Relationship of Serum Methotrexate Concentration in High-Dose Methotrexate Chemotherapy to Prognosis and Tolerability: A Prospective Cohort Study in Chinese Adults With Osteosarcoma

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ABSTRACT

BACKGROUND: Cancer that originates in the bone, termed primary bone cancer, is rare. Osteosarcoma (OS) occurs primarily in growing bone tissue and is more prevalent in children and adolescents. OS in adults is rare, with 3 to 5 cases per million population per year worldwide. There are limited data on treatment-related prognosis and adverse reactions in adults reported in the literature.

OBJECTIVES: The aims of this study were to investigate factors that influence serum methotrexate (MTX) concentrations used in chemotherapy in Chinese adult patients with OS, and to determine the correlations (based on age, sex, and dosage), if any, between MTX and prognosis, in terms of disease-free survival (DFS) and overall survival (OAS), and tolerability.

METHODS: Adult patients aged ≥30 years with OS received ≥3 courses (2 courses before surgery and 3–4 courses postsurgery) of high-dose MTX (6 or 8 g/m²) combined chemotherapy. The regimen consisted of day 1: MTX + folinic acid (herein referred to as citrovorum factor rescue); day 8: cisplatin; days 21 to 25: ifosfamide + mesna; and day 21: doxorubicin. Serum MTX concentrations were assessed immediately after the end of infusion (baseline) and at 24 and 48 hours using high-performance liquid chromatography. Changes in serum MTX concentrations, factors that influence serum MTX concentrations, and the relationship between serum MTX concentrations and prognosis and tolerability (determined by adverse reactions) were analyzed. Patients received a second course of treatment after a 3-week period.

RESULTS: Ninety patients (58 men, 32 women; age range, 30–67 years) with OS were included in the study. A total of 532 courses of combined chemotherapy were administered. The serum MTX concentrations ranged widely at baseline (244.31–929.68 mol/L, Cmin and Cmax, respectively) and at 24 hours (0.73–28.24 mol/L, respectively), suggesting that the serum MTX concentrations varied significantly between different individuals and within the same individual at different time points. The serum MTX concentrations in ~23% of cases (122/532) determined at 24 and/or

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48 hours were numerically higher than the safety values (according to Nirenberg’s reference: irreversible damage if MTX concentration was >10 μmol/L and >1 μmol/L at 24 and 48 hours, respectively). No correlation was found between high serum MTX concentration at baseline and high serum MTX concentration at 24 hours \((r = 0.401)\). The prevalences of the 3 most common adverse reactions in these patients were depressed white blood cell count (44.03%), dental ulcer (23.0%), and rash (18.0%). However, in the remaining 410 courses in which serum MTX concentrations were lower than the safety values, these prevalences were 14.6%, 3.9%, and 2.4%, respectively. Neither age nor sex was significantly associated with MTX \(C_{\text{max}}\), but dosage was \((P < 0.05)\). Patients with a serum MTX \(C_{\text{max}}\) concentration >500 μmol/L at baseline had a significantly longer DFS rate than those with ≤500 μmol/L \((P = 0.040)\). There were no significant between-group differences in the OAS rates.

**Conclusions:** In these Chinese patients with OS, serum MTX concentrations measured at different time points were varied. The findings suggest that adverse reactions occurred in patients whose serum MTX concentrations at 24 and/or 48 hours were higher than the safety values. The dosage appeared to have influenced MTX \(C_{\text{max}}\) while sex and age did not, and the \(C_{\text{max}}\) was significantly related to DFS but not OAS. *(Curr Ther Res Clin Exp. 2009;70:150–160) © 2009 Excerpta Medica Inc.*

**Key words:** osteosarcoma, MTX, serum concentration, prognosis, side effect.

**INTRODUCTION**

Cancer that originates in the bone, termed *primary bone cancer*, is rare. Osteosarcoma (OS), a malignant primary tumor, is more prevalent in children and adolescents (≥75% compared with adults).\(^1\) OS in adults is rare, with 3 to 5 cases per million population per year worldwide.\(^1\) High-dose (HD) combined chemotherapy, first used in the 1970s,\(^1\) has been associated with a significant increase of 20% to 50% in 5-year survival. Because of its utility, HD methotrexate (MTX) (6–8 g/m\(^2\)), which is 100-fold greater than that of routine MTX dose of 30 to 40 mg/m\(^2\) + folinic acid (herein referred to as citrovorum factor rescue [CFR]) in combined chemotherapy for OS has been widely used in clinical practice.\(^1\) The recommended dose is different between adults and adolescents, and the metabolic process of MTX remains unknown.\(^2,3\)

The present study sought to investigate factors that influence serum MTX concentrations used in chemotherapy in Chinese adult patients with OS, and to determine the correlations (based on age, sex, and dosage), if any, between MTX and prognosis, in terms of disease-free survival (DFS) and overall survival (OAS), and tolerability.

**PATIENTS AND METHODS**

**Inclusion Criteria and Clinical Data**

Between July 2003 and December 2007, there were 153 new cases of OS diagnosed at the Affiliated People’s 6th Hospital, Shanghai, People’s Republic of China. Eligible patients met the following inclusion criteria: age ≥30 years; presence of typical radiographic and histologic features of primary, high-grade OS; diagnosis at stage IIB according to the Enneking standard\(^4\); no history of cancer and no prior cancer treatment;
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no coexisting disease contraindicating chemotherapy; and no evidence of metastases at diagnosis. Before the start of chemotherapy, patients who met the inclusion criteria had to: reach functional capacity of ≥60% according to Karnofsky Performance Status criteria; have white blood cell (WBC) count ≥4.0 × 10^9 cells/L; platelet count ≥120 × 10^9 cells/L; and normal hepatic, renal, and cardiac function electrocardiography (ECG) test results.

**Chemotherapy Regimens**

All patients received ≥3 courses (2 courses before surgery and 3–4 courses post-surgery) of HD-MTX (6 or 8 g/m^2) combined chemotherapy. The regimen consisted of day 1: MTX + CFR; day 8: cisplatin (DDP); days 21 to 25: ifosfamide (IFO) + mesna; and day 21: doxorubicin (ADM).

**Serum MTX Concentration Analysis**

Approximately 2 mL of venous blood was collected into a glass tube immediately after the start of infusion (0 hour; baseline) and at 24 and 48 hours at the end of the 6-hour IV HD-MTX infusion. The blood was centrifuged at 3500 rpm for 10 minutes and stored at 20°C until analysis. Serum MTX concentrations were measured using high-performance liquid chromatography fitted with an IC-8A pump (Shimadzu Corporation, Kyoto, Japan). The parameters were set as follows: the chromato bar was a 150 × 460 mm Chromegabond BAS C18 (Shimadzu Corp.); sodium acetate buffer (pH, 3.5); acetonitrile (89:11 [volume ratio]); flow rate (1.5 mL/min); and mobile phase (0.15 mol/L). The mobile phase was ultrafiltrated and degassed by ultrasound. The column temperature was 35°C, the ultraviolet detection wavelength was 303 nm, and the sample particle size was 10 μL. Detailed methods appear elsewhere in the literature. The safety values of serum drug concentration were ≤10 μmol/L and ≤1 μmol/L at 24 and 48 hours, respectively.

**Chemotherapy Regimen and Observation of Adverse Reactions**

A modified Bacci’s adjuvant and neoadjuvant chemotherapy regimen (referred to as IOR [Istituto Ortopedico Rizzoli]/OS-N5) was used in the present study. A panel of senior clinical oncologists determined the dose of MTX administration. On day 1, CFR was administered 12 hours after MTX administration (MTX 6 or 8 g/m^2 [IV infusion over 6 hours]; CF 15 mg [administered intravenously q3h × 8]; and CF 12 mg [administered intramuscularly q6h × 6]). Patients with lower (<100 mL/min) glomerular filtration rate (n = 35) received the lower MTX dose (6 g/m^2).

The frequency of CFR was determined by serum MTX concentration (eg, if the MTX concentration increased 2-fold, the CFR was doubled). On day 8, IV DDP 80 to 100 mg/m^2 was administered. On day 21, a 15-minute ADM 60 mg/m^2 IV infusion was administered. Additionally, on days 21 through 25, IFO 2.0 g/m^2 IV was administered and mesna 400 to 600 mg was intravenously administered at 0, 4, and 8 hours after IFO administration. After a 3-week period, a second course of treatment was started.

During HD-MTX therapy, patients were advised on sufficient water intake, basic electrolyte (potassium, sodium, magnesium, calcium, and chlorine) balance, kidney protec-
tion, and bladder control. Prior to the initiation of chemotherapy, patients underwent routine blood and urinary tests for hepatic and renal function, as well as ECG. These laboratory values and system functions were also monitored daily for 3 consecutive days while patients were undergoing chemotherapy. Adverse reactions were assessed according to the World Health Organization guidelines for measurement during chemotherapy. Serum MTX concentrations outside of safety values, defined as >10 μmol/L at 24 hours or >1 μmol/L at 48 hours, indicated greater susceptibility to adverse reactions.

To examine the relationship between \( C_{\text{max}} \) and DFS, patients were divided into 2 groups (those with MTX \( C_{\text{max}} \leq 500 \) μmol/L and those with MTX \( C_{\text{max}} > 500 \) μmol/L).

**Follow-Up**

Patients underwent a follow-up appointment 2 to 3 weeks after chemotherapy was discontinued. By the end of June 2008, all the patients had received follow-up either on an outpatient basis or by telephone interview. The living status of all included patients was confirmed.

**Statistical Analysis**

The \( t \) test was used for statistical analysis. Mathematical computation was conducted using SPSS version 13.0 (SPSS Inc., Chicago, Illinois). Results are described as mean (SD). Significance level was set at \( P < 0.05 \). OAS was calculated from the first day of chemotherapy until death or last follow-up using the Kaplan-Meier method, and the log-rank test was used for comparison. DFS was calculated based on the date of documented detection of metastasis and/or local recurrence as the standard. The Cox regression model was used for multivariate comparisons (age, sex, and \( C_{\text{max}} \)). Group \( t \) test was used to compare between-group differences.

**RESULTS**

**Analysis of Serum Concentration**

A total of 98 patients were eligible for the study. However, 8 declined to enter the study, leaving 90 patients (58 men, 32 women; age range, 30–67 years). All patients underwent ≥3 courses of HD-MTX (6 or 8 g/m\(^2\)) therapy, totaling 532 courses among all included patients. Overall, the serum MTX concentrations ranged widely at baseline (244.31–929.68 mol/L, \( C_{\text{min}} \) and \( C_{\text{max}} \), respectively) and at 24 hours (0.73–28.24 mol/L, respectively), suggesting that the serum MTX concentrations varied significantly between different individuals and within the same individual at different time points. The present study also found that although the baseline serum MTX concentration in individual patients might have been higher than the mean (ie, >510.14 μmol/L), the concentration at 24 hours in the same patient was not necessarily higher than the mean (ie, >6.11 μmol/L). No correlation was found between high serum MTX concentration at baseline and high serum MTX concentration at 24 hours (\( r = 0.401 \)).

**Correlations Between MTX Concentration and Age, Sex, and Dosage**

Mean serum MTX concentration was obtained from patients (90) who received ≥3 courses of chemotherapy (total, 532). Age and sex were not associated with signifi-
cant MTX concentrations; however, dosage was significantly associated with $C_{\text{max}}$ (Table I). At baseline, mean (SD) MTX concentrations were 418.60 (200.47) and 595.83 (96.35) μmol/L at the 6- and 8-g/m² doses, respectively ($P = 0.02$). At 24 hours, corresponding values were 5.15 (6.25) and 5.36 (4.29) μmol/L ($P = \text{NS}$).

**Disease-Free Survival**

The 1- and 2-year DFS rates among included patients (N = 90) were 85% and 67%, respectively, with the follow-up ranging from 8 to 63 months; median, 25 months; and mean, 31.2 months. In the 2 subgroups (those with $C_{\text{max}} \leq 500$ μmol/L [n = 47] and those with $C_{\text{max}} > 500$ μmol/L [n = 43]) used to assess the relationship between $C_{\text{max}}$ and DFS, the median and mean DFSs in the first group were 27.6 and 21.0 months, respectively, whereas the median and mean DFSs in the second group were 50.0 and 38.4 months ($P = 0.040$) (Figure 1). Among multivariate (age, sex, and $C_{\text{max}}$) comparisons, the present study found that the $C_{\text{max}}$ was an independent prognostic factor ($P = 0.049$).

**Overall Survival**

The 1- and 2-year OAS rates of the patients included in the analysis were 90% and 76%, respectively. In the 2 groups combined, median and mean OASs were 33.0 and 32.7 months in the first group (those with MTX $C_{\text{max}} \leq 500$ μmol/L [n = 47]) and 47.0 and 45.2 months in the second group (those with MTX $C_{\text{max}} > 500$ μmol/L [n = 43]). Although the data found that the OAS rate in the second group was numerically higher than that of the first group, there was no statistical difference between the 2 groups (Figure 2).

**Table I. Correlation between serum methotrexate (MTX) concentration and age, sex, and dose at baseline and 24 hours in Chinese adults undergoing chemotherapy for osteosarcoma. Data are mean (SD).**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>24 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum MTX Concentration, μmol/L</td>
<td>$P$</td>
<td>Serum MTX Concentration, μmol/L</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30–50 (n = 71)</td>
<td>493.59 (91.39)</td>
<td>0.09</td>
<td>4.47 (3.10)</td>
</tr>
<tr>
<td>&gt;50 (n = 19)</td>
<td>532.90 (104.47)</td>
<td></td>
<td>5.60 (4.29)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n = 58)</td>
<td>489.07 (88.42)</td>
<td>0.10</td>
<td>5.79 (3.91)</td>
</tr>
<tr>
<td>Female (n = 32)</td>
<td>549.60 (104.88)</td>
<td></td>
<td>4.65 (4.30)</td>
</tr>
<tr>
<td>Dose, g/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (n = 35)</td>
<td>418.60 (200.47)</td>
<td>0.02</td>
<td>5.15 (6.25)</td>
</tr>
<tr>
<td>8 (n = 55)</td>
<td>595.83 (96.35)</td>
<td></td>
<td>5.36 (4.29)</td>
</tr>
</tbody>
</table>
Among the 90 patients who received ≥3 rounds of HD-MTX (6 or 8 g/m²) chemotherapy, in 122 of 532 cases, the serum MTX concentration was higher than the safety value (>10 μmol/L at 24 hours or >1 μmol/L at 48 hours). As shown in Table II, the patients whose MTX concentrations were higher than the safety value at 24 and/or 48 hours had significantly higher incidences of some adverse reactions than those who had MTX concentrations below the safety value (ie, normal values). However, there were no significant differences between the subgroups with MTX concentrations above or below safety values with regard to the incidences of hepatic impairment and nausea and vomiting (Table II). In 13 of 122 cases, patients experienced severe adverse reactions (bone marrow suppression [11.1%], dental ulcer [5.08%], and rash [4.5%]). In these cases, the MTX concentrations were higher than the safety values at both 24 and 48 hours, requiring additional CFR in these patients. These patients experienced complete recovery within 10 days of the discharge date.

**DISCUSSION**

MTX, an antifolate drug, has been widely used in the treatment of various tumors and immune suppression. It acts as a dihydrofolate reductase (DHFR) inhibitor that...
Figure 2. Analyses in patient subgroups (those with methotrexate [MTX] C\textsubscript{max} \leq 500 μmol/L \[n = 47\] and those with C\textsubscript{max} >500 μmol/L \[n = 43\]) used to assess the relationship between C\textsubscript{max} and overall survival (OAS).

Table II. Correlation between serum methotrexate (MTX) concentration and adverse reactions in Chinese adult patients undergoing chemotherapy for osteosarcoma. Data are no. (%) of patients.

<table>
<thead>
<tr>
<th>Adverse Reactions</th>
<th>Serum MTX Concentration</th>
<th>Serum MTX Concentration Higher Than Safety Values* at 24 and/or 48 h, μmol/L</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Values at 24 and 48 h, μmol/L [n = 410]</td>
<td>Higher Than Safety Values* at 24 and/or 48 h, μmol/L [n = 122]</td>
<td></td>
</tr>
<tr>
<td>Impaired hepatic function</td>
<td>206 (50.2)</td>
<td>72 (59.0)</td>
<td>0.454</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>172 (42.0)</td>
<td>66 (54.1)</td>
<td>0.295</td>
</tr>
<tr>
<td>Depressed white blood cell count</td>
<td>60 (14.6)</td>
<td>54 (44.3)</td>
<td>0.002</td>
</tr>
<tr>
<td>Dental ulcer</td>
<td>16 (3.9)</td>
<td>28 (23.0)</td>
<td>0.005</td>
</tr>
<tr>
<td>Rash</td>
<td>10 (2.4)</td>
<td>22 (18.0)</td>
<td>0.008</td>
</tr>
<tr>
<td>Fever</td>
<td>0</td>
<td>16 (13.1)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Safety values: ≤10 μmol/L at 24 hours and ≤1 μmol/L at 48 hours.\(^7\)
inhibits the metabolism of folic acid, DNA synthesis, and cell growth.\textsuperscript{10} MTX enters cells with the aid of a reduced folate carrier and generates polyglutamate methotrexate (MTXPG) by the catalysis of folylpolyglutamate synthase. Both MTX and MTXPG are competitors of dihydrofolic acid to bind with DHFR. MTXPG has a higher affinity to DHFR and dissociates slowly, making it a better inhibitor of DHFR than MTX.\textsuperscript{10} Eventually, the synthesis of DNA and RNA becomes restricted, causing the proliferating cancer cells to die. The sensitivity of tumor cells to MTX depends on intracellular MTX concentration. An appropriate concentration is crucial to achieve the best effect. Some studies have investigated a dose-response curve between HD-MTX and tumor cell cytotoxicity.\textsuperscript{11,12}

The number of destroyed tumor cells has been found to exponentially increase when a double dose of chemotherapeutic agent is used.\textsuperscript{12} The international standard dose of MTX ranges from 8 g/m\textsuperscript{2} (adults) to 12 g/m\textsuperscript{2} (children).\textsuperscript{12} In this study, 90 Chinese adult patients aged ≥30 years received HD-MTX (6 or 8 g/m\textsuperscript{2}) chemotherapy. The analysis found significant differences between patients, possibly because of variations in MTX renal clearance. It also found variable differences in the same patient at different time points. Bacci et al\textsuperscript{13} conducted a study in 272 patients with OS who received HD-MTX (> 10 g/m\textsuperscript{2}) by IV infusion for 6 consecutive hours. The study found that complete historic MTX response rate in the group with a high mean \(C_{\text{max}}\) (≥700 μmol/L) was significantly higher than that in the group that had a low mean \(C_{\text{max}}\) (<700 μmol/L) (28% and 9.9%, respectively; \(P = 0.001\)), and that the 4-year OAS rate in the group with the higher mean \(C_{\text{max}}\) was significantly higher (88% and 58%; \(P < 0.007\)). Therefore, to obtain a serum \(C_{\text{max}}\) ≥700 μmol/L, an MTX dose adjustment was recommended by those authors. Because the patients involved in the present study were adults aged ≥30 years and the MTX dosage was 6 or 8 g/m\textsuperscript{2}, the serum MTX concentration resulted in a relatively lower mean (SD) \(C_{\text{max}}\) (510.14 [119.43] μmol/L) compared with the concentrations found by Bacci et al.\textsuperscript{13} Therefore, a higher MTX dosage was possibly used in these Chinese adult patients with OS in the effort to receive an enhanced therapeutic effect.

Much is still unknown regarding MTX pharmacokinetics, such as the exact metabolic process, key factors that influence the metabolic process, and the effects, if any, of age and sex. In another study conducted by Bacci et al\textsuperscript{14} in 336 patients with OS of the extremities who were treated with 3 neoadjuvant protocols of chemotherapy, including HD-MTX (different doses for each protocol), MTX dose, not age or sex, was found to be a key factor influencing the mean serum \(C_{\text{max}}\). Patients treated with MTX doses of 8, 10, or 12 g/m\textsuperscript{2} had \(C_{\text{max}}\) values of 578, 735, and 1114 μmol/L, respectively, demonstrating a significant difference between groups (\(P < 0.001\)). The results of the present study support the findings from the study by Bacci et al.\textsuperscript{14} In the present study, when MTX was administered at 6 and 8 g/m\textsuperscript{2}, MTX concentrations at baseline were 418.60 and 595.83 μmol/L, respectively, suggesting a significant difference in \(C_{\text{max}}\) at different doses (\(P < 0.05\)). However, no significant difference was found in MTX concentrations at 24 or 48 hours at different doses, suggesting that the dose did not affect the serum MTX concentrations at 24 or 48 hours, probably due to the higher serum MTX concentration at baseline hav-
ing produced an accelerated discharge, which is consistent with the findings in literature.\textsuperscript{15,16}

Selecting 700 or 1000 \(\mu\)mol/L as the effective MTX \(C_{\text{max}}\) has been controversial among researchers worldwide.\textsuperscript{17–21} In a study by Zelcer et al\textsuperscript{22} conducted in 48 patients with OS (adults and children) treated with HD-MTX, no correlation was found between DFS and \(C_{\text{max}}\) or between a serum MTX concentration >1000 \(\mu\)mol/L and extended DFS. This study, however, was limited by its small sample size. In a separate study by Crews et al\textsuperscript{23} conducted in 140 children and adults with OS treated with HD-MTX, patients with a \(C_{\text{max}}\) >1500 \(\mu\)mol/L appeared to have a poorer prognosis, in terms of 5-year survival, compared with those who had a \(C_{\text{max}}\) of \(\leq\)1500 \(\mu\)mol/L. In the present study, 4 patients (0.04\%) had a high \(C_{\text{max}}\) (>700 \(\mu\)mol/L) at baseline. The mean \(C_{\text{max}}\) was 505.83 \(\mu\)mol/L and the median \(C_{\text{max}}\) was 499.56 \(\mu\)mol/L. DFS in the group with a \(C_{\text{max}}\) of >500 \(\mu\)mol/L was significantly longer than that in the group with a \(C_{\text{max}}\) of \(\leq\)500 \(\mu\)mol/L (\(P = 0.040\)). OAS in the group with the higher \(C_{\text{max}}\) at baseline was numerically longer than that in the group with the lower \(C_{\text{max}}\) but this difference was not statistically significant, likely because of the small population and brief follow-up. Further studies investigating these parameters are warranted.

Although HD-MTX therapy was associated with effectiveness survival, the incidences of some adverse reactions were significantly higher than those associated with routine dose MTX (30–40 mg/m\(^2\)) as well.\textsuperscript{24,25} The chemotherapeutic mortality rate was found to be \(~\)6\%. Nirenberg et al\textsuperscript{7} reported that irreversible damage would take place if serum MTX concentration was >10 \(\mu\)mol/L and >1 \(\mu\)mol/L at 24 and 48 hours, respectively. Impaired hepatic function, bone marrow depression, gastrointestinal mucositis, and ulcer were all common adverse reactions experienced, probably due to the short cell cycle and rapid proliferation associated with MTX use, resulting in cell damage.\textsuperscript{19} Therefore, it was necessary to minimize these reactions in chemotherapy with HD-MTX in the present study, and CFR was crucial in decreasing the incidence of adverse reactions associated with HD-MTX use. Routine CFR was administered 12 hours after HD-MTX administration in many cases. The dosage of CFR accounted for 3\% to 5\% of MTX. If the serum MTX concentrations at 24, 48, and 72 hours were higher than the safety values, CFR would need to be administered more frequently based on the CFR dosing schedule. At the same time, water and basic electrolytes would be required to obtain 3000 mL of urinary output each day and a urinary pH >7.0. In addition, oral allopurinol administration was necessary to prevent the inhibition of MTX and its metabolite crystal in the nephric tubule due to severe renal function damage. Among the patients who received HD-MTX (6 or 8 g/m\(^2\)) in this study, 122 of 532 cases had serum MTX concentrations that were higher than the safety values at 24 hours (10 \(\mu\)mol/L) and/or 48 hours (1 \(\mu\)mol/L). Compared with the patients whose serum MTX concentrations were lower than the safety values, patients with serum MTX concentrations higher than the safety values were more likely to experience bone marrow suppression, dental ulcer, rash, and fever, but the incidences of hepatic impairment and gastrointestinal reaction were not significantly different between the 2 subgroups. In 13 of 122 cases in which MTX concentrations were higher than safety values, at both 24 and 48 hours, severe adverse reactions (bone marrow sup-
pression [11.1%], dental ulcer [5.1%], and rash [4.5%]) occurred. CFR administration was particularly important in these patients, and they experienced complete recovery within 10 days of the discharge date. In addition to increasing CFR administration frequency and dosage, other measures undertaken were elevating the WBC and platelet counts via granulocyte colony-stimulating factor and interleukin-11, proper blood component transfusion, oral care with CFR, confinement in a sterile room, and antibiotic administration. Patients with mild or moderate adverse reactions usually recovered within 3 to 5 days.

LIMITATIONS
The present study used a retrospective analysis of serum MTX concentrations at different dosages. It provides information on relationships between serum MTX concentration and prognosis and tolerability in these Chinese adult patients with OS. Because this study was not blinded or randomized, the findings might have been subject to bias. A prospective study is currently under way at the Affiliated People’s 6th Hospital.

CONCLUSIONS
The present study found that serum MTX concentrations measured at different time points were variable within and between these Chinese adult patients. Adverse reactions occurred at a higher frequency in patients whose serum MTX concentrations at 24 and 48 hours were higher than the safety values (10 μmol/L and 1 μmol/L, respectively). The dosage, but not sex and age, appeared to influence MTX C_{max}, and C_{max} was significantly related to DFS but not OAS in this study.

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