

Risk Factors for Delayed Elimination of Methotrexate in Children, Adolescents and Young Adults With Osteosarcoma

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Abstract. *Background/Aim:* High-dose methotrexate (HD-MTX) is pivotal chemotherapy in the treatment of patients with osteosarcoma. Blood concentrations of MTX are associated with several side effects, but there are large individual differences in the elimination of MTX. The aim of this study was to explore risk factors for delayed elimination of MTX in children, adolescents and young adults with osteosarcoma. *Patients and Methods:* We conducted a retrospective study on Japanese patients with osteosarcoma who were treated with HD-MTX at Kanazawa University Hospital from April 2006 to March 2015. Risk factors for delayed elimination of methotrexate were identified by multiple logistic regression analysis. *Results:* A total of 92 cycles of HD-MTX therapy were analyzed. Female and lower creatinine clearance (CCr) were identified as independent risk factors for delayed elimination of MTX. *Conclusion:* Knowing the factors associated with delayed elimination of MTX could lead to safer and optimized chemotherapy for patients with osteosarcoma.

Osteosarcoma is the most commonly diagnosed primary bone tumor in children and adolescents (1). Half of patients with osteosarcoma are diagnosed by the age of 20, and the peak of occurrence is 11-20 years (2). Treatment for osteosarcoma consists of surgery and systemic chemotherapy for prevention and treatment of distant metastasis. Due to advances in treatment combined with systemic chemotherapy and surgical resection, the cure rate for osteosarcoma

exceeds 70% (3, 4). Anti-tumor drugs such as cisplatin (CDDP), doxorubicin (DXR), and methotrexate (MTX) are used in the treatment of osteosarcoma. Among them, high-dose MTX (HD-MTX) therapy is considered essential for adequate treatment of osteosarcoma (5).

In HD-MTX therapy, monitoring serum concentrations of MTX is indispensable because delayed elimination of MTX (DEM) leads to an increased incidence of side effects such as severe myelosuppression, renal dysfunction, liver dysfunction, oral stomatitis (6). To promote excretion of MTX, appropriate regimen management is important such as infusing sufficient hydration with urine alkalization and avoiding concomitant use of drugs that may inhibit the excretion of MTX (6). Nevertheless, there is a large individual difference of more than 10 times in the concentration of MTX during the process of elimination (7, 8). Identifying factors associated with DEM will allow implementation of early measures to avoid adverse effects. In addition, it has been reported that higher MTX concentrations are associated with poor outcome in children and young adults with osteosarcoma (9). Therefore, it is extremely important to identify factors associated with DEM to provide safe and more effective chemotherapy.

There are few studies that focused on factors associated with delayed elimination of HD-MTX in children, adolescents and young adults with osteosarcoma. Fukuhara *et al.* have reported that creatinine clearance (CCr) and the MTX dose were the most significant factors that affected clearance of MTX in adult patients with malignancies (10). However, in that study, only 23 adult patients with osteosarcoma in 51 malignancies were included. Furthermore, it is not appropriate to apply results obtained from adult patients to younger patients, because it has been reported that DEM is more likely to occur in adults with osteosarcoma compared with children with osteosarcoma when HD-MTX therapy is used (11). Moreover, detailed information of DEM has not been fully elucidated in children, adolescents and young adults with osteosarcoma.

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Therefore, we performed an exploratory study of children, adolescents and young adults to clarify factors associated with DEM in patients with osteosarcoma treated with HD-MTX therapy at Kanazawa University Hospital.

Patients and Methods

Data collection. We retrospectively analyzed the medical records of children, adolescents and young adults with osteosarcoma (under 40 years old) who were treated with HD-MTX at Kanazawa University Hospital from April 2006 to March 2015. The following baseline data were collected: age, gender, height, body weight, body mass index (BMI), MTX dose, the presence of metastases, previous history of chemotherapy, serum creatinine (sCr), urine creatinine (uCr), alanine aminotransferase, aspartate aminotransferase, total bilirubin, blood urea nitrogen (BUN), albumin, urine N-acetyl- β -D-glucosaminidase, brain natriuretic peptide, and left ventricular ejection fraction. CCr, as a measure of renal function, was calculated as follows: CCr (ml/min)=uCr (mg/dl) \times urine volume (ml/day)/sCr (mg/dl)/24/60. We analyzed the data collected from the first to the third course of HD-MTX therapy in each patient. We excluded patients who underwent dialysis due to acute renal failure during chemotherapy, or for whom any of the clinical data required for this study were missing.

Chemotherapy. The standard dose of MTX was 12 g/m² in patients younger than 10 years, 10 g/m² in those aged 10-19 years, and 8 g/m² in those aged 20 and older. The dose of MTX was infused over 5 h after 1.5 mg/m² (max 2 mg) vincristine was infused. Leucovorin rescue was started 12 h after the end of MTX infusion. The standard dose of leucovorin was 15 mg every 3 h and increased according to the serum concentration of MTX at 24, 48, 72 h after starting MTX infusion; thereafter, from 72 h after the end of MTX infusion, 15 mg every 6 h was used until the concentration of MTX fell to 0.1 μ mol/l. The serum concentrations of MTX were measured by the TDx/FLx analyzer (Abbott Diagnostics, Chicago, IL, USA). The patients were hydrated using sodium bicarbonate to keep the urine pH >7, and acetazolamide to obtain sufficient urine flow during treatment.

Study protocol. We defined patients with median or higher concentrations of MTX at 24 h after start of MTX infusion (C24) as the DEM (+) group. To identify risk factors associated with DEM, univariate analysis and multivariate logistic regression analysis were performed. Based on multivariate logistic regression analysis, we also examined the relationship between the number of risk factors and the incidence of DEM.

Statistical analyses. Univariate analysis was performed using Mann-Whitney *U*-test or Pearson's chi-square test. Factors with a *p*-value<0.05 in the univariate analysis were evaluated for correlation with each other to avoid multicollinearity. Each correlation diagram was created using the R software version 3.5.1 (The R Foundation for Statistical Computing, Vienna, Austria). When there was a strong correlation (Spearman correlation coefficient: ρ >0.7) between them, one factor was selected based on clinical importance. For these factors, multivariate logistic regression analysis was performed to detect independent risk factors for DEM. Cutoff values for continuous variables were determined by receiver operating characteristic (ROC) curve analysis. All statistical analyses were

Table I. Demographic and clinical characteristics of patients.

Characteristic	Data (total cycles=92)
Gender (male/female)	43/49
Age (yr)	14 (6-38)
Height (cm)	157 (116-180)
Body weight (kg)	39.5 (19.2-87.1)
Body surface area (m ²)	1.33 (0.81-2.02)
Body mass index (kg/m ²)	16.6 (11.4-28.6)
Metastases (+/-)	28/64
Methotrexate dose (g)	11.6 (5.2-18.4)
Prior methotrexate ^a (g/m ²)	9.2 (0-25.1)
Prior cisplatin ^a (mg/m ²)	619.8 (0-1038.7)
Prior doxorubicin ^a (mg/m ²)	296.6 (0-490.8)
Prior ifosfamide ^a (g/m ²)	9.0 (0-54.0)
Serum creatinine (mg/dl)	0.66 (0.25-1.66)
Urine creatinine (mg/dl)	51 (12-144)
Creatinine clearance (ml/min)	73.5 (22.5-199.5)
Aspartate aminotransferase (U/l)	20 (11-233)
Alanine aminotransferase (U/l)	23 (4-137)
Total bilirubin (mg/dl)	0.4 (0.2-1.1)
Blood urine nitrogen (mg/dl)	15 (5-56)
Albumin (g/dl)	3.9 (3.0-7.0)
Urine N-acetyl- β -D-glucosaminidase (U/l)	6.7 (1.0-17.6)
Brain natriuretic peptide (pg/ml)	9.6 (4.0-59.3)
Ejection fraction (%)	62 (47-80)

Data are expressed as median (range) unless otherwise noted.
^aCumulative dose.

performed using IBM® SPSS® Version 24.0 (IBM Japan Ltd., Tokyo, Japan).

Ethics statement. This study was conducted in accordance with the Declaration of Helsinki and ethical principles for clinical research and the protocol was approved by the Ethics Committee at Kanazawa University (approval no.: 1752-1).

Results

Patient characteristics. We extracted data from 45 patients who received a total of 92 cycles of HD-MTX therapy. There were 23 males and 22 females with a median age of 15.0 years (quartiles 1-3, 12.0-19.0 years). Table I shows baseline data before HD-MTX therapy. Most patients had been treated with combination therapy with CDDP and DXR before MTX therapy. No patient had used non-steroidal anti-inflammatory drugs, or had edema or pleural effusion, each of which can cause DEM (12-14).

Univariate and multivariate analyses of risk factors for DEM. In the 92 cycles of HD-MTX therapy, the median C24 was 42.2 μ M (range=1.42-193.00 μ M). Thus, we defined patients with a C24 >42.2 μ M as the DEM (+) group.

Table II shows comparisons of patient characteristics between the DEM (+) group and DEM (-) group. Body weight, body surface area, and BMI were similar in both groups. The

Table II. Comparison of characteristics between subjects with (+) and without (–) delayed elimination of methotrexate.

Characteristics	Delayed elimination (–) n=46	Delayed elimination (+) n=46	p-Value
Gender (male/female)	29/17	14/32	0.002 ^{b*}
Age (yr)	14 (6-38)	14 (6-30)	0.589 ^c
Height (cm)	159 (115-180)	157 (120-176)	0.227 ^c
Body weight (kg)	39.1 (20.9-87.1)	40.1 (19.2-74.6)	0.767 ^c
Body surface area (m ²)	1.35 (0.82-2.02)	1.33 (0.83-1.89)	0.507 ^c
Body mass index (kg/m ²)	15.6 (12.6-28.6)	17.3 (11.4-27.6)	0.353 ^c
Metastases (+/–)	12/34	16/30	0.365 ^b
Methotrexate dose (g)	12.5 (7.4-17.7)	11.1 (5.2-18.4)	0.015 ^{c*}
Prior methotrexate ^a (g/m ²)	8.0 (0-23.0)	9.5 (0-25.1)	0.373 ^c
Prior cisplatin ^a (mg/m ²)	513.2 (0-1038.7)	712.1 (340.1-1006.9)	<0.001 ^{c*}
Prior doxorubicin ^a (mg/m ²)	256.0 (0-490.8)	328.1 (167.9-461.8)	<0.001 ^{c*}
Prior ifosfamide ^a (g/m ²)	16.7 (0-45.0)	7.5 (0-54.0)	0.105 ^c
Serum creatinine (mg/dl)	0.60 (0.25-1.45)	0.74 (0.55-1.04)	0.003 ^{c*}
Urine creatinine (mg/dl)	53 (18-144)	45 (12-142)	0.116 ^c
Creatinine clearance (ml/min)	90.3 (40.7-199.5)	51.2 (22.5-129.2)	<0.001 ^{c*}
Aspartate aminotransferase (U/l)	21 (12-233)	20 (11-60)	0.678 ^c
Alanine aminotransferase (U/l)	24 (5-137)	21 (4-119)	0.656 ^c
Total bilirubin (mg/dl)	0.4 (0.2-0.8)	0.4 (0.2-1.1)	0.782 ^c
Blood urine nitrogen (mg/dl)	13 (7-28)	17 (5-56)	0.001 ^{c*}
Albumin (g/dl)	3.8 (3.0-4.9)	4.0 (3.3-7.0)	0.169 ^c
Urine N-acetyl-β-D-glucosaminidase (U/l)	6.4 (1.0-17.6)	7.1 (2.3-13.0)	0.554 ^c
Brain natriuretic peptide (pg/ml)	8.9 (4.0-59.3)	9.8 (4.0-59.3)	0.213 ^c
Ejection fraction (%)	64 (47-80)	61 (50-76)	0.110 ^c

Data are expressed as median (range) unless otherwise noted. ^aCumulative dose; ^bPearson's chi-squared test; ^cMann-Whitney *U*-test; *Statistically significant.

proportion of female patients was significantly higher in the DEM (+) group compared with the DEM (–) group (69.6% and 37.0%, respectively). MTX dose in the DEM (+) group was significantly lower than that in the DEM (–) group. Doses of CDDP and DXR as prior chemotherapy were significantly higher in the DEM (+) group than in the DEM (–) group.

SCr and BUN of patients in the DEM (+) group were significantly higher than in DEM (–) group. CCr of the DEM (+) group was 51.2 ml/min compared with 90.3 ml/min in the DEM (–) group ($p < 0.001$).

In univariate analysis, significant differences were observed between groups in regards to gender, the dose of MTX, the doses of CDDP and DXR used for prior chemotherapy, sCr, BUN, and CCr. We examined the correlation between these seven potential factors to avoid multicollinearity (Figure 1). The cumulative doses of CDDP and DXR were strongly correlated with each other ($\rho = 0.914$, $p < 0.001$). We selected the dose of CDDP instead of DXR as a possible risk factor of DEM because it was reported that MTX clearance was reduced after CDDP therapy (9, 15). Table III shows the results of the multiple logistic regression analysis with these factors, which indicated that female gender and lower CCr were independent risk factors associated with DEM.

Relationship between number of risk factors and incidence of DEM. Figure 2 shows results of the ROC curve analyses for the CCr cutoff value. $\text{CCr} \leq 73.5$ ml/min was regarded as an independent risk factor for DEM. The incidence of DEM was 22.2% in patients with no risk factors (males with $\text{CCr} > 73.5$ ml/min), 37.1% in those who had one risk factor (males with $\text{CCr} \leq 73.5$ ml/min or females with $\text{CCr} > 73.5$ ml/min), and 90.0% in those who had two risk factors (females with $\text{CCr} \leq 73.5$ ml/min). Patients with two risk factors had a significantly increased incidence of DEM compared with those who had ≤ 1 risk factor (Figure 3).

Discussion

In this study, lower CCr and female gender were shown to be independent risk factors for DEM in children, adolescents and young adults with osteosarcoma. These results indicate that it is necessary to establish a stricter monitoring system and assessment of adverse effects when starting HD-MTX therapy in female patients with osteosarcoma with decreased renal function.

Multiple logistic regression analysis showed that lower CCr was an independent risk factor for DEM. C24 is likely to be affected by renal function, because 95% of

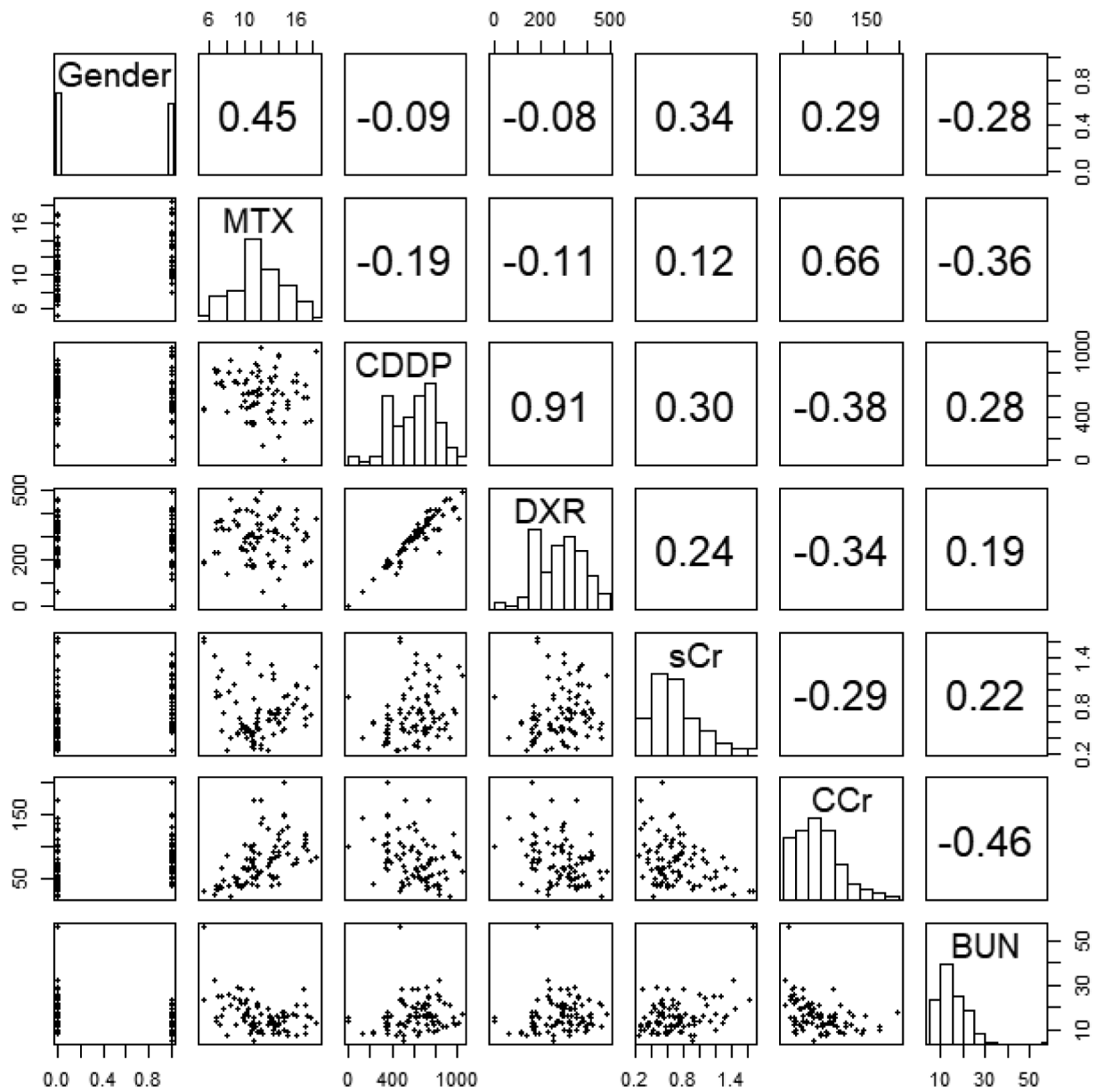


Figure 1. Correlation matrix among some covariates. Gender (0, female; 1, male), MTX: Dose of methotrexate (g), CDDP: cumulative dose of cisplatin (mg/m^2), DXR: cumulative dose of doxorubicin (mg/m^2), sCr: serum creatine (mg/dl), CCr: measured creatinine clearance (ml/min), BUN: blood urea nitrogen (mg/dl). Upper panels indicate Spearman's rank correlation coefficient between each variable. Diagonal panels indicate histograms of each covariates.

MTX is excreted into the urine during the first 30 h after initiation of infusion in children with malignant tumors (16). In fact, several reports have shown that the decrease in CCr leads to the delayed elimination of MTX in adult patients with osteosarcoma (10, 17, 18). Through this study, it was confirmed that CCr is an important marker for DEM in children, adolescents and young adults with osteosarcoma.

In our data, the median CCr was 51.2 ml/min in the DEM (+) group. Because anti-tumor drugs used in chemotherapy for osteosarcoma, such as CDDP and ifosfamide (IFO), are associated with potent nephrotoxicity, cumulative doses of these agents can lead to decreased renal function (19, 20). A study in pediatric patients with osteosarcoma found that the minimum cumulative doses affecting MTX clearance were 200 mg/m^2 for CDDP alone, 32 g/m^2 for IFO alone, and 100

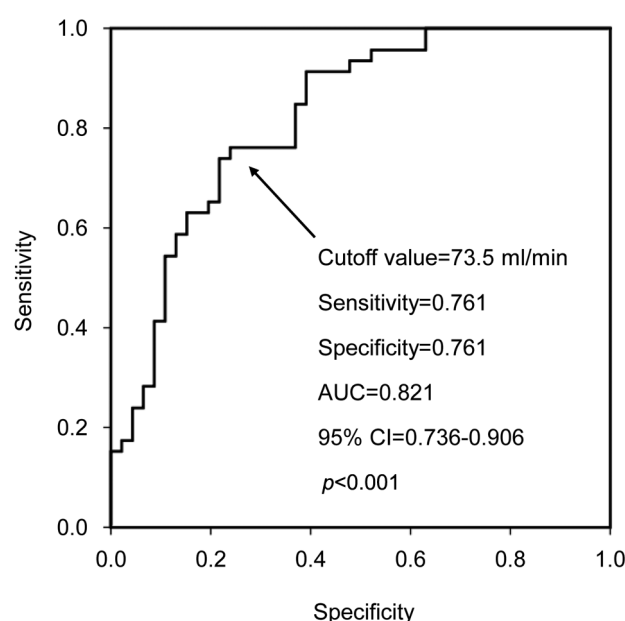


Figure 2. Receiver operating characteristic curve for predicting risk of delayed elimination of methotrexate based on creatinine clearance. AUC: Area under the curve; CI: confidence interval.

Table III. Multivariate analysis.

Characteristics	Adjusted odds ratio	95%CI	p-Value
Gender (female/male)	9.638	2.238-41.516	0.002*
Methotrexate dose (g)	1.258	0.940-1.683	0.122
Prior cisplatin ^a (mg/m ²)	1.002	0.999-1.005	0.139
Serum creatinine (mg/dl)	12.101	0.959-125.653	0.054
Creatinine clearance (ml/min)	0.958	0.928-0.988	0.007*
Blood urine nitrogen (mg/dl)	1.019	0.923-1.108	0.747

Data are expressed as median (range) unless otherwise noted. ^aCumulative dose; *Statistically significant.

mg/m² for CDDP in combination with IFO (9). In our study, these drugs were used before MTX treatment, and the cumulative dose of CDDP was significantly higher in the DEM (+) group than in the DEM (−) group. In addition, a weak negative correlation was seen between the cumulative dose of CDDP and CCr ($\rho = -0.380$, $p < 0.001$). Therefore, a possible reason for the decrease in renal function was the administration of CDDP, which led to DEM. On the other hand, in the results of our logistic analysis, the direct impact of these nephrotoxic drugs was not significant. A possible explanation is that the median dose of the prior CDDP was 619.8 mg/m² in our patients, so most patients were thought to have already received more than 200 mg/m² of CDDP,

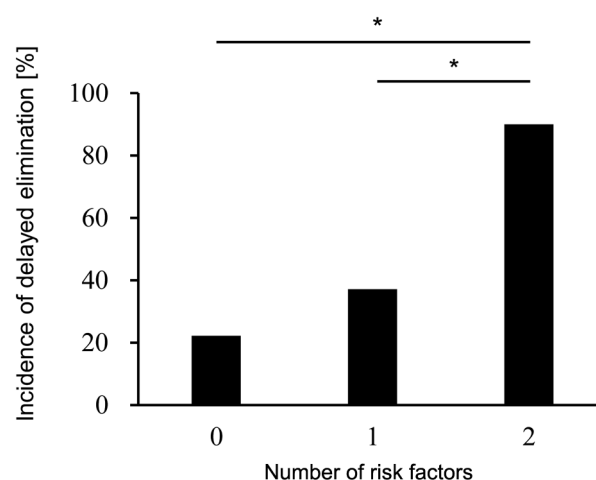


Figure 3. Correlation between incidence of delayed elimination of methotrexate and number of risk factors. Females with creatinine clearance (CCr) > 73.5 or males with CCr ≤ 73.5 patients have one risk factor and females with CCr ≤ 73.5 have two risk factors. * $p < 0.001$ (Pearson's chi-square test).

which reduced MTX clearance (9). Therefore, when treatment with CDDP is repeated prior to HD-MTX in patients with osteosarcoma, it seems that the reduction of CCr is more important than the cumulative dose of anti-tumor drugs in terms of DEM. As a result of ROC analysis, the cut-off value of CCr that indicates a risk for DEM was 73.5 ml/min. This value indicates that even mild renal dysfunction can cause DEM, and this laboratory value must not be ignored.

Fukuhara *et al.* reported that MTX clearance was affected most by CCr and MTX dose (whether or not ≥ 10 g) in adult patients with malignancies (10). In our logistic analysis, the influence of CCr on MTX clearance was consistent, whereas the influence of MTX dose on C24 was not clear. This discrepancy may be due to differences in dose distribution. MTX is administered intravenously at 8–12 g/m² for osteosarcoma, which is higher than the 3–5 g/m² generally used for lymphoblastic leukemia (21, 22). The median dose of MTX in Fukuhara's data was 3 g (10). Because the dosage of MTX differs between osteosarcoma and other diseases, the influence of dose on the blood concentration of MTX needs to be considered.

Our analysis also showed that female gender is an independent risk factor for DEM. Several studies in children and young adults or adults with osteosarcoma have reported that the elimination of MTX is not affected by gender (9, 17, 23). On the other hand, in a study including children and adults, Zhang *et al.* reported that among patients with DEM, which was defined as a concentration of MTX at 24 h of > 5 μ M, no significant difference was observed between males

and females, but the incidence of severe DEM, which was defined as a concentration of MTX at 24 h of $>20 \mu\text{M}$, was significantly higher in female patients than in male patients (24). In our study, DEM was defined as a blood concentration of MTX at 24 h of $>42.2 \mu\text{M}$. Considering these results, female gender may be a factor that causes a severe DEM such that C24 greatly exceeds the general clinical limit value of $10 \mu\text{M}$. In an *in vivo* study, male wildtype mice, but not Oat3 knockouts or females, demonstrated significantly accelerated methotrexate clearance in response to reduced folate levels (25), suggesting that sex differences exist in the transporter related to the excretion of MTX. However, gender differences regarding OAT3 in humans have not been studied.

It has been reported that delayed elimination of MTX is associated with co-administration of proton pump inhibitors (PPIs) (26). In our study, the effect of PPIs on elimination of MTX could not be investigated, because most of the subjects in this study were children and adolescents for whom use of PPIs had not been approved at the time of the survey. Since the use of a PPI in children has been approved, it is necessary to further study the effects of PPIs on DEM in children, adolescents and young adults with osteosarcoma.

The present study has some limitations. First, it is a single-center retrospective survey; thus, multiple biases are possible and no causal effect can be proved. Second, C24 of most patients exceeded the generally defined C24 limit of $10 \mu\text{M}$. The risk at this limit could not be assessed. Still, the identification of risk factors for DEM in children, adolescents and young adults with osteosarcoma is a new finding.

In conclusion, our results indicate that female gender and lower renal function ($\text{CCr} \leq 73.5 \text{ ml/min}$) are risk factors for DEM in children, adolescents and young adults with osteosarcoma. Patients with these risk factors should be monitored closely and may require enhanced hydration, alkalization to excrete MTX, and dosage reduction if necessary. These findings could lead to safer and optimized chemotherapy for patients with osteosarcoma. Future studies are needed to confirm our findings regarding the relationship between gender and elimination of MTX.

Conflicts of Interest

The Authors declare that they have no conflicts of interest in relation to this study.

Authors' Contributions

K. Misaka designed the protocol, carried out the study, and drafted the manuscript. Y. Staub and A. Tsubata collected data from clinical records and analyzed the data. Y. Suga helped to design and carry out the study and draft the manuscript. T. Shimada, Y. Sai, and R. Matsushita coordinated the study and helped to draft the manuscript. All Authors revised the manuscript for intellectual content and approved the final manuscript.

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