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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

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

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19 **Short running title:** Renal toxicity after high-dose methotrexate

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25 **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
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34 **ABSTRACT**

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

39 **METHODS:** Pharmacokinetic data was retrospectively collected on 218 Danish children with acute
40 lymphoblastic leukemia treated with HD-MTX 5 or 8 g/m² on the NOPHO2000 protocol. Moderately
41 delayed MTX elimination was defined as 42-hour plasma MTX ≥ 4.0 -9.9 μM , and severely delayed
42 elimination was defined as 42-hour plasma MTX ≥ 10 μM .

43 **RESULTS:** Median 42-hour plasma MTX was 0.61 μM (IQR: 0.4-1.06 μM). Of 1295 MTX
44 infusions with 5 g/m² (n=140 patients) or 8 g/m² (n=78 patients) 5.1% were severely (1.5%) or
45 moderately (3.6%) delayed. The risk of having delayed elimination was highest in the first of eight

46 infusions with MTX 5 g/m² (7.4% vs 0.0 to 4.1% for subsequent MTX infusions) (p<0.02). A 25 μM
47 increase or a 1.5 fold increase in plasma creatinine within 36 hours from start of the MTX infusion
48 had a sensitivity of 92% (95%CI: 82%-97%) and specificity 85% (95%CI: 83-87%) for predicting
49 42h MTX ≥4.0 μM

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

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55 11 INTRODUCTION

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

63
64 MTX is primarily eliminated by renal filtration and nephrotoxicity is seen in up to 20% of all HD-
65 MTX infusions.[9-12] Nephrotoxicity reduces the MTX elimination and results in life threatening
66 systemic MTX exposure.[4,11,13] Early detection of MTX induced nephrotoxicity is important
67 because increased hydration and urine alkalinisation can promote the renal elimination of MTX and
68 prevent further damage to the kidneys.[10,11] An increase in plasma creatinine has in some protocols
69 been used as a biomarker to detect MTX induced nephrotoxicity.[9,12]

70
71 Folinic acid is used as a rescue drug to counteract MTX induced intracellular toxicity.[14] In case of
72 severely delayed MTX elimination, the dose of folinic acid has to be increased in proportion to the
73 MTX concentration but this could theoretically circumvent the antileukemic effects of MTX.[15-17]
74 Severely delayed MTX elimination is defined as a plasma MTX concentration $\geq 10 \mu\text{M}$ at 42 hours
75 after start of the HD-MTX infusion.[18] In this study we examine how an increase in plasma
76 creatinine (1.5 fold or $25 \mu\text{M}$) and end of infusion plasma MTX can be markers of severely and
77 moderately delayed MTX elimination (42-hour plasma MTX $\geq 4 \mu\text{M}$).

78 2 | MATERIALS AND METHODS

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

82

83 2.1 Risk grouping

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

91

92 2.2 High-dose MTX infusions

93 The complete NOPHO2000 protocol has been described in detail elsewhere.[2] In the consolidation
94 phase, patients with standard or intermediate risk ALL received oral 6-mercaptopurine (6MP) 25
95 mg/m²/day in combination with three courses of 5 g/m² HD-MTX at three weeks intervals. Patients
96 with high-risk ALL received two or four courses of 8 g/m² HD-MTX in the consolidation phase
97 without concomitant 6MP. During the first year of oral MTX/6MP maintenance treatment, patients
98 with standard-risk and intermediate-risk ALL received further five courses of 5 g/m² HD-MTX at
99 eight weeks intervals. High-risk patients were not treated with HD-MTX in the maintenance phase.
100 The starting maintenance dose of oral 6MP was 75 mg/m²/day, and subsequently adjusted to a target

101 WBC of $1.5-3.5 \times 10^9/L$. During the HD-MTX infusions the patients received one dose of intraspinal
102 MTX (dose 8, 10 or 12 mg depending on age). Prehydration 150 ml/m²/h was started four hours
103 before the HD-MTX infusion. After the prehydration 10% of the HD-MTX dose was infused over an
104 hour, and the remaining 90% of the dose was given during the next 23 hours. The first dose of folinic
105 acid 15 mg/m² was given at 42 hours after start of the 5 g/m² HD-MTX infusion (after 36 hours for 8
106 g/m²) and was repeated every 6th hour until the plasma MTX concentration was below 0.2 μmol/L.
107 The dose of folinic acid was increased in case of delayed MTX elimination (Supplemental table 2).
108 During and after the MTX infusion the hydration volume was 3000 ml/m²/day. Plasma creatinine was
109 measured at baseline, 23 hours and 36 hours after start of the HD-MTX infusion. The total hydration
110 volume was elevated to 4500 ml/m²/day if plasma MTX was $\geq 3 \mu M$ at 36 hours or $\geq 1 \mu M$ at 42
111 hours after start of the HD-MTX infusion; or if plasma creatinine increased ≥ 1.5 fold within the first
112 42 hours after start of the HD-MTX infusion. Additional bicarbonate was given if urine pH was below
113 7.0 anytime before, during and after the HD-MTX infusion.

114
115 At 42 hours after start of the HD-MTX infusion, moderately delayed MTX elimination was defined as
116 plasma MTX 4.0-9.99 μM, and severely delayed MTX elimination was defined as plasma MTX ≥ 10
117 μM.[18] Acute kidney injury stage one is according to the NCI Common Terminology Criteria for
118 Adverse Events (CTCAE) defined as an increase in plasma creatinine of 0.3 mg/dl (26.4 μM) or 50%
119 from baseline.[20]

120

121

122 **2.3 Statistics**

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

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

130

131 3 | RESULTS

132 3.1 Risk of moderately and severely delayed MTX elimination

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

141

142 Of the 1052 infusions with 5 g/m² HD-MTX, 2.5% (n=26) in 23 patients were moderately delayed
143 with plasma MTX 4-9.99 μ M at 42 hours after start of the HD-MTX infusion (Fig. 1a). Only three
144 (13%) of these 23 patients had more than one HD-MTX course with moderately delayed MTX
145 elimination. HD-MTX lower infusion number ($P < 0.02$) (7.4% vs 0.0 to 4.1% for subsequent MTX
146 infusions) and older age (≥ 10 years) ($P < 0.001$) were significantly associated with a higher risk of
147 having one or more courses with moderately delayed MTX elimination, but gender had no significant
148 impact (male to female ratio was 1:1).

149

150 Severely delayed MTX elimination with plasma MTX $\geq 10 \mu\text{M}$ 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 $\geq 10 \mu\text{M}$ 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 $\geq 4.0 \mu\text{M}$ in the following HD-MTX infusions

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

175

176 **3.2 Plasma creatinine as predictor of delayed MTX elimination**

177 At 42-hour, 5% of all HD-MTX infusions had plasma MTX $>4.0 \mu\text{M}$. The area under the ROC was
178 high for a 1.5 fold and a $25 \mu\text{M}$ ($\sim 0.3 \text{ mg/dl}$) increase in plasma creatinine at 36-hour, meaning that
179 these cut-off points would have high sensitivity and specificity for predicting severely and moderately
180 delayed MTX elimination plasma MTX $\geq 4.0 \mu\text{M}$ (Supplement Fig. 2a-b).

181

182 Only 31% (n=13) of the HD-MTX infusions with moderately or severely delayed MTX elimination
183 were identified by a 1.5 fold increase in plasma creatinine at 23 hours after start of the infusion. At the
184 23-hour time-point, a 1.5 fold increase in plasma creatinine was seen in 5.1% (n=39) of all 5 g/m^2
185 HD-MTX infusions, corresponding to a positive predictive value of only 13%. For infusions with 8
186 g/m^2 HD-MTX, the positive predictive value was 50% at the 23 hour time-point. Not all patients had
187 the plasma creatinine measured at end of the HD-MTX infusion and this could potentially be a
188 selection bias. However, there was no difference in age, gender, HD-MTX infusion number or
189 baseline plasma creatinine when infusions with 23-hour and 36-hour plasma creatinine was compared
190 to infusion where only the 36-hour plasma creatinine was measured.

191

192 At 36 hours after start of the HD-MTX infusion, a 1.5 fold increase in plasma creatinine identified
193 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**

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

225

226 4 | DISCUSSION

227 When the 42-hour plasma MTX is above 5 μM in infusions with HD-MTX, the dose of leucovorin
228 has to be increased proportionately to prevent systemic toxicity and this can theoretically rescue some
229 of the leukemia cells.[15-17] When the 42-hour plasma MTX is above 10 μM in infusions with HD-
230 MTX, it is recommended to start treatment with the enzyme glucarpidase, that cleaves MTX into non-
231 toxic metabolites.[13] In this analysis, “delayed HD-MTX elimination” was defined as 42-hour
232 plasma $\text{MTX}_{\geq 4}$ μM , because this cut-off would include the 5% of HD-MTX infusions with the
233 slowest MTX elimination and need for the largest doses of rescue leucovorin or even treatment with
234 glucarpidase.

235

236 We found that despite the use of vigorous hydration and urine alkalization, moderately or severely
237 delayed MTX elimination occurred in 3.2 % of all infusions with 5 g/m^2 HD-MTX and 4.2 times as
238 often in 8 g/m^2 HD-MTX infusions. An increased plasma creatinine in relation to the HD-MTX
239 infusion has in other studies been associated with decreased MTX clearance in children with
240 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 μ M 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 μ M 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

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

269

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

281

282 In conclusion, the risk of having severely delayed MTX elimination was highest in the first of eight
283 infusions with HD-MTX and correlated to older age and MTX dose. It was not possible to find an end
284 of infusion plasma MTX cut-off value, which had both high sensitivity and specificity as a predictor
285 of delayed MTX elimination. An absolute increase (25 μM) in creatinine at 36 hours after start of the
286 infusion had higher specificity compared to a relative increase (1.5 fold), thus could be used as a
287 predictor for moderately and severely delayed MTX elimination and allowing increased hydration and
288 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.

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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 μ M).

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 μ M and the total no. of HD-MTX infusions.

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

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

	5 g/m²	8 g/m²
	N=34	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, Factor and MTX dose	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	LR+ (95% CI)	Delta
Cr 50% Cr increase					
5 g/m ²	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/m ²	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
Cr 25µM Cr increase					
5 g/m ²	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/m ²	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
Cr 100µM Cr increase					
Infusion MTX>100 µM					
5 g/m ²	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/m ²	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

Time-points are defined as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 hours after start of the MTX infusion. Cr, creatinine. MTX, methotrexate. PPV, positive predictive value. LR+, positive likelihood ratio, CI, confidence interval, N, number of MTX infusions.

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