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Plasma creatinine as predictor of delayed elimination of high-dose methotrexate in childhood acute lymphoblastic leukemia – a Danish population-based study

Diana Schmidt, MD,[1] Kim Kristensen, PhD[1,7] Henrik Schroeder, MD, DMSc,[2] Peder Skov Wehner, MD, PhD,[3] Steen Rosthøj, MD,[4] Jesper Heldrup, MD,[5] Linn Damsgaard, B.Sc,[1] Kjeld Schmiegelow, MD, DMSc,[1,6] and Torben Stamm Mikkelsen, MD, PhD[2]

[1]Department of Pediatrics and Adolescent Medicine, University Hospital Rigshospitalet, Copenhagen, Denmark

[2]Department of Pediatric Oncology, Aarhus University Hospital, Aarhus, Denmark

[3]Department of Pediatric Hematology and Oncology, H.C. Andersen Children's Hospital, Odense University Hospital, Denmark

[4] Department of Pediatric Oncology, Aalborg University Hospital, Aalborg, Denmark

[5] Department of Pediatric Oncology, Lund University Hospital, Lund, Sweden

[6] Institute of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

[7]Development DMPK – PKPD, Novo Nordisk A/S, Maaløv, Denmark

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Corresponding author: Dr. Torben Stamm Mikkelsen, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, DK-8200 Aarhus N, Denmark. Phone: +45 53566513; Fax: +45 78451710; e-mail: torben.mikkelsen@clin.au.dk

Abbreviations

MTX	methotrexate
HD-MTX	high-dose methotrexate
ALL	acute lymphoblastic leukemia
95%CI	95 percent confidence interval
NOPHO	Nordic Society for Pediatric Hematology and Oncology
CNS	Central nervous system
WBC	White blood cell count
6MP	6-mercaptopurine
CTCAE	Common Terminology Criteria for Adverse Events
ROC	Receiver operating characteristic curves
AUC	Area under the curve
ROC	Receiver operating characteristic curves

IQR	Interquartile range

ABSTRACT

BACKGROUND: Severely delayed elimination of methotrexate (MTX) is difficult to predict in patients treated with high-dose MTX (HD-MTX), but may cause life-threatening toxicity. It has not been defined how an increase in plasma creatinine best can be used as a predictor for severely delayed MTX elimination thus a guide for therapeutic interventions to minimize renal toxicity.

METHODS: Pharmacokinetic data was retrospectively collected on 218 Danish children with acute lymphoblastic leukemia treated with HD-MTX 5 or 8 g/m^2 on the NOPHO2000 protocol. Moderately delayed MTX elimination was defined as 42-hour plasma MTX ≥4.0-9.9 µM, and severely delayed elimination was defined as 42-hour plasma MTX ≥10 µM.

RESULTS: Median 42-hour plasma MTX was 0.61 µM (IQR: 0.4-1.06 µM). Of 1295 MTX infusions with 5 g/m^2 (n=140 patients) or 8 g/m^2 (n=78 patients) 5.1% were severely (1.5%) or moderately (3.6%) delayed. The risk of having delayed elimination was highest in the first of eight

infusions with MTX 5 g/m² (7.4% vs 0.0 to 4.1% for subsequent MTX infusions) (p<0.02). A 25 μM increase or a 1.5 fold increase in plasma creatinine within 36 hours from start of the MTX infusion had a sensitivity of 92% (95%CI: 82%-97%) and specificity 85% (95%CI: 83-87%) for predicting 42h MTX ≥4.0 μM.

CONCLUSIONS: A 25 μM increase or a 1.5 fold in plasma creatinine within 36 hours after start of a HD-MTX infusion can predict delayed MTX elimination, thus allowing intensification of hydration and alkalization to avoid further renal toxicity and promote the elimination of MTX.

1 | INTRODUCTION

Methotrexate (MTX) is an important chemotherapeutic drug used in the treatment of acute lymphoblastic leukemia (ALL).[1] High-dose MTX (HD-MTX) 1-8 g/m² is used to overcome cellular resistance and promote transport into pharmacological sanctuaries (e.g. testes and central nervous system (CNS)).[1-3] The MTX elimination vary significantly between HD-MTX courses, and extremely slow MTX elimination is seen in up to 5% of patients with ALL.[4,5] The variation in MTX elimination is difficult to predict and can only to some extend be explained by age, gender, treatment protocol, and germline DNA polymorphisms.[6-8]

MTX is primarily eliminated by renal filtration and nephrotoxicity is seen in up to 20% of all HD-MTX infusions.[9-12] Nephrotoxicity reduces the MTX elimination and results in life threatening systemic MTX exposure.[4,11,13] Early detection of MTX induced nephrotoxicity is important because increased hydration and urine alkalisation can promote the renal elimination of MTX and prevent further damage to the kidneys.[10,11] An increase in plasma creatinine has in some protocols been used as a biomarker to detect MTX induced nephrotoxicity.[9,12]

Folinic acid is used as a rescue drug to counteract MTX induced intracellular toxicity.[14] In case of severely delayed MTX elimination, the dose of folinic acid has to be increased in proportion to the MTX concentration but this could theoretically circumvent the antileukemic effects of MTX.[15-17] Severely delayed MTX elimination is defined as a plasma MTX concentration ≥10 μM at 42 hours after start of the HD-MTX infusion.[18] In this study we examine how an increase in plasma creatinine (1.5 fold or 25 μM) and end of infusion plasma MTX can be markers of severely and moderately delayed MTX elimination (42-hour plasma MTX≥4 μM).

2 | MATERIALS AND METHODS

From January 2002 until June 2008, a total of 218 children were diagnosed with B-cell precursor or T-lineage ALL in Denmark and treated with HD-MTX on the ALL 2000 protocol from the Nordic Society for Pediatric Hematology and Oncology (NOPHO) (Supplemental Fig. 1).

2.1 Risk grouping

Patients were classified based on ALL subtype, age, white blood cell count (WBC), response to induction therapy and a number of unfavorable features as described elsewhere.[2] Patients with unfavorable features such as a high white blood cell counts WBC at diagnosis, T-cell immunophenotype, hypodiploid karyotype or cytogenetic rearrangements, CNS-involvement and testicular leukemia were classified as high-risk patients and received intensive therapy that included 8 g/m² HD-MTX. Patients without unfavorable features were treated with either standard intensive therapy (SI) or intermediate intensive therapy (II) that included infusions with 5 g/m² HD-MTX.[2,19]

2.2 High-dose MTX infusions

The complete NOPHO2000 protocol has been described in detail elsewhere.[2] In the consolidation phase, patients with standard or intermediate risk ALL received oral 6-mercaptopurine (6MP) 25 mg/m^2/day in combination with three courses of 5 g/m^2 HD-MTX at three weeks intervals. Patients with high-risk ALL received two or four courses of 8 g/m^2 HD-MTX in the consolidation phase without concomitant 6MP. During the first year of oral MTX/6MP maintenance treatment, patients with standard-risk and intermediate-risk ALL received further five courses of 5 g/m^2 HD-MTX at eight weeks intervals. High-risk patients were not treated with HD-MTX in the maintenance phase. The starting maintenance dose of oral 6MP was 75 mg/m^2/day, and subsequently adjusted to a target

WBC of 1.5-3.5x10^9/L. During the HD-MTX infusions the patients received one dose of intraspinal MTX (dose 8, 10 or 12 mg depending on age). Prehydration 150 ml/m²/h was started four hours before the HD-MTX infusion. After the prehydration 10% of the HD-MTX dose was infused over an hour, and the remaining 90% of the dose was given during the next 23 hours. The first dose of folinic acid 15 mg/m² was given at 42 hours after start of the 5 g/m² HD-MTX infusion (after 36 hours for 8 g/m²) and was repeated every 6th hour until the plasma MTX concentration was below 0.2 µmol/L. The dose of folinic acid was increased in case of delayed MTX elimination (Supplemental table 2). During and after the MTX infusion the hydration volume was 3000 ml/m^2/day. Plasma creatinine was measured at baseline, 23 hours and 36 hours after start of the HD-MTX infusion. The total hydration volume was elevated to 4500 ml/m²/day if plasma MTX was ≥ 3 µM at 36 hours or ≥ 1 µM at 42 hours after start of the HD-MTX infusion; or if plasma creatinine increased ≥ 1.5 fold within the first 42 hours after start of the HD-MTX infusion. Additional bicarbonate was given if urine pH was below 7.0 anytime before, during and after the HD-MTX infusion.

At 42 hours after start of the HD-MTX infusion, moderately delayed MTX elimination was defined as plasma MTX 4.0-9.99 µM, and severely delayed MTX elimination was defined as plasma MTX ≥10 µM.[18] Acute kidney injury stage one is according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) defined as an increase in plasma creatinine of 0.3 mg/dl (26.4 µM) or 50% from baseline.[20]

2.3 Statistics

Receiver operating characteristic curves (ROC) were used to study the association between delayed MTX elimination and increase in plasma creatinine (Supplement Fig. 2a-b). A large area under the

curve indicates that the cut-off value has both a high sensitivity and specificity as a predictor. All statistics were calculated using the statistical program STATA14. The HD-MTX treatment courses were considered as unrelated events. Analysis of variance (ANOVA) or Chi-squared tests were used to compare differences in means between groups. McNemar's test was used to evaluate the differences in sensitivity and specificity between the predictors for delayed MTX elimination.

3 | RESULTS

3.1 Risk of moderately and severely delayed MTX elimination

A total of 140 patients (51% females) with a mean age of 4.9 years (range 1.4-17.0 years) were treated with 5 g/m² HD-MTX according to the NOPHO2000 protocol for standard and intermediate risk ALL (missing data are given in Supplemental Fig. 1a). The mean MTX clearance was 117.4 ml/min/m² but HD-MTX infusions with delayed MTX elimination at 42-hour had a significantly lower MTX clearance 66.9 ml/min/m² compared to the infusions that were not delayed 119.1 ml/min/m² (difference in mean 52; 95%CI 35-68 ml/min/m²; P<0.001). The median end of infusion plasma MTX was 69 µM (IQR: 51-87 µM) and the median 42-hour plasma MTX was 0.53 µM (IQR: 0.37-0.89 µM) for 5 g/m² HD-MTX.

Of the 1052 infusions with 5 g/m² HD-MTX, 2.5% (n=26) in 23 patients were moderately delayed with plasma MTX 4-9.99 µM at 42 hours after start of the HD-MTX infusion (Fig. 1a). Only three (13%) of these 23 patients had more than one HD-MTX course with moderately delayed MTX elimination. HD-MTX lower infusion number (P<0.02) (7.4% vs 0.0 to 4.1% for subsequent MTX infusions) and older age (≥ 10 years) (P<0.001) were significantly associated with a higher risk of having one or more courses with moderately delayed MTX elimination, but gender had no significant impact (male to female ratio was 1:1).

149

150 Severely delayed MTX elimination with plasma MTX ≥ 10 μM at 42 hours after start of the 5 g/m²
151 HD-MTX infusion was seen in 0.76% (n=8) of the infusions. One of these seven patients had two
152 courses with severely delayed MTX elimination. The median age of patients with severely delayed
153 MTX elimination was 10.2 years (range 3.2-14.7 years), and 57% of them were females.

154

155 A total of 78 patients (44% females) with a mean age of 5.0 years (range 1.7 - 15.0 years) were treated
156 with 8 g/m² HD-MTX according to the NOPHO2000 protocol for high risk ALL. The mean MTX
157 clearance was 107.7 ml/min/m² but HD-MTX infusions with delayed MTX elimination at 42-hour had
158 a significantly lower MTX clearance 69.5 ml/min/m² compared to the infusions that were not delayed
159 113.3 ml/min/m² (difference in mean 43.8, 95%CI 27-60 ml/min/m²; P<0.001). The median end of
160 infusion plasma MTX was 114 μM (IQR: 93-146 μM) and the median 42-hour plasma MTX was 1.1
161 μM (IQR: 0.69-1.5 μM) for infusions with 8 g/m² HD-MTX.

162

163 Of the 243 infusions with 8 g/m² HD-MTX, 8.6% (n=21) were moderately delayed with plasma MTX
164 4-9.99 μM at 42 hours after start of the HD-MTX infusion, and 4.9% (n=12) were severely delayed
165 with plasma MTX ≥10 μM at 42 hours. Fifty seven percent of the 33 courses with moderately or
166 severely delayed MTX elimination occurred in the first of the four HD-MTX courses given to patients
167 with high risk ALL (Fig. 1b).

168

169 Of the 67 HD-MTX courses (5 and 8 g/m²) with moderately and severely delayed MTX elimination,
170 50 patients were re-challenged with a by protocol dose of HD-MTX. Only 8.0% (n=4) of these
171 patients had delayed elimination with plasma MTX ≥4.0 μM in the following HD-MTX infusions

(Table 1). Before the re-challenge with HD-MTX all patients had plasma creatinine within the normal range, but 28% (n=14) of the patients experienced a 1.5 fold increase in plasma creatinine within 36 hours from the start of the infusion as a result of the re-challenge with HD-MTX.

3.2 Plasma creatinine as predictor of delayed MTX elimination

At 42-hour, 5% of all HD-MTX infusions had plasma MTX >4.0 µM. The area under the ROC was high for a 1.5 fold and a 25 µM (~0.3 mg/dl) increase in plasma creatinine at 36-hour, meaning that these cut-off points would have high sensitivity and specificity for predicting severely and moderately delayed MTX elimination plasma MTX ≥4.0 µM (Supplement Fig. 2a-b).

Only 31% (n=13) of the HD-MTX infusions with moderately or severely delayed MTX elimination were identified by a 1.5 fold increase in plasma creatinine at 23 hours after start of the infusion. At the 23-hour time-point, a 1.5 fold increase in plasma creatinine was seen in 5.1% (n=39) of all 5 g/m² HD-MTX infusions, corresponding to a positive predictive value of only 13%. For infusions with 8 g/m² HD-MTX, the positive predictive value was 50% at the 23 hour time-point. Not all patients had the plasma creatinine measured at end of the HD-MTX infusion and this could potentially be a selection bias. However, there was no difference in age, gender, HD-MTX infusion number or baseline plasma creatinine when infusions with 23-hour and 36-hour plasma creatinine was compared to infusion where only the 36-hour plasma creatinine was measured.

At 36 hours after start of the HD-MTX infusion, a 1.5 fold increase in plasma creatinine identified 87.1% (n=54) of all infusions with moderately or severely delayed MTX elimination. At this time-

194 point, the positive predictive value was 20% for 5 g/m² HD-MTX, and 42% for 8 g/m² HD-MTX
195 infusions (Table 2).

196

197 A 25 μM increase in plasma creatinine at 36 hours after start of the HD-MTX infusion identified
198 79.0% (n=49) of the infusions with moderately delayed MTX elimination (Table 2). In 7.9% (n=91)
199 of all HD-MTX infusions there was a 25 μM increase in plasma creatinine, and 54% of these courses
200 were moderately delayed. Thus, at 36 hours after start of the HD-MTX infusion, a 25 μM increase in
201 creatinine had higher specificity as a predictor of delayed MTX elimination compared to a 1.5 fold
202 creatinine increase (P<0.001) but the sensitivity was equal for the two tests. All of the HD-MTX
203 infusions with severely delayed MTX elimination were identified at 36 hours after start of the infusion
204 because of either a 1.5 fold or 25 μM increase in plasma creatinine.

205

206 Although a 25 μM increase in plasma creatinine had similar sensitivity as a 1.5 increase, the two tests
207 identified slightly different groups of patients with delayed MTX elimination (Fig. 2). In the youngest
208 age group, a 1.5 fold increase in plasma creatinine identified some of the patients with delayed MTX
209 elimination who did not have a 25 μM increase. Patients with delayed MTX elimination who had a
210 1.5 fold increase in plasma creatinine but not a 25 μM (n=8), were younger (median age 3.8 years)
211 than the patients with delayed MTX elimination who had a 25 μM increase in plasma creatinine
212 (median age 9.5 years) (n=49; p=0.03). A combination of a 25 μM and 1.5 fold increase in plasma
213 creatinine had a sensitivity of 93.8 (95%CI: 79.2–99.2) and specificity 87.2 (95%CI: 84.9–89.3) in
214 predicting delayed MTX elimination.

215

216 **3.3 End of infusion plasma MTX as a predictor of delayed MTX elimination**

End of infusion plasma MTX was available in 1027 HD-MTX infusions; median 74 μM (IQR: 54-96 μM). There was no linear association between end of infusion plasma MTX and 42-hour MTX (Supplement Fig. 3). When end of infusion plasma MTX was tested as a predictor for delayed MTX elimination it was not possible to find a cut-off value that had both high sensitivity and specificity. This was illustrated by the small area under the ROC curve for end of infusion plasma MTX as a predictor of delayed MTX elimination (Supplement Fig. 2c). Supplemental table 1 shows the sensitivity and specificity for end of infusion plasma MTX 70 μM and 100 μM as predictors of delayed MTX elimination.

4 | DISCUSSION

When the 42-hour plasma MTX is above 5 μM in infusions with HD-MTX, the dose of leucovorin has to be increased proportionately to prevent systemic toxicity and this can theoretically rescue some of the leukemia cells.[15-17] When the 42-hour plasma MTX is above 10 μM in infusions with HD-MTX, it is recommended to start treatment with the enzyme glucarpidase, that cleaves MTX into non-toxic metabolites.[13] In this analysis, "delayed HD-MTX elimination" was defined as 42-hour plasma MTX≥4 μM, because this cut-off would include the 5% of HD-MTX infusions with the slowest MTX elimination and need for the largest doses of rescue leucovorin or even treatment with glucarpidase.

We found that despite the use of vigorous hydration and urine alkalization, moderately or severely delayed MTX elimination occurred in 3.2 % of all infusions with 5 g/m² HD-MTX and 4.2 times as often in 8 g/m² HD-MTX infusions. An increased plasma creatinine in relation to the HD-MTX infusion has in other studies been associated with decreased MTX clearance in children with ALL.[9,21] Plasma creatinine increases when the glomerular filtration rate declines and can therefore

241 serve as an indicator for the kidneys capability to eliminate MTX.[22] To translate this knowledge
242 into clinical practice we explored if a 25 µM or 1.5 fold increase in plasma creatinine could be used as
243 early markers for severely and moderately delayed MTX elimination.

244

245 This study, which included both 5 and 8 g/m² HD-MTX infusions showed that, at end of the HD-
246 MTX infusion, a 1.5 fold increase in plasma creatinine could only identified 14% of the infusions
247 with severely delayed MTX elimination. This suggests, that the MTX induced nephrotoxicity
248 occurred late during the HD-MTX infusion and it reflects the fact that plasma creatinine does not
249 increase until the glomerular filtration rate is decreased significantly.[22] Similar, the end of infusion
250 MTX concentration could not be used as a predictor with sufficiently high sensitivity and specificity.
251 Not all patients had the plasma creatinine measured at end of the HD-MTX infusion and this could
252 potentially have led to a selection bias.

253

254 At 36 hours after start of the HD-MTX infusion, almost all (93.8%) HD-MTX infusions with severely
255 or moderately delayed MTX elimination were identified by an increase in plasma creatinine. The
256 much higher sensitivity at 36 hours vs 23 hours after start of the HD-MTX infusion strongly suggests
257 that a significant number of patients with delayed MTX elimination could have been identified earlier
258 than 36 hours after start of the HD-MTX infusion. For patients predisposed to develop delayed MTX
259 elimination (eg. due to older age, or genetic background) it could therefore be relevant to measure the
260 plasma creatinine at 30 hours after start of the HD-MTX infusion to evaluate if this could identify
261 delayed MTX elimination at an even earlier time point.

262

263 A 25 µM and a 1.5 fold increase in plasma creatinine had similar sensitivity in predicting delayed
264 MTX elimination, but the two tests identified slightly different groups of patients with delayed MTX

elimination. In the oldest age group of patients, an absolute increase in plasma creatinine identified some patients with delayed MTX elimination who did not have a 1.5 fold increase in plasma creatinine. This is most likely because the youngest patients with a small muscle mass have low plasma creatinine concentrations.

The risk of having severely or moderately delayed MTX elimination was strikingly higher in the first HD-MTX infusion compared to the HD-MTX infusions given later in the consolidation and maintenance treatment phases. Others have similarly found, that the MTX clearance is lowest in the first HD-MTX infusion,[8] and it was recently shown that treatment with carboxypeptidase for patients with severely delayed MTX elimination was primarily needed in the first HD-MTX infusion given to patients with ALL.[23] The first HD-MTX infusion was given shortly after the induction therapy, suggesting that factors, such as tumor lysis, or nephrotoxicity during the induction phase could have reduced the kidneys ability to eliminate MTX in the first HD-MTX infusion. Nephrotoxicity and reduced MTX clearance can also be caused as a result of concomitant use of other drugs (e.g. proton pump inhibitors and antibiotics).[24] However, plasma creatinine can theoretically also be used as biomarker for nephrotoxicity in these situations.

In conclusion, the risk of having severely delayed MTX elimination was highest in the first of eight infusions with HD-MTX and correlated to older age and MTX dose. It was not possible to find an end of infusion plasma MTX cut-off value, which had both high sensitivity and specificity as a predictor of delayed MTX elimination. An absolute increase (25 μM) in creatinine at 36 hours after start of the infusion had higher specificity compared to a relative increase (1.5 fold), thus could be used as a predictor for moderately and severely delayed MTX elimination and allowing increased hydration and alkalization to avoid further kidney toxicity.

289

290 **Conflict of Interest Statement:** The authors whose names are listed below attest that they have NO
291 affiliations with or involvement in any organization or entity with any financial interest or non-
292 financial interest in the subject matter discussed in this manuscript.

293 Diana Shabaneh, Kim Kristensen, Henrik Schroeder, Peder Skov Wehner, Steen Rosthøj, Jesper
294 Heldrup, Kjeld Schmiegelow, Linn Damsgaard, Torben Stamm Mikkelsen.

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LEGENDS

TABLE 1 42-hour plasma MTX in the re-challenge infusions.

TABLE 2 Creatinine as predictor of delayed MTX elimination (42 hour MTX ≥4 μM).

FIGURE 1 Distribution of 42-hour plasma MTX concentrations versus HD-MTX infusion number for (A) HD-MTX 5 g/m² and (B) HD-MTX 8 g/m². MTX, methotrexate; HD-MTX, high-dose methotrexate. Numbers above the columns denote the number of HD-MTX infusions with 42-hour plasma MTX > 4 μM and the total no. of HD-MTX infusions.

FIGURE 2 HD-MTX infusions with and without an increase in plasma creatinine at the 36-hour. Outer circle includes 932 infusions, white background: infusions with 42-hour plasma MTX<4 μM, and grey background: infusions with 42-hour plasma MTX≥4 μM. Inner left circle includes infusions with ≥50% increase in plasma creatinine. Inner right circle includes infusions with ≥25 μM increase in plasma creatinine. Numbers in the circles denotes number of infusions. Cr, plasma creatinine; MTX, methotrexate; HD-MTX, high-dose methotrexate. The table displays the risk of delayed MTX elimination with the different combinations of increase in plasma creatinine.

TABLE 1 42 hour plasma MTX in the re-challenge infusions.

	5 g/m² N=34	**8 g/m²** N=33
42-hour MTX (μM)		
<1	17	11
1-1.99	7	8
2-2.99	1	1
3-3.99	0	1
≥ 4	2	2
Not re-challenged	6	9
Missing data	1	1
***Renal toxicity**	5 (18%)	7 (30%)

MTX, methotrexate. *Renal toxicity defined as 1.5 or 25 μM increase in plasma creatinine.

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Time-point, tor and MTX dose	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	LR+ (95% CI)	Dela
r 50% Cr increase					
5 g/m²	87.5 (71.0 - 96.5)	87.2 (84.9 - 89.3)	19.6 (13.4 - 27.0)	6.8 (5.5 - 8.5)	3
8 g/m²	86.7 (69.3 - 96.2)	81.2 (74.9 - 86.4)	41.9 (29.5 - 55.2)	4.6 (3.3 - 6.4)	3
r 25µM Cr increase					
5 g/m²	81.3 (63.6 - 92.8)	96.2 (94.8 - 97.4)	43.3 (30.6 - 56.8)	21.5 (14.9 - 31.1)	3
8 g/m²	76.7 (57.7 - 90.1)	95.8 (91.9 - 98.2)	74.2 (55.4 - 88.1)	18.3 (9.0 - 37.1)	3
usion MTX>100 µM					
5 g/m²	51.7 (32.5 – 70.6)	91.7 (89.7 – 93.4)	16.5 (9.5 - 25.7)	6.2 (4.1 – 9.4)	2
8 g/m²	78.6 (59.0 – 91.7)	37.1 (30.1 – 44.5)	15.8 (10.2 – 23.0)	1.3 (1.0 – 1.6)	2

rs after start of the MTX infusion. Cr, creatinine. MTX, methotrexate. PPV, positive predictive value. LR+, positive likelihood ratio, C

e interval, N, number of MTX infusions.

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