Journal of Hematology and Blood Disorders
Volume 11 | Issue 1
ISSN: 2455-7641

Research Article Open Access

Early Urinary Potassium Level Predicts High-Dose Methotrexate Elimination Delay in Primary Central Nervous System Lymphoma

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Citation: V Harlay, A Bertucci, C Boucard, G Petrirena, C Campello et al. (2024) Early Urinary Potassium Level Predicts High-- Dose Methotrexate Elimination Delay in Primary Central Nervous System Lymphoma, J Hematol Blood Disord 11(1): 102

Received Date: May 25, 2024 **Accepted Date:** June 25, 2024 **Published Date:** June 29, 2024

Abstract

Background: First-line treatment of primary central nervous system lymphoma (PCNSL) includes high-dose methotrexate-based polychemotherapy (HD-MTX). Nevertheless, HD-MTX is associated with renal toxicity, potentially compromising chemotherapy completion and prognosis. Our objective was to identify early predictive factors of HD-MTX delayed elimination.

Methods: We prospectively included all patients for newly diagnosed PCNSL. Daily serum and urinary creatinine and ionogram were collected from the day before HD-MTX, until elimination. Standard elimination was defined by plasmatic methotrexate (MTX) level 0.05 µmol/L before 72 hours. We conducted 2 independent cohorts, training (TC) and confirmation (CC).

Results: We included 64 cures (20 patients) in the TC and 59 cures (22 patients) in the CC. At inclusion, median ages were 71 years and 74 years, median Karnofsky Performance Status (KPS) were 70% and 80% and median elimination time of MTX was 95 hours (range 47-205) and 96 hours (range 62-264) in the TC and the CC respectively. In multivariate analysis, older age (p 0.004), low KPS (p 0.036) and high urinary potassium level at day 1 (uK+D1) (p 0.001) were associated with delayed MTX elimination. An optimal cutoff for uK+D1 was defined at 17 mmol/L. In the CC, we confirmed that high uK+D1 (p 0.004) remained associated with delayed MTX elimination. After merging the cohorts, we were able to predict a standard MTX elimination probability based on age and uK+D1. Patients younger than 70 years had a standard MTX elimination probability of 84% versus 33%, depending on low and high uK+D1, respectively.

Conclusion: uK+D1 may be predictive of delayed MTX elimination. Its relevance as a decision-making factor needs to be validated in a larger prospective cohort.

Keywords: Primary central nervous system lymphoma; PCNSL; High-dose methotrexate; biomarker; toxicity

List of Abbreviations: primary central nervous system lymphoma: PCNSL; high-dose methotrexate-based polychemotherapy: HD-MTX; methotrexate: MTX; training cohort: TC; confirmation cohort: CC; Karnofsky Performance Status: KPS; urinary potassium level at day 1: uK+D1; complete response: CR; autologous stem-cell transplantation: ASCT; blood-brain barrier: BBB; cerebrospinal fluid: CSF; carboxypeptidase-G2: CPDG2;

Introduction

Primary central nervous system lymphoma (PCNSL) is a rare and aggressive form of extra nodal non-Hodgkin's lymphoma which is limited to brain, cranial nerves, leptomeninges, spinal cord, and retina. PCNSL represents approximately 2-4% of all primary brain tumors. No risk factor is clearly identified, and it can appear at any age, with incidence increasing in the elderly population [1–3]. Age is the main prognostic factor in PCNSL. Karnofsky Performance Score (KPS) and achievement of a complete response (CR) represent other prognostic factors [4].

Standard first line treatment is composed by high-dose methotrexate-based polychemotherapy (HD-MTX). For young patients (age <65-70 years), induction treatment with HD-MTX is followed by high-dose chemotherapy with autologous stem-cell transplantation (ASCT) as consolidation treatment if patient achieved a CR [5–7]. For elderly patients, oncogeriatric assessment is mandatory to select the optimal treatment. For fit patients, HD-MTX-based polychemotherapy still represents the best option with good efficiency and feasibility. For unfit patients, chemotherapy monotherapy is feasible (HD-MTX or Temozolomide) [8].

MTX is an antimetabolite and antifolate drug inhibiting purine and thymidine nucleotide synthesis. MTX can suppress DNA synthesis, leading to an antiproliferative and anti-inflammatory activity. HD-MTX is defined with a dose superior or equal to 500 mg/m2. In PCNSL, the recommended dose is superior or equal to 3 g/m^2 to cross the blood-brain barrier (BBB) and to reach cytotoxic levels in cerebrospinal fluid (CSF). HD-MTX is administrated intravenously for 2-3 hours and the number of cycles ranges from 4-6 cycles including 2 injections per cycle [4]. HD-MTX is associated with a high risk to develop toxicities. The main toxicity is nephrotoxicity, which ranges from increase of plasmatic creatinin to tubular necrosis and acute kidney injury. This nephrotoxicity is related to the precipitation of MTX and its metabolites in the renal tubules, favored by acidic pH and volume depletion. To prevent nephrotoxicity, urine alkalinization and hyperhydration are required but they remain frequently unsufficient. In addition to the morbidity and prolonged hospitalization stay, nephrotoxicity could result in treatment delay or suspension that may compromise chemotherapy completion, and then seriously affecting patients' prognosis and future consolidation treatment such as ASCT. Thereby, it is crucial to predict and prevent this frequent adverse event leading to patient outcome deterioration. Currently, MTX administration is systematically associated with acid folic administration, the dose of which can be adapted according to the MTX level [9]. The carboxypeptidase-G2 (CPDG2) or glucarpidase is a recombinant bacterial rescue enzyme that can catabolize MTX into inactive metabolites, reducing plasma MTX concentrations within few minutes [10]. This drug is currently evaluated in a phase I trial to determine the safety and impact of its early administration on MTX completion and patient response (METHOGLU, NCT 05135858). Nevertheless, its current high cost limits its use to rare and particularly severe MTX overdose. Then, the determination of an early and easy-to-use predictive biomarker of MTX delay and toxicity would be determining in PCNSL patient management. If several blood biomarkers [11] were previously analyzed with no robust results, urinary markers, that may be directly related to renal function, were not previously explored.

Our objective was to identify early urinary predictive biomarkers of MTX delayed elimination to derive predictive algorithms for clinical practice.

Patients and Methods

Study Design and Participants

Between November 2020 and May 2021, we included all patients treated in our institution with HD-MTX based chemotherapy for PCNSL. This first cohort was called "training cohort". The second cohort called "confirmatory cohort" included all patients treated between January 2022 and May 2022 using the same inclusion criteria than the previous cohort. The time between May 2021 and January 2022 allowed us to ensure the independence of the 2 cohorts. At each treatment, daily blood and urinary creatinine and ionogram were prospectively collected from the day before MTX administration until its elimination. Standard elimination of MTX was defined by plasmatic MTX level ≤ 0.05 μmol/L before 72 hours.

The present study was conducted in accordance with the declaration of Helsinki. All patients were prospectively included into the LOC network and provided their written consent for clinical data collection and genetic analysis according to national and LOC network policies.

Data Collection

For all patients demographic profile (age and gender), presenting symptoms, radiological characteristics on MRI, KPS score were prospectively collected in real time. All first-line treatments were composed by a polychemotherapy with HD-MTX. Young patients (age ≤ 60 years) received rituximab, MTX, carmustine, etoposide, prednisone and aracytine (R-MBVP-A regimen) and older patients (age > 60 years) received rituximab, MTX, procarbazine, vincristine and aracytine (R-MPV-A regimen). Concerning toxicity, treatment dose reductions, chemotherapy delay and treatment discontinuation for toxicity were reported. Adverse events were scored using Common Toxicity Criteria scale for adverse events version 5.0 (CTCAE v5.0). Finally, the use of CPDG2 was recorded. All blood and urinary analyses were carried out during routine care and no specific dosage has been requested in the protocol.

Statistical Analysis

The clinical, histological, biological and treatment data were collected. Data are presented as median, range, mean and standard error of mean (se). For correlation analysis, the chi-square test (or Fisher's exact test) was used to compare qualitative variables. Continuous variables were compared using the Mann–Whitney U test. In the training cohort, sensitivity and specificity of potential blood or urinary biomarkers were analyzed using receiver operating characteristic (ROC) curve analysis after dichotomization of MTX elimination delay (MTX at 72 hours level was ≤ 0.05 μmol/L or not). In the confirmatory cohort, for biomarkers of interest established during the training cohort, we analyzed only the pre-established cutoff. For both cohorts, univariate and multivariate analyses were performed. All statistical tests were two-sided, and the threshold for statistical significance was $p = 0.05$. Analyses were conducted using PASW Statistics version 22 (IBM SPSS Inc., Chicago, IL, USA).

Results

Patient Characteristics

The TC included 64 methotrexate courses from 20 patients. The CC included 59 methotrexate courses from 22 patients. Median age at inclusion were 71 (range 33-86) and 74 years (range 47-87) in the training and confirmatory cohort, respectively. Median KPS were 70% (range, 40-80) and 80% (range, 40-100) in the training and confirmation cohort, respectively. Median

number of prior courses per patients were 4 (range, 1-14) and 3 (range, 1-15) in the training and confirmation cohort, respectively. Table 1 summarizes patient's characteristics and the main urinary or plasmatic biomarkers in both cohorts. Comparing the two cohorts, we found a lower KPS (p<0.001) and a higher number of previous MTX courses (p=0.047) in the training cohort. Both cohorts were similar for the other characteristics. Only 1 patient in the training cohort received CPDG2 after the 7th MTX administration for a grade 3 of creatinine increase.

Table 1: Characteristics of the population in the 2 cohorts

Characteristics	Training cohort (n=20)	Confirmatory cohorts(n=22)	P value
Age (Median, range)	71 (33-86)	74 (47-87)	
KPS (Median, range)	70 (40-80)	80 (40-100)	<0.001
Weight (median, range)	70 (45-91)	66 (50-103)	
MTX dose (mg/m2)	2.8	3.0	
MTX dose reduction (%)	17.4 %	13.4%	
Number of cycle (Median, range)	4 (1-14)	3 (1-15)	0.047
MTX delayed elimination (>0.05 µmol/L) at 72h (%)	34.4 %	32.8%	
Plasmatic creatinin D0 (umol/L, Median)	74.0	73.0	
Plasmatic creatinin D3 (umol/L, Median)	67.0	73.0	
Na+ u D0 (mmol/L, Median)	132.5	110	
K+ u D0 (mmol/L, Median)	21.8	31.8	
Na+ u D1 (mmol/L, Median)	126.0	104.5	
K+ u D1 (mmol/L), Median	16.4	23.9	
AE/SAEs Hepatic impairment	62.7%/5.1%	NA	
AE/SAEs creatinin	40.0%/0%	NA	
Voraxaze use (cure)	1.6%	0%	

KPS: Karnofsky performans status; MTX: methotrexate; D0: day before MTX infusion; D3: day 3 after MTX infusion; Na+: sodium; K+: potassium; AE: adverse event; SAE: serious adverse event; NA: not available

Urinary Factors Variation under Treatment

In the TC, all factors, except urinary osmolarity, significantly varied under treatment, between hydration initiation (Day -1), methotrexate administration (Day 0) and follow-up (Day 1). Urinary potassium (p<0.001, Figure 1a), urea (p<0.001) and creatinine (p<0.001) significantly decreased under treatment, while urinary sodium (p<0.001, Figure 1b) initially increased and then slightly decreased. In contrast, urinary osmolarity remained stable (p=0.677, Figure 1b).

No significant difference was observed depending on the cycle number, between early *versus* late cycles notably. Moreover, for a same patient, these factors had significant variability between cycles. As example, urinary potassium level at day 1 presented with a mean individual standard deviation of 10.7mmol/l between each cycle.

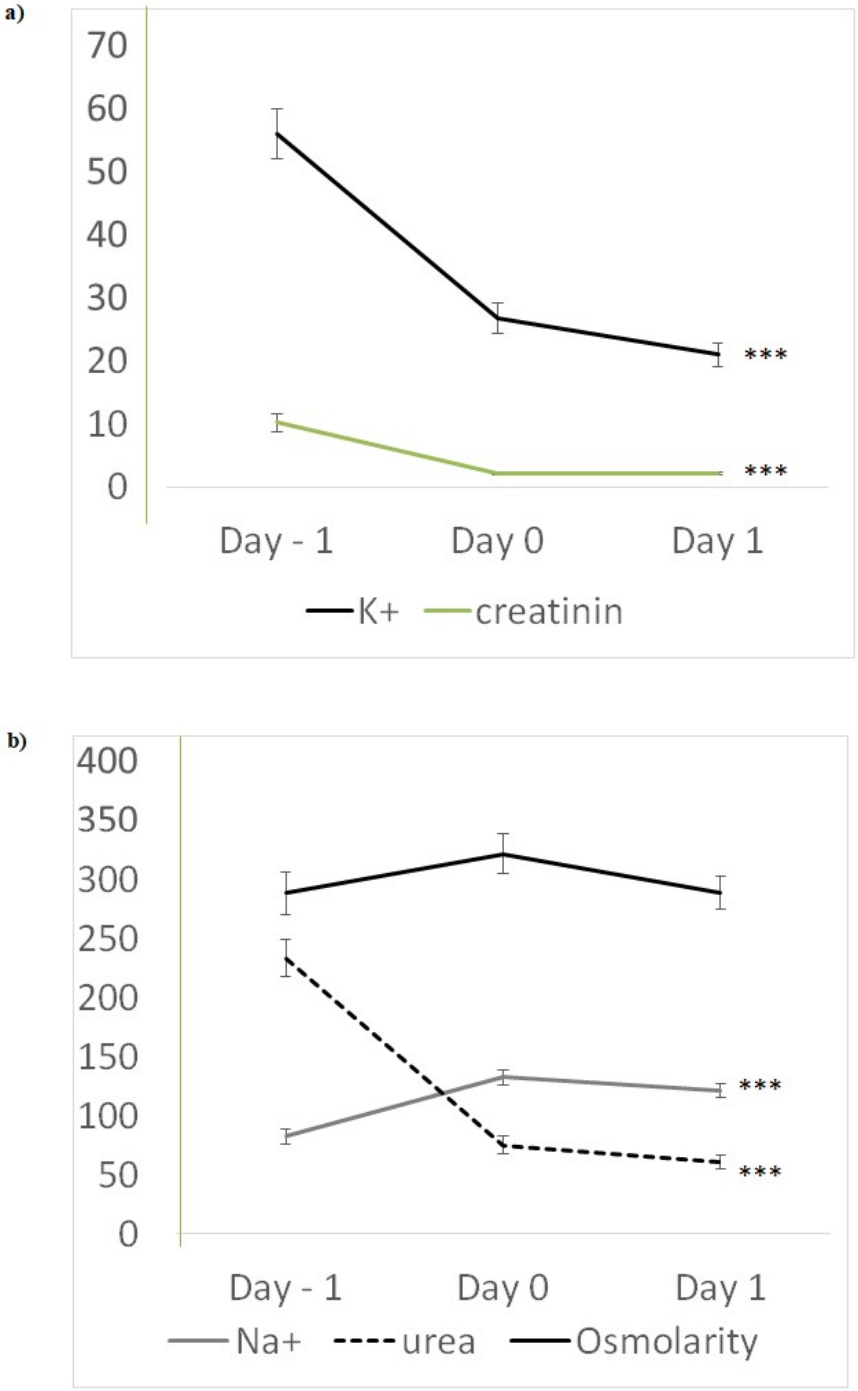

Figure 1: Urinary factors variation under treatment at D-1; D0 and D1. a) Evolution of potassium (K+) and creatinin. b) Evolution of sodium (Na+), urea and urinary osmolarity.

Day 0 (D0) represents the day of methotrexate infusion, alkaline hyperhydration begins the day before (D-1). The Y axis is expressed as mmol/L. ***: $p<0.001$

Predictive Impact of Urinary Factors on Methotrexate Elimination

Median delay of methotrexate elimination (< 0.05 µmol/L) was 95 hours (range, 47 - 205) in the TC and 96 hours (range, 62 - 264) in the CC. Only 34.4 % and 32.8% of patients have eliminated at 72 hours, in the TC and CC respectively.

In the TC, the factors associated with delayed methotrexate elimination (over 72 hours) were an older age (p<0.004), a lower KPS (p<0.036) and a higher urinary potassium level at day 1 (uK+D1) (p=0.001) in univariate analysis. Using a ROC analysis (p=0.008, AUC=0.712), an optimal cutoff for urinary potassium level was defined at 17 mmol/L with a specificity of 81%, a sensitivity of 57%, a positive predictive value of 84% and a negative predictive value of 52%. In the same way, for patient age (p<0.001, AUC: 0.825) an optimal cutoff was determined at 70 years with a sensitivity of 78%, a specificity of 72%, a positive predictive value of 84% and a negative predictive value of 64%.

In the CC, focused on day 1 only, and with the predetermined cutoff at 17 mmol/L for urinary potassium level, we confirmed the association between urinary potassium level and methotrexate elimination delay (p=0.004), as well as age (p=0.002) and functional status (p=0.007) impact.

Predictive Score of Methotrexate Elimination Delay

Finally, we merged both cohorts to confirm the impact of the age, the functional status and the early urinary potassium level on methotrexate elimination delay. Using multivariate analysis, we confirmed the significant correlation between MTX elimination delay and uK+D1 (p=0.001), functional status (p=0.036) and the patient age (p=0.004). Based on age and urinary potassium early level, we were able to derive 4 predictive sub-groups with probabilities of methotrexate elimination at 72 hours of 84%, 33%, 26% and 14%, respectively (Figure 2).

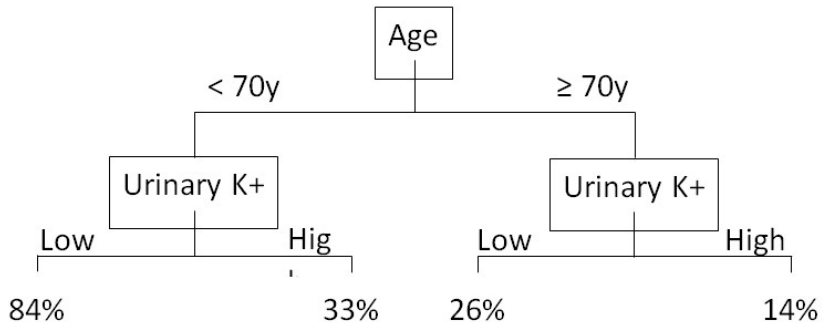

Figure 2: Standard MTX elimination probability

In the combined cohorts, we were able to predict standard MTX elimination probability based on patient age and urinary potassium (K+) level at D1.

Discussion

Our study highlights that early urinary potassium level 24 hours after HD-MTX administration may predict delayed MTX elimination in PCNSL adult patients. In combination with classical predictive markers like patient age and autonomy we were able to identify a specific population with a higher risk of delayed MTX elimination, opening personalized patient management opportunities.

HD-MTX is used worldwide in the treatment of PCNSL and for other adult or pediatric malignancies. Its main side effects are well known, including serious and sometimes irreversible nephrotoxicity. To prevent renal injury, hyperhydration, urine alkalinization and leucovorin rescue are recommended [12]. However, despite these treatments, acute renal injury is still commonly observed [13]. It has long been established that renal toxicity is directly related to urinary MTX concentration that is related to plasmatic MTX concentration and time of exposure. That's why our objective was to identify early and easy-to-use predictive biomarkers able to predict delayed MTX elimination to anticipate and prevent irreversible renal alteration [14]. Currently,

the follow up of HD-MTX chemotherapy requires daily blood MTX concentration and creatinin evaluation until elimination [15–17]. Early indications of CPDG2 administration are currently restricted due to its very expensive cost, and renal function protection remains to be improved. Nevertheless, the optimization of first line HD-MTX is critical to allow patient complete response, autologous stem cell transplantation and then patient remission.

Few studies explored potential early markers associated with acute renal injury under HD-MTX. Wiczer *et al.* retrospectively analyzed 140 patients with leukemia or lymphoma treated with HD-MTX. They found that male gender, a low baseline plasmatic albumin level, number of drug interactions and furosemide treatment were associated with nephrotoxicity. In this study, plasmatic or urinary ionogram were not evaluated [18]. Some studies have demonstrated drug interactions between commonly used medication and MTX, including proton pump inhibitors, levetiracetam, penicillins [19]. In clinical practice, these drugs are now systematically suspended during MTX administration, until elimination, to avoid delayed MTX elimination. In 2014, May *et al.* published that older age, male sex, decreased baseline creatinine clearance and proton pump inhibitor use were responsible for a higher renal toxicity in lymphoma and osteosarcoma patients treated with HD-MTX [20]. In 2013, Xu *et al.* reported that blood creatinin and creatinine clearance, both at 24 and 48 hours after MTX administration, were correlated with plasma MTX concentration in 105 patients with childhood lymphoblastic malignancies [21]. In 2014, Mao *et al.* also demonstrated a positive correlation between blood creatinine concentrations at 24 and 48 hours and blood MTX levels at 48 hours, in the same context [22]. In 2014, Ylinen *et al.* proposed plasma cystatin C concentration as a sensitive marker to monitor renal function during and after HD-MTX infusion in pediatric acute lymphoblastic leukemia patients, but its impact to predict delayed MTX elimination and renal toxicity is not demonstrated [23]. Other studies suggested that genetic polymorphism in genes coding for enzymes involved in folate metabolism may predict MTX elimination delay but these polymorphisms were different in the two studies [24, 25]. Taken together, different biomarkers were previously evaluated to predict delayed MTX elimination and potential toxicity, but none was validated and transfer to routine use.

In our study, At the initiation of hyperhydration, all urinary factors were modified: we observed that potassium, creatinin and urea levels decreased, and sodium level increased. Nevertheless, urinary osmolarity remained stable. These variations could be related to the preventive hyperhydration associated with HD-MTX administration. Several studies showed that urine alkalinization during MTX administration increased the activities of Na-K-2Cl-cotransporters (NKCC2) which are located on the ascending branch of the loop of Henle. NKCC2 increased both urinary sodium and chlorine levels and decreased urinary potassium level [26, 27]. Moreover, hyperhydration decreased both urea and creatinin levels. Taken together, these results support the osmolarity stability we observed in our study. Regarding the biological rational of the predictive value of urinary K+ level, we propose to focus the discussion on hypokalemia management. To determine hypokalemia etiology, urinary K+ level is required. If this level is less than 15 mmol/L, we consider that the cause is of extra-renal origin and if the level is greater than 15 mmol/L then the cause is of renal origin. In the context of renal origin, bicarbonate level is required to separate renal tubular acidosis from other potential etiologies [28]. In our study, even though we do not have the dosage of bicarbonate, we hypothesized that the increase in urinary K+ reflects the onset of tubular acidosis and therefore the increased risk of developing acute tubular necrosis.

The determination of the urinary potassium level is a simple dosage that can be carried out in current clinical practice. Indeed, the daily collection and quantification of diuresis is part of the monitoring of patients during treatment with HD-MTX. Moreover, it's an affordable analysis which allows an easy and fast generalization to all tertiary centers treating PCNSL patients. The interest of this dosage seems to predominate for younger patients (<70 years). Indeed, our multivariate analysis showed that the integration of patient age, KPS and early potassium urinary level may help the clinicians to predict delayed HD-MTX elimination (84% versus 33% at 72 hours). The questions that could be subsequently asked could be how can we adapt the management of these young patients who have a high early rate of urinary potassium? Should we increase the folinic rescue and/or should we prematurely use the CPDG2? While awaiting the results of the current clinical trial METHOGLU, it seems that the

use of CPDG2 would be the best option to limit renal toxicities, allowing an optimal HD-MTX administration and patient prognosis. Nevertheless, the remaining socio-economic limitation of the systematic use of CPDG2 is its expensive cost. Then, its indication must be first assessed by clinical trials to validate a significant and robust clinical benefit to its administration. Concerning the population of patients older than 70 years, early urinary potassium level does not seem to be relevant enough to justify its use in routine. There must be other physiological (or pathological) factors to integrate to improve delayed HD-MTX elimination prediction. For instance, the association of several blood or urinary markers could be discussed. On the other hand, comorbidities or concurrent medication intake may also influence urinary ionogram during chemotherapy in this older population (diuretics, antihypertensive therapy). Moreover, the clinical impact of early prediction of HD-MTX toxicity has also to be tempered in this population, rarely candidate to intensification at the end of HD-MTX induction.

This study has some limitations: the rate of renal toxicity observed is relatively low, which limits the interpretation of our results. However, in view of previous studies which showed a relationship between elimination of MTX and occurrence of toxicity, we could assume a potential association between the increase in early urinary potassium and the occurrence of toxicity. This study has been carried out in a single center. Nevertheless, our center is the tertiary regional center for the management of PCNSL, within the RENOCLIP-LOC French network, including around 10% of all newly diagnosed primary CNS lymphoma. Moreover, data were prospectively collected, and we validated our results in an independent cohort, increasing the robustness of our results.

Conclusion

To our knowledge, this is the first study exploring urinary factors as potential biomarkers of delayed MTX elimination. We found that early urinary potassium level at 24 hours may predict delayed MTX elimination and opens new perspectives for personalized care in young adult patients with PCNSL. Its relevance as a decision-making element needs to be validated in a larger prospective and multicentric cohort. Prompt identification and effective treatment of acute renal injury mitigate further toxicity, improve renal recovery, and allow patients to resume HD-MTX therapy or receive other chemotherapy. Varying the dose of folinic rescue or improving early CPDG2 rescue use according to the early urinary potassium level may be one of the research opportunities.

Statements and Declarations

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript

Conflict of Interest

Author’s contributions

Study conception and design [Harlay, Bertucci, Chinot and Tabouret]. Material preparations were performed by [Boucard, Harlay, Bertucci, Petrirena, Campello, Barrié, Autran, Chinot and Tabouret], data collection was performed by [Harlay], and analysis were performed by [Tabouret]. The first draft of the manuscript was written by [Bertucci] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics Approval

All patients were prospectively included into the LOC network and provided their written consent for clinical data collection according to national and LOC network policies (CPP OUEST II ANGERS - 19 April 2018)

Informed Consent

All patients provided their written consent for clinical data collection.

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