The influence of impaired kidney function at start of the therapy on the hematologic and renal toxicity of high-dose methotrexate: a retrospective cohort study in the University Medical Center Utrecht

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Abstract

Background: High-dose methotrexate (HD-MTX) is mainly excreted by the kidneys and this causes delayed methotrexate (MTX) clearance in patients with impaired kidney function. It is expected that this will also cause more hematologic and renal toxicity compared to patients with normal renal function. Studies examining effect of renal impairment on toxicity are currently lacking.
Objective: To measure the effect of renal impairment, represented as the estimated glomerular filtration rate (eGFR), on the hematologic toxicity (neutropenia and thrombocytopenia) and nephrotoxicity after the first HD-MTX infusion.
Methods: This is a retrospective cohort study performed in the University Medical Care Center Utrecht. Data was extracted from the Utrecht Patient Orientated Database (UPOD). Four different treatment 3 g/m^2 protocols for hemato-oncology indications were included. Individuals were classified in one of the following five groups, based on their kidney function: eGFR $\geq$ 90, eGFR 81-89, eGFR 60-80, eGFR 59-30 or eGFR < 30 ml/min/1,73m^2. The primary outcome was to assess the relation between kidney function at start of the therapy and the occurrence of grade 3 or 4 hematologic toxicity due to MTX administration. Secondary outcomes were differences in neutrophil- and platelet counts between baseline and nadir, time in days until hematologic toxicity and renal toxicity.
Results: This study showed no significant difference in the prevalence of grade 3 or 4 neutropenia and thrombocytopenia compared to patients with normal renal function ($\geq$ 90 ml/min). The difference in the mean delta platelet and neutrophil counts of the eGFR groups were also not significantly different compared to the patients with a kidney function ≥ 90 ml/min. The time until hematologic toxicity was comparable in all eGFR groups. A trend towards more nephrotoxicity in patients with lower renal function was seen. The results showed increased creatinine values in the lower kidney function groups compared to patients with normal kidney function. However, this results was not significant.
Conclusion: Hematologic toxicity seems independent of kidney function at start of the therapy. There is a trend toward more nephrotoxicity in patients with lower renal function in the main analysis, however no significant relation between kidney function at baseline and nephrotoxicity was found. Conclusions should be taken with caution because of the study design.

Introduction

Methotrexate (MTX) is a folate antagonist and has immunomodulatory, anti-inflammatory and anticancer properties. High-dose methotrexate (HD-MTX), defined as ≥ 500 mg/m^2, has a prominent role in the treatment of a range of adult and childhood cancers, including osteosarcoma, primary central nervous system lymphoma and acute lymphoblastic leukemia. [1,2,3]

The kidneys play an important role in the excretion of MTX. [1,2] After administration 80-90% is excreted unchanged by glomerular filtration and to a lesser extent by tubular secretion. [4] There is good correlation between MTX clearance and the endogenous creatinine clearance. [5] In patients with renal impairment the excretion of MTX will therefore be delayed, which results in increased exposure (AUC) and related toxicity. [6,7,8,9,10] HD-MTX is associated with multiple toxicities, including hematologic toxicity and nephrotoxicity. [1,2,3,11,12,13,14,15,18] Hematologic toxicity includes myelosuppression and this can result in thrombocytopenia, neutropenia and leucopenia. [3,13] Severe myelosuppression is a life-threatening condition and is related to prolonged MTX exposure. [14] It occurs in about 28% of patients receiving HD-MTX [13,15] Nephrotoxicity develops in 2%-12% of the patients using HD-MTX. These percentages of toxicity were found despite appropriate supportive care measures such as folinic acid. Nephrotoxicity is caused by precipitation in the tubules. [2,16] Risk factors for precipitation are an acidic environment, volume depletion and prolonged high MTX concentrations. Hydration and alkalizing is therefore recommended before starting and during methotrexate infusion. [17]

At this moment, relatively little is known about dosing HD-MTX in patients with renal impairment at start of HD-MTX therapy. The Food and Drug Administration (FDA) does not advise on dosing HD-MTX in patients with impaired kidney function but the Medicines and Healthcare products Regulatory Agency (MHRA), the Renal Drug Handbook and Aronoff et al. suggest to reduce the dose to 50% in patients with an estimated glomerular filtration rate (eGFR) of 20-50 ml/min and to avoid use when below 20 ml/min. [19,20,21] However, there is also literature which already recommends reducing the dose to 75% in patients with a kidney function <80 ml/min. [17,22] This is followed by varying recommendations in literature on when to reduce the dose to 50%, having a range 10-50 ml/min. [17,22,23] In addition, there is also literature that provides intervening recommendations, such as dosing 65% in patients with a kidney function 45-60 ml/min or to reduce the dose proportional to creatinine clearance. [23,24,25] Opinions on when to avoid HD-MTX also differ and have a range <10-59 ml/min. [17,20,21,22,23,24,25] The Dutch guideline (KNMP/IM) does not advise on dosing HD-MTX but only recommends to monitor (adverse) effects in patients with renal function 10-50 ml/min. [4] The consequence of the conflicting literature is that there is currently no structural adjustment in the dosage of HD-MTX based on kidney function. Krens et al. (2019) recently published a review in which they discuss the effect of hepatic and renal impairment on pharmacokinetic parameters and give recommendations for dose adjustments of cytotoxic agents, including HD-MTX. [19] Their advice is to reduce the methotrexate dose with 50% in patients with an eGFR 20-50 ml/min and to avoid use below 20 ml/min. However, the recommendation in Krens et al. for HD-MTX has its limitations because it is only based on Summary of Product Characteristics (SmPC) from the FDA and MHRA. It is unknown why the other available literature about dose reductions are not included in this review.

Unequivocal advice about dosing HD-MTX in patients with impaired kidney function is lacking and this may cause suboptimal treatment in this population. Studies which examine the direct effect of renal impairment on hematologic toxicity and nephrotoxicity are missing. This retrospective study therefore aims to examine the influence of impaired renal function at start of HD-MTX 3 g/m^2 therapy on the hematologic and renal toxicity of MTX and to see how this relates to previous findings in literature.

Methods

Study design and study population

This is a retrospective cohort study performed in University Medical Centre, Utrecht, the Netherlands, to evaluate the effect of kidney function at start of HD-MTX therapy on the hematologic toxicity and nephrotoxicity of intravenous 3 g/m^2 HD-MTX. The pseudonymized data were collected from the Utrecht Patient Orientated Database (UPOD). [26] The information extracted for this study were patient characteristics (age, gender), medication data (HD-MTX, dose, administration date), laboratory data (neutrophil count, platelet count, liver lab results, date of measurement) and other measurements (length, weight, body mass index [BMI], body surface area [BSA]). The MTX information in UPOD was extracted from CATO, a software system used for prescribing, preparing and registering oncolytic treatments. Data was collected in the period between January 2011 and May 2021.

Four different protocols in which patients are treated with MTX 3 g/m2 were included. These protocols contained either methotrexate as monotherapy or combined with other (oral) chemotherapeutics and were all used for hemato-oncology indications. See appendix figure 1. The interval between the first and second methotrexate dose was around 14 days in all protocols. Data on the hematologic toxicity and nephrotoxicity after the first MTX dose was collected up to 13 days after the first therapy date. Lab data closest to the day of the first MTX dose, with a range of maximum 14 days prior to administration, were used for the baseline characteristics.

Other protocols containing HD-MTX were excluded. Protocol exclusion criteria were a different MTX dose than 3 g/m2 (except for dose reductions $\leq$ 10%), concomitant use of other nephrotoxic chemotherapeutics (cisplatin), an interval of more than 14 days between the first two MTX infusions and a small patient population (<10 participants during the study period).

Included study participants had a minimum of one administration of intravenous 3 g/m2 methotrexate between January 2011 and May 2021, were aged ≥ 18 years and had renal and hematologic measurements at baseline and hereafter. Individuals were excluded in the following order:

1. Methotrexate dose-escalation > 3 g/m^2 in the included protocols
2. Dose reduction (>10%)
3. A new dose of 3 g/m^2 methotrexate within 13 days

Study participants were classified in one of the five following groups, based on baseline renal function: eGFR $\geq$ 90, eGFR 81-89 (group 1), eGFR 60-80 (group 2), eGFR 59-30 (group 3) or eGFR < 30 ml/min/1,73m^2 (group 4), chosen based on recommendations in literature. [17,20,21,22,23,24,25] The group with a kidney function $\geq$ 90 was used as the reference group.

Primary and secondary outcomes

The primary outcome was to assess the relation between kidney function at start of the therapy and the occurrence of hematologic toxicity (neutropenia and thrombocytopenia) due to MTX administration (3g/m^2). Hematologic toxicity was defined as grade 3 or 4 neutropenia or thrombocytopenia based on the Common Terminology Criteria for Adverse Events (CTCAE) [27]. For this, the lowest neutrophil and platelet counts after baseline were taken into account. Leukocyte lab results were lacking in the database and thus could not be evaluated.

Secondary outcomes were the differences in neutrophil- and platelet counts between baseline and nadir, time in days until hematologic toxicity occurs and to assess the relation between kidney function

at start of the therapy and renal toxicity, using the difference in creatinine values between baseline and the highest creatinine value. The delta in neutrophil and platelet counts were seen a predictor of hematologic toxicity, while the delta creatinine values were seen a predictor of nephrotoxicity.

Data analysis:

The primary independent predictor of hematologic and renal toxicity was baseline renal function, represented as eGFR. In October 2013, the UMCU switched from the MDRD equation to the CDK-EPI equation to determine the eGFR. The eGFR values before this date were therefore recalculated using the CDK-EPI equation. Besides that, due to a change in analytical method in April 2013, a correction of 7 µmol/l was applied for creatinine values measured before this date based on method validation parameters.

Data analysis was performed for all four treatment protocols with HD-MTX combined. Furthermore, a subgroup analysis was performed, including only the HD-MTX monotherapy protocol. This subgroup analysis included less participants but did not have the interference of other cytotoxic co-medications.

Patients with missing hematologic data at baseline or nadir could not be analyzed on the difference in neutrophil and platelet counts, while both the previous described patients and the patients with hematotoxicity at baseline were not taken into account for the analysis on grade 3 or 4 hematologic toxicity. Analysis on nephrotoxicity could be performed on all individuals because there were no missing creatinine values.

Statistical Analysis

Data analysis was performed using SPSS version 16.0.0.1. [28] The descriptive statistics present the baseline characteristics. The normality of the variables were tested with the Shapiro-Wilk test. The Kruskal Wallis test was used to compare non-normally distributed continuous variables between the four groups. The Independent Samples T-test compared the normally distributed continuous variables. The comparison between categorical variables between the groups was tested with the Chi-Square Test. A p-value < 0.05 was considered statistically significant. Logistic regression analysis was performed to investigate the association between kidney function and grade 3 or 4 hematologic toxicity. For the analysis of the differences between baseline and the lowest hematologic lab results/ highest creatinine lab results, linear regression was performed. The Kruskal Wallis analysis was performed to evaluate the difference in time in days between the eGFR groups until grade 3 or 4 hematologic toxicity or nadir. Forward inclusion was used to adjust for potential confounders in the logistic and linear regression methods. Covariates were included in the analysis if there was a change in the regression coefficient of 10% or more.

Results

Within the four HD-MTX treatment protocols a total of 191 individuals received HD-MTX during the study period. Figure 1 shows a diagram of the study inclusion for both the main analysis and the subgroup analysis. The final study population included 103 individuals. No patients were excluded based on dose reductions >10% at start of the therapy. At baseline 62% of the study

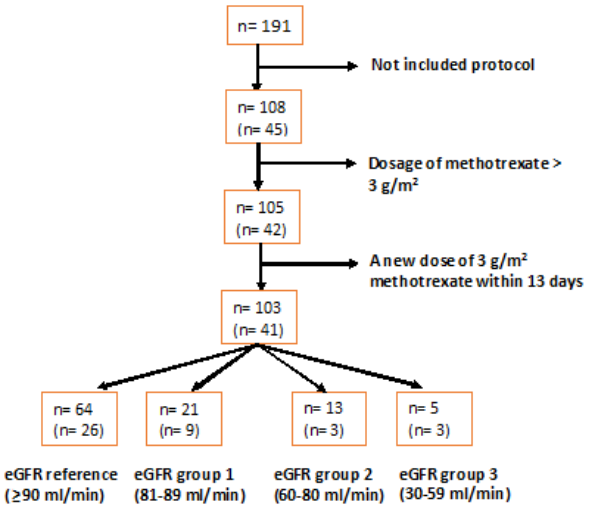

Figure 1: *Flowchart of patient inclusion. The data in the brackets shows the inclusion of the subgroup analysis.*

population was classified as the reference eGFR group (≥90 ml/min), 20% as group 1 (81-89 ml/min) and 12% as group 2 (60-80 ml/min). Only 5 individuals were classified as group 3 (30-59 ml/min). None had an eGFR < 30 ml/min so this group was excluded for further analysis. Within the final study population, 41 participants received MTX monotherapy and were eligible for the subgroup analysis. The distribution of participants in the reference group and group 1 was similar to the main analysis, 63% and 22% respectively. The group with an eGFR of 60-80 ml/min (group 2) and the group with an eGFR of 30-59 ml/min (group 3) both contained 7.3% of the study population.

Table 1: Patient characteristics at start of the therapy

			eGFR ≥90 Reference	eGFR 81-89 Group 1	eGFR 60-80 Group 2	eGFR 30-59 Group 3	p-value[a]
Total no of patients (n)	All protocols Monotherapy	103 41	64 26	21 9	13 3	5 3	
Age, yr Median (IQR)	All protocols	65.0 (50.0)	63.5 (50.0)	69.0 (22.0)	69.0 (22.0)	75.0 (11.0)	0.001
	Monotherapy	66.0 (50.0)	63.5 (46.0)	68.0 (17.0)	69.0 (4.0)	76.0 (11.0)	0.048
Gender, Male, n (%)	All protocols	66.0 (64.1%)	42.0 (65.6%)	12.0 (57.1%)	9.0 (69.2%)	3.0 (60.0%)	0.875
	Monotherapy	28.0 (68.3%)	19.0 (73.1%)	4.0 (44.4%)	3.0 (100.0%)	2.0 (66.7%)	0.258
Weight, kg Mean (SD)	All protocols	77.2 (13.9)	76.9 (14.6)	78.2 (13.5)	77.2 (12.8)	77.0 (14.6)	0.987
	Monotherapy	75.7 (13.9)	75.8 (15.1)	74.8 (13.9)	77.1 (4.6)	75.7 (14.8)	0.995
Length, cm Mean (SD)	All protocols	173.1 (9.3)	173.6 (9.7)	173.1 (9.6)	170.4 (7.0)	173.6 (8.2)	0.724
	Monotherapy	173.5 (7.6)	173.9 (7.1)	172.6 (9.6)	169.0 (3.6)	177.7 (7.8)	0.552
Body mass index, kg/m² Mean (SD)	All protocols	25.8 (3.9)	25.5 (3.7)	26.0 (3.5)	26.7 (4.8)	25.6 (5.5)	0.793
	Monotherapy	25.2 (4.0)	25.3 (4.5)	25.0 (3.5)	27.0 (1.6)	23.8 (3.1)	0.818
Body surface area (m²) Mean, (SD)	All protocols	1.9 (0.2)	1.9 (0.2)	1.9 (0.2)	1.9 (0.2)	1.9 (0.2)	0.969
	Monotherapy	1.9 (0.2)	1.9 (0.2)	1.9 (0.2)	1.9 (0.1)	1.9 (0.2)	0.969
ALAT, U/l Median (IQR)	All protocols	34.0 (594.0)	40.0 (594.0)	26.0 (468.0)	27.0 (50.0)	30.0 (45.0)	0.066
	Monotherapy	30.0 (345.0)	36.0 (345.0)	21.0 (20.0)	21.0 (15.0)	30.0 (22.0)	0.087
ASAT, U/l Median (IQR)	All protocols	21.0 (132.0)	22.5 (132.0)	20.0 (84.0)	21.0 (29.0)	22.0 (17.0)	0.186
	Monotherapy	21.0 (106.0)	22.5 (106.0)	18.0 (22.0)	22.0 (7.0)	22.0 (14.0)	0.167
Albumin, g/l Mean (SD)	All protocols	36.2 (4.3)	35.3 (4.2)	38.2 (4.4)	37.9 (3.1)	34.5 (4.9)	0.016
	Monotherapy	35.5 (4.3)	34.4 (4.3)	38.3 (3.4)	37.3 (3.6)	33.6 (5.1)	0.093
Bilirubin, µmol/l Median (IQR)	All protocols	10.0 (24.0)	10.0 (23.0)	12.0 (24.0)	9.0 (17.0)	9.0 (6.0)	0.334
	Monotherapy	9.0 (24.0)	8.0 (21.0)	10.0 (24.0)	6.0 (11.0)	9.0 (5.0)	0.678
Creatinine, µmol/l Median (IQR)	All protocols	65.0 (118.0)	59.0 (46.0)	72.0 (28.0)	86.0 (41.0)	105.0 (61.0)	<0.001
	Monotherapy	65.0 (115.0)	58.0 (40.0)	69.0 (28.0)	87.0 (12.0)	105.0 (59.0)	<0.001
GFR, ml/min,1,73m²	All protocols*	92.0 (111.0)	100.5 (58.0)	87.0 (8.0)	74.0 (20.0)	53.0 (22.0)	<0.001
	Monotherapy**	93.8 (19.6)	103.9 (13.9)	85.6 (3.2)	74.7 (7.6)	50.3 (11.7)	<0.001
Neutrophil count Median (IQR)	All protocols	7.7 (20.7)	7.7 (19.4)	8.2 (20.7)	5.4 (13.7)	6.3 (5.0)	0.653
	Monotherapy	4.5 (20.6)	4.6 (15.6)	9.2 (20.6)	2.0 (4.2)	4.5 (5.0)	0.349
Platelet count Mean (SD)	All protocols	251.5 (113.3)	249.6 (112.4)	277.5 (123.1)	236.8 (118.3)	200.5 (41.3)	0.559
	Monotherapy	250.1 (133.6)	234.1 (131.3)	291.1 (143.7)	318.6 (184.7)	197.7 (50.1)	0.495

eGFR= estimated glomerular filtration rate, ALAT= Alanine-Amino-Transferase, ASAT = Aspartate-Amino-Transferase, IQR= interquartile range, SD= standard deviation

a. The p-value describes any difference between the eGFR groups ≥ 90, 81-89, 60-80 and 30-59 ml/min
The p value for gender is based on the Pearson Chi-Square test. The Kruskall Wallis test is used to calculated the p values for age, ALAT, ASAT, bilirubin, creatinine, GFR (all protocols) and neutrophil count. The p value for weight, length, BMI, BSA, GFR(monotherapy) and platelet count are based on the one-way-ANOVA.
***Median(IQR)**
**** Mean (SD)**

Table 1 shows the demographic, laboratory and hematologic baseline characteristics by eGFR group for both the main analysis and the subgroup analysis. Normally distributed variables are presented as the mean and standard deviation, while not normally distributed variables are presented as the median and interquartile range. There was a significant difference in median age between patients with an eGFR 30-59 ml/min and the other eGFR groups (P=0.001). Patients with impaired renal function were older compared to patients with normal renal function. The median age difference between the patients with a kidney function ≥90 ml/min and patients with a kidney function 30-59 ml/min was 11.5

years in the main analysis and 12.5 years in the subgroup analysis. In all groups, there were more man than woman but the distribution was not significantly different between the kidney function groups. The mean albumin value was only in the main analysis significantly different between the four eGFR groups (P=0.016). There were no statistically significant differences in neutrophil and platelet count between the groups in both the main and subgroup analysis. However, the overall median neutrophil count in the monotherapy cohort was lower than the neutrophil count in the cohort with all protocols combined (4.5 vs 7,7).

Main analysis – all protocols

Hematologic toxicity

Grade 3 or 4 adverse events

Table 2 shows the prevalence of grade 3 or 4 adverse events for both neutropenia and thrombocytopenia. The prevalence of grade 3 or 4 neutropenia in the reference group (eGFR ≥ 90 ml/min), group 1 (eGFR 81-89 ml/min) and group 2 (eGFR 60-80 ml/min**)** were respectively 25.0%, 15.8% and 20.0%. The prevalence of grade 3 or 4 thrombocytopenia was 21.1% in the patients with a kidney function ≥ 90 ml/min. In both group 1 and 2, only one participant developed grade 3 or 4 thrombocytopenia. There were no grade 3 or 4 events in patients with a kidney function 30-59 ml/min for both neutropenia and thrombocytopenia.

Table 2. Prevalence of grade 3 or 4 adverse events, all protocols

Neutrophils n=85 Platelets n=94	**eGFR ≥ 90 ml/min (Reference group) n= 52 n= 59**		**eGFR 81-89 ml/min (Group 1) n= 19 n= 19**		**eGFR 60-80 ml/min (Group 2) n= 10 n= 12**		**eGFR (30-59) ml/min (Group 3) n= 4 n= 4**	
Grade 3 or 4 neutropenia	13	25.0%	3	15.8%	2	20.0%	0	0.0%
Grade 3 or 4 thrombocytopenia	11	21.2%	1	5.3%	1	10.0%	0	0.0%

The results showed no statistically significant differences in the prevalence of events between the different groups (neutropenia p= 0.600, thrombocytopenia p= 0.353). According to the multivariate logistic regression, kidney function was not associated with the occurrence of grade 3 or 4 neutropenia and thrombocytopenia. No confounders were included in this analysis. Results of the logistic regression with kidney function as a continuous parameter are shown in table 1 in the appendix. Group 3 was excluded because no events had occurred.

Difference in neutrophil and platelet counts (delta value)

The linear regression analysis evaluating the relation between kidney function at start of the therapy and differences in neutrophil and platelet counts between baseline and nadir within 14 days included 97 individuals. The results are shown in table 3. The patient group with a kidney function 30-59 ml/min had an increase of 2.95 in neutrophil count and an increase of 40.11 in platelet count compared to the mean difference of the patient group with a kidney function ≥ 90 ml/min. The mean difference in neutrophil count for the group ≥ 90 ml/min was -6.01 and the mean difference in platelet count was -85.62. This shows that there is an increase in neutrophil and platelet counts in patients with a kidney function 30-59 ml/min. However, this increase was not statistically significant. Comparing group 1 and 2 with the reference group also showed no significant differences in the delta neutrophil and platelet counts. Age was included as a confounder only in the analysis examining the delta neutrophil outcome.

Table 3: linear regression outcomes, all protocols

Kidney function	**Mean difference between baseline neutrophil count and nadir (delta value) ± SD**	**Mean difference between baseline platelet count and nadir (delta value) ± SD**

≥ 90 ml/min	-6.01 ± 5.40		-85.62 ± 26.24	
Kidney function	**Difference in the mean delta neutrophil count compared to the reference group (≥90 ml/min)** ± SD**	**p-value***	**Difference in the mean delta platelet count compared to the reference group (≥90 ml/min)** ± SD**	**p-value***
81-89 ml/min (group 1)	-0.44 ± 2.66	0.742	-15.67 ± 52.81	0.557
60-80 ml/min (group 2)	-0.07 ± 3.19	0.964	36.91 ± 64.73	0.260
30-59 ml/min (group 3)	2.95 ± 5.28	0.270	40.11 ± 105.79	0.453
* The p-value describes the difference in delta values of the kidney function groups 81-89, 60-80 or 30-59 ml/min compared to the mean delta value of the reference eGFR group (≥90 ml/min) ** The increase/decrease in platelet or neutrophil count compared to the mean delta value of the reference eGFR group (≥ 90 ml/min)				

Time until hematologic toxicity

The overall median time until the lowest neutrophil count was 8 days. There was no significant difference in the median time between the different eGFR groups (p = 0.317). The overall median time until the lowest platelet count was 9 days. The median time until the lowest platelet count was also not significantly different between the eGFR groups (p= 0.374). The analysis was performed in all patients with lab results after the first therapy date. Results can be found in table 2 in the appendix.

Nephrotoxicity

Results on the relation between kidney function at start of the therapy and the difference in creatinine value is shown in table 4. Age was included as a covariate in the analysis. The difference in creatinine tends to be higher in patients with lower kidney function at start of the therapy when group 1, 2 and 3 were compared with the reference group. Table 6 shows an increase of 4.09 µmol/l in the creatinine value in patients with a kidney function 81-89 ml/min, an increase of 18.92 µmol/l in patients with a kidney function 60-80 ml/min and an increase of 26.38 µmol/l in patient with a kidney function 30-59, all compared to the mean difference in creatinine value (-7.92 ± 43.48) of the reference group (≥90 ml/min). However, all results were not statistically significant.

Table 4. linear regression nephrotoxicity outcomes, all protocols

Kidney function	**Mean difference between baseline creatinine value and the highest value after baseline ± SD**	
≥ 90 ml/min	-7.92 ± 43.48	
Kidney function	**Difference in the mean creatinine value compared to the reference group (≥90 ml/min)** ± SD**	**p-value***
81-89 ml/min (group 1)	4.09 ± 17.30	0.640
60-80 ml/min (group 2)	18.92 ± 20.35	0.069
30-59 ml/min (group 3)	26.38 ± 31.95	0.105
* The p-value describes the difference in delta creatinine values of the specific kidney function group compared to the reference group (≥90 ml/min) ** The increase/decrease in creatinine value compared to the mean delta value of the reference eGFR group (≥ 90 ml/min)		

Subgroup analysis monotherapy

Neutropenia and thrombocytopenia

Grade 3 or 4 adverse events

The prevalence of grade 3 or 4 adverse events in the different eGFR groups is shown in table 5. The final population included 30 participants for neutropenia and 37 participants for thrombocytopenia. Within this protocol neutropenia grade 3 or 4 did not occur. Grade 3 or 4 thrombocytopenia was seen

in 5 individuals (29.4%) with a kidney function ≥ 90 ml/min. There were no thrombocytopenia grade 3 or 4 events in the other eGFR groups. The relation between kidney function at start of the therapy and grade 3 or 4 hematologic toxicity could therefore not be evaluated.

Table 5. Prevalence of grade 3 or 4 adverse events, monotherapy

Neutrophils n=30 Platelets n=37	eGFR ≥ 90 ml/min (Reference group) n= 17 n= 23		eGFR 81-89 ml/min (Group 1) n= 8 n= 8		eGFR 60-80 ml/min (Group 2) n= 2 n= 3		eGFR (30-59) ml/min (Group 3) n= 3 n= 3	
Grade 3 or 4 neutropenia	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Grade 3 or 4 thrombocytopenia	5	29.4%	0	0.0%	0	0.0%	0	0.0%

Difference in neutrophil and platelet counts (delta value)

The results on the relation between kidney function at start of the therapy and the difference in neutrophil and platelet count are shown in table 6. Patients with a kidney function 81-89 ml/min showed a decrease in neutrophil (-1.30 ± 2.69) and platelet count (-25.27 ± 101.40) compared to the mean difference of the reference group, while the patients with a kidney function 60-80 and 30-59 ml/min both show an increase in neutrophil (60-80 ml/min: 3.20 ± 4,12 / 30-59 ml/min: 2.11 ± 4.15) and platelet count (60-80 ml/min: 34.45 ± 155,30 / 30-59 ml/min: 22.66 ± 156,38) compared to the mean difference of the reference group. However, the difference in neutrophil and platelet count was not statistically significant comparing the 3 kidney function groups with the reference group (≥ 90 ml/min). Age and gender were included as covariates in the analysis. One individual did not have hematologic lab results after the first MTX infusion and was therefore excluded from the analysis.

Table 6: linear regression outcomes, monotherapy

Kidney function	**Mean difference between baseline neutrophil count and nadir (delta value) ± SD**		**Mean difference between baseline platelet count and nadir (delta value) ± SD**	
≥ 90 ml/min	2.81 ± 6,62		-101,42 ± 249,59	
Kidney function	**Difference in the mean delta neutrophil count compared to the reference group (≥90 ml/min)** ± SD**	**p-value***	**Difference in the mean delta platelet count compared to the reference group (≥90 ml/min)** ± SD**	**p-value***
81-89 ml/min (group 1)	-1.30 ± 2.69	0.332	-25.27 ± 101.40	0.616
60-80 ml/min (group 2)	3.20 ± 4.12	0.124	34.45 ± 155.30	0.655
30-59 ml/min (group 3)	2.11 ± 4.15	0.309	22.66 ± 156.38	0.770

* The p-value describes the difference in delta values of the kidney function groups 81-89, 60-80 or 30-59 ml/min compared to the mean delta value of the reference eGFR group (≥90 ml/min)
** The increase/decrease in platelet or neutrophil count compared to the mean delta value of the reference eGFR group (≥ 90 ml/min)

Time until the lowest neutrophil or platelet value

There was no statistically significant difference in time in days until the lowest neutrophil or platelet value was reached between the four baseline eGFR groups (p = 0.294 and p = 0.167). Results can be found in table 3 in the appendix. The overall median time until the lowest neutrophil count was 5 days, while the overall median time until the lowest platelet count was 8 days.

Nephrotoxicity

All 41 individuals were included in the subgroup analysis to evaluate nephrotoxicity of MTX monotherapy. There were no significant differences in the delta creatinine values comparing eGFR group 1 (81-89 ml/min), 2 (60-80 ml/min) and 3 (30-59 ml/min) with the mean difference (-2.58 ± 51.01) of the reference group (≥ 90 ml/min) when adjusted for gender and age. The results can be found in table 7.

Table 7. linear regression nephrotoxicity outcomes, monotherapy		
Kidney function	**Mean difference between baseline creatinine value and the highest value after baseline ± SD**	
≥ 90 ml/min	-2.58 ± 51.01	
Kidney function	**Difference in the mean creatinine value compared to the reference group (≥90 ml/min)** ± SD**	**p-value***
81-89 ml/min (group 1)	14.78 ± 21.14	0.165
60-80 ml/min (group 2)	-2.32 ± 32.37	0.885
30-59 ml/min (group 3)	-6.45 ± 32.62	0.691
* The p-value describes the difference in delta creatinine values of the specific kidney function group compared to the reference group (≥90 ml/min) ** The increase/decrease in creatinine value compared to the mean delta value of the reference eGFR group (≥ 90 ml/min)		

Discussion

Literature has shown that HD-MTX is associated with hematologic toxicity. [1,2,3,11] It was expected that impaired kidney function at baseline would cause more hematologic toxicity. However, in this study it seems that hematologic toxicity is independent of kidney function at start of the therapy in patients receiving HD-MTX. Both the prevalence of grade 3 or 4 hematotoxicy and the difference in neutrophil and platelet counts were not significantly different between the eGFR groups compared to the reference group (≥ 90 ml/min). The influence of impaired kidney function on the hematologic toxicity of HD-MTX can be examined best in patients who were administered HD-MTX according to the 3 g/m^2 monotherapy protocol because they do not have concomitant use of other possible hematologic and nephrotoxic chemotherapeutics. However, a disadvantage of this subgroup analysis is that the study population became even smaller (n=41). The results on the monotherapy protocol also showed that there was no relation between baseline kidney function and the difference in platelet and neutrophil count between start of the therapy and nadir within 13 days. The relation between kidney function and the prevalence of grade 3 or 4 adverse events could not be evaluated within the monotherapy protocol because no grade 3 or 4 neutropenia events had occurred and because thrombocytopenia events only occurred in the reference group (≥ 90 ml/min). For both the analysis with all protocols combined and the analysis with only the monotherapy protocol, there was no significant difference in the time until the lowest neutrophil or platelet value. A decrease in neutrophil and platelet count was expected in patients with lower renal function at baseline. However, it could be possible that the patients in our study with impaired renal function at baseline did not have delayed clearance of HD-MTX and that this is the reason why we saw no relation between kidney function at start of the therapy and hematologic toxicity. Besides that, this study only investigated the effect of the first MTX dose on the renal and hematologic toxicity. The results in the present study show that the chance of developing toxicity after one dose is not higher in patients with impaired renal function compared to patient with healthy renal function. However, there is a possibility that toxicity will arise after several MTX infusions. The length of the therapy and the frequencies of MTX infusions were different between the four protocols. We therefore made the decision to only look at the effect of the MTX first dose, which was the equal component in all protocols. The small number of patients in each eGFR group may also have contributed to the non-significant results.

The difference in the creatinine value between the first therapy date and the highest value after baseline was chosen as the outcome for nephrotoxicy because an increase in creatinine is directly related to deterioration of the kidney function. Besides that, Tiwari et al. showed that the difference in creatinine values could effectively predict hematological toxicities. [18] Based on previous literature, we would expect a relation between kidney function at start of the therapy and the occurrence of nephrotoxicity. [29] The results of this study showed that the occurrence of nephrotoxicity seems independent of the kidney function at baseline in patients receiving HD-MTX. However, in the analysis with all four protocols, although not significant, we did see a trend towards a higher chance in

developing nephrotoxicity in patients with lower renal function at start of the therapy. The results on the monotherapy protocol can fully be attributed to HD-MTX because there is no interference of other cytotoxic comedication. This indicates that the trend seen in the main analysis is caused by other co-medication used in that protocol. However, it is hard to draw firm conclusions on this because the number of patients was low in the different eGFR groups. We also had no information about the use of other co-medication. This unknown co-medication could also have had an effect on the kidney function. However, if co-medication has an effect on the outcome you would already expect an abnormal creatinine value at baseline. We corrected for this by looking at the differences between the creatinine value at start of the therapy and the highest creatinine value after baseline. The prevalence of grade 1 up to grade 4 kidney impairment based on the CTCAE was not included as an outcome because most patients with an event at the lowest kidney function already had grade 1, 2 or 3 impaired renal function at baseline. We recommend investigation of the worsening of these grades in a future study.

Literature has shown that delayed clearance of MTX due to renal impairment is associated with the occurrence of myelosuppression and nephrotoxicity. [6,10,11,14] Multiple articles recommend reducing the MTX dose in patients with renal impairment. [17,19,20,21,22,23,24,25] However, these recommendations are only based on pharmacokinetic parameters and also differ between the studies. Combining all literature, a dose reduction of HD-MTX below an eGFR of 80 ml/min seemed reasonable. Furthermore, the majority of the available literature recommended avoiding HD-MTX in patients with a kidney function below 30 ml/min [23,24,25]. The eGFR groups in this study were chosen based on these conclusions. The outcomes of this study for both hematologic toxicity and nephrotoxicity do not support the advice found in literature to give dose reductions in patients with impaired kidney function at start of the therapy.[17,19,20,21,22,23,24,25] We couldn't compare our results to the recommendation in literature to avoid HD-MTX in patients with a kidney function < 30 ml/min because there wasn't any participant who fell in this kidney function range at baseline.

As already mentioned, the dataset contained very few individuals with low kidney function at baseline. This caused an unequal distribution of participants among the eGFR groups. Besides that, the overall patient population was small in both the analysis with all protocols and the analysis which only included the monotherapy protocol (n=103, n=41). The results in this study should thus be interpreted with caution. A second limitation is that, because of the retrospective design, there was missing data about leukocyte count as hematological outcome and occurrence of adverse events that cannot be expressed in one of the lab parameters. Mucositis is one of such known toxicities in HD-MTX which could not be included as outcome. Also the use of hematotoxic or nephrotoxic co-medication and other factors around the first MTX dose with a potential effect on the outcome were unknown in the dataset. [11,30] This was partly due to the use of a pseudonymized, retrospective study design. The last limitation is that plasma levels of MTX were not included in this study. Plasma levels of MTX are determined every 12-24 hours, up to 72 hours post-infusion and provide insight into potentially prolonged exposure in patients with renal impairment. [31] We recommend to include MTX plasma concentrations in future research to examine MTX clearance and renal toxicity in patients with impaired kidney function at start of the therapy. Furthermore, when interpreting the results it is important to consider that patients with abnormal neutrophil counts, platelet counts or creatinine values at baseline were taken into account for the analysis on the differences of these parameters (delta values). Worsening of these abnormal parameters after a HD-MTX infusion cannot be attributed to HD-MTX alone because there might have been other factors, co-medication for example, that attributed to this abnormal value at baseline and that still have an influence on the outcome.
Another strength is that this is the first study to look at the effect of renal impairment on the hematologic and renal toxicity of HD-MTX. Therefore, this study provides more insight whether there is indeed more change of developing hematotoxicity and nephrotoxicity in patients with impaired kidney function at start of the therapy

In the current study, we only looked separately at patients using the MTX 3 g/m^2 monotherapy protocol. We recommend performing the different analysis separately for the other three protocols included in this study. This could not be examined in this study due to an insufficient number of patients in a 10 years period of time. We would also recommend examining the influence of kidney function as a continuous variable on hematologic toxicity and nephrotoxicity in future research. Another interesting end point for future research is the increase in creatinine values within a patient described as percentages. A creatinine value of 80 µmol/l indicates a good kidney function. However, when this patient started with a creatinine value of 40 µmol/l, there is a still 50% decrease in kidney function within this patient. This endpoint will provide better information about nephrotoxicity. These recommendation should be combined in a prospective study with more complete data and inclusion of more participants to validate the results of our study.

In conclusion, hematologic toxicity seems independent of kidney function at start of the therapy. The results on nephrotoxicity were not significant but show a trend towards more nephrotoxicity in patients with lower renal function at baseline in the main analysis. The results do not support the advice of dose reductions in patients with impaired kidney function found in literature. However, the conclusions should be taken with caution because the results were not significant and because the study was performed in a small patient population. Prospective research in a larger patient population needs to be performed to validate the conclusions of this study.

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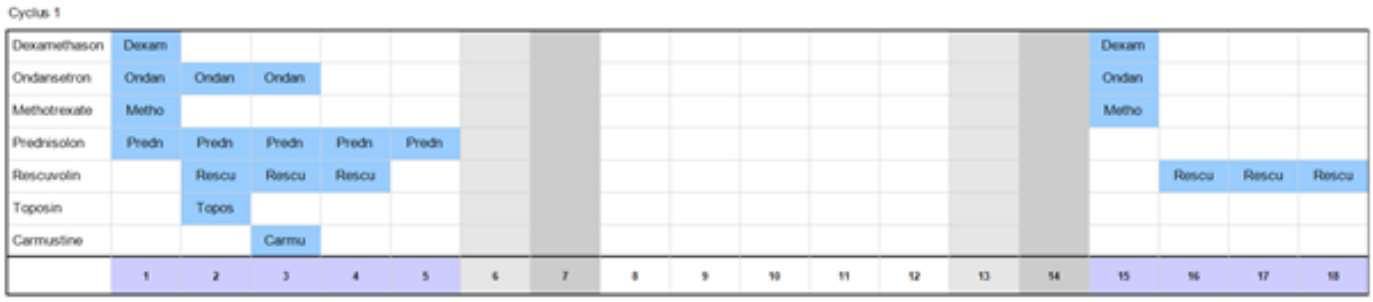
Appendix

MTX monotherapy 3 g/m^2 protocol

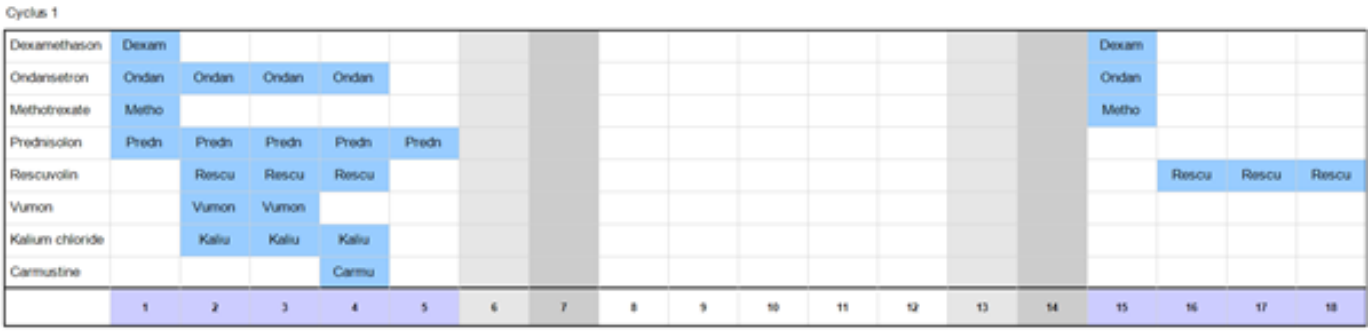
Cyclus 1

Dexamethason	Dexam						
Ondansetron	Ondan						
Methotrexate	Metho						
Rescuvolin		Rescu	Rescu				
	1	2	3	4	5	6	7

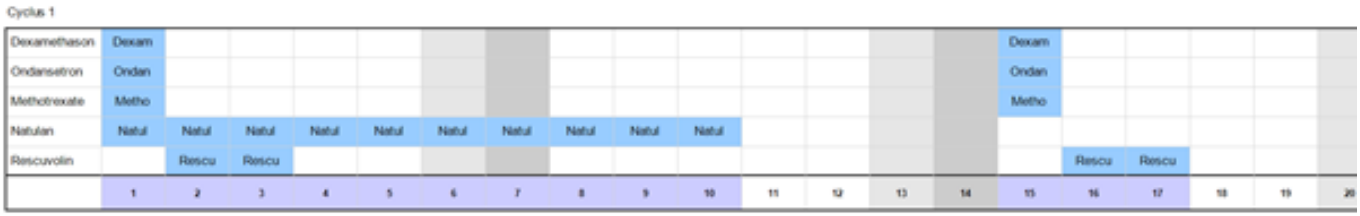
MBTP protocol (MTX 3 g/m^2)



MBVP protocol (MTX 3 g/m^2)



MTX 3 g/m^2 with procarbazine 60 mg/m^2



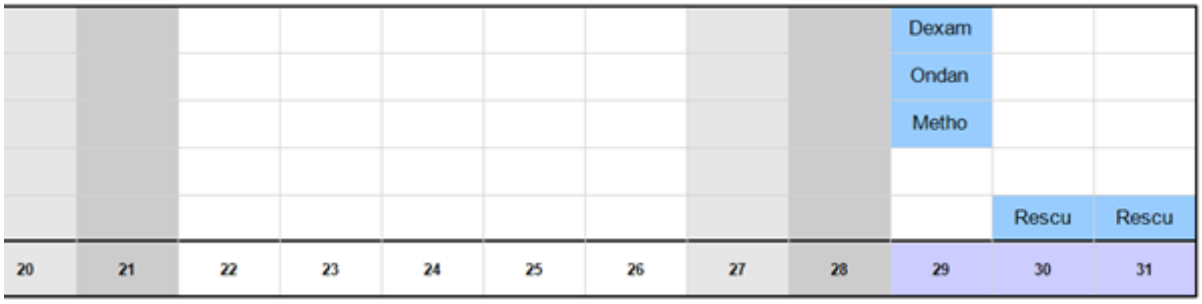


Figure 1: *Description of HD-MTX and co-medication therapy days of one cycle of the included protocols.*

Table 1. Logistic regression outcomes with kidney function as continuous variable, all protocols				
	Odds	**95.0 % CI for Exp(B)** Lower	Upper	**p-value**
Grade 3 or 4 neutropenia	1.021	0.985	1.059	0.259
Grade 3 or 4 thrombocytopenia	1.037	0.998	1.077	0.061

Table 2. Time difference between the 4 eGFR groups, all protocols				
eGFR group	Median time until lowest neutrophil count (IQR)	p-value*	Median time until lowest platelet count (IQR)	p-value*
Overall	8.0 (12.0)	0.317	9.0 (12.0)	0.374
≥ 90	9.0 (12.0)		8.0 (12.0)	
81-89	6.5 (12.0)		8.5 (12.0)	
60-80	6.5 (11.0)		9.0 (11.0)	
30-59	3.5 (9.0)		2.0 (7.0)	
*The p-value describes any difference in median neutrophil or platelet count between the eGFR groups				

Table 3. Time difference between the 4 eGFR groups, monotherapy				
eGFR group	Median time until lowest neutrophil count (IQR)	p-value*	Median time until lowest platelet count (IQR)	p-value*
Overall	5.0 (12.0)	0.294	8.0 (12.0)	0.167
≥ 90	6.0 (12.0)		9.0 (12.0)	
81-89	3.0 (11.0)		8.0 (12.0)	
60-80	6.0 (6.0)		6.0 (6.0)	
30-59	2.0 (5.0)		2.0 (0.0)	
*The p-value describes any difference in median neutrophil or platelet count between the eGFR groups				