Letter to the Editor

Franz L. Knörnschild, Sven Liebig, Peggy Kießling, Monika Prpic, Tehyung Kim, Ulrich Keller, Kai Kappert, Stefan Schwartz and Amir Jahic*

Simplified preanalytical laboratory procedures for therapeutic drug monitoring (TDM) in patients treated with high-dose methotrexate (HD-MTX) and glucarpidase

https://doi.org/10.1515/cclm-2024-0523
Received April 25, 2024; accepted June 25, 2024; published online July 15, 2024

Keywords: drug monitoring; glucarpidase; kidney failure; methotrexate

To the Editor,

High-dose methotrexate (HD-MTX) therapy is a pivotal element of chemotherapy regimens applied in various malignancies such as acute lymphoblastic leukemia, central nervous system lymphoma and osteosarcoma. Using well-standardized supportive measures (i.e., hyperhydration, urine alkalization) and pharmacokinetically–guided leucovorin rescue, HD-MTX can be administered safely in most patients. However, HD-MTX occasionally causes acute crystal nephropathy by drug precipitation in addition to direct renal tubule damage, resulting in impaired renal clearance and thereby prolonged exposure to toxic MTX blood levels [1, 2]. In such emergency situations, MTX-cleavage by the recombinant enzyme glucarpidase offers an alternative route of rapid and effective MTX removal from circulation [3]. Glucarpidase has been evaluated in clinical trials exploring patients with HD-MTX associated renal failure and has been approved for this purpose in the United States and European Union [4, 5]. Glucarpidase rapidly cleaves MTX and, to a minor extent, its natural metabolite 7-OH-MTX into glutamate and the non-toxic metabolites 2,4-diamino-N10-methylpetroic acid (DAMPA) and 7-OH-DAMPA [6]. In HD-MTX treated patients, therapeutic drug monitoring (TDM) of MTX is routinely performed by immunoassays. However, immunoassays produce unreliable laboratory findings since these results mirror the sum of MTX concentration plus the concentration of enzyme–induced MTX-metabolite DAMPA, due to the cross-reactivity of DAMPA with immunoassays [7]. Thus, chromatography-based methods are required for reliable TDM in patients treated with glucarpidase [8]. Theoretically, MTX might be further cleaved ex vivo by glucarpidase after blood sampling, thus potentially underestimating MTX concentration in vivo. To mitigate this potential confounding factor, preanalytical glucarpidase inactivation through blood specimen heating or acidification using hydrochloric...
acid (HCl) has been recommended [4, 5]. However, currently no consensus about preanalytical blood sample processing, intended for laboratory MTX/7-OH-MTX/ DAMPA monitoring post HD-MTX and glucarpidase rescue, has been established. For the detection of MTX and its metabolites in blood specimens, combined liquid chromatography–tandem mass spectrometry (LC-MS/MS) could serve as a gold standard technique [9]. While HD-MTX is frequently used, LC-MS/MS is not widely available, and thus, simplified procedures for blood sample processing and shipment from centers without appropriate laboratory facilities are required for optimal clinical care.

For this study, 96 specimens from four lymphoma patients (age: 73–85 years), who had received HD-MTX and glucarpidase, were collected (Figure 1). Routine measurements of (serum) MTX were carried out by an immunoassay (ARK Diagnostics Inc., Fremont, USA) applying an automated clinical chemistry analyzer (cobas® 8000, Roche Diagnostics GmbH, Mannheim, Germany) and were further compared to the LC-MS/MS (MTX plasma) results. Quantification of MTX using an immunoassay vs. LC-MS/MS disclosed differences as anticipated. The immunoassay consistently showed higher values compared to LC-MS/MS (median: 1.054 μmol/L; range 0.051–8.825 μmol/L; Figure 2A), which is considered the diagnostic gold standard. This finding is consistent with the known cross-reactivity of the MTX metabolite DAMPA with established MTX immunoassays [8].

For LC-MS/MS measurements, 96 EDTA-plasma samples were processed with additive 1 N HCl (1:10 vol ratio) or without (i.e., native), and were stored at different temperatures, −80 °C, +4 °C or room temperature (RT), prior to laboratory analyses. All patient samples were collected within a time frame of 0.25–18 h post glucarpidase administration, with no specific effect of early vs. late enzymatic MTX cleavage (Supplementary Material, Figure 1). Glucarpidase cleaves MTX into its non-toxic metabolites DAMPA and glutamate in vivo. There is no evidence that glucarpidase further metabolizes MTX ex vivo, i.e. after blood collection. It therefore remains unclear whether this putative, ex vivo metabolic process should mandatorily be inhibited by preanalytical sample processing such as acidification. To investigate the effects of acidification on ex vivo MTX cleavage, we compared paired, acidified and non-acidified samples. Furthermore, we investigated the effects of various storage conditions in paired samples (−80 °C, n=30; +4 °C, n=9; room temperature, n=9). For MTX, 7-OH-MTX and DAMPA quantification, a LC-MS/MS system (HPLC: Shimadzu Deutschland GmbH, Duisburg, Germany; MS: Sciex LLC, Framingham, MA, USA) with an adapted analysis protocol [9] was used. Statistical analysis were conducted using R software (version 4.2.2, R Foundation for statistical Computing, Vienna, Austria). Except for MTX in paired samples stored at +4 °C (n=9; p=0.008; Figure 2B4), there were no significant differences in MTX, 7-OH-MTX or DAMPA concentrations between acidified and non-acidified samples at any other storage condition (Figure 2B). However, differences of MTX concentrations in paired, acidified and non-acidified samples were minor and are unlikely to have a significant impact on toxicity (maximum range of concentrations: 0.036–1.445 μmol/L for acidified samples.

Figure 1: Patient characteristics and sample processing conditions. Schematic drawing of patient characteristics including gender (indicated as ♂, female, numbers represent sample size), age, disease, and sample processing conditions. CNSL, central nervous system lymphoma; HCL, hydrochloric acid.
Figure 2: Concentrations of MTX in serum as well as MTX, 7-OH-MTX and DAMPA in EDTA-plasma. (A, B) values are show on a logarithmic scale and dashed lines represent identity line. For statistical analysis the Wilcoxon signed rank test was used, p<0.0001. (A) Concentrations of MTX in patient serum and EDTA-plasma paired samples quantified by an immunoassay (IA) and LC-MS/MS (n=28). (B) Concentrations of MTX, 7-OH-MTX and DAMPA quantified by LC-MS/MS in samples stored at −80 °C (1–3, n=60), at 4 °C (4–6, n=18), and at room temperature (RT; 7–9, n=18).
and 0.043–1.021 μmol/L for native samples; range of deviations 0.002–0.424 μmol/L; data not shown). However, these differences might have an effect on specific aspects of clinical management, such as leucovorin dosing. Considering all tested sample processing and storage conditions (−80 °C, +4 °C, room temperature), median differences between acidified and non-acidified samples ranged from 0.002 μmol/L to 0.007 μmol/L for MTX, from 0.051 μmol/L to 2.086 μmol/L for 7-OH-MTX, and from 0.611 μmol/L to 2.707 μmol/L for DAMPA (data not shown), indicating no decisive deviations which might impact clinical decisions. This reveals that preanalytical sample acidification is not required in clinical routine TDM of patients treated with glucarpidase post HD-MTX. However, the observed differences for MTX and its metabolites may be at least partially due to the expected methodological LC-MS/MS precision. Hence, the methodological LC-MS/MS precision, based on the coefficient of variation (CV), was determined using the laboratory data obtained by measuring manufactured quality control (QC) material. Two different concentration levels (QC low and high) of all three compounds MTX/7-OH-MTX/DAMPA were re-evaluated. This process was conducted over a two-month period, which is equivalent to the time frame for patient sample analysis with regard to clinical trials. In addition, considering various temperature-processing conditions, the medians for ratios calculated for paired acidified and non-acidified patient samples were determined and compared to the CV precision data set. Importantly, when comparing the QC data with patient sample data, the deviations between acidified and non-acidified samples were within the range of methodological LC-MS/MS precision for samples stored at −80 °C (MTX, 7-OH-MTX, DAMPA) and +4 °C (MTX and 7-OH-MTX), while storage at room temperature exceeded the methodological precision for all three compounds MTX, 7-OH-MTX and DAMPA (Supplementary Material, Table 1).

In conclusion, a simplified preanalytical blood sample processing without an acidification step is reasonable and appears sufficient for LC-MS/MS-based TDM in patients treated with HD-MTX and glucarpidase. This is an essential preanalytical factor that contributes to reducing turnaround times, enabling clinical decisions to be made within an acceptable time frame. However, sample storage conditions could impact quantification of MTX and MTX metabolites, indicating that sample storage at −80 °C or +4 °C is appropriate prior to LC-MS/MS analysis. In particular, storage at +4 °C is deemed appropriate for clinical decision-making, while storage at −80 °C might be more relevant for research purposes. In sum, these results are of relevance for preanalytical sample processing in routine clinical care of patients with delayed MTX elimination and glucarpidase treatment after HD-MTX therapy.

Acknowledgments: Sebastian Kühn (Labor Berlin – Charité Vivantes Services GmbH) is acknowledged for excellent technical support. The authors are grateful to all patients who participated in this study. We thank Dr. Katharina Friedrich, Institute of Diagnostic Laboratory Medicine, Clinical Chemistry and Pathobiocchemistry, Charité – Universitätsmedizin Berlin, for her support in formatting the manuscript and critical input for result interpretation. FLK is MD candidate at Charité – Universitätsmedizin Berlin. This work is submitted in fulfillment of the requirement for the medical dissertation.

Research ethics: This study was approved by the Ethics Committee of the Charité – Universitätsmedizin Berlin (ref. No. EA4/258/21) and was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.

Author contributions: FLK, SL, SS, AJ, UK, and KK contributed to the conception and design of the study, acquisition of data, data analysis and interpretation, and revised the article critically. PK, MP, IM and TK performed acquisition of data and data analysis. FLK, SS and AJ drafted the article, and revised the article critically for important intellectual content. The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Stefan Schwartz has received honoraria from SERB SAS and a research grant from Protherics Medicines Development Ltd for the present study. All other authors declare no competing financial interests.

Research funding: Stefan Schwartz has received honoraria from SERB SAS and a research grant from Protherics Medicines Development Ltd for the present study.

Data availability: The raw data can be obtained on request from the corresponding author.

Trial registration: This study is registered at ClinicalTrials.gov: NCT05250869.

References


Supplementary Material: This article contains supplementary material (https://doi.org/10.1515/ccm-2024-0523).