White Paper

Methotrexate Monitoring for Oncology and Autoimmune Disorders

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Key messages

- High-dose methotrexate (MTX) therapy is a key therapeutic agent for treating numerous types of cancer in both children and adults.
- High-dose methotrexate therapy can be highly cytotoxic and carries risk of severe or fatal damage to the kidneys, liver, heart, GI tract and skin.
- Laboratory evaluation plays a key role in patient care:
  - Monitoring serum MTX concentrations for 24–48 hours after initiation of infusion is important for determining timing and dosing of leucovorin rescue.
  - Monitoring serum MTX concentrations during and after infusion can detect delayed elimination and toxicity risk earlier than using other elimination markers such as serum creatinine.
  - Evaluating the elimination rate using serum MTX concentrations pre- and post-chemotherapy cycles may allow for individualized dosing decisions for subsequent cycles to maximize therapeutic efficacy and minimize toxicity and other adverse events.

Introduction

Methotrexate is a pleiotropic drug. As a folic acid analog, MTX is classified as an antimetabolite and antineoplastic agent, but also has antirheumatic, antipsoriatic, and immunosuppressant activity. Due to its multiple methods of action it is used in high doses as a chemotherapeutic agent for treating several forms of cancer and in low doses to treat a wide range of inflammatory autoimmune diseases including rheumatoid arthritis, Crohn's Disease, and psoriasis.[1,2]

Abbreviations

AKI	Acute kidney injury
ALL	Acute lymphoblastic leukemia
AUC	Area under the curve
csDMARD	Conventional synthetic disease-modifying antirheumatic drug
DME	Delayed MTX elimination
GFR	Glomerular filtration rate
HDMTX	High-dose methotrexate therapy
MTX	Methotrexate
RA	Rheumatoid arthritis
ROC	Receiver operator characteristic
sCr	Serum creatinine
SNPs	Single nucleotide polymorphisms
WHO	World Health Organization

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High-Dose MTX as a Chemotherapeutic Agent in Oncology

In 2019, approximately 200,000 patients worldwide had a need for high-dose MTX therapy.[3] On-label uses of MTX include treatment of some types of solid tumors, blood cancers, and lymphomas (Table 1).

Table 1. Examples of cancers treated with MTX.

Solid Tumors	Blood Cancers	Lymphomas
Breast	Meningeal leukemia	Relapsed non-Hodgkin's lymphoma
Osteosarcoma	Acute lymphoblastic leukemia (ALL)	Refractory non-Hodgkin's lymphoma
Gestational trophoblastic neoplasia		Cutaneous T-cell lymphoma
Head/neck squamous cell carcinoma		

Mechanism of Action

As a folic acid (folate) analog, MTX has a very similar chemical structure to folate (vitamin B9) and functions as an antimetabolite by competitively binding to the dihydrofolate reductase enzyme with 1000 times the affinity of the natural substrate, folate (Figure 1).[4] Thus, MTX prevents conversion of folate from its inactive form (dihydrofolate) to its active form (tetrahydrofolate), which is an essential component required for many physiological and cellular functions.[5] Nucleic acid synthesis is one such function, thus inhibition of folate conversion impedes or prevents both DNA synthesis required for cell division and RNA synthesis necessary for protein synthesis, tissue growth, and cellular messaging (Figure 2). Rapidly dividing cells have the greatest need for folate metabolism, making MTX a very useful agent for treating many forms of cancer by curtailing neoplastic cellular replication and functions necessary for neoplastic growth and migration, such as inter- and intracellular signaling. Additionally, blocking folate metabolism impedes DNA methylation of oncogenes, preventing them from turning on and converting healthy cells to neoplastic ones.[2]

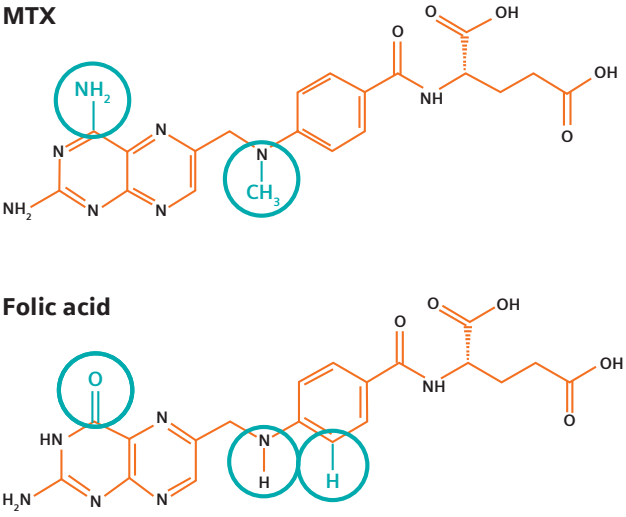

Figure 1. Similarity of MTX and folate (folic acid) structures. Significant differences between the two molecules are noted and circled in blue (adapted from Rudin et al[6]).

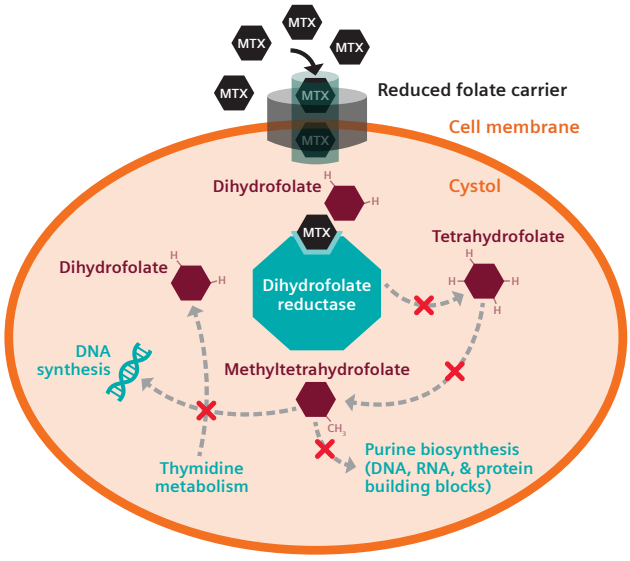

Figure 2. MTX binds to the active site of dihydrofolate reductase with 1000 times the affinity of the enzyme for dihydrofolate (DHF). This prevents conversion to tetrahydrofolate, which is required for DNA, RNA, and protein synthesis (adapted from Howard et al[4]).

High-dose MTX Therapy and Leucovorin Rescue

The most receptive tissues to MTX uptake are the intestines and bone marrow; hence MTX is utilized for blood cancers and lymphomas. Transport of MTX into solid tumors is less reliable than for other cancers due to the chaotic and dysfunctional characteristics of their vascularization resulting in inefficient blood supply.[2,7] Consequently, MTX treatment of solid tumors necessitates use of high drug doses to ensure adequate diffusion into cells through leaky vessels and the extracellular milieu. Additionally, many types of cancers can become resistant to MTX, mainly due to the balance of the import and export rates imposed by folate import/export channels, requiring additional cellular exporters and high dosage to overcome this. For this reason, high-dose MTX therapy (HDMTX) has become the norm for treating MTX-receptive cancers to ensure that the intracellular MTX levels are high enough to be effective.

HDMTX can be administered intrathecally, orally, intramuscularly or intravenously and is defined as doses of ≥500 mg/m^2.[4] At these levels and higher, MTX can be highly cytotoxic to many other types of cells and organs and can cause side effects ranging from mild (e.g., diarrhea, nausea, fatigue) to severe or fatal (e.g., necrotizing skin rash, or renal-, hepatic-, or cardiac injury or failure,).[1,2,4] To counteract this, HDMTX infusion is followed by rescue of noncancerous cells by infusing low levels of leucovorin (5-formyl tetrahydrofolate)—another folate analog which is preferentially taken up by the importer and out competes MTX—allowing tissues to resume normal cellular functions dependent on folate.[2]

Monitoring MTX Levels during HDMTX Therapy Cycles

MTX antineoplastic activity is based on use at cytotoxic levels. Once that level has been exceeded the length of exposure is a critical factor in causing significant harm since prolonged exposure—even at low plasma concentrations—may result in serious toxicity and increased cytotoxicity. Acute kidney injury (AKI) is a particular concern during HDMTX as MTX is primarily eliminated renally. If injury reduces the glomerular filtration rate (GFR), MTX elimination can be substantially delayed, resulting in toxic accumulation, further renal damage, and damage to other organ systems, as mentioned above.[4] Consequently, the need for direct monitoring of the serum or plasma concentration of MTX and its polyglutamate metabolites during HDMTX has been well recognized for over four decades.[8,9] More recently, the World Health Organization (WHO) recommended monitoring of HDMTX for measurement of plasma concentration on the advice of the Strategic Group of Experts on In Vitro Diagnostics (SAGE IVD).[10] This recommendation includes monitoring to interpret plasma concentration with respect to the concentration-effect relationship and to propose individualized dose adjustment to ensure safety and effectiveness, particularly for patients with clinical complications, such as impaired renal function.[11] Many methods can be used for evaluating MTX levels. One widely used method is immunoassay, and most commercial products are generally robust, cost-effective, and their automation enables rapid turn-around while handling high workloads.[12] Although there are some limitations that can potentially impact accuracy (such as cross-reactivity with metabolites) and detection of very low MTX concentrations in comparison to chromatographic or spectrometric methods, sensitivity of immunoassays is sufficient for accurately detecting concentrations typical of HDTMX during the most crucial first 96 hours following initiation of infusion (see Table 4 in "Expected Therapeutic Concentrations").[4,13-15]

Although some protocols from low-resource countries suggest it is safe to monitor only a single consistent time point of 54 hours after beginning infusion,[16] protocols vary by institution and generally require monitoring at multiple time points depending on the dosing level and duration for the type of cancer being treated, as well as to accommodate potential patient biovariability in clearance rate and already existing renal dysfunction.[4,17-20] Adjusting individual dosing was shown to be effective by Evans et al over two decades ago and this study is still widely quoted as the source for current practice today.[21] In this and an earlier study, the MTX clearance rate was estimated based on plasma MTX measured one- and six-hours following initial administration. MTX dosage was then adjusted depending on whether the patient demonstrated slower or faster MTX clearance based on a range set between the 50th and 90th percentile of a time curve determined in a previous trial.[22] In ALL patients treated with individualized vs. non-individualized regimens, the percentage of treatment courses in which the MTX therapeutic target range was within the therapeutic target range was significantly greater than in those receiving conventional regimens with no differences in the number of severe toxic events (Figure 3. See also Table 4 in the "Expected Therapeutic Values" section). Moreover, the percentage of patients achieving complete remission at five years was higher in those receiving individualized treatment (72 ± 6%) than in those receiving conventional treatment (66 ± 6%, Figure 4).[21]

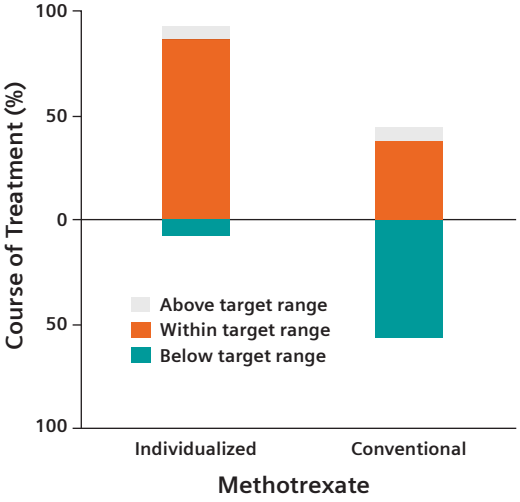

Figure 3. Percentage of treatment courses falling above, within, and below the targeted clearance range in individualized vs. conventional therapy (adapted from Evans et al Figures 2 and 4[21]).

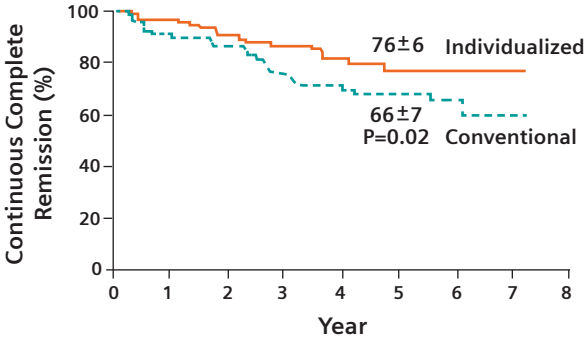

Figure 4. Kaplan-Meier curve demonstrated greater rate of continuous complete remission in individualized vs. conventional treatment regimens (adapted from Evans et al Figures 2 and 4[21]).

Evans' study was seminal, however it was limited to direct observation of each child, running the risk of toxicity in the initial treatment course. Acevedo et al. generalized individualized MTX dosage methods using a high-dose MTX monitoring algorithm to anticipate MTX dosage reduction in real time for children with ALL (ages 1–13 years). The method was developed to address the needs of low- to middle income healthcare environments where rescue with leucovorin or other methods is not an option due to drug access and/or cost. The algorithm checked intrainfusion MTX 2 hours after induction began and reduced the infusion dosage by 50% if the current serum level was high enough to trigger concern over toxicity (at least 100 µmol/L). A second measurement at either 6 or 8 hours post-induction was used to decrease the current dosage by 20–50% if the serum level predicted a toxic or high MTX concentration for the patient (Table 2). In ALL patients, only one child in the study experienced significant acute kidney injury using this method, and other significant adverse reactions occurred at rates similar to non-algorithm-based monitoring schemes used with leucovorin rescue.[17]

Pauley et al advanced Evans' study by demonstrating that individualizing HDMTX dosage based on the clearance rate in the prior course of therapy on a person-by-person basis resulted in MTX being within 20% of the target range in 70% of courses vs. only 63% of the courses when a non-individualized protocol was used.[23] Cao et al extrapolated beyond Pauley's study to develop a population based two-compartment pharmacokinetic model that showed that the serum concentration of MTX 24 hours post-infusion can be used to determine if toxicity risk will be high in subsequent treatment cycles, necessitating use of decreased MTX dosage at the beginning of infusion to prevent severe adverse effects.[19]

Table 2. Suggested ALL MTX dosing and monitoring scheme based on population pharmacokinetics (adapted from Acevedo et al, Figure 1[17]).

	Time post infusion inception[a]									
	+2 hours		+3 hours	+6 hours		+7 hours	+8 hours		+9 hours	+24 hours
Inception	Measure sMTX	Action	Action	Measure sMTX	Action	Action	Measure sMTX	Action	Action	Action
Infuse 5g/m² for 24 hours	<100 µM	Continue at (5g/m²)	----→	<75 µM	Continue at (5g/m²)	----→	----→	----→	----→	End infusion, even if MTX remains in the bag.
				75–<100 µM	Reduce current infusion rate by 20%[a]					
				>100 µM	Pause infusion	Resume at 50% of initial infusion rate[a]				
	>100 µM	Pause infusion	Resume at 50% of initial infusion rate[a]	----------→			<75 µM	Continue at +3 hour rate	----→	
							75–<100 µM	Reduce +3 hour rate by 20%[a]	----→	
							Pause infusion	Pause infusion	Resume at 50% of +3 hours rate[a]	

a. Reduction in rate refers ONLY to the rate of MTX infusion. Increase IV fluids to maintain the original flow rate for the full 24 hr of treatment.

Howard et al notes that monitoring is important for preventing significant toxicity, which can not only irreparably harm the patient but also result in patient noncompliance with therapy.[4] The risk of toxicity is especially high in patients who experience delayed excretion of the drug, which can result in AKI, liver injury, neurologic toxicity, myelosuppression leading to pancytopenia (low levels of red and white blood cells and platelets), inflammation of the mucous membranes in the mouth and digestive tract (mucositis), potentially fatal dermatologic toxicity, multiorgan failure, and increased infection risk.[4,24] Delayed MTX elimination (DME) has been identified as a key cause of HDMTX-related AKI and occurs in ~15% of treatment cycles. Delayed clearance increases crystallization of MTX and its metabolites in the renal tubules, prolonging exposure to toxic levels.[4,24]

A group of nine European oncology experts published a consensus document emphasizing the importance of direct monitoring of MTX during and after HDMTX infusion to detect DME and apply prompt interventions to manage toxicity and neutralize MTX.[24] They note that serum MTX ≥10 µmol/L at 24 hours following short MTX infusions, ≥1 µmol/L at 42 or 48 hours, or ≥0.3 µmol/L at 72 hours following longer infusions is generally indicative of DME. A number of pre-existing factors and medications are associated with increased risk of DME and warrant frequent and careful monitoring (Table 3. See also Table 4 in the "Expected Therapeutic Values" section.)[24] They recommend determining MTX levels at regular intervals starting at 24, 42, 48, or 72 hours after infusion and repeated at least every 24 hours until MTX levels have decreased to <0.1 µmol/L and other institution-specific criteria have been met, since individual institutions may set their own protocols for MTX use and monitoring.[24]

Table 3. Factors associated with high risk of DME and development of renal or other toxicity related to HDTMX (adapted by Bielak et al, Tables 2 and 3[24]).

HDTMX risk factors	Drug interactions
Pre-existing renal impairment	Acetylsalicyclic acid
BMI ≥25 kg/m^2	Nonsteroidal anti-inflammatory drugs (NSAIDs)
Frailty	Penicillin and sulfonamides
Third spacing- pleural effusion or ascites	Tyrosine kinase inhibitors (e.g., imatinib, dasatinib)
Fever	Probenecid and weak organic acids (e.g., pyrazoles)
Infection	Proton pump inhibitors
Tumor lysis	Radiographic contrast agents
Diabetes or hypoalbuminemia	Other nephrotoxic drugs

In addition to the risk factors cited by Howard et al, the European consensus document, and others, there is substantial variability among patients with respect to both MTX efficacy and its degree of toxicity. For example, Trevino et al determined that single nucleotide polymorphisms (SNPs) affecting the ARID5B gene encoding a transcription modulator are associated with MTX resistance and reduced efficacy, while others are associated with increased MTX intracellular accumulation in children with ALL.[25] Subsequently, Csordas et al found that children with specific ARID5B SNPs had higher serum MTX when receiving a dose of 2 g/m^2, while others had lower serum MTX concentrations at an MTX dosage of 5 g/m^2. Still other ARID5B variants were associated with increased hepatoxicity risk or nephrotoxicity when using 5 g/m^2 MTX.[26] More recently, a 2021 meta-analysis by Song et al evaluated 34 studies to determine which SNPs within the genes controlling MTX cellular transport, elimination, and targeting impact MTX toxicity and efficacy. They found two polymorphisms within the MTHFR and ABCB1 genes were associated with increased hepatotoxicity, mucositis, and renal toxicity, while three others conferred a tendency toward reduced hepatotoxicity, mucositis and renal toxicity.[27] These studies imply the need for individualizing MTX dosage and monitoring based on pretreatment patient genotyping to increase alertness to elevated serum MTX and delayed elimination to initiated prompt intervention to prevent or ameliorating toxicity.

A multicenter retrospective study conducted by Tentoni et al investigated whether AKI could be detected earlier by monitoring MTX for DME than was possible using serum creatinine (sCr) for this purpose. The study evaluated MTX elimination half-life in patients with ALL (67% <18 years old) who had received multiple HDMTX infusions lasting either 4 hours or 24 hours. MTX concentration was determined using an immunoassay at either two (older patients) or three time points within 16 hours of completing infusion.[28] Using receiver operator characteristic (ROC) analysis and accounting for potential causal confounding factors (age, sex, dose, infusion duration), they determined that the area under the curve (AUC) for DME for ascertaining high-risk of AKI was 0.79 (interquartile range [IQR] 0.73–0.84) using MTX plasma concentration (MTXc) at a cutoff of >1µM at any time point taken 42 hours from the beginning of infusion, and was 0.84 (IQR 0.73–1.00) if MTXc was >2 standard deviations (SD) from the study's population mean at either 42 or 48 hours from the start of the infusion cycle.[28] In contrast, the AUC for using sCr >50% of baseline as a biomarker of AKI was 0.65 (IQR 0.54–0.77). Notably, the authors pointed out that sCr elevation >50% might not be reached until 48–72 hours following completion of infusion, and thus using MTXc to determine DME would enable earlier initiation of injury-mitigating intervention, such as increasing hydration, augmenting leucovorin rescue, or administering glucarpidase as recommended by a European expert consensus document.[24] This study took advantage of https://mtxpk.org/, a freely available online pharmacokinetic modeling tool developed at Cincinnati Children's Hospital (see box).[29-30]

MTXPK.org is a useful tool designed to help clinicians understand the pharmacokinetics of high-dose methotrexate, especially with regard to delayed clearance. The calculator uses an appropriate pharmacokinetic model for the dose of methotrexate to display the concentration vs. time curve for an individual patient overlaid upon the population predicted curve for that dose. Required input includes the age, sex, height, and weight of the patient, the dose and infusion time, at least one methotrexate plasma concentration measurement and one creatinine measurement. Full step-by-step instructions for its use can be found on its site.

Monitoring MTX can also be used to inform leucovorin dosage and duration during HDMTX. For example, leucovorin rescue usually begins 24 hours after initiation of MTX infusion and is stopped once monitoring indicates MTX has decreased to below 5 µmol/L.[31,32] In the event of serum MTX >5 µmol/L 24 hours after administration or > 0.9 µmol/L 48 hours after administration, leucovorin dosage can be substantially increased until the serum MTX drops below 0.08 µmol/L.[31]

Expected MTX Therapeutic Concentrations

The peak serum concentration observed during HDTMX varies depending on the regimen applied for the disease being treated, the patient's renal function, metabolism, and genetic biovariability. Some target MTX concentrations are shown in Table 4.

Table 4. MTX concentrations targeted during HDMTX for various cancers and clinical situations.

Condition	Target MTX serum level (µmol/L)	Hours after first administration	Reference
Intermediate-/high-risk pediatric ALL	56–75	20	Chen et al, 2024[13]
Osteosarcoma (children and young adults)	700–1000	1–6	Nagamine et al, 2025[14]
Pediatric brain tumor: Very young infants (median age = 0.24yr) Older infants (median age = 1.9yr)	 0.4–2.4 0.1–3.6	24	Panetta et al, 2019[15]
Normal Elimination	~10 ~1 <0.2	24 48 72	Howard et al, 2016[4]
High toxicity risk (delayed MTX elimination)	~10–100 ~5–50 ~5–40	48 72 96	Howard et al, 2016[4]

Low-Dose MTX Therapy for Autoimmune Disorders

In addition to its use in oncology, MTX is often considered a first-line treatment for many autoimmune inflammatory disorders. Approximately 5% of the worldwide population >18 years old (~1.1B) has some form of autoimmune disease treatable with MTX, and an estimated 85 million have one of the five most common conditions treatable with MTX (inflammatory bowel disease/Crohn's Disease/ulcerative colitis, psoriasis, rheumatoid arthritis [RA], psoriatic arthritis, systemic lupus erythematosus).[33-38] In the United States alone, over 4.6 million prescriptions were provided to ~1.1 million patients in 2023.[39]

Mechanism of Action: Autoimmune Diseases

MTX is classified as a conventional synthetic disease-modifying antirheumatic drug (csDMARD). This class of drugs helps relieve pain, inflammation, and disease progression. As a folate antagonist, MTX curtails proliferation of various immune cell lineages and is also thought to trigger apoptosis (programmed cell death) of activated lymphocytes participating in inflammatory cytokine production and signaling.[34,38,40,41] In addition, MTX appears to restore CD73 expression and downstream accumulation of adenosine, thereby reducing inflammation.[40,42]

Monitoring Low-Dose MTX During Treatment for Autoimmune Diseases

In contrast to its use in oncology, low-dose MTX is administered for the treatment of autoimmune diseases. While there is still possibility of toxicity, immunoassays are neither sensitive nor specific enough to detect low concentrations of MTX in serum and plasma for direct evaluation. Instead, mass spectrometric methods and assays which quantitate MTX polyglutamate in erythrocytes are generally recommended. Potential toxicity is monitored via routine laboratory biomarkers to assess organ injury and disease activity (Table 5). The timing and frequency of testing is dependent on regional, local, or society guidelines followed. [5,11,43,44]

Table 5. Recommended testing for monitoring physiological parameters at initiation and during low-dose MTX therapy. [5,11,43,44]

Infectious diseases	Blood	Liver	Kidney
• HIV	• CBC • WBC differential • Platelet count • Erythrocyte sedimentation rate • Antinuclear antibody • Anticyclic citrullinated peptide • Creatine kinase • Rheumatoid Factor • C reactive protein • Anti-dsDNA • Anti-Sm • Electrolytes • B12 • Iron • pANCA • ASCA • Serum calprotectin	• Hepatitis A (HAV) • Hepatitis B (HBV) • Hepatitis C (HCV) • Metabolic associated steatotic liver disease (MASLD) • Liver function tests (ALT, AST) • Serum albumin • Serum bilirubin • Liver biopsy after 10 years	• CrCl • Serum creatinine • BUN • Serum albumin

Conclusion

MTX is a decades-old therapy utilized both on- and off-label for a wide range of oncologic and autoimmune diseases. Due to its capacity for severe and potentially fatal toxicity, monitoring is essential during HDTMX to determine the rate of renal clearance and appropriate timing of leucovorin rescue therapy or glucarpidase infusion in the event of accidental overdose. Early monitoring during therapy to adjust MTX dose while infusion is ongoing can be used successfully to personalize and optimize treatment efficacy while reducing toxicity. Immunoassays provide rapid, sensitive, and specific testing of MTX serum and plasma concentrations during infusion therapy, while other standard assays are used to monitor physiological functions that can indicate mild toxicity during low dose usage.

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