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Adriamycin–Methotrexate High Dose versus Adriamycin–Methotrexate Moderate Dose as Adjuvant Chemotherapy for Osteosarcoma of the Extremities: a Randomized Study

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Abstract—*Adjuvant chemotherapy comprising Adriamycin (ADM) and Methotrexate (MTX) with Citrovorum Factor (CF) was administered on a randomization basis to 2 groups of patients with osteosarcoma after surgical ablation of the primary tumor. One group received high dose MTX (regimen I) and the other moderate dose MTX (regimen II). In both groups a short period of heparin treatment was also administered to prevent neoplastic emboli during surgery. All patients were free of metastasis at the beginning of therapy. The efficacy of therapy was determined by recording the percentage of continuously disease-free patients. This was compared to the disease-free survival in 132 patients previously treated with other ADM or ADM-MTX regimens and to a group of 39 patients treated during this period with amputation only. The latter did not receive adjuvant chemotherapy for a variety of reasons and are equated to a concurrent control group. Over the ensuing 27–66 months, 31 of 56 patients (55%) treated with regimen I and 25 of 50 (50%) treated with regimen II were disease-free. The overall disease-free survival in both regimens was 53%. This is similar to the 132 patients treated with previous adjuvant chemotherapy protocols (45-50%). However, the percentage of continuously disease-free patients treated with adjuvant chemotherapy was significantly better than the 39 patients (12%) treated contemporaneously with surgery only ($P < 0.0005$). Survival in the latter is similar to that of historical control patients. These results do not suggest any change in the natural history of osteosarcoma and reveal benefits which may accrue with adjuvant chemotherapy. These results also demonstrate that in adjuvant treatment of osteosarcoma performed with ADM and MTX the high and the moderate doses of MTX are equally efficacious.*

INTRODUCTION

BETWEEN 1970 and 1980 many independent uncontrolled studies suggested that adjuvant chemotherapy improved the disease-free survival and overall survival of patients with localized osteosarcoma of the extremities [1–9]. High dose methotrexate (MTX), alone or in combination with other agents, was widely acclaimed as an effective treatment and formed the basis of several adjuvant or neoadjuvant regimens for this disease [2, 5, 9, 10]. However, in the adjuvant treatment of osteosarcoma, the alleged superiority of high dose of MTX over moderate dose has never been tested.

The purpose of this paper is to report the results in 106 consecutive patients with localized osteosarcoma of the extremities between 1980 and 1983 treated at our Institute by surgery and adjuvant chemotherapy performed with Adriamycin (ADM) and MTX. ADM was delivered at a fixed dose (75 mg/m^2) while MTX was delivered on a randomization basis: high (2000 mg/m^2) or moderate (200 mg/m^2) dose. In both groups a short period of heparin treatment was also administered to prevent neoplastic emboli during surgery [11].

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MATERIALS AND METHODS

Patient selection

Between January 1980 and March 1983, 179

histologically confirmed cases of osteosarcoma were seen at the Bone Tumor Center of the Istituto Ortopedico Rizzoli (IOR) in Bologna. The patients had the following characteristics:

1. Typical histologic features of osteosarcoma (central and high grade);
2. No previous treatment (surgery, radiotherapy or chemotherapy);
3. Tumor located in the extremities;
4. Age under 50 years;
5. Absence of evident metastases;
6. Absence of associated disease contraindicating the use of ADM or MTX.

Diagnostic procedures to exclude the presence of metastasis other than routine history and physical examination, included full chest tomograms and total body scan (99Tc). Sixty-nine of the 179 patients were excluded from the study for the following reasons: other osteosarcoma varieties (24 cases),, site of the tumour: pelvis, spine or jaws: (12 cases), metastatic disease (14 cases), age over 50 years (3 cases), surgery refused (1 case), chemotherapy refused (8 cases), contraindications to perform chemotherapy (5 cases) and first treatment performed at other Institutions (2 cases).

Of the 110 remaining cases who entered the study, four patients (randomized for regimen II) were not evaluable, two because of interruption of chemotherapy (local infection in one case and refusal to continue chemotherapy after the first cycle of ADM in the other), one due to early lethal MTX toxicity, and the fourth because the patient refused amputation after a resection that histologically demonstrated marginal persistence of tumor.

The clinical features of the patients entered on study are reported in Fig. 1.

Treatment of the primary tumor

The local control was always achieved surgically according to the site of the primary tumor as follows: radical surgery or wide amputation (distal femur, tibia or fibula); hip disarticulation (proximal or diaphyseal femur); forequarter or hindquarter amputation (proximal humerus/femur).

In only 24 selected cases, where the tumor was totally intraosseous or there was a small soft tissue extension without involvement of major neurovascular structure, a resection was performed. Bone reconstruction was usually accomplished with Kuntscher rod and cement (distal femur or proximal tibia) or with prosthetic devices (proximal femur or proximal humerus) [12]. No reconstruction was done for resection of expendible bones (fibula).

Adjuvant chemotherapy

Patients who entered the study were randomized into two groups, according to the initial site of the tumor (proximal vs distal): one group was treated with regimen I and the other with regimen II. In regimen I, MTX was administered *in bolus* in 15–20 min with Citrovorum Factor (CF) rescue commencing after 24 hr; in regimen II MTX was delivered over a period of 6 hr with CF rescue commencing 6 hr from the end of the infusion. The choice of 200 mg/m^2 as low dose MTX was made because this was the dose of MTX we used in a previous protocol [13]. CF rescue was given per os or i.m. if vomitus was present: the dosage was 15 mg every 6 hr for 12 cycles commencing 24 hr after MTX. Vincristine (VCR) was administered 30 min before MTX (1.5 mg/m^2); top dose: 2 mg) (Fig. 2).

Hydration was delivered for 1 day in regimen I and for 2 days in regimen II as follows: Glucosate solution 5%, 2000 ml/m^2; NaCl 20%, 20 cc/m^2; KCl 2mEq/cc, 20 cc/m^2; and $NaHCO_3$ 1mEq/cc, 2–4 cc/kg.

Due to the high incidence of cardiotoxicity (see

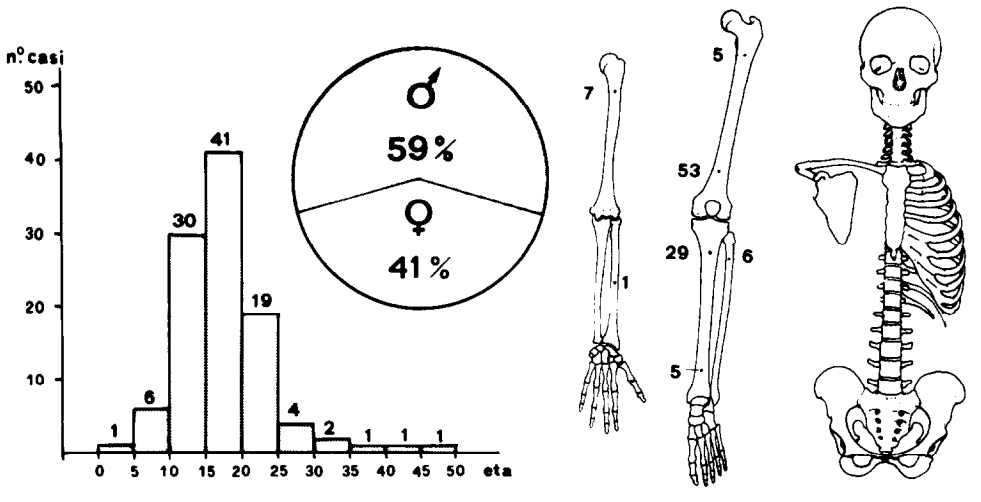

Fig. 1. Clinical characteristics of the 106 patients evaluable for study.

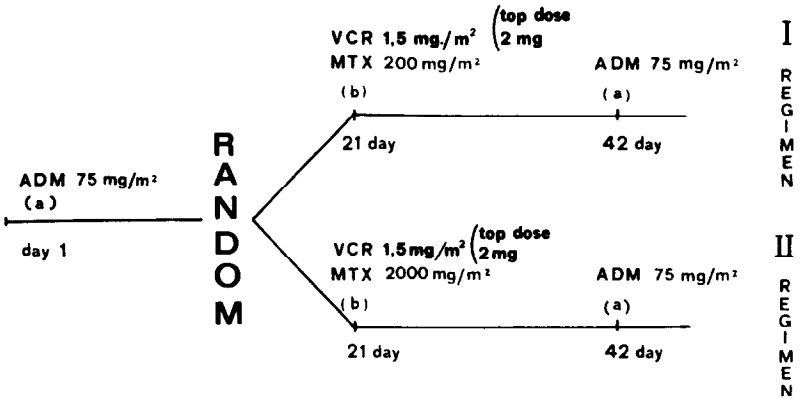

Fig. 2. Adjuvant chemotherapy schedule (Protocol "D"): regimen I (moderate dose MTX) and regimen II (high dose MTX).

later), from April 1981 ADM cycles were reduced from 7 to 6; this was administered to the last 61 patients who entered the study.

Adjuvant chemotherapy started 2 or 3 days after surgery, but was delayed 2–3 weeks in patients treated by resection. A short period of heparin (Calciparine 5000u subcutaneous every 8 hr for 10 days starting 3 hr before surgery) was also administered to prevent possible metastatic development of neoplastic emboli during surgery [11].

Follow-up

Initial work-up included ECG, haemogram, platelet count, alkaline phosphatase, transaminases and creatinine clearance. The same tests were performed before each cycle of cytotoxic drug treatment and were repeated, at 2 month intervals, for 1 year after completion of chemotherapy. Clinical evaluation including X-rays of the stump (or resected bone) and four views of the chest (or tomograms if indicated) were performed every 2 months for 3 years and subsequently every 6 months. Additional investigations were performed if there was clinical or radiographic suspicion of relapse.

Evaluation of treatment

The effect of treatment was evaluated by the following criteria:

1. Percentage of continuously disease-free patients;
2. Duration of disease-free interval before the appearance of metastases or local recurrence;
3. Evaluation of the initial site and extent of metastases;
4. Evaluation of side effects attributable to adjuvant chemotherapy;
5. Post metastatic survival.

The results were compared with those achieved at our Institution in 132 patients with osteosarcoma of the extremities treated between 1972 and 1979 with three different adjuvant chemotherapy protocols as previously reported [13] and with 39 patients observed in the period (1972–1982) and treated with surgery alone. The latter patients, for a variety of reasons refused adjuvant chemotherapy (32 cases) or had contraindications to treatment with chemotherapeutics agents, e.g. heart disease (7 cases). This cohort of patients includes those who refused chemotherapy or had contraindications to it in the 1980–1983 period as originally described.

N.E.D. curves analysis was performed with log-rank test proposed by Mantel as extension of Mantel and Haenszel test [14].

RESULTS

Fifty-six patients were randomized to regimen I, and 54 to regimen II (50 evaluable). The two treatment groups (and patients treated with surgery alone) were virtually superimposable for clinical characteristics, sex, interval between onset of symptoms and surgery and type of local treatment, but not for the age (Table 1). Of the total number of evaluable patients (106), 70 completed the chemotherapy program, 29 with 7 cycles of ADM and 41 with 6 cycles. In the remaining 36 patients scheduled chemotherapy was interrupted or terminated for toxicity (4 cases) or relapse (32 cases). Sixteen of the 39 patients in regimen I completed the scheduled chemotherapy with 7 cycles of ADM; similarly 13 of 27 patients had 7 cycles of ADM with regimen II.

Therapeutic effects

Fifty-six of the 106 evaluable patients (53%) remained continuously disease-free with a median follow-up of 38 months (27–66) (Table 2). The percentage of continuously disease-free patients was 55% (31/56) in the group treated with moderate dose of MTX (Regimen I) and 50% (25/50) with high dose of MTX (Regimen II). The

Table 1. Comparison of the characteristics of the patients treated with regimen I and regimen II

		Regimen I	Regimen II	P
Sex	Male	55%	62%	NS
	Female	45%	38%	NS
Age	< 20 yr	80%	62%	< 0.05
	⩾ 20 yr	20%	38%	< 0.05
Onset of symptom	<3 month	48%	56%	NS
	⩾ 3 month	52%	44%	NS
Site	Proximal	8%	12%	NS
	Distal	92%	88%	NS
Extension	⩽ 1/3 of bone length	84%	80%	NS
	> 1/3	16%	20%	NS
Surgery	Amputation	71%	84%	NS
	Resection	29%	16%	NS
Surgery performed	Immediately after frozen section	45%	54%	NS
	2–21 days after biopsy	55%	46%	NS
Serum Alkaline Phosphatase	Normal	29%	22%	NS
	Elevated	71%	78%	NS

NS: not statistically significant.

difference is not statistically significant (Fig. 3).

The overall percentage of continuously disease-free patients was not different from the percentage of 132 patients previously observed at our Institution treated with other adjuvant chemotherapy regimens: ADM alone (regimen "B"), or ADM and MTX delivered in different schedules (regimen "A" and "C"), as previously reported (45–50%) (Fig. 4) [13].

However, the percentage of continuously disease-free patients with both the regimens was significantly better than the survival observed in the 39 patients (12%) treated between 1972 and 1982 with surgery only ($P < 0.0005$) (Fig. 5). The clinical features of those patients are reported in Table 3.

Fifty patients treated with regimen I and regimen II relapsed with metastatic disease: two of these patients also had a local recurrence; these two patients were treated initially by amputation

Table 2. Results according to the two regimens.

		Regimen I	Regimen II	Total
No. pts		56	50	106
Pts continuously disease-free		31 (55%)	25 (50%)	56 (53%)
Follow-up (mos)			x = 38 (27–66)	
Metastases	No	25	25	50
	median time (months)	11.1	11.6	11
Local recurrence	No	–	2	2
	Time (months)		6 and 11	
Death	No	17	18	35
	Median time (months)	19 (9–36)	22.3 (7–41)	20.6 (9–41)

*4 patients lost at follow-up after relapse.

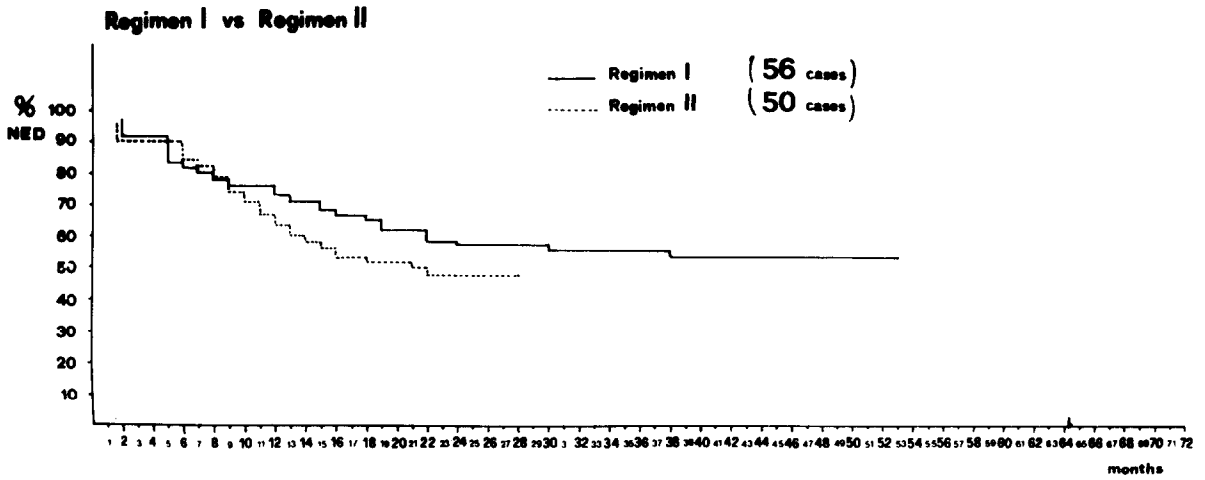

Fig. 3. Disease-free survival curve comparing regimen I and regimen II results. The difference is not statistically significant.

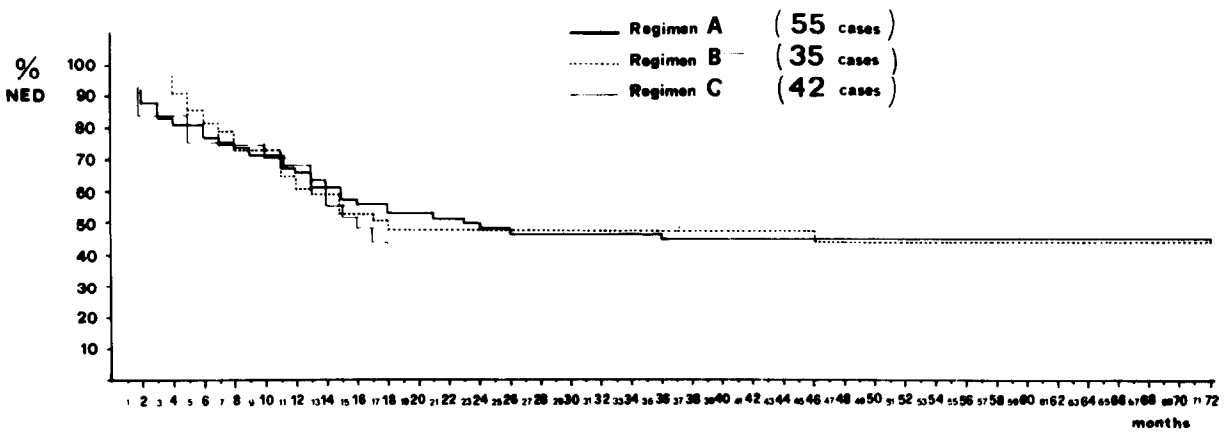

Fig. 4. Disease-free survival curve of patients treated between 1972 *and* 1979 *at the Istituto Ortopedico Rizzoli with* 3 *different adjuvant chemotherapy protocols as previously reported* [11].

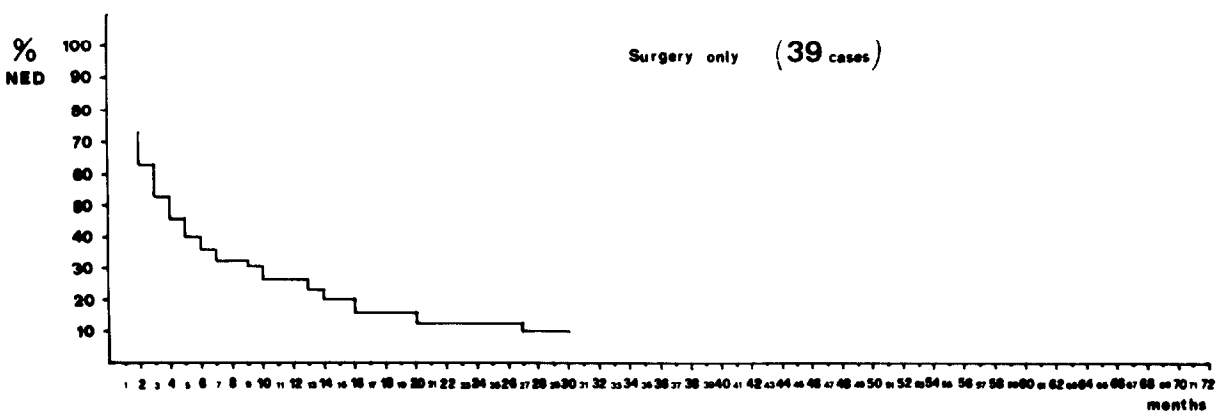

Fig.5. Disease-free survival curve of patients treated between 1972 *and* 1982 *at the Istituto Ortopedico Rizzoli with surgery alone.*

and local recurrence occurred after they had developed lung metastasis.

The site of first metastasis was lung in 44 cases followed by bone in five, and bone and skin simultaneously in one case. The time to metastases was 11 months for the lung and 12 months for the bone. The time to relapse is essentially superimposable to the time observed with the regimens A, B and C previously used in our Institution (12, 10 and 11 months, respectively) and slightly longer

Table 3. Characteristics of the patients treated only with surgery between 1972–1983.

Sex	Male	22 (56%)
	Female	17 (44%)
Age	< 20 yr	25 (65%)
	21–50 yr	14 (35%)
Onset of symptoms	< 3 months	20 (51%)
	> 3 months	19 (49%)
Site	Femur	23 (59%)
	Tibia	11 (28%)
	Fibula	2 (5%)
	Humerus	3 (8%)
Extension	< 1/3 bone length	28 (72%)
	> 1/3 bone length	11 (28%)
Surgery	Resection	7 (18%)
	Amputation	32 (82%)
Biopsy	Frozen section	18 (46%)
	Traditional biopsy	21 (54%)

than the interval observed in the patients treated with surgery alone (7.5 months).

Initially pulmonary metastasis were single in nine cases, multiple and unilateral in 25 cases and bilateral in 6. No differences were observed between regimen I and regimen II in regard to site of first relapse or number of lung metastasis. In 32 patients, the metastases appeared during the adjuvant chemotherapy period; in the remaining 18 they appeared after the end of chemotherapy. The median time to metastases was 11 months [3–24]. No differences in the median time to relapse were observed in the two groups: 11.1 months in regimen I and 11.6 in regimen II (Table 2).

Relapses were unrelated to patients age and sex, duration of symptoms before surgery, preoperative serum alkaline phosphatases levels, type of biopsy (traditional or frozen), radiologic features, size and extension of the tumor or to the number of courses of ADM (6 vs. 7). The percentage of patients who relapsed was higher for the distal femur in comparison with the proximal tibia (47% of 34 cases vs. 40% of 53 cases). However this difference was not significant.

With respect to the type of initial surgery, no significant difference in the percentage of relapses was observed in 82 patients treated by amputation and 24 treated by resection (37/82 vs. 13/24). The time to metastases in the group treated by resection was slightly delayed in comparison with patients treated by amputation (16.1 vs. 11.8 months).

Of the 50 patients who relapsed, four are alive and disease-free 13.4 months (6–38) after thoracotomy, seven are alive with uncontrolled disease (5–43 months after relapse), four are lost to follow-up after relapse and 35 are dead at a median time of 10 months (2–16) from relapse. Most of these patients were referred after relapse to other institutions and the post-metastatic treatment was not uniform.

Toxicity

One patient in regimen II died of acute nephrotoxicity and pancytopenia following the first cycle of MTX. Serum levels of the drug were not determined and the CF rescue was initiated 6 hr after MTX.

Eight patients developed clinical cardiomyopathy manifested with signs and symptoms of congestive heart failure. This occurred after 5 cycles of chemotherapy in 2, after 6 cycles in 4 and after 7 cycles in the remaining 2. In these last 2 cases the time to development of symptoms after the end of chemotherapy was 4 and 11 months, respectively. In all cases chemotherapy was stopped immediately with the appearance of clinical signs of congestive heart failure.

Two patients died of ADM congestive heart failure 12 and 14 months after the beginning of treatment (1 and 3 months after onset of symptoms of cardiomyopathy). Both patients received 7 cycles of ADM: these two patients did not have clinical or radiological signs of recurrence but autopsy was not performed. Two other patients with ADM induced cardiomyopathy died of metastatic disease 8 and 15 months after commencement of adjuvant chemotherapy. The last four patients who developed cardiotoxicity are alive and disease-free; their cardiomyopathy is well controlled with medical therapy, 8, 12, 14 and 40 months, respectively, after onset. The overall incidence of cardiomyopathy was 11% (8/70) in the total number of patients who completed the scheduled chemotherapy programs.

A single patient treated with regimen II developed a wound infection after the first cycle of ADM and all chemotherapy was discontinued: the infection was controlled with conservative surgery, drainage and antibiotics after 4 months and pulmonary metastasis developed 23 months later.

Three patients treated with regimen II developed spontaneous pneumothorax after the third, fourth and sixth cycle of MTX, respectively. In one of these patients X-rays revealed also pulmonary metastases; in the other two pulmonary metastases became radiologically evident 2 and 3 months later.

In most patients there was a transient leukopenia (leucocyte count below 2500 cu/mm) with both regimens, more pronounced after ADM. No patient had reduced doses of drugs or the next course of chemotherapy delayed due to severe and persistent leukopenia. The only protocol deviations

were those due to cardiomyopathy (4 cases) or to wound infection (1 case), as reported above.

DISCUSSION

In the past 10 years, ADM and MTX in various doses and schedules, were investigated as adjuvant or neoadjuvant chemotherapy in an effort to improve the disease-free and overall survival in osteosarcoma [1, 2, 5–9]. Commonly MTX has been delivered at a dose of about 7.5 g/m^2 or more.

The justification for the use of high dose MTX derived from dose-related responses achieved in overt disease. Graduated escalations of the MTX dose achieved responses only after reaching a MTX dose of 4.5 g/m^2 [15]. In addition subsequent reports revealed that in metastatic osteosarcoma the therapeutic efficacy of MTX could possibly be enhanced by escalating the MTX dose even further (10–12.5 g/m^2), particularly in young patients who appeared to have developed resistance to "conventional high dose" [16, 17].

While there is no doubt that in metastatic osteosarcoma the conventional (low) dose MTX is ineffective, up to now there has been no published study concerning the therapeutic efficacy of the intermediate range of MTX dosage (200–500 mg/m^2) in adjuvant treatment of localized osteosarcoma.

In the present study the percentage of continuously disease-free patients was not different when the adjuvant chemotherapy was administered as ADM plus MTX moderate dose or ADM plus MTX high dose. In our study the schedules of MTX differed not only in dosage but also in time to administration: a *bolus* for the moderate dose and a 6 hr infusion for high doses. In literature there is no data referring to the pharmacokinetics of low dose MTX (200 mg/m^2 *in bolus*); although we did not perform pharmacological studies, it is probable that with the regimen with high dose we had a plateau shape of MTX serum levels while with the regimen with moderate dose we had a peak concentration immediately after the *bolus* which then drops down. The possible significance of the difference between a uniformly lower concentration and that of a higher peak concentration is difficult to establish.

The high dose of MTX of the present study was not as high as the dose commonly administered in other studies (2 vs. 7–12 g/m^2) [1, 2, 5, 7–9]. However the "Children's Cancer Study Group" (cited by Lange and Levine [20]) has also failed to demonstrate any advantage of high dose MTX over moderate dose when this drug was used in association with Vincristine and ADM for adjuvant treatment of osteosarcoma. In this study the MTX doses were respectively 7.5 g/m^2 (high dose) and 750 mg/m^2 (moderate dose). In addition, in our study, the percentage of patients continuously disease-free appears no different from the percentage reported by other Authors who performed adjuvant chemotherapy of osteosarcoma with high dose MTX associated with Vincristine and ADM, or with Vincristine, ADM and Cyclophosphamide [2, 4, 7–10].

It seems therefore probable that when MTX is used in association with Vincristine and ADM for adjuvant treatment of osteosarcoma, high doses of the drug are just as effective as moderate doses. This fact is important because the use of high dose of MTX is approximately 7 times more expansive, more uncomfortable for the patient, requires facilities for monitoring serum levels of the drug and, most important, is potentially more toxic. In fact, even with the most careful and prudent use, with high dose MTX drug related deaths may occur [18, 19].

The percentage of patients continuously disease-free at 3 years in the current study is similar to the percentage observed in 132 patients previously treated (1972–1979) at our Institute utilizing three different adjuvant chemotherapy protocols (Fig. 4). One of these consisted of ADM only, while in the other two ADM, Vincristine and MTX were administered in a different schedule than used in the present study [13]. The fact that several different regimens of adjuvant chemotherapy for osteosarcoma seem to be equally effective has been the reason why some authors questioned the real effetiveness of adjuvant chemotherapy in osteosarcoma [10, 18–21]. For some investigators the reported improvement in disease-free survival rate may not be the result of adjuvant chemotherapy but the consequence of alterations in the natural history of this tumor [22], or of indirect manifestations of changes in selection of patients and/or of local treatment [20, 21, 23].

This led to the claim that in osteosarcoma the administration of adjuvant chemotherapy could only be justified if its efficacy is demonstrated by means of a randomized trial utilizing surgery without chemotherapy as a concurrent control arm consisting of surgery plus chemotherapy [20, 21, 23].

The data collected at our Institute in the past 10 years do not support this hypothesis. The disease-free survival in 39 patients treated in the same period with surgery only was 12% (Fig. 5). Amongst these only five patients were not given chemotherapy because it appeared medical contraindication. All other patients rejected adjuvant chemotherapy on their own accord. The survival of the latter patients treated with surgery only paralleled that of our historical controls [24], while patients treated with chemotherapy achieved a superior response equivalent to that reported with

other regimens of adjuvant therapy [1–9]. This data seems to be confirmed by the preliminary results of two of three recent studies [25–27].

Analysis of these data therefore does not reveal any change in the biological behavior of the osteosarcoma and reinforces the validity of historical controls in this tumor.

The complications encountered in our study included a fatal episode of MTX toxicity, ADM-induced myelosuppression, infection and cardiac failure (with two deaths). Despite the ADM associated leucopenia and the early start of chemotherapy after surgery, infection did not constitute a major problem; the incidence of cardiotoxicity was reduced by curtailing the number of ADM courses. Whether an alteration in the schedule of ADM to a continuous infusion, as has been claimed by others [28, 29] will also reduce the incidence of cardiotoxicity remains to be determined.

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