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Long-term outcome for patients with non-metastatic Ewing's sarcoma treated with adjuvant and neoadjuvant chemotherapies. 402 patients treated at Rizzoli between 1972 and 1992

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Abstract

We evaluated the long-term results obtained in 402 patients with non-metastatic Ewing's sarcoma (ES) of the bone treated in a single institution with adjuvant and neoadjuvant chemotherapies between 1972 and 1992. Multivariate analyses showed male gender, age older than 14 years, high serum lactate dehydrogenase (LDH) level, axial location of the tumour, use of radiotherapy alone as a local treatment, and poor histological response to chemotherapy, to be independent, adverse prognostic factors for event-free survival (EFS). At a mean follow-up of about 18 years (10–30 years), 177 patients (44.0%) remained continuously free of disease, 2 died of doxorubicin-induced cardiotoxicity and 8 developed a second neoplasm (5 died, and 3 are alive and free of disease). 215 patients relapsed with metastases and/or local recurrence: 14 are alive and free of disease, 1 is alive with uncontrolled disease, and 200 died. The overall survival (OS) at real follow-ups of 5-, 10-, 15- and 20-years was 57.2, 49.3, 44.9 and 38.4%, respectively. We conclude that since local or systemic relapses, treatment-complications and second malignancies are more common after 5 years or more from the beginning of treatment; a long-term follow-up is mandatory for patients with ES.

Keywords: Ewing's sarcoma; Adjuvant chemotherapy; Neodjuvant chemotherapy; Follow-up; Outcome; Late relapse

1. Introduction

Many uncontrolled studies have demonstrated that adjuvant and neoadjuvant chemotherapies dramatically improve the prognosis of patients with non-metastatic Ewing's sarcoma (ES), increasing the long-term survival rate from 10–15% [1–3] to 60–70% [4–15]. However, most studies reported their results only in terms of the probability of 5-year event-free survival (EFS) calculated on study populations whose minimum follow-up was often less than 3 years [4–10,13]. Only a few studies have updated these results with a longer follow-up [14,16–19].

A long-term follow-up for ES patients treated with adjuvant or neoadjuvant chemotherapies is very important for several reasons. Firstly, adjuvant and neoadjuvant chemotherapies, besides improving the cure rate, might alter the natural history of this disease, by delaying recurrence. Second of all, in patients followed for an adequate period of time, second malignant neoplasms were reported by several authors, especially radio-induced bone sarcomas [20–24]. Thirdly, the late toxic effects of chemotherapy, for instance doxorubicin (DX)-induced cardiotoxicity [25,26] and sterility [27,28], are also adverse late side-effects. Lastly, in most patients with tumours located in the extremities and treated locally with limb salvage surgery, reconstruction devices

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can produce poor functional results, which require new operations [4,28]. Further late complications, such as pathological fractures, are possible for patients treated locally by radiotherapy, and also radio-induced tumours [3–5,7,15].

This study aimed to evaluate: the EFS, the post-relapse outcome of relapsing patients, the overall survival (OS), the incidence of second malignancy, the late toxic effects of chemotherapy, and the late surgical and radiotherapeutic local complications in 402 patients with non-metastatic ES treated at the authors' institution between 1972 and 1992, with different protocols of adjuvant and neoadjuvant chemotherapies and followed-up for at least 10 years. The preliminary results of these studies, previously reported in other single papers [3,14,28,29], have been updated.

2. Patients and methods

2.1. Patient population

Patients with non-metastatic ES of the bone treated at the authors' institution between April 1972 and December 1992 with adjuvant or neoadjuvant chemotherapies were reviewed retrospectively. Eligibility criteria for entering these studies of combined treatment were: histological diagnosis of ES, no previous tumours, age under 40 years, no metastases at diagnosis, no previous treatment, interval from biopsy to beginning of therapy no longer than 4 weeks.

2.2. Diagnosis and pretreatment evaluation

The diagnosis of ES was made on representative specimens obtained by open biopsy. Standard histological investigation and immunohistochemistry studies were performed in 310 patients seen from 1984. In patients treated prior to 1984, the diagnosis was based only on histological and histochemical data. Each histopathological diagnosis was based on the presence of a small round-cell tumour occurring in the bone with no histological or immunohistological features of lymphoma, rhabdomyosarcoma or neuroblastoma. No attempts were made to differentiate typical ES from peripheral malignant neuroectodermal tumour (PNET).

The histology of all the cases and the histological response to induction treatment in patients treated with neoadjuvant chemotherapy and surgery was retrospectively evaluated by two pathologists with special expertise in bone tumours.

Initial evaluation included medical history, physical examination, haematological studies, and several chemical laboratory tests, including serum lactate deydrogenase (LDH).

Diagnostic imaging varied according to changes in radiological techniques over the 20-year period covered by our study. Local imaging of the lesion included radiographs for all patients, computed tomography (CT) in 258 and magnetic resonance imaging (MRI) in 128. To exclude metastatic disease, the first 43 patients treated had chest radiographs and, depending on the clinical evaluation, other skeletal radiographs. In patients treated after 1976, a bone scan was also used to exclude bone metastases. The 102 patients observed between 1976 and 1979 also had full chest tomography and isotope bone total body scan, while the 300 most recent patients underwent a CT scan of the chest. A bone marrow aspiration from sites distant from the tumour was performed in 176 patients.

Tumour size was estimated, using plain X-rays, on the three diameters of the lesion and calculated according to the method reported by Gobel and colleagues in Ref. [30].

2.3. Chemotherapy

Chemotherapy consisted of five different protocols that were successively activated. These protocols, reported in detail in previous papers [3,14,28,29], can be summarised as follows: in the first protocol REA-1 (April 1972–December 1978), 86 patients were treated for 24 months with a 3-drug regimen (cyclophosphamide (CP), DX and vincristine (VC)). In the second protocol, REA-2 study (January 1979 to December 1982, 59 cases), chemotherapy involved 45 weeks of treatment with a four-drug regimen (VC, dactinomycin (DT), CP and DX). In these two adjuvant studies, the local treatment was surgery performed before chemotherapy and/or radiation therapy delivered with chemotherapy. In the third protocol, REN-1 study (March 1983 to April 1987), 109 patients received two months of induction chemotherapy with three cycles of VC, DOX, CP (VAC regimen). After local treatment, patients received maintenance chemotherapy with five cycles of VC/DX, six cycles of VC/DT and six cycles of VC/CP. In the fourth protocol, REN-2 study (May 1987–July 1990; 81 cases), two cycles of VAC regimen were administered as induction chemotherapy, and four cycles of the same regimen were administered in maintenance chemotherapy, intercalated with three cycles of VC/ifosfamide (IF)/DT, followed by 3 cycles of etoposide (ET)/IF and two cycles of VC/DC/CP. In the last protocol, REN-3 study (August 1990–December 1993; 67 cases), induction chemotherapy consisted of two cycles of VAC intercalated with one cycle of VC/IF/DT. Maintenance chemotherapy was the same as that used in the REN-2 protocol.

2.4. Local treatment

Local treatment (surgery, surgery followed by radiotherapy or radiotherapy alone) was individually planned

for each patient after consultation among the radiotherapist, surgeon and medical oncologist. However, the type of local treatment was also changed over the study period. In patients treated before 1980, surgery was suggested only for tumours located in the expandable bones. After 1980, all patients were generally offered surgery as local treatment. In tumours located in the extremities, amputation was recommended in cases of pathological fractures that could not be differently treated, as well as lesions of the distal femur, leg or foot in children, since radiation therapy would cause them limb length discrepancy with a worse functional impairment than would result from amputation. In patients treated with conservative surgery, postoperative radiation therapy was given at full doses to all cases with inadequate surgical margins. In cases of resections without reconstructions in the expandable bones, postoperative radiation therapy was added at a reduced dose (40–45 Gy). The degree of histological response was not taken into account in deciding whether to use postoperative radiation therapy. After 1980, radiation therapy was used alone for unresectable tumours or in patients who refused surgery (usually amputation in adults), and when the tumour required reconstruction leading to poor functional results. Radiation therapy alone was administered at a dose of 60 Gy, as reported in detail in other papers [3,14,28,29]. Before 1991, patients received conventionally fractionated irradiation, whereas hyperfractionated irradiation was used after 1991 [31].

In patients treated with neoadjuvant protocols, local treatment was scheduled 3 weeks after the completion of induction chemotherapy. When postoperative radiotherapy was required, it was started 3 weeks after surgery. In the adjuvant protocol, chemotherapy was delivered 3 weeks after surgery, and it was contemporary to radiotherapy.

2.5. Pathological evaluation of surgically-treated patients

After surgery, all gross specimens were examined, and surface labelled histological sections were taken. Surgical margins were evaluated according to Enneking and colleagues [32]. Radical and wide margins were considered adequate, while marginal and intralesional margins were considered inadequate. Surgery through oedematous tissue was classified as marginal.

The response to chemotherapy was determined by a thorough histological examination of an entire coronal section of the tumour according to a method previously reported in Ref. [33] and classified as 'grade I' (evidence of macroscopic foci of viable tumour cells), 'grade II' (only isolated microscopic nodules of viable tumour cells), and 'grade III' (no nodules of viable cells).

2.6. Follow-up

During and after the combined treatments, patients were followed-up by a physical check-up and standard radiographs (CT scan after 1980) of the chest and involved bones. Additional studies, including biopsies, were done if indicated by the clinical and radiological examinations. These tests were carried out every 3 months for 4 years, and then twice a year for up to 10 years. After this time, for this review, patients were contacted by phone or letter. Only 2 patients, alive and free of disease at 8 and 11 years, respectively, were lost at this follow-up.

2.7. Statistical analysis

The results of treatment were evaluated in terms of EFS and OS. Both, relapse and side-effects were classified as 'early' when they happened in the first 5 years after the beginning of treatment and 'late' when they presented after 5 years.

Relapses were classified as local (local recurrence alone), systemic (metastasis alone) and combined (local recurrence and metastasis). EFS was calculated from the first day of treatment until relapse, death from toxicity or secondary malignancy. OS was calculated from the first day of treatment until death.

The following pretreatment variables were considered potential prognostic factors able to influence the pattern and time of relapse: gender, age, tumour size, site, and volume, LDH serum values at diagnosis. The cut-off values for age (± 14 years) and tumour volume (± 150 ml), were chosen to allow a comparison of our data with other authors [34,35]. However, considering the long study period over which treatments intensified, it must be remembered that the prognostic significance of these cut-off points remained constant over time. As regards treatment-associated factors, the EFS and OS were analysed according to the type of systemic chemotherapy, local treatment, and, in patients of the neoadjuvant protocols who were treated locally by surgery, also according to the surgical margins and the response to chemotherapy.

EFS and OS curves were calculated using the Kaplan–Meier method. It must be underlined that the 10-year interval data refer to real results (not projections). P values were calculated with the Brestlow and Mantel–Cox methods. Relationships between variables (patients' characteristics or type of treatment) were evaluated using the Cox proportional hazard model (stepwise). A P value <0.05 was considered significant.

3. Results

Of the 566 newly-diagnosed cases of Ewing's sarcoma of bone observed at the authors' institution between

March 1972 and December 1992, 412 were eligible, while 154 were excluded for the following reasons: metastases at presentation (123), age older than 40 years [13], previous treatment [10]. In 8 patients, there was more than one reason for exclusion.

Of the 412 eligible patients, 10 preferred to move elsewhere for treatment after diagnosis. The remaining 402 patients, treated according the protocols of chemotherapy reported before, were reviewed for this study. Their characteristics are reported in Table 1.

3.1. Local treatment

The primary tumour was treated with radiotherapy in 186 patients (46.3%), with surgery in 102 (25.4%), with surgery followed by radiotherapy in 113 (28.1%). In the remaining patient (an 18-month-old child with ES of the proximal femur whose parents refused amputation), no local treatment was performed. The percentage of surgically-treated patients was not the same in the five studies, but it increased progressively from the first (32.9%) to the last (64.1%) study.

For tumours located in the extremity, surgery consisted of limb salvage in 131 patients (86.2%), amputation in 18 (11.8%) and rotationplasty in 3 (2.0%).

In the 215 patients treated locally by surgery, the surgical margins were adequate in 183 (85.1%) (radical 18, wide 165) and inadequate in 32 (14.9%) (marginal 30, and intralesional 2). In the 158 patients treated with neoadjuvant chemotherapy and locally with surgery, the histological response to chemotherapy was good (i.e. grades II and III) in 83 patients (55.3%), and poor (grade I) in 67 (44.7%). For 8 patients, data for the histological response to chemotherapy were missing.

3.2. Outcome

The mean follow-up at 31 December 2002 was 17.7 years (10–30 years), with 187 patients (46.5%) followed-up for more than 15 years and 113 (28.1%) for more than 20 years.

3.3. Event-free survival

177 patients (44.0%) remained continuously event-free, 215 relapsed (53.5%), 8 patients (2.0%) developed a second neoplasm, and 2 patients died of DX cardiotoxicity. The adverse events were classified as 'early' in 191 patients (85.7%) and 'late' in 32 (14.3%).

The 10-year EFS and OS were 45.4% (Confidence Interval (CI) 0.480–0.460%) and 48.9% (CI 0.457–0.507%), respectively.

As shown in Table 1, by univariate analyses, the 10-year OS rate was significantly higher in females (55.6% versus 44.4%; $P<0.01$), those aged less than 14 years (55.3% versus 44.7%; $P<0.02$), with tumours located in the extremities (55% versus 45.1%; $P<0.04$), with a normal serum level of LDH at presentation (60.5% versus 39.5%; $P<0.0001$). OS was not related to tumour volume, at least at a cut-off of 150 ml.

According to local treatment, the OS was significantly higher in patients treated with surgery than in patients treated with radiotherapy (64.7% versus 36.6%; $P<0.0007$), while patients treated with surgery followed by radiotherapy had an intermediate prognosis (10-year OS = 56.6%). However, it must be stressed that radiotherapy was more often used in patients with axile lesions than in patients with extremity locations, as well as in patients treated between 1972 and 1990. In fact, a further analysis of the interactions between local treatment and other variables (Table 2), revealed significance according to site (axile versus extremity location, $P=0.0001$), and to the treatment period (1972/1990 versus 1991/1992, $P=0.0001$). In patients treated with neoadjuvant chemotherapy and surgery, the 10-year OS was significantly higher in good responders than in poor responders (80.1% versus 31.3%; $P<0.0001$).

According to the protocol of chemotherapy, OS was significantly better in patients treated with a six-drug regimen than in patients treated with a three-drug regimen (56.8% versus 34.8% $P<0.0001$), while patients treated with a four-drug regimen had an intermediate prognosis (50.0%).

Then, the following variables were included in the Cox regression model: patients' gender and age, tumour site, serum LDH values at presentation and type of local treatment. Protocols of chemotherapy were excluded because of the small numbers in each subgroup. A complete set of data was available for all patients. The following variables were significantly related to a better prognosis: normal LDH level at diagnosis ($P<0.0001$), female gender ($P<0.03$), age less than 14 years ($P<0.04$). The Odds Ratio and 95% CIs are shown in Table 3.

By multivariate analyses, only LDH serum level, and patients' gender had kept their independent significance (Table 3).

3.4. Pattern of relapse

215 patients relapsed: 141 (65.6%) with metastases, 70 (32.6%) with metastases and local recurrence, and 4 (1.9%) with local recurrence alone. The total rates of local recurrence and metastases were 18 and 52%, respectively.

According to local treatment, the rate of local relapses, was significantly higher ($P<0.0001$) in patients treated with radiotherapy alone (29.3%), than in patients treated with surgery (8.8%) or surgery followed by radiotherapy (8.9%). In surgically-treated patients, there were five local recurrences among the 32 patients

with inadequate surgical margins (15.6%) and 14 local recurrences among the 183 patients (7.7%) with adequate surgical margins. This difference was not statistically significant. However, it must been remembered that in patients with inadequate surgical margins, surgery was followed by radiotherapy.

The 70 patients who had both metastases and local recurrence had contemporary events in 31 cases; in 24 cases, local recurrence developed 2–22 months before the evidence of metastatic disease, and in 15 patients local recurrence appeared 3–30 months after metastases.

The first site of metastasis was the lung in 93 patients, bone in 79, and lung and bone in 38. In the remaining 5 patients, the first site of metastasis was the central nervous system (CNS) in 4 patients and the skin in the remaining case.

3.5. Time to relapse

The mean time to relapse was 28.1 months (2–240 years). In detail, among the 215 patients who relapsed, 187 (87.0%) did so during the first 5 years after the beginning of treatment, 25 (11.6%) between 6 and 10

Table 1
Event-free survival and 10-year overall survival according to several variables

	No. cases	% EFS	*P* value	% OS	*P* value
All cases	402	44.5		49.3	
Gender					
Male	257	40.1		44.4	
			0.01		<0.01
Female	145	52.4		55.6	
Age					
⩽14 year	190	51.6		55.3	
			0.01		<0.02
>14 year	212	38.2		44.7	
Site					
Extremity	253	49.8		55.3	
			0.01		<0.004
Other sites	149	35.6		45.1	
Serum LDH					
Normal	248	54.4		60.5	
			0.0001		<0.0001
Elevated	144	26.4		39.5	
Tumour volume (ml)					
⩽150 ml	228	46.9		52.2	
		0.22		0.09	
>150 ml	147	38.9		47.8	
Local treatment[a]					
Radiotherapy	186	32.8		36.6	
Surgery	102	59.8	0.0007	64.7	<0.0001
Surgery + radiotherapy	113	50.4		56.6	
Histological response to chemotherapy[a]					
Poor	67	26.8		31.3	
			0.0001		<0.0001
Good	83	74.6		80.1	
Protocol of chemotherapy					
Three-drug regimen	86	26.7		34.8	
Four-drug regimen	168	44.0	0.001	50.0	<0.005
Six drug regimen	148	55.4		56.8	
Protocol of chemotherapy					
REA-1	86	26.7		34.9	
REA-2	59	50.8		57.6	
REN-1	109	40.4	0.002	45.9	<0.002
REN-2	81	49.4		50.6	
REN-3	67	62.7		64.2	

LDH, lactate dehydrogenase; EFS, event-free survival; OS, overall survival; see text for definitions of the chemotherapy regimens.
[a] Some of the data are missing in these subgroups.

Table 2
Local treatment according to different variables

Variables	Radiotherapy (%)	Surgery (%)	Surgery + radiotherapy (%)	*P* value
Gender				
Male	48	28	24	<0.10
Female	43	22	35	
Age (years)				
⩽14	41	33	26	
>14	51	19	30	
Site				
Axile	64	6	30	<0.0001
Extremity	36	37	27	
Serum LDH				
Normal	58	63	69	<0.36
Elevated	39	37	28	
Tumour volume (ml)				
⩽150	39	30	31	<0.003
>150	56	20	24	
Treatment period				
1972–1990	64	5	31	<0.0001
1991–1992	38	35	27	

Table 3
Multivariate analyses of the variables significant at the univariate examination

Variable	Odds Ratio	95% CI	*P* value
Gender			
Female	1		
			<0.03
Male	1.23	1.02–1.45	
Site			
Other sites	1		
			<0.09
Extremities	0.86	0.72–1.03	
Age (years)			
>14	1		
			<0.04
⩾14	0.84	0.70–0.99	
Local treatment			
Surgery alone or + radiotherapy	1		
			<0.09
Radiotherapy	1.17	0.97–1.40	
Serum LDH			
Elevated	1		
			<0.0001
Normal	0.64	0.54–0.76	

95% CI, 95% Confidence Interval.

years, 1 between 11 and 15 years, and 2 after 15 years. Therefore, according to our classifications, there were 187 'early relapses' (87.0%) and 28 'late relapses' (13.0%). As shown in Table 4, the rate of late relapse was significantly higher in patients with normal serum levels of LDH (18.6% versus 6.9%; $P<0.01$). According to local treatment, the rate of late relapse was significantly higher in patients treated locally with surgery or surgery + radiotherapy, than in patients treated with radiotherapy alone (20.8% versus 6.7%; $P<0.004$). Considering the histological response to chemotherapy (available only for 67 patients), the rate of late relapse was significantly higher in the good responders than in the poor responders (31.6% versus 4.2%; $P<0.006$). The rate of late relapse in the 10 patients with a good response to chemotherapy and normal LDH serum value was 40% (4/10). We further analysed interactions of late relapses with the same variables in patients who were free of disease at a real follow-up of 5 years, and we found no significant correlations (Table 5).

The mean time of local recurrence was 26.6 months (2–84 months) and the mean time to the appearance of metastases was 28.8 months (2–240 months), significantly longer for patients who had lung metastases in comparison with patients whose first metastases were located in other bones or in the bones and lung simultaneously (33.3 months versus 20.3 and 23.9 months; $P<0.003$). Time to relapse was almost the same (34.4 months) for patients with first metastases in the lung, and outside of the lungs and in the bones.

Serum level of LDH, type of local treatment and histological response to chemotherapy were significantly related, not only to EFS and OS, but also to the time to relapse.

3.6. Postrelapse treatment and final outcome

The type of treatment for relapsing patients was not standard, but was chosen on an individual basis, considering the site and the number of metastases, the length of the event-free interval and the type of chemotherapy previously given.

The postrelapse outcome of the 215 relapsing patients was: 14 (6.5%) are alive and free of disease 14–121 months (mean: 79 months) after the last treatment, 200 died from their tumour 2–148 months after relapse (mean: 13.1 months), and 1 is alive with uncontrolled disease, 28 months after treatment for their third relapse. Therefore, among the 220 patients who were alive after 5 years, 30 (13.6%) have died. In detail: the OS rates at 5, 10, 15 and 20 years are reported in Table 6 (real data, not projections). The OS rate reaches a plateau at 5 years (57.2%) and progressively decreases thereafter (20 years: 38.4%). It is interesting to note that 6 patients alive at the 15-year interval died from their tumour afterwards.

After relapse, 14 patients are free of disease: 4 had had local recurrence and 10 metastases. The treatment of relapses was the following: the four recurrences were treated by surgery + further chemotherapy in 2 cases. The 10 cases of metastases, all in the lungs, were treated with a thoracotomy (5), thoracotomy + chemotherapy (4), and radiotherapy + chemotherapy (1). All 10

Table 4
Late relapses and time to relapse according to several variables

	Late relapses	(%)	*P* value	Mean time to relapse (months)	*P* value
All cases	28/215	(13.4)		28.1 (2–240)	
Gender					
Male	21/151	(13.9)		29.2 (2–240)	
			0.56		0.49
Female	7/64	(10.9)		25.6 (2–156)	
Age (years)					
⩽14	15/87	(17.2)		29.5 (3–240)	
			0.30		0.56
>14	13/128	(10.1)		27.2 (2–180)	
Site					
Extremity	14/120	(11.6)		29.4 (3–240)	
			0.78		0.57
Axile	14/95	(14.7)		27.1 (2–180)	
Serum LDH					
Normal	21/113	(18.5)		33.7 (2–240)	
			0.01		0.002
Elevated	7/102	(6.8)		21.7 (2–108)	
Tumour volume (ml)					
⩽150	20/115	(17.4)		31.3 (4–108)	
			0.20		0.68
>150	8/100	(8)		29.2 (2–240)	
Local treatment					
Radiotherapy	8/119	(6.7)		24.0 (2–180)	
			0.004		0.02
Surgery or surgery + radiotherapy	20/96	(20.8)		32.7 (2–240)	
Histological response					
Poor	2/48	(4.1)		24.0 (3–84)	
			0.006		0.02
Good	6/19	(31.5)		32.7 (2–240)	

patients who relapsed with metastases and are free of disease were among those 93 patients with metastases located in the lung, while none of the 122 patients with a different location are still alive. This difference (10/93, 10.8% versus 0/118, 0%) was highly significant ($P < 0.0009$).

In addition, the probability of rescue after relapse was strictly related to the time of relapse: 85 months for the 14 patients presently alive and free of disease, only 19 months for the 187 patients who died ($P < 0.0001$). Moreover, if we consider the patients who died from their tumour, the interval between the time of relapse and the death was significantly longer for those 21 who had a late relapse than for those 180 who had an early relapse (24.2 months versus 11.9 months; $P < 0.0003$).

3.7. Second malignancy

8 patients developed a second malignancy. These patients' features are summarised in Table 7. The primary tumour was located in the extremities in 7 cases and in the scapula in 1 patient. All had been treated locally with radiotherapy, in 2 cases combined with surgery. The second malignancies were six radio-induced bone sarcomas (five osteosarcomas and one malignant fibrous histiocytoma) (MFH) arising in the irradiation field and two acute lymphoblastic leukaemias. The 4 patients treated with radiotherapy alone who developed bone sarcomas were given a radiation dose of 60 Gy and the 2 patients treated by surgery + radiotherapy were given 44 Gy.

The rate of radio-induced sarcoma was 2.1% for the 186 patients who were treated locally with radiotherapy alone and who received between 55 Gy and 60 Gy, and 1.9% for the 102 patients treated with surgery and radiotherapy and who therefore received between 40 and 45 Gy. If we consider only the patients who survived 5 years or more, their percentages were almost the same (5/84, 5.4% versus 2/74, 2.7%).

The interval between the beginning of treatment and the appearance of a second neoplasm ranged between 4 and 20 years for the secondary sarcomas, and was only 10 and 32 months for the systemic diseases.

Table 5
Rate of relapses according to different variables, in patients disease-free at a real follow-up of 5 years

Variables	Rate of relapse (%)	*P* value
Gender		
Male	17.3	<0.23
Female	10.5	
Age (years)		
⩽14	15.9	<0.94
>14	13.8	
Site		
Axile	21.2	<0.06
Extremity	11.4	
Serum LDH		
Normal	13.6	<0.53
Elevated	15.6	
Tumour volume (ml)		
⩽150	15.7	<0.88
>150	13.9	
Local treatment		
Radiotherapy	16.6	
Surgery	9.2	<0.08
Surgery + radiotherapy	16.2	
Histological response[a]		
Poor response	14.2	<0.14
Good response	9.8	

[a] For patients treated with neoadjuvant protocols and locally by Surgery or Surgery + radiotherapy.

Table 6
Patients exposed at risk and alive at different times

	No. of patients at risk	No. of patients	(%)
5 years	402	230	(57.2)
10 years	402	198	(49.3)
15 years	243	109	(44.9)
20 years	125	48	(38.4)

The six radio-induced sarcomas were treated at our institution by surgery (amputation in 3 cases and limb salvages in 3 cases). In 3 patients, surgery was followed by further chemotherapy. The two leukaemias were treated by chemotherapy in other hospitals. 3 of the 6 patients with bone sarcomas are alive and free of disease 4, 15 and 23 years after treatment, respectively; 1 is alive with uncontrolled disease 4 years from their last treatment and 2 died after 13 and 18 months. Both patients with leukaemia died 6 and 13 months from diagnosis.

3.8. Late toxic events

The late toxic events, i.e. those occurring 5 or more years after the beginning of treatment, were the following: (a) 1 patient developed a severe doxorubicin-induced cardiotoxicity 8 years after the end of chemotherapy. This patient is now alive and well, 8 years after a heart transplantation. (b) 6 patients underwent a spermatogram that showed azoospermia 5–8 years after the end of treatment. All of them had received chemotherapy after puberty, and 4 had received a six-drug regimen, i.e. a regimen with IF and ET in addition to VC, DX, CP and DT.

There were 82 female long-term survivors, and only one of the 34 who had received chemotherapy after the time of puberty experienced a permanent amenorrhoea. However, it must be remembered that this woman was 38 years old. Menarche occurred normally in the 48 patients who had received chemotherapy before puberty. All the 17 women who desired to have a pregnancy succeeded (there were two pregnancies which ended with an early spontaneous abortion and 15 healthy newborns).

3.9. Late local complications and functional results

There were five late major local complications, all in patients with tumours located in the extremities: four concerning surgery (limb salvage); two prosthetic loosenings, one graft fracture, and one deep infection. These complications were diagnosed after 6, 8, 9 and 11 years, respectively, and were treated by surgery without the loss of the involved limb or removal of the reconstructive device.

The other late local complication was a pathological fracture of the diaphysis of the femur in a patient treated locally with radiotherapy alone (5500 r). This fracture, diagnosed 7 years after the initial treatment, was successfully treated with surgery.

4. Discussion

Over the past three decades, the outcome of patients with ES of bone that is non-metastatic at presentation has improved considerably. Progress has been possible due to the development of effective chemotherapy regimens to prevent distant metastases that were the main cause of relapse in patients with this tumour. Moreover, local control has become more effective thanks to a wider use of surgery [24,29] and the implementation of radiotherapeutical techniques [31,36]. With a multimodal approach, combining surgery and/or radiotherapy, for the local control of the primary tumour, with systemic chemotherapy to treat micrometastatic disease, several cooperative studies [5,6,8,9–13,15,34,35] as well as single institution studies [3,7,14,17,19,29,37] reported 5-year progression free survival rates of 40–60%, whereas, in the past, when the tumour was mainly

Table 7
Secondary malignancies: clinical characteristics, treatment and outcome

Patient	Gender/age (years)	Protocol of chemotherapy	Local treatment	Second neoplasm	Latency time (months)	Treatment	Outcome (m = months, y = years)
1	M/20	REA-1	RxT (60 Gy)	Radio-induced	48	Amputation	Dead after 12 m
2	F/10	REA-1	RxT (50 Gy)	ALL	10	Chemotherapy	Dead after 13 m
3	F/6	REN-1	Surgery + RxT (40 Gy)	Radio-induced osteosarcoma	48	Amputation + chemotherapy	Dead after 18 m
4	M/9	REA-1	RxT (60 Gy)	Radio-induced osteosarcoma	86	Limb salvage	NED at 20 y
5	M/17	REA-1	RxT (60 Gy)	Radio-induced MFH of bone	93	Amputation	NED at 22 y
6	M/10	REN-1	RxT (60 Gy)	Radio-induced osteosarcoma	120	Limb salvage + chemotherapy	Dead after 48 m
7	F/14	REA-2	RxT (56 Gy)	ALL	32	Chemotherapy	Dead after 6 m
8	F/17	REA-2	Surgery + RxT (44 Gy)	Radio-induced osteosarcoma	240	Limb salvage + chemotherapy	NED at 5 y

M, male; F, female; RxT, radiotherapy; NED, no evidence of disease; MFH, malignant fibrous histiocytoma; ALL, acute lymphoblastic leukaemia.

treated with radiotherapy alone, the cure rate was less than 15% [1,2]. However, most recent adjuvant and neoadjuvant studies have reported their results in terms of the probability of EFS up to 5 years, often calculated on study populations whose maximum follow-up was less than 3 years [4–10,13]. For this reason, it is still uncertain whether these promising results reflect a real, reliable final cure rate. The data of the few papers with updated results with a minimum of more than 5 years follow-up are contrasting: some authors [9,16] have reported that, even if late events occurred, the projected 5-year disease-free survival (DFS) equated to the cure rate, whereas other authors [3,19] have found that more than 10% of patients could relapse or die of a second neoplasm after that time.

The updated results of our adjuvant and neoadjuvant studies indicate that a long-term DFS can be achieved in approximately 50% of patients. None the less, the data based on a 5-year follow-up are not reliable. In fact, 37 out of 212 patients who were alive and free of disease at the real 5-year follow-up had a recurrence or a second neoplasm after that time, and 36 of them died.

In other words, the OS rate was the highest at 5 years, but progressively declined up to 20 years. Considering real data and not projections, we had the following rates: 57.2% at 5 years, 49.3% at 10 years, 44.9% at 15 years and 38.4% at 20 years. It may be suggested that patients with a longer follow-up who were treated with less effective protocols of chemotherapy have affected these rates. However, this is not true because patients treated according to the first protocol were all early relapses. Moreover, it is important to notice that only in the first 83 patients, treated before 1978, was the 5-year OS significantly lower than in patients treated with the following protocols.

With our follow-up, the 10-year OS was significantly correlated with patients' gender and age, with tumour site, with serum LDH levels at presentation, and, in patients treated with neoadjuvant chemotherapy + surgery, with the histological response to preoperative treatment. In other words, female gender, age less than 14 years, the extremity location of the tumour, normal serum values of LDH and a good histological response to preoperative chemotherapy, are all independent favourable prognostic factors. In contrast to other reports, modalities used for local control and tumour volume were not significant predictors of outcome for the entire group of patients. Perhaps the two decades of accrual in several 'treatment eras', as well as the different radiographical methods available during the time these patients presented may explain these discrepancies. These data confirm other authors' results [26,19] and some of our previous observations [14], in patients followed for shorter periods of time. It is interesting to note that the presence of prognostic factors such as a normal serum level of LDH at presentation

and a good histological response to chemotherapy, not only increases the long-term survival rates, but it also significantly delays the time of relapse. This is important because it shows that there is a strict correlation between the time of the first relapse and the probability to be rescued by further therapies in ES, as has been observed for other tumours. In fact, in our study, the rate of patients that were rescued after a late relapse and who are presently free of disease was significantly higher than in patients who relapsed early (28.5% versus 0.3%, $P < 0.0001$). We also observed that in patients who died from their tumour, the interval between the time of relapse and the death was significantly longer for late relapses in comparison with early relapses. So it is possible to say that an ES-patient with a normal LDH serum level and a good histological response has more chance of being cured, and if relapse occurs, this is usually late and it can still be cured.

We would also like to focus on the fact that all 14 patients who were long-term event-free survivors after treatment of their recurrence did not have metastases located in the bone or in the bone + lung. The very poor prognosis of patients with metastases outside of the lung has also been recently reported by other authors [38,39].

The occurrence of a second malignant neoplasm in successfully treated ES patients has become an area of increasing concern. From early reports [20–22], it appears that the rate of second malignancies in ES patients was alarmingly high. While there is no doubt that cancer treatment increases the risk of developing second malignancies, from a recent study, the second cancer-risk in patients with ES seems to be in the range expected for all cancer survivors [24]. In fact, in 690 patients treated with multimodal therapy between 1992 and 1999, only 6 (0.9%) developed a second neoplasm. However, it must be stressed that the median observation time for survivors was less than 3 years. In our study, with a follow-up of at least 10 years, the percentage of second malignancy was more than double this: eight second tumours in 402 patients (2.0%).

Two of these second tumours were leukaemias, which appeared early, and six were radio-induced bone sarcomas, diagnosed 4–20 years after the initial treatment. It is interesting to note that two radio-induced sarcomas occurred in patients treated locally with surgery + radiotherapy, with relatively low doses of radiation (44 Gy). This contrasts with the data by Kuttesk and colleagues [23] who reported a radiation dose-dependency in 10 radio-induced bone sarcomas, in 226 long-term survivors of ES; none of these tumours was observed among patients who had received a dose of less than 48 Gy.

There is no doubt about the radiation dose-dependency of radio-induced sarcomas, while further studies are needed to determine whether low doses, as was observed in two cases in our series, can cause this catastrophic event. Our positive observation is that, despite the very poor prognosis of the radio-induced sarcomas, [40] 3 of our 6 patients with secondary sarcomas are alive and free of disease after 5, 20 and 22 years, respectively; only 1 of them had an amputation, and only one received further chemotherapy.

The major chemotherapy-induced late complication was a case of severe DX cardiomyopathy, successfully treated by a heart transplantation. Other authors have previously reported cases of late DX-induced cardiotoxicity [25,26].

Another important chemotherapy-related complication is male infertility. This is not a new finding, since infertility has been reported as a late effect in many long-term survivors treated with chemotherapy [26,27], as well as the possibility of its reversibility. Infertility is probably not a late event, but it is usually diagnosed late, since spermatograms are not routinely performed, and ES patients are generally young, and are not thinking about the possibility of procreation at that time. In addition, in our group of patients, it was not possible to exactly evaluate the extent of this side-effect because a sperm-count was not routinely measured. According to the literature, infertility due to chemotherapy is mainly a male problem, and females are less severely affected. This was also confirmed by our study, where there were no problems for females, whereas 6 male patients who performed a spermatogram showed azoospermia, 5–8 years after completion of chemotherapy.

Major late local complications are also possible. We observed 5 cases requiring further surgery (in 1 case an amputation was performed due to a deep infection).

In ES, as in other tumours, it is generally accepted that patients with no evidence of disease (NED) 5 years from the beginning of treatment are expected to live as long as someone without a history of cancer. According to our results, this 5-year cut-off point should be extended to ensure reliability. In fact, 35 (15.2%) of our 231 patients who were alive after 5 years died afterwards, and 10 (5.1%) of the 197 patients alive after 10 years later died of relapses or second tumours. In conclusion, the updated results of our studies show that patients with ES of bone who were successfully treated must be carefully followed-up for a long period of time in order to identify late relapses, late treatment-related complications and second neoplasms, especially radiation-induced sarcomas, at an early stage.

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