Methotrexate Pharmacokinetics and Survival in Osteosarcomat

Irene Aquerreta, PharmD, PhD,[1]* Azucena Aldaz, PharmD, PhD,[1] Joaquín Giráldez, PharmD, PhD,[1] and Luis Sierrasesúmaga, MD, PhD[2]

[1]Department of Pharmacy, University Hospital of Navarra, Pamplona, Spain
[2]Department of Pediatrics, University Hospital of Navarra, Pamplona, Spain

*Correspondence to: Irene Aquerreta, Department of Pharmacy, University Hospital of Navarra. Avda. Pío XII 36, 31008 Pamplona, Spain. E-mail: iaquerreta@unav.es

Background
The aim of this study was to analyze the relationship between exposure to high-dose methotrexate (HDMTX) and tumor response in terms of survival in children with osteosarcoma.

Procedure
This study included 44 patients (479 courses) who received a median dose of 5.92 g/m^2 of MTX (interquartile range (IQR) 2.37 g/m^2) in a 4-hr infusion. The mean area under the concentration–time curve (AUC) estimated by parametric methods (non-parametric expectation maximization, NPEM), and the mean concentration at the end of the infusion were considered to be the exposure parameters. Tumor response was recorded as disease-free survival (DFS), overall survival (OS), and histologic tumor response. The relationship between MTX exposure and survival parameters was analyzed by Cox regression.

Results
The group of 11 patients who were the least exposed to MTX (AUC < 2,400 μmol/Lhr) presented a high DFS, probably due to the shorter interval of time between MTX courses that led to a higher dose density. In patients with AUC > 2,400 μmol/Lhr, an increase in the AUC was related to an increase in the DFS. Significant differences were observed in the DFS between patients whose mean AUC was below or above 4,000 μmol/Lhr (P = 0.024), such that 4,000 μmol/Lhr was considered as the minimum AUC to be aimed at for future patients.

Conclusions
Dose density seems to be an important factor in osteosarcoma response, but this must be confirmed in further studies. In order to improve the response to osteosarcoma in children, it is recommended that the dose of MTX to be increased such as to obtain an AUC higher than 4,000 μmol/Lhr.

Key words
High-dose methotrexate; osteosarcoma; pharmacokinetics; tumor response

INTRODUCTION

High-dose methotrexate (HDMTX) is a drug therapy useful in the treatment of diverse pediatric tumors such as osteosarcoma [1–5] and acute lymphocytic leukemia (ALL) [6,7]. Its role in hematological tumor responses has been widely documented [6–11] but few studies describe the relationship between exposure to MTX and tumor response in solid tumors such as osteosarcoma. To some extent this reflects the difficulty in measuring responses in solid tumors due to delayed growth in time following Gompertzian kinetics and the clonal heterogeneity of the tumor, which generates different responses to chemotherapy [12].

Different studies have questioned the role of HDMTX in the treatment of osteosarcoma [13,14]. In contrast, various authors have described a significant correlation between osteosarcoma tumor response and HDMTX peak level [15–18]. A highly significant correlation between tumor response and a MTX serum level greater than 1,000 μmol/L at the end of the MTX 4-hr infusion was reported by Delepine et al. [17], and was confirmed in 1994 by Graf et al. [3]. Bacci et al. [19] recommended that a serum peak of 700 μmol/L or greater should be attained when MTX is infused over 6 hr in order to improve the response of osteosarcoma tumors.

To date, no study has correlated the response of osteosarcoma tumors with the area under the concentration–time curve (AUC) as the parameter to be considered for exposure to MTX. The mechanism of action suggests that MTX exposure is the true factor that determines the tumor response in osteosarcoma. It has been shown that the cytotoxic effects of HDMTX depend both on MTX concentration and duration of exposure [20].

The aim of this study was to establish a relationship between the exposure to HDMTX measured either as the AUC or the peak level, and the response of osteosarcoma tumors in terms of patient survival.

MATERIALS AND METHODS

Patients

Forty-four children with high-grade osteosarcoma (19 males and 25 females) who received high-dose MTX therapy between 1986 and 1993 have been studied. The total number of MTX courses administered was 479. The inclusion criterion was the availability of the relevant data in the patient's medical charts.

Data Collection

The following clinical data were collected by retrospective chart review: age, sex, height, weight, lean body weight, body surface area (BSA), diagnosis, pathologic subtype of tumor, location of the tumor, presence of distant metastases, creatinine clearance estimated by the Schwartz formula [21], significant delay in MTX elimination defined as MTX concentration at 24 hr after the end of the infusion ($Cp_{24\ hr}$) >3.5

μmol/L, and plasma half-life for the initial elimination phase ($t_{1/2}\alpha$) > 3.5 hr, or MTX concentration 48 hr after the end of the infusion ($Cp_{48\ hr}$) ≥ 0.35 μmol/L and plasma half-life for the terminal elimination phase ($t_{1/2}\beta$) > 12.5 hr (Aldaz et al., personal communication); serum creatinine; baseline leukocyte count; volume of hydration; lowest recorded urine pH; concurrent use of other drugs (anti-emetics, trimethoprim/sulfamethoxazole, penicillins, aminoglucosides, NSAID's, diuretics, cisplatin, carboplatin, or vancomycin); disease-free survival (DFS); and overall survival (OS).

Chemotherapy Protocol

The chemotherapy regimen administered before the surgery was: cisplatin 40 mg/m^2/day intraarterial (IA), days 1, 2, 3, 21, 22, and 23; doxorubicin 30 mg/m^2/day intravenous (IV), days 1, 2, 22, and 23; MTX 6 g/m^2 IV, days 7,14, 29, and 36; cisplatin 40 mg/m^2/day IA, days 43, 44, and 45. All patients underwent radical excision surgery. Two weeks after the surgery the patients received: (A) MTX 6 g/m^2 IV; (B) cisplatin 40 mg/m^2/day IV for 3 days; doxorubicin 25 mg/m^2/day IV for 3 days; (C) bleomycin 10 mg/m^2/day IV for 3 days; dactinomycin 0.5 mg/m^2/day IV for 3 days; ifosfamide 1.5 g/m^2/day IV for 3 days; and Mesna 300 mg/m^2 administered before and 4 and 8 hr after the ifosfamide. Cycles B and C were repeated every 21 days. Cycle A was administered between cycles B and C, days 7 and 14.

MTX Administration

The dose of MTX ranged from 3 to 12 g/m2 (median 5.92 g/m^2, interquartile range (IQR) 2.37 g/m^2). MTX dose administered in our hospital increased with the publication of new articles that found a significant correlation between MTX peak level and survival. Hydration consisted of 3 L/m^2/24 hr of 5% dextrose in water with 300 mEq of $NaHCO_3$ and 60 mEq of KCl per L. Hydration was begun 12 hr before MTX administration and maintained for 3 days. Urine pH was checked throughout the hydration period. MTX administration was performed when urine pH was between 7 and 8 in two consecutive voids and urine flow was at least 120 ml/min. If necessary, $NaHCO_3$ dose was adjusted to obtain a urine pH between 7 and 8. MTX dose was diluted in 500 ml of 0.9% NaCl and infused over a 4-hr period. The standard leucovorin rescue was 15 mg/m^2 every 6 hr for 3 days, beginning 24 hr after the end of MTX administration.

Plasma Samples

MTX concentrations were measured at specific times to determine adequate leucovorin rescue therapy or supportive care. High-risk concentrations of MTX had been previously determined in our hospital in a preliminary study in pediatric patients (Aldaz et al., personal communication): $Cp_{24\ hr}$ > 3.5 μmol/L or $Cp_{48\ hr}$ > 0.35 μmol/L. Therefore, in most cases MTX plasma concentrations were determined at the end of the MTX infusion and at 24 and 48 hr. Crom and Evans [22] guidelines for modification of leucovorin dosage were followed in patients with high-risk MTX concentrations, at time ≥ 42 hr after the beginning of MTX infusion.

Sample Analysis

MTX plasma concentrations were measured by fluorescence polarization immunoassay (FPIA) with a TDxFLx^{TM} analyzer (Abbott Laboratories, Abbott Park, IL) [23].

Pharmacokinetic Analysis

Pharmacokinetic parameters of MTX were estimated by parametric (IT2B, Iterative Two-stage Bayesian) [24] and non-parametric methods (non-parametric expectation maximization, NPEM) [25] using the software package USC*PACK 10.7a (University of Southern California, Los Angeles, CA) and assuming a two-compartment pharmacokinetic model. First of all, pharmacokinetic parameters were estimated by IT2B in order to obtain a range of variability of the parameters and estimate the value of σ (intraindividual variability) to be applied on NPEM. The assay error pattern was determined by the Jelliffe and Tahani method [26]. The pharmacokinetic parameters estimated by NPEM were chosen for their lower log-likelihood, lower entropy, and similar predictive performance when compared with that obtained by IT2B. Pharmacokinetic parameters estimated were: elimination rate constant (k_{el}); rate constants for MTX's movement from central to peripheral compartment (k_{cp}); and peripheral to central (k_{pc}) and volume of distribution for the central compartment (V_c). MTX systemic clearance (CL) and AUC were estimated according to the following equations [27]:

$$CL = k_{el} \times V_c \quad \text{and} \quad AUC = dose/CL$$

Pharmacokinetics–Pharmacodynamics Analysis

As parameters for MTX exposure, the mean peak defined as the mean value of all plasma peak values of the patient, and the mean AUC as the mean value of all the AUC's obtained in the patient in each course of MTX were estimated. The different values of MTX peak concentrations and AUC for the same patient were very similar in the different MTX courses. As parameters for the treatment response, histologic tumor response (%), DFS, and OS were recorded. To compare the influence of AUC in osteosarcoma response, patients were divided in four groups depending on the value of the mean AUC recorded, with 11 patients in each group (group 1: <2,400 μmol/Lhr; group 2:2,400–3,675 μmol/Lhr; group 3: 3,700–4,800 μmol/L; group 4: > 4,800 μmol/Lhr).

Statistical Analysis

The relationship between exposure to MTX and survival parameters was analyzed by Cox regression. Coxs F-test was applied to compare final events of two groups in terms of survival. To compare multiple samples, a nonparametric test that is an extension of Gehans generalized Wilcoxon test, Peto and Petos generalized Wilcoxon test, and the log-rank test was used. To compare multiple samples two by two, Coxs F-test with Bonferroni adjustment [28] was used. Cumulative proportional survival graphs were realized by Kaplan–Meier curves. The statistic program used was STATISTICA

(edition 99, Stat Soft^{TM}, Inc., Tulsa, OK) [29]. The alpha level considered was 0.05 except in multiple comparisons with Bonferroni adjustment in which a value of 0.05/k was used, being k the number of comparisons performed.

RESULTS

We have studied the relationship between exposure to HDMTX and response of osteosarcoma tumors in terms of patient survival in 44 children. The clinical and treatment variables for the children, who received a total number of 479 HDMTX courses, are listed in Tables I and II. No significant variation was observed in MTX doses administered to the same patient in the different MTX courses.

Urine pH lower than 7 was detected only in four MTX courses. In three cases, the acid urine pH (5–6.5) resulted in a significant delay in MTX elimination ($Cp_{24\ hr} > 3.5$ μmol/L and $t_{1/2}\alpha > 3.5$ hr, or $Cp_{48\ hr} \geq 0.35$ μmol/L and $t_{1/2}\beta > 12.5$ hr).

A large variability was observed in both the mean doses of MTX administered (from 3.51 to 10.33 g/m^2) and in the mean AUC (from 1,073 to 9,266 μmol/Lhr). The DFS was 42.50 (64.50) months and the OS 72.50 (52.75) months, both expressed as media (IQR). At a follow-up of 5 years, 21 patients (47.7%) remained free of disease and 32 (72.7%) alive.

The characteristic of the treatments received and the clinical responses of the different groups depending on the mean AUC are presented in Table III, and their diagnostics in Table IV. No significant differences were found between the four AUC groups in the different variables of osteosarcoma prognostics [30], such as the osteosarcoma subtype, tumor location, histologic tumor response, sex, age, and MTX dose in g/m^2 per week. Four patients presented distant metastases at diagnosis considered being a poor prognosis factor. However, these patients were evenly distributed between the four groups. In Figures 1 and 2 the DFS and the OS of the different AUC groups are shown. Highly significant differences were observed in the DFS between the different mean AUC groups by Cox regression (P = 0.0055) (Fig. 1), although this was not the case in terms of the OS (P=0.33) (Fig. 2). Both the DFS and the OS are correlated [r = 0.68, P < 0.001; OS (months) = 37,098 + 0,65 x DFS (months)] such that patients with a longer DFS, lived longer than those who developed early distant metastases or had a local progression of the illness.

In general, an increase in the mean AUC was reflected by an increase in the DFS and in the OS. However, an exception was observed in the group exposed to the lowest MTX AUC (group 1), that showed a high DFS and OS. The DFS of group 1 was comparable to that obtained in the group 4 (P = 0.23). One variable that could explain the response observed in group 1 was the shorter interval of time between MTX courses. In this group, the mean interval between MTX courses was 28 days while in the rest of the groups was about 7 days longer. In the analysis of the relationship between MTX exposure and tumor response, this group was analyzed independently in order to avoid the possible influence of the interval of time between courses in the results.

In patients with AUC >2,400 μmol/Lhr, a highly significant relationship was found between DFS and the AUC (P = 0.0037), and between DFS and the mean peak level (P = 0.010) by Cox regression. On the contrary, no significant relationship was detected between OS and AUC (P = 0.19) or OS and mean peak level (P = 0.69). By Coxs F-test with Bonferroni adjustment, none of the comparisons two by two between groups was significant in DFS (P < 0.017): group 2 and 3, P = 0.041; group 3 and 4, P = 0.034; and group 2 and 4, P = 0.019. In the same way, none significant differences were detected in OS: group 2 and 3, P = 0.038; group 3 and 4, P = 0.019; and group 2 and 4, P = 0.048. The group of patients that received the highest MTX exposure (group 4) also presented the best histologic tumor response, considered as an index of the response of the tumor to chemotherapy prior to surgery, mean 89.29% (SD 13.36).

In patients with AUC > 2,400 μmol/Lhr, significant differences were detected by Coxs F-test in DFS considering the mean AUC of 4,000 μmol/Lhr as the cut-off point (P = 0.024) (Fig. 3). A close and linear correlation was observed between the MTX peak concentration and the AUC (Spearman R = 0.91, P < 0.001, Fig. 4), with a highly significant relationship between a mean AUC of 4,000 μmol/Lhr and a mean peak concentration of 700 μmol/L.

DISCUSSION

In this study of 44 children with osteosarcoma, a significant relationship was found between the DFS and the mean AUC of MTX. The threshold mean AUC where significant differences in DFS were detected was a mean AUC value of 4,000 μmol/Lhr.

A high DFS was also observed in the group of patients exposed to the lowest levels of MTX (group 1, AUC < 2,400 μmol/Lhr), a result that could not be explained by a difference in the prognosis for these patients. Since these 11 patients received the lowest mean dose of MTX, media 4.84 (IQR 1.19) g/m^2 (Table III), they consequently developed less toxic effects than the rest of the patients. This fact allowed the administration of MTX courses every 28 days whereas the patients who received higher doses frequently developed mucositis, leukopenia, or renal toxicity that delayed the administration of MTX course by a mean of 7 days (Table III). Thus, one possible explanation for the good response observed in group 1 might be the shorter interval between MTX courses, with the importance that it may have in solid tumors response. In this group, the dose density (amount of drug administered during a defined period of time) was higher although dose intensity (dose administered per cycle) was lower.

The influence of the time interval between MTX ad-ministration on the response observed in earlier studies might support this hypothesis. In a study published by Frei et al. [31], the response in osteosarcoma was improved by increasing the dose of MTX and was made worse by increasing the period of time between MTX administration. The French Tumor Study Group [1] found that the delay in MTX course administration was a negative factor in the prognosis of osteosarcoma. Bacci et al. [32] have described that avoid reductions of doses and/or delays in performing the courses of chemotherapy is crucial for outcome in osteosarcoma. Whether shorter intervals between MTX courses might improve osteosarcoma response should also be addressed. On the other hand, it cannot be disregarded that the high DFS observed in group 1 was due to a different

exposure to other agents used in the treatment, as cisplatin, doxorubicin, bleomycin, dactinomycin, or ifosfamide. Also other factors that were not assessed in the study could have contributed to the good response observed.

In those patients with AUC > 2,400 μmol/L, a significant relationship was found between DFS and AUC whereby an increase in the mean AUC led to a longer DFS. It has been reported that the cytotoxic effects of HDMTX depend on the drug concentration and the duration of exposure above a threshold concentration, being the duration of exposure the determinant factor once the minimum threshold concentration has been achieved [20].

The threshold mean AUC required to generate significant differences in DFS was a mean AUC value of 4,000 μmol/Lhr, which correlates with a peak of 700 μmol/ L. In contrast, Graf et al. [3] found that a threshold MTX peak level of 1,000 μmol/L needs to be surpassed for MTX treatment in osteosarcoma to be effective. The corresponding AUC (4,000 μmol/Lhr) abolished the differences observed in DFS of osteosarcoma. They also found a highly significant correlation between MTX peak level and AUC (R = 0.66). It can be deduced from the slope of the linear relationship between the AUC and the MTX peak level that the concentration was higher in our study than in COSS-80 [3] and the same values of AUC were obtained with different peak MTX concentrations.

Different MTX peak levels (700 and 1,000 μmol/L) could generate the same AUC value. Renal characteristics were not described in the COSS-80 study and therefore it was not possible to compare the differences at this level. However, it is worthy noting that in 1982, Breithaupt and Küenzlen [33] found that an AUC of 4,000 μmol/Lhr was correlated with a peak level of 700 μmol/L in osteosarcoma patients, data that correspond with the result obtained in our study.

In an in vitro study in lymphoblasts, Keefe et al. [20] found that exposure of cells to 1 μmol/L MTX for 36 or 42 hr was significantly more effective than shorter exposures of 3 or 6 hr at 100 μmol/L. This result can be explained by intracellular metabolism of MTX to its polyglutamate derivates, which is related to the cellular retention and cytotoxicity of MTX. Polyglutamation of MTX depends on the presence of free intracellular MTX. Once an intracellular MTX concentration that maximally inhibits dihydrofolate reductase has been reached, polyglutamation is dependent on the duration of exposure. For that reason, the continued presence of intracellular MTX has been related to the accumulation of intracellular polyglutamates.

The relationship between histologic tumor response and DFS and OS has been described in different studies [34]. In our study, OS was significantly longer in patients that demonstrated an histologic tumor response over 90% (P = 0.017). The group of patients who received the highest exposure of MTX (group 4, AUC > 4,800 μmol/ Lhr) presented the highest mean histologic response, mean 89.29% (SD 13.36). This result indicates that the higher the MTX exposure, the higher the histologic tumor response and consequently the patient survival.

Although in COSS-80 Graf et al. [3] did not find significant differences in DFS with the AUC, the threshold peak level of 1,000 μmol/L was correlated with an AUC of 4,000 μmol/Lhr. The coincidence between the threshold AUC values obtained in our study and that of Graf et al., despite the different peak levels, confirms the importance of the

length of exposure above a critical concentration in the response of osteosarcoma. The optimal parameter when considering MTX exposure is the AUC because it reflects the real exposure, including both the concentration reached and the time of exposure. When the AUC is not available, the peak level can be used due to the high correlation between both variables.

The fact that no significant relationship was detected between the OS and patients with AUC > 4,000 μmol/Lhr could be explained because of the inherent limitations of the survival analysis. This method penalizes the absence of final events (death of the patient) and therefore is not able to obtain information from group 4, where only one patient died during the period of time of the study. Besides, there were few patients in each group (only 11).

CONCLUSIONS

The results obtained in this study indicate that MTX dose should be adapted in osteosarcoma patients in order to obtain an AUC > 4,000 μmol/Lhr in order to improve the DFS. Moreover, we have shown that the highest levels of exposure to MTX are associated with the highest levels of tumor necrosis. On the other hand, the group that was least exposed MTX (AUC < 2,400 μmol/Lhr) showed a high DFS, probably due to the higher dose density. Besides dose intensity, dose density seems to be an important factor in osteosarcoma response. This must be confirmed in further studies. The possibility that other factors could influence this response cannot be disregarded.

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Table 1. Anthropometric and Clinical Variables (44 Patients)

Variable	Mean	SD	CV (%)	Range
BSA (m^2)	1.40	0.33	23.48	0.67–2.28
Weight (kg)	46.67	15.99	34.38	15.32–96.43
WBC (/mm^3)	5,167.64	2,286.20	44.24	1,240–15,900
CrCL (ml/min)	120.80	46.23	38.27	46.23–216.69
	Median	IQR		
Age (years)	14	7	37.88	4–21
Height (cm)	158	30	14.38	105–192
SCr (mg/dl)	0.6	0.2	25.25	0.3–1.50

BSA, body surface area [21]; WBC, baseline leukocyte count; CrCL, creatinine clearance estimated by Schwartz formula [21]; SCr, serum creatinine; SD, standard deviation; CV, coefficient of variation; IQR, interquartile range.

Table 2. Treatment Variables Considering the Mean Values in the Patient in the Different Methotrexate (MTX) Courses (44 Patients)

Variable	Median	IQR	Range
MTX dose (g/m^2)	4.87	2.90	3.51–10.33
MTX peak level (μmol/L)	450	320	125–1,725
MTX $Cp_{24\ hr}$ (μmol/L)	0.85	1.092	0.04–32
MTX $Cp_{48\ hr}$ (μmol/L)	0.14	0.14	0.0081–6.67
MTX AUC (μmol/L • h)	3,296	1,748	1,073–9,266
Volume of hydration (ml/m^2/24 hr)	2,352.5	844	1,355–4,687
Minimum urine pH	7.5	0.5	5–8
Leucovorin dose (mg/m^2)	177.20	71.14	94.46–2,460

$Cp_{24\ hr}$, MTX plasma concentration 24 hr after the end of the infusion; $Cp_{48\ hr}$, MTX plasma concentration 48 hr after the end of the infusion; AUC, area under the concentration–time curve.

Table 3. Characteristics of the Different AUC Groups
(44 Patients, 11 Patients in Each Group)

Variable	Group 1	Group 2	Group 3	Group 4
Mean AUC (μmol/Lhr)	1,879 (415)	3,194 (404)	4,283 (386)	6,253 (1,167)
Mean peak level (μmol/L)	381.42 (114.96)	579.76 (84.77)	767.47 (111.85)	968.42 (191.33)
Mean MTX dose (g/m^2)	4.84 (1.19)	6.30 (1.12)	7.99 (1.12)	8.87 (0.75)
Dose intensity (g/m^2/week)	1.20 (0.46)	1.60 (0.56)	1.89 (0.66)	2.05 (0.76)
Number of MTX courses	10.73 (4.29)	13.91 (7.15)	10.27 (5.83)	8.64 (3.56)
Mean number of days between MTX courses	28.64 (8.81)	35.38 (15.44)	33.69 (11.59)	35.091 (9.45)
Histologic tumor response (%)	70.71 (34.09)	83.75 (12.46)	67.86 (23.78)	89.29 (13.36)
DFS (months)	63.64 (85.00)	18.18 (20.00)	36.36 (58.00)	72.73 (52.50)
OS (months)	81.82 (69.00)	63.64 (65.00)	54.55 (52.00)	90.91 (48.33)

DFS, disease-free survival; OS, overall survival.
Group 1, mean AUC < 2,400 μmol/Lhr; group 2, mean AUC 2,400–3,675 μmol/Lhr; group 3, mean AUC 3,700–4,800 μmol/Lhr; group 4, mean AUC > 4,800 μmol/Lhr. Data are expressed as median (IQR).

Table 4. Osteosarcoma Subtype and Tumor Location Within the Different AUC Groups (44 Patients)

Mean AUC group	Subtype	N (%)	Tumor location	N (%)
Group 1	Osteoblastic	6 (54.55)	Femur	5 (45.45)
	Chondroblastic	3 (27.27)	Tibia	3 (27.27)
	Fibroblastic	1 (9.09)	Humerus	2 (18.18)
	Mixed	1 (9.09)	Calotte	1 (9.09)
Group 2	Osteoblastic	6 (54.55)	Tibia	5 (45.45)
	Chondroblastic	2 (18.18)	Femur	3 (27.27)
	Osteogenic	2 (18.18)	Humerus	2 (18.18)
	Telangiectasic	1 (9.09)	Fibula	1 (9.09)
Group 3	Osteoblastic	7 (63.64)	Tibia	5 (45.45)
	Chondroblastic	2 (18.18)	Femur	4 (36.36)
	Mixed	2 (18.18)	Humerus	1 (9.09)
			Costovertebral	1 (9.09)
Group 4	Osteoblastic	7 (63.64)	Femur	6 (54.55)
	Fibroblastic	2 (18.18)	Tibia	3 (27.27)
	Osteogenic	1 (9.09)	Humerus	2 (18.18)
	Telangiectasic	1 (9.09)		

Group 1, mean AUC<2,400 µmol/Lhr; group 2, mean AUC 2,400– 3,675 µmol/Lhr; group 3, mean AUC 3,700–4,800 µmol/L; group 4, mean AUC>4,800 µmol/Lhr. Results expressed as number of patients (percentage of patients of the total number of patients in each AUC group).

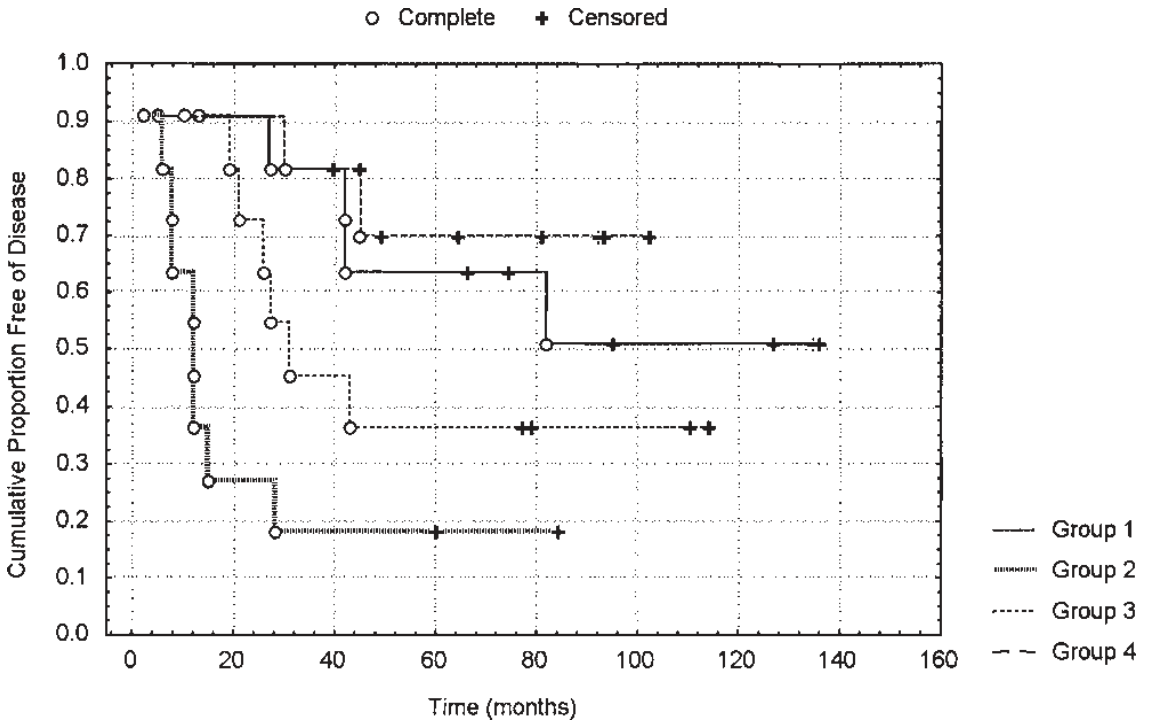

Figure 1. Disease-free survival (DFS) in the mean area under the concentration–time curve (AUC) groups. Group 1: mean AUC < 2,400 µmol/ Lhr; group 2: mean AUC 2,400–3,675 µmol/Lhr; group 3: mean AUC 3,700–4,800 µmol/L; group 4: mean AUC > 4,800 µmol/Lhr.

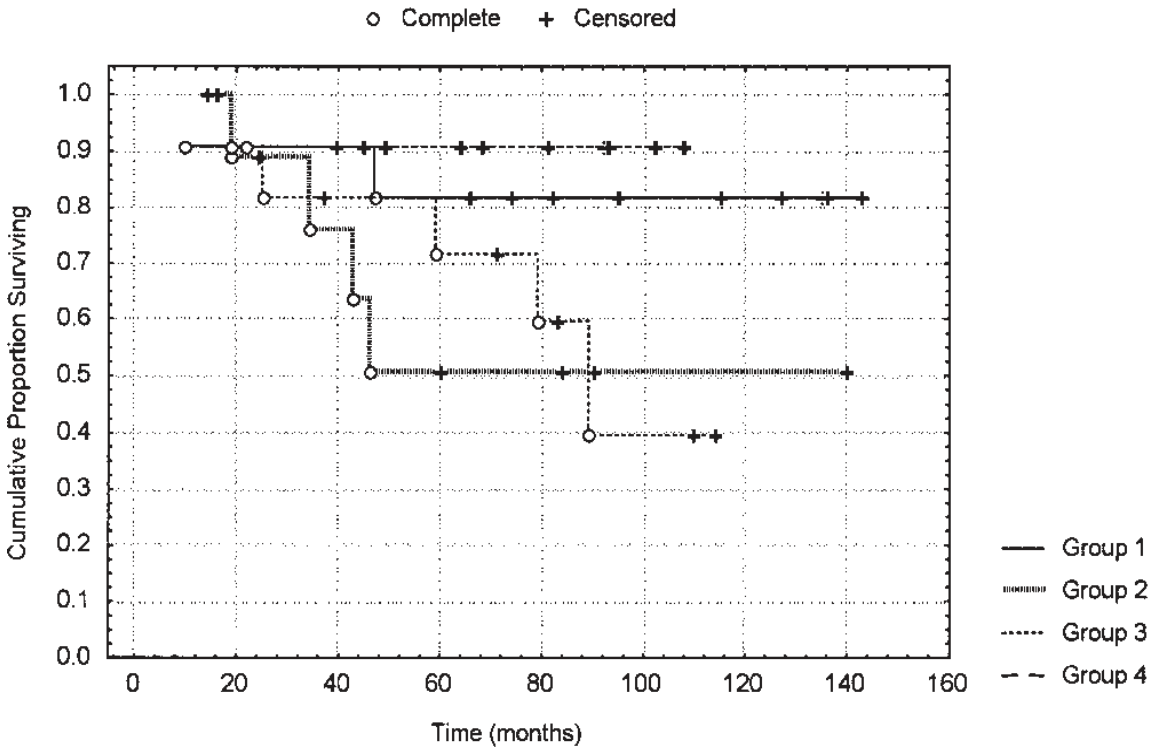

Figure 2. Overall survival (OS) in the mean AUC groups. Group 1: mean AUC < 2,400 µmol/Lhr; group 2: mean AUC 2,400–3,675 µmol/Lhr; group 3: mean AUC 3,700–4,800 µmol/L; group 4: mean AUC > 4,800 µmol/Lhr.

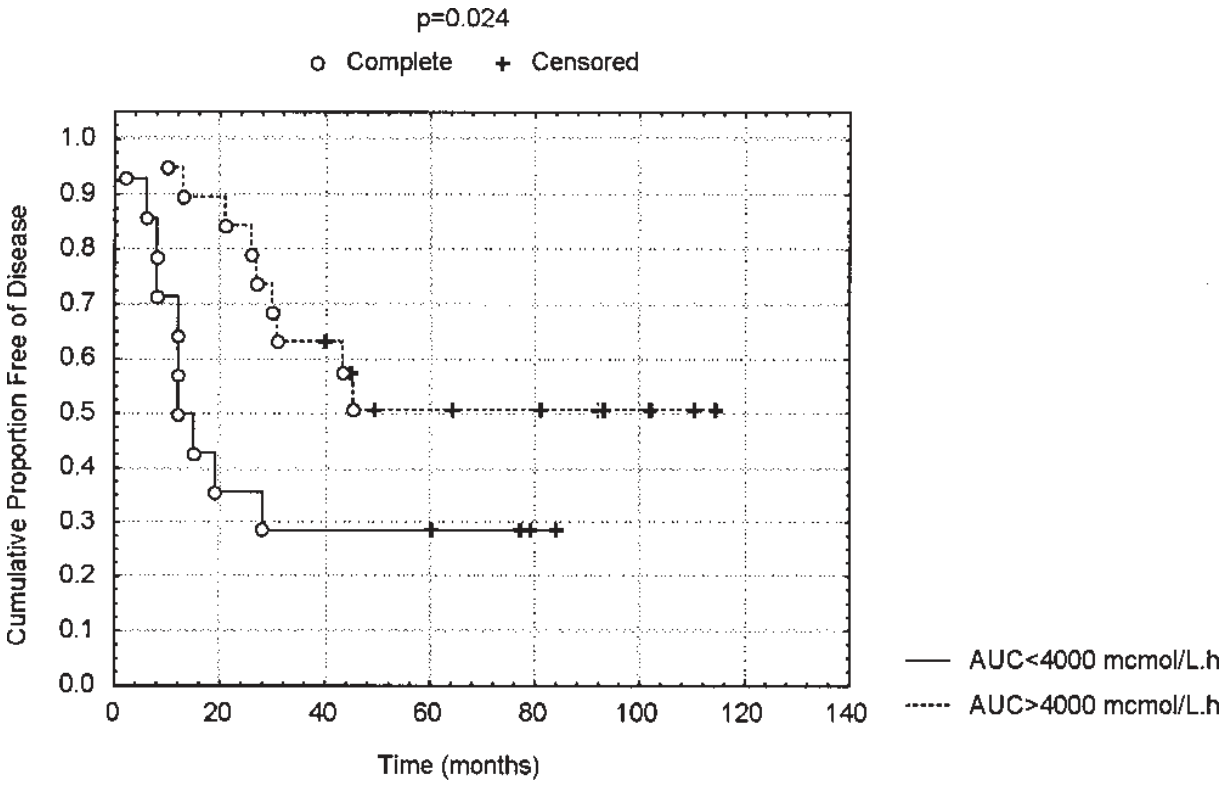

Figure 3. DFS in the cut-off point of 4,000 µmol/Lhr including patients with mean AUC > 2,400 µmol/Lhr (n = 33) (Coxs F, P = 0.024).

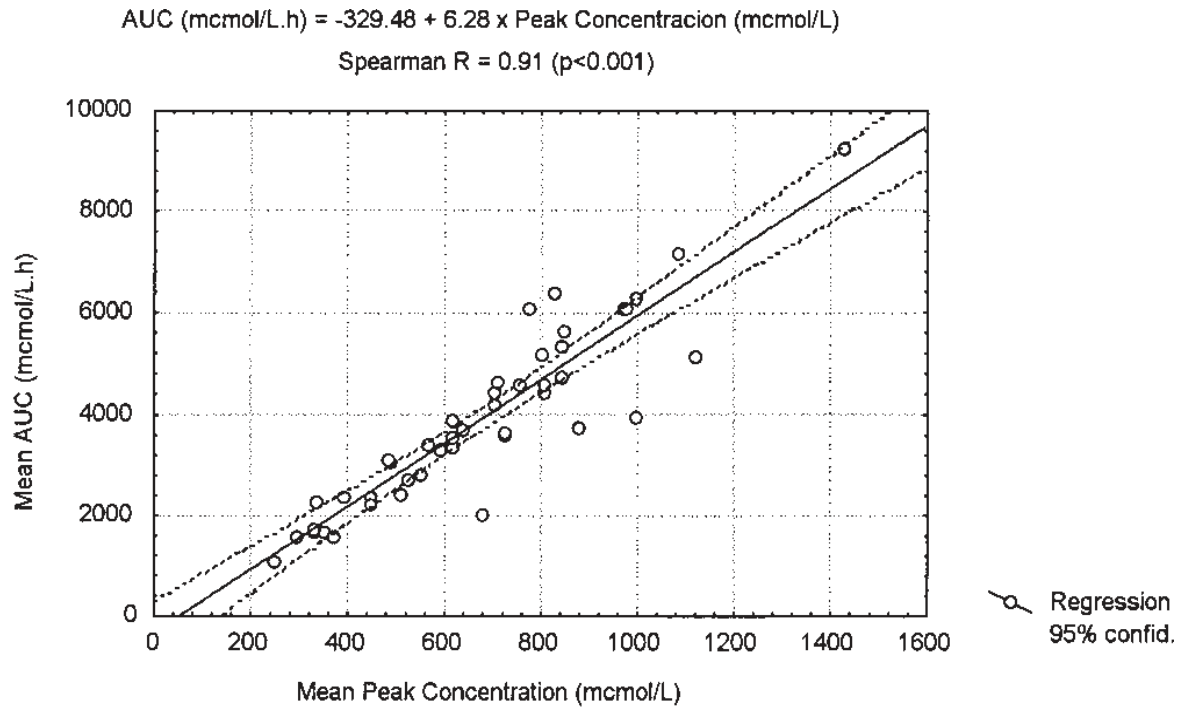

Figure 4. Correlation between mean AUC and mean methotrexate (MTX) peak concentration (44 patients). Spearman R = 0.91, P < 0.001.