies did not report covariates [24, 32, 60]. The most frequently identified covariates were CrCL, SCr, BSA, and weight. CrCL significantly influenced MTX PK. Fifteen studies retained CrCL as a significant predictor of MTX CL [25, 26, 28, 30, 31, 35, 39, 40, 45, 53–55, 57, 58, 61]. For adults, most of these applied the Cockcroft–Gault formula, while Yang et al. [35] used the Chronic Kidney Disease Epidemiology Collaboration 2009 SCr formula; Johansson et al. [52] used the Rhodin formula [65]; and Batey et al. [58] used an estimate of CrCL obtained from the EDTA PPK model

[66]. For pediatric patients, most of the studies applied the Schwartz formula, while Panetta et al. [25] used the St. Jude equation, and Johansson et al. [52] used a linear extrapolation based on the oldest pediatric group predicted by Ceriotti et al. [67]. For patients with renal impairment (CrCL 20 mL/min), the median CL (range) was 52.5% (40–85%), lower than for those with median renal function.

Eight studies of HDMTX that retained SCr as significant revealed that HDMTX CL decreased as SCr increased [27, 29, 34, 36, 38, 49, 50, 52]. Taylor et al. [49] reported a nonlinear relationship between SCr and MTX CL that varied with time. Some studies were unable to detect a nonlinear relationship between SCr and CL, possibly owing to the narrow distribution of SCr or small sample size. Three studies revealed an effect of SCr on CL exceeding 40%. Based on these studies, for patients with renal failure (SCr > 3 mg/dL), the median estimated CL was 58% lower than in patients with median SCr (Fig. 7).

HDMTX dosage is calculated based on the patient's BSA. Eight studies retained BSA as a covariate; in all cases, it was positively correlated with CL [25, 34, 38, 44, 45, 47, 48, 53]. Five studies of pediatric patients revealed that BSA significantly influenced CL, with a median decline of CL 51% (range 37–77.8%). Therefore, it is necessary to consider the influence of BSA influence on CL in pediatric patients.

Seven studies retained gene polymorphism as a covariate and identified several genes that contribute to the vast variability in MTX PK. These included *MTHFR 677C > T* (rs1801133) [43], a folate pathway gene associated with MTX; MTX-related transporter genes including *ABCC2* (rs717620) [30], *ABCB1* (rs1045642) [61], *ABCG2* (rs13120400) [46], *ABCC3* (rs4148416) [51], the *SLCO1B1 521 T > C* (rs4149056) and *SLCO1B1 388A > G* (rs2306283) variants which encodes OATP1B1 [56], *SLC19A1* (rs17004785) [51], and *OATP1B1* (rs2306283) [62]. Our results revealed that a mutation in *MTHFR* reduced enzyme activity, thus reducing MTX CL by ca. 26.2%. Mutations in *ABCG2*, *SLCO1B1 388A > G*, and OATP1B1 reduced MTX CL. Patients carrying the *OATP1B1* 388A > G mutation exhibited a 35.3% reduction in CL, which has clinical significance. Expression of *ABCC2*, *ABCC3*, and *ABCB1* increased CL by 43%, 48%, and 30%, respectively, demonstrating that all of these genes have clinically significant effects.

Seven articles reported the effects of bodyweight on CL; of these, six addressed HDMTX in children and young adults [26, 41–43, 52, 56, 57], and one addressed LDMTX in adults [57]. These studies all revealed that MTX CL increased with bodyweight. Aumente et al. [41] showed that, for children, age is closely related to weight. Our results revealed that, for the same weight (40 kg), children younger than 10 years had 24% higher CL than those older than 10 years. This is consistent with the findings of Donelli et al. [66], who reported that in children older than 10 years, the drug reached higher plasma concentrations and was cleared at a lower rate.

Seven studies retained age as a covariate, and six studies of adults found that age was negatively associated with CL [23, 27, 36–38, 40, 42]. One study of children and one of infants revealed that age was positively correlated with CL [23].

Four studies revealed that co-medication influenced MTX CL, with inhibition of transporters involved in MTX uptake as the potential mechanism. HDMTX combined with both benzimidazoles and β-lactams [31] reduced MTX CL by 11%, whereas co-administration of penicillin reduced it by 38.7% [61]. Dexamethasone [25, 26] and vancomycin [25] were associated with a small increase in MTX clearance (ca. 20%).

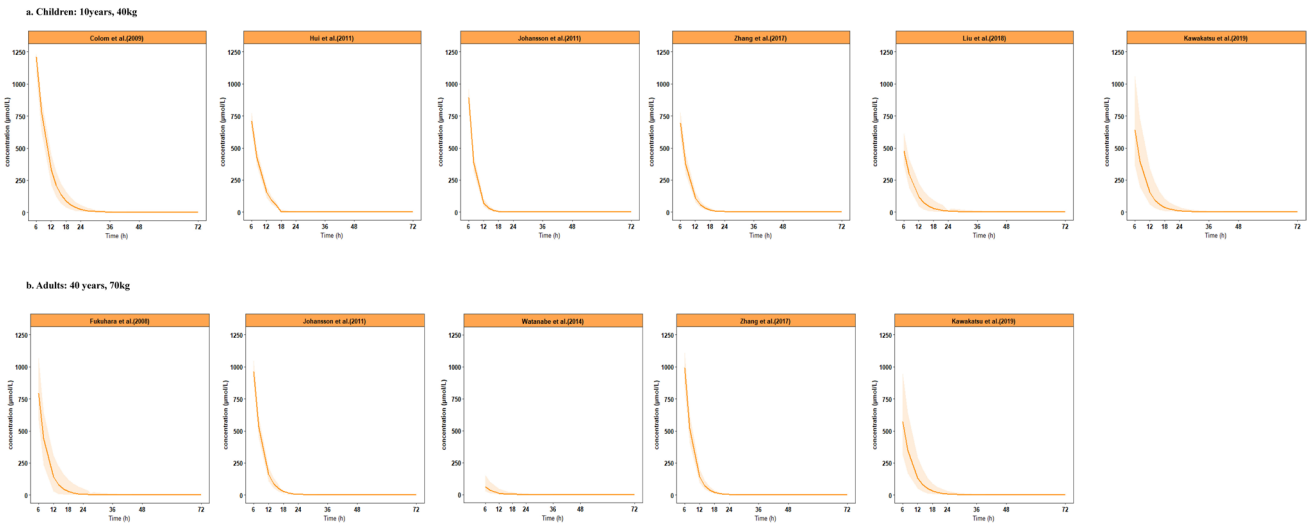

Fig. 3 Concentration–time profiles of HDMTX for **a** children and **b** adults with OS patients in retrieved studies. The solid line represents the median of the simulated concentration–time profile, and the light shadows represent the 10th–90th percentiles of the simulated concentration–time profiles

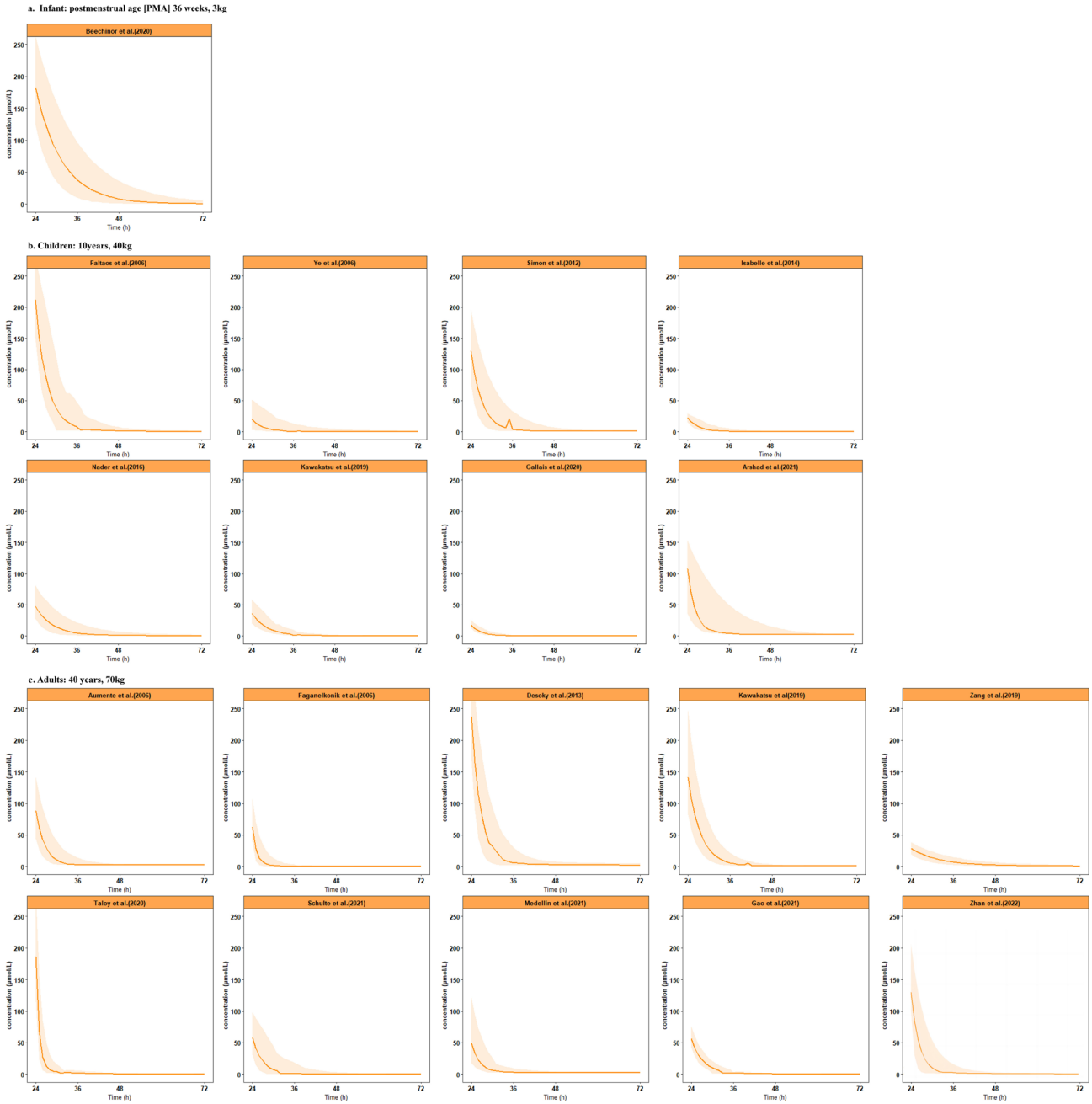

Fig. 4 Concentration–time profiles of HDMTX for **a** infant, **b** children, and **c** adults with ALL in retrieved studies. The solid line represents the median of the simulated concentration–time profile, and the light shadows represent the 10th–90th percentiles of the simulated concentration–time profiles

Three studies retained sex as a significant covariate, finding that CL was higher for males than females. Two of these included patients with OS. One LDMTX study found that MTX dosage increased the clinical significance of MTX CL.

Other covariates retained as significant for CL included hematocrit (HCT) [33], urine volume to hydration (UV/HV) [39], vertebral body height (VBH) [36], difference in the urinary coproporphyrin I (UCP I): UCP I + III ratio between hospital discharge and methotrexate infusion (DP3) [33], and albumin (ALB) [36, 40].

Seven articles retained bodyweight as a covariate of MTX CL [29, 33, 41–44, 48]. Bodyweight was positively correlated with V_d; therefore, bodyweight affected both MTX distribution and elimination. One study fixed V_d at 32.8 L/kg [26] because it could not be accurately estimated owing to the lack of distribution of blood collection points. The

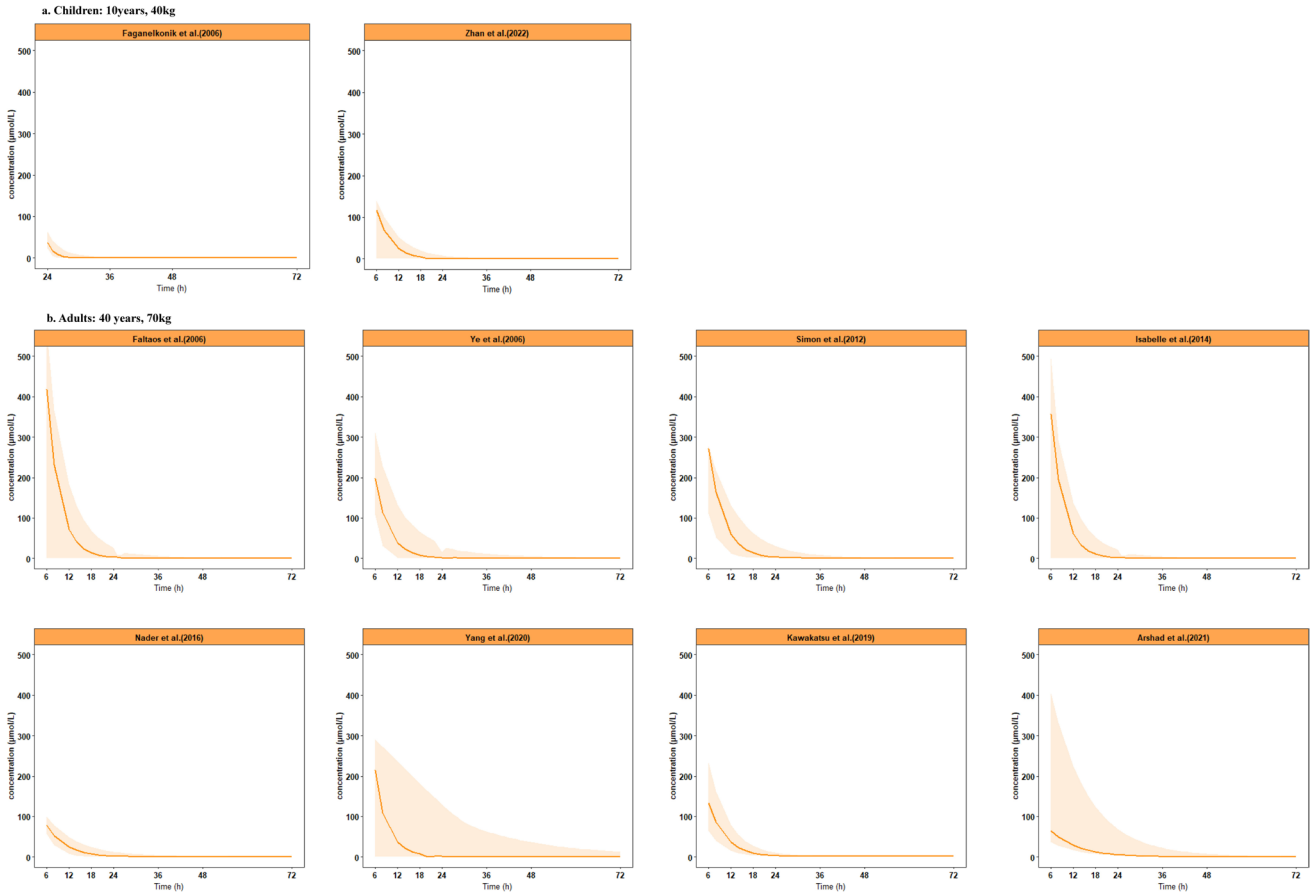

Fig. 5 Concentration–time profiles of HDMTX for **a** children and **b** adults with Lymphomas in retrieved studies. The solid line represents the median of the simulated concentration–time profile, and the light shadows represent the 10th–90th percentiles of the simulated concentration–time profiles

inter-individual variability in V_d was partially explained by the fact that this parameter was weight-dependent, supporting the need for dose adjustment following significant weight gain or loss. Other covariates of V_d included age [34, 41] BSA [25, 45], alanine aminotransferase (ALT) [55], and co-medication with dexamethasone and vancomycin [50].

Several age-dependent factors can influence the distribution of drugs in the body, most notably of drugs with high plasma protein levels [68]. Age positively influenced V_d in the study of Mei et al. [34], with a median age of 57.16 years. In the study by Kawakatsu et al. [55] (median age 15 years), age was negatively correlated with V_d. The reason for this inconsistency may be that bodyweight increases with age in patients ≤ 50 years, decreasing after 50 years. The distribution of MTX and accumulation of MTX-polyglutamate in erythrocytes increases with age [69]. Two studies of pediatric patients by Hui et al. [45] and Panetta et al. [25] found that V_d was significantly related to BSA because of the rapid increase in body size as children grow. Hui et al. [45] found that height influenced V_d in patients with OS. Panetta et al. [25] found that dexamethasone and vancomycin use influenced V_d, probably as an indirect result of the patient's clinical care and conditions, such as increased fluid administration and increased MTX V_d.

Kawakatsu et al. [55] found that ALT significantly affected V_d and V_p, suggesting that ALT is an indicator of liver dysfunction and that increased ALT could lead to decreased albumin production. Changes in liver function may affect MTX binding and affinity in plasma and tissue. Gallais et al. [37] and Schulte et al. [56] found that V_p was directly correlated with weight, implying that the higher the weight, the longer the MTX half-life. Simon et al. [30] found that *ABCC2 24C > T* was involved in MTX elimination and distribution, although the mechanism underlying the association between *ABCC2* and MTX PK remains unclear. For the HDMTX peripheral clearance rate (Q_P), age influenced Q_p in patients with ALL, and weight influenced Q_p in those with OS. Yang et al. [35] and Zhang et al. [54] found that BSA was positively correlated with Q_p, and Hui et al. [45] found that age influenced Q_p in patients with ALL.

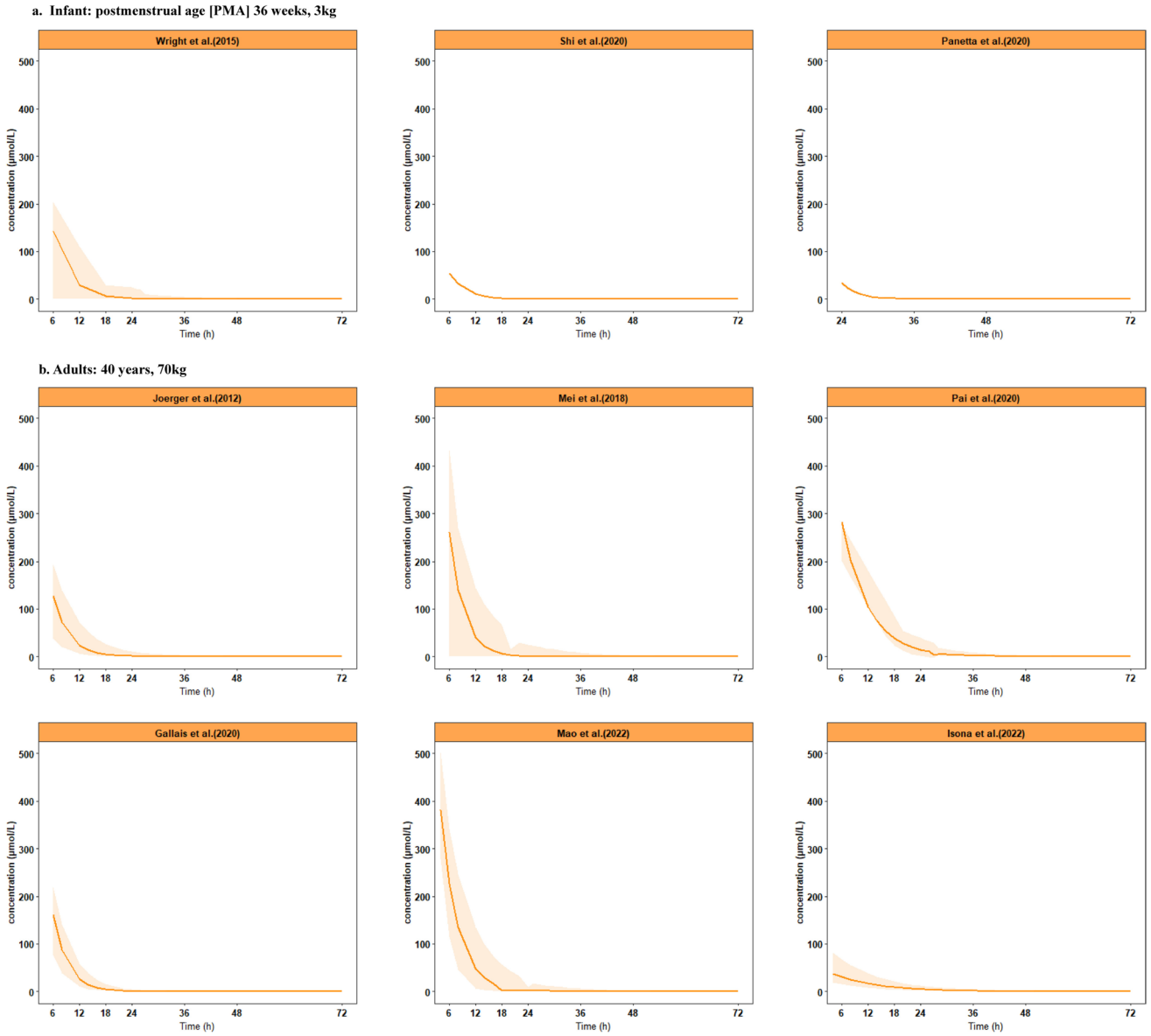

Fig. 6 Concentration–time profiles of HDMTX for **a** infants and **b** adults with PCNSL in retrieved studies. The solid line represents the median of the simulated concentration–time profile, and the light shadows represent the 10th–90th percentiles of the simulated concentration–time profiles

Discussion

Our study reveals that, after administering MTX at a fixed dose for the same diagnosis, MTX CL exhibited substantial inter- and intra-patient variability, varying tenfold in patients with normal renal function, and MTX plasma concentration varied substantially between individuals. The selected HDMTX studies, of infants, children, and adults, reported different concentration–time profiles. MTX CL varied substantially between the LDMTX and HDMTX studies. Since MTX is contraindicated during pregnancy, none of the studies included this special population.

Based on our findings, HDMTX should be applied in conjunction with TDM to optimize initial dosing regimens to reach target concentrations and achieve elimination-phase concentrations. This will guide rescue strategies to prevent MTX-induced toxicity. In contrast, it is unnecessary to perform TDM routinely for LDMTX, except in cases of MTX-related toxicity and suspected ineffective MTX treatment.

Although some PPK studies have been conducted for adult and pediatric patients, most of these were single-center studies that were limited by not fully investigating the factors that may affect MTX PK. Our study is one of the most comprehensive evaluations of MTX PK to date, representing

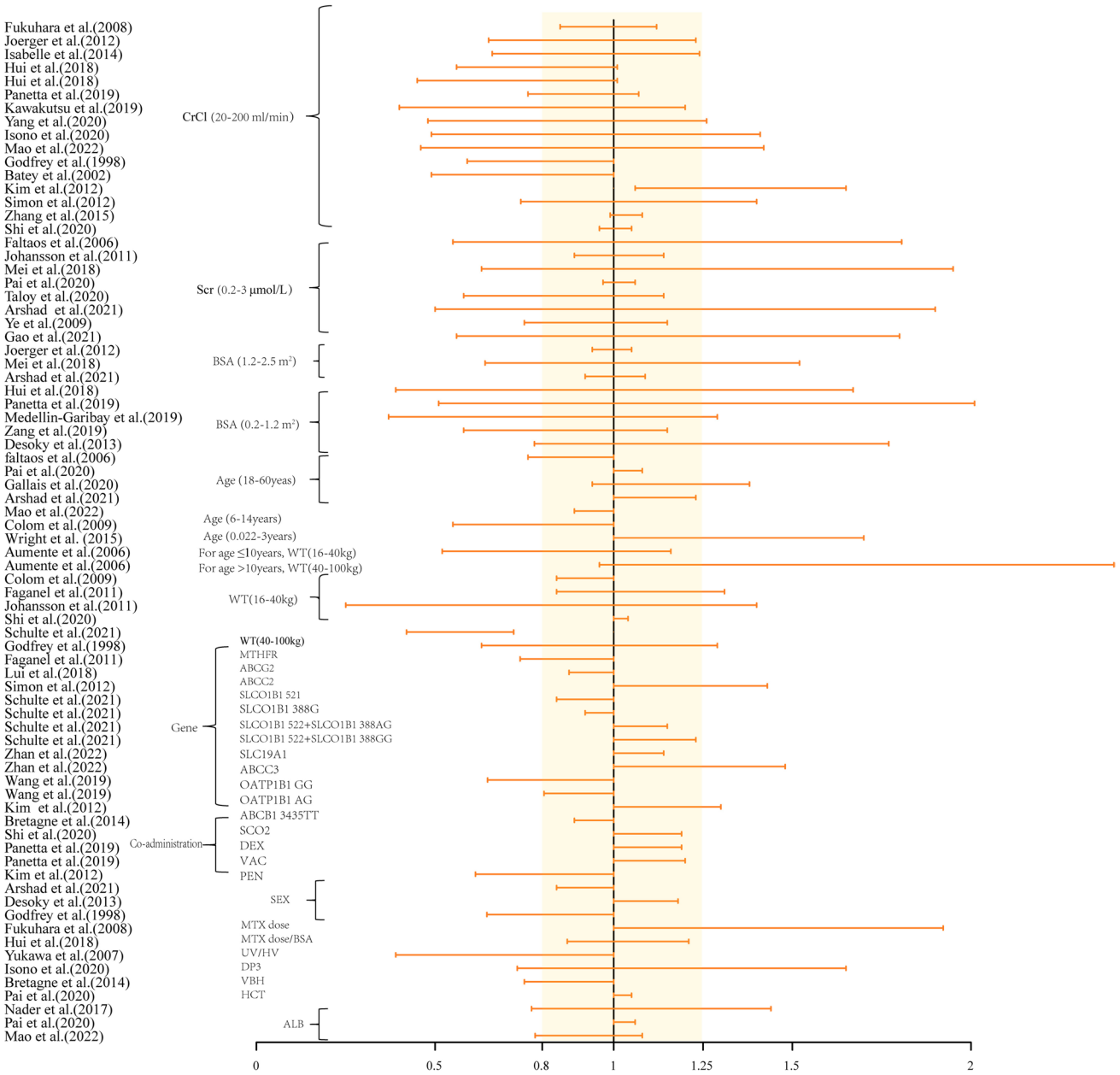

Fig. 7 Covariate effect on the clearance of MTX. The horizontal bars represent the covariate effect on clearance in each study. The typical value of clearance in each study was considered to be 1. The effect of each covariate for clearance is displayed by the ratio of clearance in the range each covariate to the typical clearance value. The shade area ranges from 80 to 125%

a wide range of ages and disease types. Our findings can potentially help in optimizing initial MTX dosing regimens for patients with different physical characteristics.

Our results revealed that HDMTX CL was higher in infants than in children and adults, because renal excretion is its primary route of elimination, accounting for ca. 70–90% of MTX clearance [8]. The reduction in HDMTX CL with age is due to the maturation of renal tubule function, glomerular filtration rate, and renal blood flow [24], and to the increase in CL in the first year of life as the metabolic pathways mature. CL gradually declines after the first year of life, reaching the adult level in adolescence [70]. However, for infants, the concentration profiles illustrate that BSA influences MTX pharmacokinetics.; infants had significantly lower MTX elimination than children and adults, with a greater possibility of metabolic delays. For infants aged < 1 year, further PK data is required to describe MTX disposition in this vulnerable population. Future studies are therefore needed to elucidate the relationship between MTX efficacy and toxicity in infants, and the use

of BSA-dependent dosing for infants should be re-evaluated. Children exhibit higher renal function than adults, potentially explaining the difference in CL between children and adults [70]. Our Monte Carlo simulations revealed considerable variation in blood MTX levels within individuals. Therefore, to ensure that all patients achieve similar plasma MTX, we propose individualizing MTX dosing via TDM, which involves determining the individual MTK PK.

We found that adults had lower V_d than children, potentially because body water content declines with age. Moreover, substantial amounts of MTX can be distributed into third-space fluids [8], potentially affecting its disposition in infants, who are known to undergo significant changes in total body water content, from ca. 75% during the neonatal period to 55% in adulthood [25]. Furthermore, MTX is 50% protein-bound, and glomerular filtration is limited to unbound drugs; as infants' physiological parameters potentially alter the plasma protein-binding of drugs, they exhibit lower total plasma protein than adults [70]. Therefore, prior knowledge of age- and weight-related changes would be useful for designing pediatric clinical trials and defining the initial MTX dose to be administered in the pediatric population.

The aim of reviewing these PPK studies was to identify potential covariates describing the BSV of MTX PKs. Two of the selected studies included ethnicity as a covariate, finding no significant associations [25, 56]. However, CL and V_d were reportedly higher in Middle Eastern populations than in other populations [33], indicating that ethnicity may influence the MTX PK; this requires further study.

Renal function parameters (CrCL and SCr) significantly influence MTX CL. One of the selected trials (of adults receiving HDMTX for ALL) recommended that the HDMTX dose should be based on SCr on the day of treatment [71] and suggested reducing the dose to 50% of the usual dose if SCr is greater than 2.0 mg/dL. Our results showed that, for adults, when SCr was increased from 1 to 3 mg/dL, the CL of MTX for Faltaos et al. [27] and Mei et al. [34] decreased by 33% and 36%, respectively. The optimal CrCL-based MTX dose adjustment is unclear because there is no consensus on the optimal dose-reduction scheme and institutional practices vary. Our results reveal that MTX CL was 24% lower in patients with renal insufficiency (eGFR < 60 mL/min/1.73 m^2) and 47.5% lower in those with renal impairment (eGFR < 20 mL/min/1.73 m^2) than in those with normal renal function (eGFR > 90 mL/min/1.73 m^2). Therefore, for patients with renal impairment, the MTX dosing regimen must be adjusted before infusion. A pooled HDMTX PPK model is needed to analyze the relationship between age and renal function to achieve individualized MTX dosing. Monitoring of SCr alone is inadequate for this purpose because there are large inter-individual variations in MTX clearance. If the patient develops HDMTX-related nephrotoxicity during treatment, SCr should be serially monitored, and MTX administration should be paused until SCr has returned to baseline.

Until now, dose adjustment for hepatic impairment has not been provided in the MTX drug insert. Hepatic elimination is estimated to account for only 5–10% of MTX elimination, and no studies have considered the effects of hepatic function on CL (such as by measuring serum bilirubin or transaminase). The liver plays a key role in the metabolic conversion of MTX to 7-hydroxy-MTX via aldehyde oxidases [3]. Two studies conducted on children found that HDMTX co-administration with dexamethasone increased HDMTX CL by 19%, potentially because dexamethasone may induce aldehyde oxidase. The *SLCO1B1* gene encoding the basolateral hepatocellular transporters (OATPs) is almost exclusively expressed in the liver. Schulte et al. [56] found that genetic variation in *SLCO1B1* was associated with reduced MTX clearance, consistent with the findings of a systematic review by Taylor et al. [49], which revealed that *SLCO1B1* was the only gene that influenced MTX PK. These results indicate that for patients co-administrated dexamethasone and MTX and possessing the *SLCO1B1* gene mutation, hepatic function may be impaired; such patients, and especially children, may require MTX dosage adjustment.

Among the selected studies, only a study addressing HCT [33] revealed that it had a clinically significant impact on CL. Our simulation results revealed that MTX C_{72h} was highest in adults with ALL. Because MTX can be distributed into red blood cells, erythrocytes could be considered storage compartments for MTX, with several pharmacological implications [72]. Hemoglobin is highly correlated with HCT levels. Similar to MTX, 7-OH-MTX is also polyglutamylated in cells, and the retention of these polyglutamated forms in erythrocytes may contribute to MTX toxicity. Differences in HCT levels between patients, or changes in HTC within the same patient during treatment, may necessitate dose adjustments. The effects of HCT and hemoglobin on MTX CL require further study [73, 74].

Of the selected studies, two studies of patients with OS found that MTX dosage influenced CL. Hui et al. [45] found that the ratio of the MTX dose to BSA was positively associated with MTX CL. Fukuhara et al. [28] showed that MTX dosage was the most influential categorical covariate for MTX CL; MTX dose ≥ 10 g resulted in a difference in non-renal clearance of MTX. First, patients receiving higher doses of MTX are likely to frequently require extra hydration and urine alkalinization, which may increase CL. Second, HDMTX increases CL via a compensatory clearance mechanism caused by saturation of the process involving active tubular secretion [75], indicating that MTX is secreted, and not reabsorbed, by the renal tubules. However, tubular secretion is saturated at higher plasma concentrations, playing only a minor role in MTX elimination during and

immediately after HDMTX infusion [76]. Further exploration of the potential nonlinearity of HDMTX PK is required. An LDMTX study of patients with RA found that the CL was lower after single-dose administration than multiple doses (single-dose, 0.39 L/kg; multiple doses, 1 L/kg).

Our results confirm that HDMTX PK parameters are influenced by the type of disease, MTX dosage, and the duration of infusion [55]. It demonstrates that the treatment response at the end of infusion and HDMTX toxicity in the terminal elimination phase vary significantly with MTX serum concentration, thus emphasizing the need to develop adaptive dosing methods [77].

Our study had some limitations. The primary limitation is that our study is based on sparse data. Furthermore, only articles published in English were included, and we excluded articles that were missing PK parameters. In most of the selected studies, the data were collected retrospectively from routine TDM.

Conclusion

MTX PK differed among infants, children, and adults with different diagnoses. Infants showed higher CL and V_d than adults and children following HDMTX administration at the same dosage per kilogram bodyweight. These findings reveal that MTX dose individualization should depend on both renal function and BSA. Further PPK studies are required to characterize the MTX PK in infants. Prospective MTX PK/PD studies should be conducted to clarify how the MTX exposure–response relationship varies between patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00228-023-03579-0.

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Author contribution YYY, ZW, and ZJ designed the review and planned the work that led to the manuscript. ZL and CW performed the literature search and data analysis. YYY, ZW, and ZJ drafted and revised the manuscript. All authors approved the final version of this manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval No ethical approval is required/exemption granted.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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