Original Article

Late Effects of Chemotherapy and Radiotherapy in Osteosarcoma and Ewing Sarcoma Patients

The Italian Sarcoma Group Experience (1983-2006)

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BACKGROUND: Patients with osteosarcoma and Ewing sarcoma have achieved longer survival over the past decades, but late side effects of chemotherapy and radiotherapy have become important concerns. **METHODS:** The authors reviewed all patients with localized osteosarcoma or Ewing sarcoma who had been enrolled in the Italian Sarcoma Group neoadjuvant protocols from 1983 through 2006. Data were updated in December 2010 to determine 3 endpoints: the incidence of a secondary primary cancer (designated as "second malignant neoplasm" [SMN]), infertility, and cardiotoxicity. **RESULTS:** Data were available on 883 patients with osteosarcoma and 543 patients with Ewing sarcoma. In the osteosarcoma group, there were 39 SMNs (4.4%) in 36 patients; in the Ewing sarcoma group, 15 patients (2.8%) experienced a single SMN each. The cumulative 10-year and 20-year incidence of an SMN (±standard error) was 4.9% ± 0.9% and 6.1% ± 1.2%, respectively, in the osteosarcoma group and 3.4% ± 0.9% and 4.7% ± 1.6%, respectively, in the Ewing sarcoma group. The most common SMN in the osteosarcoma group was breast cancer (n = 11), and the most common SMN in the Ewing sarcoma group was radiotherapy-induced osteosarcoma (n = 6). After 20 years, the risk of developing an SMN increased, whereas the risk of a recurrence of the primary tumor decreased. Permanent sterility was more common in males than in females. Doxorubicin cardiotoxicity occurred in 18 patients with osteosarcoma (2%) and in 7 patients with Ewing sarcoma (1.3%). **CONCLUSIONS:** The awareness of late side effects in long-term survivors of primary bone cancers should encourage longer follow-up. ***Cancer* 2012;118:5050–9.** © *2012 American Cancer Society.*

KEYWORDS: bone sarcoma, second malignant neoplasm, childhood cancer survivors, cardiotoxicity, infertility.

INTRODUCTION

Osteosarcoma and Ewing sarcoma are rare tumors that are more common in pediatric and adolescent age groups. In the past few decades, long-term survival in affected patients has improved as a result of the success of multimodal therapy; today, the 10-year survival rate has reached approximately 50%.[1] However, long-term survival is sometimes complicated by the late side effects of chemotherapy and radiotherapy.

Investigators with the Childhood Cancer Survivor Study[2] calculated the overall effects of primary childhood cancer and its treatment on the life expectancy of survivors. They calculated that the life expectancy of a group of patients aged 15 years who had survived for 5 years was 50.6 years. They determined that this figure represented a loss of life expectancy of 10.4 years (17.1%) compared with the general population. The average reduction in life expectancy ranged from 4 years among kidney cancer survivors to >17 years among brain and bone cancer survivors. In addition, those investigators estimated that 1 in 4 survivors would be expected to die of either a late recurrence of the primary cancer, a second malignant neoplasm (SMN), or a cardiac condition. We previously reported the results from long-term side effects of earlier adjuvant treatments in patients with osteosarcoma and Ewing sarcoma.[1,3] In this article, we report the incidence of SMN, infertility, and doxorubicin cardiotoxicity in patients with osteosarcoma and Ewing sarcoma patients who had localized disease at diagnosis after treatment with chemotherapy and radiotherapy.

MATERIALS AND METHODS

Study Population

Seven different protocols for localized osteosarcoma[4-10] (Table 1) and 4 protocols for localized Ewing sarcoma[11-14] (Table 2) were received by patients at our Institute (Rizzoli Orthopedic Institute [IOR]) in Bologna, Italy and in other Italian

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Table 1. Summary of Osteosarcoma Protocols

Protocol	No. of Patients	Drugs and Dosage	Comments
IOR-OS1, 1983-2986	127	Doxorubicin, 360-450 mg/m^2; cisplatin, 300-750 mg/m^2; methotrexate, 3750-37 500 mg/m^2	Duration of doxorubicin infusion, 8 h; SMN, 5 patients; cardiomyopathy, 1 patient
IOR-OS2, 1986-1989	164	Doxorubicin, 480 mg/m^2; cisplatin, 600 mg/m^2 (240 mg intra-arterially); methotrexate, 40,000 mg/m^2; ifosfamide, 30,000 mg/m^2 (for poor responders); etoposide, 1080 mg/m^2	Duration of doxorubicin infusion, 8 h; SMN, 13 patients [a]; cardiomyopathy, 7 patients
IOR-OS3, 1990-1993	138	Doxorubicin, 300-360 mg/m^2; cisplatin, 600 mg/m^2; methotrexate, 50,000 mg/m^2; ifosfamide, 30,000 mg/m^2	Duration of doxorubicin infusion, 8 h; SMN, 6 patients[a]; cardiomyopathy, 1 patient
IOR-OS4, 1993-1995	133	Doxorubicin, 390-480 mg/m^2; cisplatin, 600 mg/m^2; methotrexate, 60,000-72,000 mg/m^2; ifosfamide, 32,000-42,000 mg/m^2	Duration of doxorubicin infusion: 8 h; SMN, 4 patients; cardiomyopathy, 4 patients
Pilot ISG-OS, 1996-1997	68	Doxorubicin, 420 mg/m^2; cisplatin, 480 mg/m^2; methotrexate, 60,000-72,000 mg/m^2; ifosfamide, 60,000-75,000 mg/m^2	Duration of doxorubicin infusion, 24 h; SMN, 3 patients; cardiomyopathy, 1 patient
ISG/SSG, 1997-2000	125	Doxorubicin, 330 mg/m^2; cisplatin, 480-600 mg/m^2; methotrexate, 48,000-60,000 mg/m^2; ifosfamide, 60,000-75,000 mg/m^2	Duration of doxorubicin infusion: 24 h; SMN, 3 patients; cardiomyopathy, 1 patient
ISG-OS1, 2001-2006	128	Doxorubicin, 420 mg/m^2; cisplatin, 600 mg/m^2; methotrexate, 60,000 mg/m^2; ifosfamide, 40,000 mg/m^2	Duration of doxorubicin infusion: 24 h; SMN, patients 5[a]; cardiomyopathy, 3 patients

Abbreviations: IOR, Rizzoli Orthopedic Institute; ISG, Italian Study Group; OS, osteosarcoma; SMN, second malignant neoplasm; SSG, Scandinavian Study Group.
[a] Includes 2 SMNs in a single patient.

Table 2. Summary of Ewing Sarcoma Protocols

Protocol	No. of Patients	Drugs and Dosage	Comments
REN-1, 1983-1987	91	Doxorubicin, 480 mg/m^2; vincristine, 39 mg/m^2; dactinomycin, 7.5 mg/m^2; cyclophosphamide, 18,000 mg/m^2	RT, 45-55 Gy; SMN, 2 patients; cardiomyopathy, 1 patient
REN-2, 1988-1991	78	Doxorubicin, 400 mg/m^2; vincristine, 18 mg/m^2; dactinomycin, 6.25 mg/m^2; cyclophosphamide, 8400 mg/m^2; ifosfamide, 54,000 mg/m^2; etoposide, 1500 mg/m^2	RT, 44-60 Gy; SMN, 2 patients; cardiomyopathy, 1 patient
REN-3, 1991-1999	186	Doxorubicin, 400 mg/m^2; vincristine, 19.5 mg/m^2; dactinomycin, 6.5 mg/m^2; ifosfamide, 54,000 mg/m^2; etoposide, 1500 mg/m^2	RT, 45-60 Gy; SMN, 8 patients; cardiomyopathy, 2 patients
ISG/SSG III, 1999-2006	188	*Good responders:* Doxorubicin, 400 mg/m^2; vincristine, 21 mg/m^2; dactinomycin, 6 mg/m^2; cyclophosphamide, 6000 mg/m^2; ifosfamide, 72,000 mg/m^2; etoposide, 1800 mg/m^2; *poor responders:* doxorubicin, 320 mg/m^2; vincristine, 15 mg/m^2; dactinomycin, 1.5 mg/m^2; cyclophosphamide, 6400 mg/m^2; ifosfamide, 21,000 mg/m^2; etoposide, 1700 mg/m^2; busulfan/melphalan	*All responders:* RT, 45-54 Gy; SMN, 3 patients; cardiomyopathy, 3 patients; *poor responders:* busulfan/melphalan followed by HDCT-PBSCR[a] Busulphan 4 mg/kg/d d1-4, Melphalan 140 mg/m^2 d5

Abbreviations: Gy, grays; HDCT, high-dose chemotherapy; ISG, Italian Study Group; PBSCR, peripheral blood stem cell rescue; REN, Rizzoli Ewing neoadjuvant; RT, radiotherapy; SMN, second malignant neoplasm; SSG, Scandinavian Study Group.
[a] HDCT with busulfan and melphalan plus PBSCR.

Sarcoma Group (ISG) centers (Mayer Pediatric Oncology Institute, Florence; Pediatric Oncology Department, National Cancer Institute, Milan; Department of Pediatric Oncology, Regina Margherita Hospital, Turin; Department of Medical Oncology, Gradenigo Hospital, Turin; and Department of Medical Oncology, Santa Chiara Hospital, Pisa) from January 1983 through December 2006. These cooperative trials enrolled patients aged $\leq$40 years. Patients who met our inclusion criteria, which were described in the articles cited above, were registered at the operations office of the Rizzoli Institute. The diagnosis of osteosarcoma or Ewing sarcoma was

confirmed by a pathologist experienced in bone sarcoma. Eligible patients entered into active treatment protocols after providing informed consent. Member institutions submitted biannual follow-up reports on all enrolled patients. These reports included information on survival, disease status, development of a second malignancy, cardiotoxicity, and fertility status. An *SMN* was defined as a cancer of a histologic type distinct from the primary bone sarcoma histiotype. *Cardiotoxicity* was defined as a left ventricular ejection fraction (LVEF) <50%; *early toxicity* was defined as toxicity that occurred during or soon after the completion of chemotherapy (0-2 months); *late cardiotoxicity* was defined as cardiopathy that occurred after 2 months; and *infertility* was defined according to Common Toxicity Criteria for Adverse Events version 3.0—that is, oligo/azospermia for males and permanent amenorrhea for females. For patients who had not been seen during the previous 3 years, a telephone call was made by the oncologist researcher. The date of the last follow-up was December 31, 2010. The results from those trials have been published.[4-14]

The Rizzoli Ewing neoadjuvant 3 (REN-3) trial protocol for Ewing sarcoma enrolled 157 patients, and it ended in 1997. However, from 1997 to 1999, when the subsequent ISG/Scandinavian Study Group (SSG) III protocol was initiated, 29 additional patients received the same REN-3 treatment, and they were included in this analysis, bringing the total number of patients in this protocol to 186 (Table 2)

For the patients who developed an SNM, the date of diagnosis, the tumor's histologic characteristics, and the tumor site were recorded. Also recorded was the interval between the date that the primary sarcoma was diagnosed and the date that the SMN was diagnosed.

Statistical Analysis

Overall survival was calculated from the first day of chemotherapy until death or the most recent follow-up. Survival curves were calculated according to the Kaplan-Meier method. The cumulative incidences of recurrent primary tumors and SMNs were calculated separately according to the method described by Kalbfleisch and Prentice,[15] in which death from any cause that occurred before an SMN was considered a competing risk. The time during which a patient was at risk of developing an SMN was calculated from the date of the primary sarcoma diagnosis to the date of the SMN diagnosis, the date of death, or the date of the most recent follow-up, whichever was earliest.

RESULTS

Our cohort consisted of 883 patients with osteosarcoma (508 males and 375 females) who were ages 1 to 40 years at diagnosis (median age, 15 years) and 543 patients with Ewing sarcoma (337 males and 206 females) who were ages 1 to 40 years at diagnosis (median age, 16 years).

Osteosarcoma Cohort

In total, 898 patients were enrolled on the 7 osteosarcoma protocols from January 1983 through December 2006. In this group, 15 patients (1.7%) were lost to follow-up, leaving 883 evaluable patients.

At study's end, 573 patients remained alive (342 males and 231 females), and 310 patients had died (166 males and 144 females). Of the 310 patients who died, 280 died of osteosarcoma, 16 died of SMN, 6 died of acute chemotherapy toxicity, 6 died of cardiotoxicity, and 2 died of other causes (1 pulmonary embolism and 1 suicide). Of the 573 patients who remained alive, we were unable to contact 45 patients for follow-up, but we know that they indeed were alive at their last follow-up 3 years earlier. These 45 patients had a follow-up of at least of 10 years (median follow-up, 131 month; range, 41-244 months), and they were included in this analysis with the assumption that they were alive and disease free at the time of the study (December 2010).

The overall median follow-up for the entire group of 883 patients with osteosarcoma was 126 months (range, 1-327 months). The median follow-up for the 573 patients who remained alive was 142 months (range, 41-327 months), and the median overall survival for the 310 patients who died was 33 months (range, 1-253 months). The 10-year, 20-year, and 25-year cumulative mortality rates (±standard error) from recurrent osteosarcoma were 39% ± 1.8%, 40% ± 1.9%, and 50% ± 2%, respectively (Fig. 1).

Ewing Sarcoma Cohort

In total, 581 patients were enrolled in 4 Ewing sarcoma protocols from 1983 through 2006. Of these, 38 patients (6.5%) were lost to follow-up, leaving 543 evaluable patients. At study's end, 323 patients remained alive (200 males and 123 females), and 220 patients had died (137 males and 83 females). Of the 220 patients who died, 212 died of Ewing sarcoma, 5 died of SMN, and 3 died of acute complications; none died of cardiopathy. Of the 323 patients who remained alive, 19 had not made a follow-up visit during the preceding 3 years, and they could not be reached by telephone or mail. For the purposes of the current analysis, they were considered to be alive and disease-free at the time of their most recent follow-up.

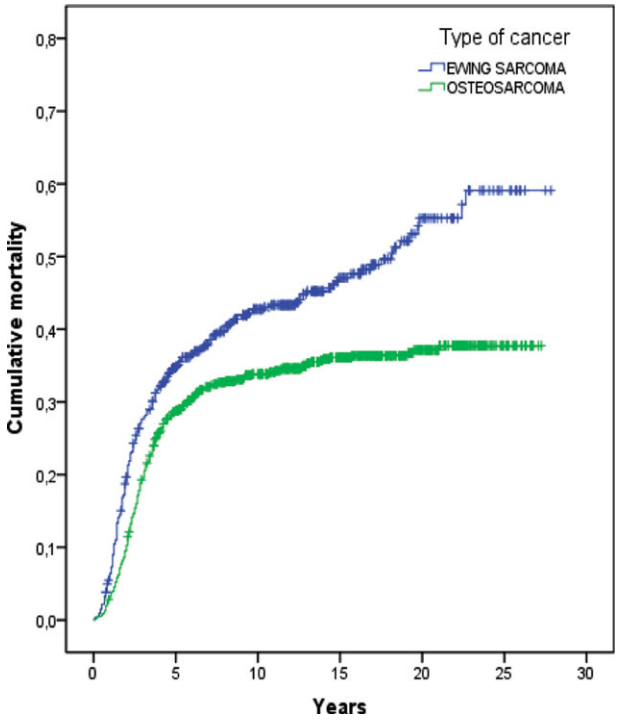

Figure 1. Overall mortality is illustrated for patients with osteosarcoma and Ewing sarcoma. SMN indicates second malignant neoplasm.

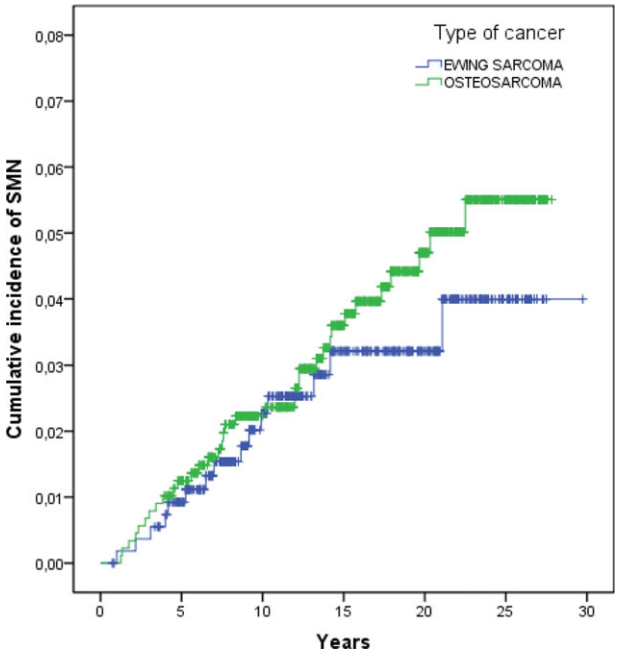

Figure 2. The cumulative incidence of secondary malignant neoplasm (SMN) is illustrated for patients with osteosarcoma and Ewing sarcoma.

The overall median follow-up for the entire group of 543 patients with Ewing sarcoma was 86 months (range, 1-330 months). The median follow-up for the 323 patients who remained alive was 141 months (range, 9-334 months), and the median overall survival for the 220 patients who died was 25 months (range, 1-22 months). The cumulative mortality rate (±standard error) from the primary cancer, including recurrence, was 54% ± 2.6% at 10 years, 58% ± 1.9% at 20 years, and 61% ± 2.4% at 25 years (Fig. 2).

Of all 543 patients, 276 received radiotherapy (145 received radiotherapy to an axial bone, and 131 received radiotherapy to an extremity) either alone or combined with chemotherapy. Among the 188 patients in the ISG/SSG III protocol, 79 received high-dose chemotherapy (HDCT) with busulfan and melphalan followed by peripheral blood stem cell rescue.

Second Malignant Neoplasms

Osteosarcoma cohort

In total, 39 SMNs were identified in 36 patients with osteosarcoma (Table 3). This group included 20 females and 16 males. Among the SMNs were 11 breast cancers (10 females and 1 male), 9 leukemias, 5 sarcomas (4 soft tissue sarcomas and 1 Ewing sarcoma), 3 parotid cancers, 2 non-Hodgkin lymphomas, 2 colon cancers, 2 central nervous system cancers, 1 squamous cell carcinoma of the skin, 1 melanoma, 1 renal cancer, 1 thyroid cancer, and 1 ovarian cancer (Table 3).

The median interval from the diagnosis of osteosarcoma to the diagnosis of an SMN was 96 months (range, 15-270 months). The median interval was 34.5 months (range, 15-165 months) for hematologic SMNs (ie, leukemias and lymphomas) and 144 months (range, 33-270 months) for solid tumors. The cumulative incidence rate (±standard error) of SMNs in the osteosarcoma group was 4.9% ± 0.9% at 10 years, 6.1% ± 1.2% at 20 years, and 6.9% ± 1.1% at 25 years (Fig. 1). In total, 16 of the 36 patients died of their SMN.

Of the 3 patients who had 2 SMNs each, 1 was a boy aged 12 years who developed acute myelogenous leukemia 26 months after the diagnosis of osteosarcoma; 10 years later, he developed a metastatic breast cancer. He was positive for Li-Fraumeni syndrome according to a DNA test that was obtained after he developed the SMN. Another of these patients was a woman aged 34 years who developed colon cancer 36 months after the diagnosis of osteosarcoma and almost simultaneously developed a glioblastoma; she died just 5 months later. The third patient with multiple SMNs was a woman aged 36 years who developed breast cancer 18 months after she was diagnosed with osteosarcoma; 2 years later, she developed

Table 3. Selected Characteristics of Patients With Osteosarcoma Who Developed a Second Malignant Neoplasm

Patient No.	Age, y	Sex	Overall Survival, mo	SMN	Interval, mo[a]	Protocol	Status
1	13	F	299	Breast cancer	98	IOR OS1	Alive
2	10	F	268	Thyroid cancer	98	IOR OS1	Alive
3	16	F	159	AML	68	IOR OS1	Dead
4	14	M	111	Liposarcoma	10	IOR OS1	Dead
5	20	F	292	NHL	126	IOR OS1	Alive
6	18	M	273	Angiosarcoma	2	IOR OS2	Alive
7	13	F	273	Breast cancer	129	IOR OS2	Alive
8	14	F	281	Breast cancer	48	IOR OS2	Alive
9	17	F	267	Breast cancer	96	IOR OS2	Alive
10	13	F	189	Breast cancer	8	IOR OS2	Dead
11	36	F	75	Breast and ovarian cancer	18	IOR OS2	Dead
12	13	M	182	Parotid cancer	102	IOR OS2	Alive
13	12	F	246	ALL	230	IOR OS2	Alive
14	11	F	282	ALL	261	IOR OS2	Alive
15	10	F	234	Ewing sarcoma	19	IOR OS2	Dead
16	19	M	267	Soft tissue sarcoma	194	IOR OS2	Alive
17	14	M	91	Soft tissue sarcoma	24	IOR OS2	Dead
18	11	F	237	Breast cancer	1	IOR OS3	Alive
19	15	F	209	Breast cancer	1	IOR OS3	Alive
20	30	M	72	Glioblastoma	18	IOR OS3	Dead
21	12	M	173	ALL and breast cancer	147	IOR OS3	Dead
22	22	M	153	NHL	5	IOR OS3	Dead
23	14	F	202	Colon cancer	12	IOR OS4	Alive
24	13	F	190	Breast cancer	29	IOR OS4	Alive
25	11	F	63	AML	17	IOR OS4	Dead
26	16	M	137	Melanoma	103	IOR OS4	Dead
27	19	F	156	Breast cancer	8	Pilot ISG	Dead
28	21	M	182	AML	38	Pilot ISG	Alive
29	11	M	76	ALL	35	Pilot ISG	Dead
30	26	M	110	SCC of skin	18	ISG/SSG	Dead
31	27	M	146	Parotid cancer	54	ISG/SSG	Alive
32	14	M	146	Renal cancer	24	ISG/SSG	Alive
33	11	F	117	Parotid cancer	29	ISG/OS1	Alive
34	34	F	41	Colon and CNS cancer	5	ISG/OS1	Dead
35	7		78	AML	50	ISG/OS1	Alive
36	16	M	25	ALL	8	ISG/OS1	Dead

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; F, female; IOR, Rizzoli Orthopedic Institute; ISG, Italian Study Group; M, male; NHL, non-Hodgkin lymphoma; OS1, Osteosarcoma Study 1; SCC, squamous cell carcinoma; SSG, Scandinavian Study Group.
[a] Interval indicates the time between the diagnosis of Ewing sarcoma and the diagnosis of SMN.

ovarian cancer, from which she died. No cluster of tumors was identified in the families of these 3 patients. Genetic testing was not performed in either of the 2 women to rule out *p53*, *BRCA1*, or *BRCA2* mutations. All patients received treatment in hospitals for their SMN except those who developed a second sarcoma as an SMN. Breast cancer, as mentioned above, was the most common SMN in the osteosarcoma group, occurring in 10 females and 1 male. The median age of these 11 patients was 13 years (range, 11-36 years) at the time of osteosarcoma diagnosis and 31 years (range, 22-41 years) at the time of breast cancer diagnosis.

Ewing sarcoma cohort

In total, 15 patients with Ewing sarcoma developed an SMN (Table 4). This group included 8 females and 7 males. Among the SMNs in this group were 6 radiotherapy-induced osteosarcomas, 2 leukemias, 2 parotid cancers, 2 thyroid cancers, 1 non-Hodgkin lymphoma, 1 breast cancer, and 1 melanoma (Table 4).

The median interval from the diagnosis of Ewing sarcoma to the diagnosis of an SMN was 84 months (range, 12-253 months). The median intervals were 37 months (range, 26-110 months) for hematologic SMNs and 94 months (range, 12-253 months) for solid tumors. At study's end, 10 of these patients remained alive, and 5 patients had died.

Among all 15 patients with Ewing sarcoma, 10 received local radiotherapy at a median dose of 57 grays (Gy) (range, 42-60 Gy). Among the 6 patients who developed radiotherapy-induced osteosarcoma, 2 received radiotherapy after surgery at a dose of 45 Gy, and the

Table 4. Selected Characteristics of Patients With Ewing Sarcoma Who Developed a Second Malignant Neoplasm

Patient No.	Age, y	Sex	Overall Survival, mo	HDCT	SMN	Interval, mo[a]	Protocol	Local Tx	RT Dose, Gy	Status
1	6	F	101	No	RT-induced OS	78	REN-1	Surgery+RT	45	Dead
2	12	F	284	No	Breast cancer	253	REN-1	RT	60	Alive
3	14	F	43	No	AML	37	REN-2	RT	60	Dead
4	Y	M	153	No	RT-induced OS	84	REN-2	RT	60	Dead
5	19	M	233	No	RT-induced OS	119	REN-2	RT	60	Alive
6	10	M	179	No	RT-induced OS	170	REN-3	RT	60	Dead
7	17	M	148	No	Melanoma	12	REN-3	Surgery		Alive
8	10	F	141	No	Parotid cancer	48	REN-3	Surgery		Alive
9	13	M	140	No	Parotid cancer	50	REN-3	Surgery		Alive
10	20	M	151	No	RT-induced OS	63	REN-3	Surgery+RT	45	Alive
11	16	F	167	No	Thyroid cancer	124	REN-3	Surgery+RT	45	Alive
12	11	F	217	No	Thyroid cancer	158	REN-3	Surgery		Alive
13	39	M	37	No	AML	26	ISG/SSG III	Surgery		Dead
14	11	F	104	Yes	RT-induced OS	104	ISG/SSG III	RT	54	Alive
15	31	F	122	No	NHL	110	ISG/SSG III	Surgery+RT	42	Alive

Abbreviations: AML; acute myelogenous leukemia; F, female; HDCT, high-dose chemotherapy; ISG, Italian Study Group; M, male; NHL, non-Hodgkin lymphoma; OS, osteosarcoma; REN, Rizzoli Ewing neoadjuvant therapy; RT, radiotherapy; SMN, second malignant neoplasm; SSG, Scandinavian Study Group.
[a] Interval indicates the time between the diagnosis of Ewing sarcoma and the diagnosis of SMN.

other 4 patients received radiotherapy as the only local treatment (median dose, 60 Gy; range, 54-60 Gy). Only 1 patient received HDCT. The cumulative incidence rate of SMN (±standard error) was 3.4% ± 0.9% at 10 years, 4.7% ± 1.6% at 20 years, and 5% ± 1.3% at 25 years (Fig. 2). In total, 5 of the 15 patients died of their SMN.

Infertility

We evaluated fertility status in males and females with osteosarcoma and Ewing sarcoma separately.

Males with osteosarcoma

Only 54 of the 342 male patients with osteosarcoma who remained alive underwent a sperm analysis after chemotherapy; and the median interval between the diagnosis of osteosarcoma and the sperm analysis was 9.5 years (range, 4-27 years). At the time they received chemotherapy, 10 of these patients were prepubertal, and 44 were postpubertal. Of these 54 patients, 36 had azoospermia (5 prepubertal patients and 31 postpubertal patients), 11 had oligospermia (<20 million spermatozoa/mL), and 7 were normospermic. In all, fertility was impaired in 47 of the tested male patients (87%). Permanent infertility was most common in patients who had received >40,000 mg/m^2 of ifosfamide.

Two patients (1 enrolled in the ISG/SSG I protocol and 1 enrolled in the ISG/OS1 protocol) who were azoospermic during the first 5 years after treatment became normospermic 10 years after their diagnosis of osteosarcoma.

Nineteen males went on to father a total of 24 children. Only 2 of these 19 fathers had undergone a sperm test after chemotherapy. These 2 patients were not able to conceive naturally, and their sperm test indicated that they were azospermic. Therefore, they underwent testicular biopsy sperm extraction and in vitro fertilization, which produced a total of 3 healthy children; neither of these patients had cryopreserved his sperm before chemotherapy (1 was still prepubertal). The other 17 males fathered a total of 21 children naturally.

Females with osteosarcoma

Of the 231 female patients with osteosarcoma who remained alive, 207 were evaluated for infertility; 53 were prepubertal and 154 postpubertal. In total, 115 postpubertal females (75%) experienced amenorrhea during chemotherapy. The median time to resumption of menstruation after chemotherapy was 4 months (range, 1-12 months). Only 6 of the females experienced permanent amenorrhea, and 4 of these were aged >35 years at the time of diagnosis. Those prepubertal patients who had menarche after chemotherapy had no delay in puberty. In all, fertility was impaired in 6 of the 207 tested females (2.8%).

Some 28 females delivered a total of 41 healthy children. The median age at first pregnancy was 28 years (range, 17-36 years). Also, 5 females had a total of 4 voluntary and 2 spontaneous abortions, and there was 1 stillbirth.

Males with Ewing sarcoma

Of the 200 male patients with Ewing sarcoma who remained alive, 23 agreed to undergo a sperm analysis a

median of 11 years (range, 4-25 years) after their diagnosis of Ewing sarcoma. At the time they received chemotherapy, 5 patients were prepubertal, and 18 were postpubertal. Only 1 of these patients had received HDCT. Testing revealed that 13 patients were azoospermic, 8 were oligospermic, and 2 were normospermic. In all, fertility was impaired 21 of the 23 tested males (91%).

After chemotherapy, 9 males had a total of 16 children. One of these patients required testicular biopsy sperm extraction, intracytoplasmic sperm injection, and in vitro fertilization; this patient was prepubertal when he was first diagnosed with Ewing sarcoma.

Females with Ewing sarcoma

Of the 123 female patients with Ewing sarcoma who remained alive, 99 had their fertility status evaluated after chemotherapy. Of these 99 patients, 36 were prepubertal, and 63 were postpubertal; their median age at diagnosis was 15 years (range, 1-40 years). Fertility evaluation revealed that 25 females had permanent amenorrhea—15 as a result of HDCT and 6 as a consequence of radiotherapy. Three of the former group had received HDCT as second-line chemotherapy after they experienced a disease relapse. Four women had permanent amenorrhea, although they had not undergone any radiotherapy or HDCT; the ages of these 4 patients were 28 years, 30 years, 35 years, and 40 years. In all, early iatrogenic menopause occurred in 29 of the 99 tested patients (29.2%). The other patients had menstruation, but this does not exclude infertility (capability to conceive), because only pregnancy is proof of fertility.

Older age at the time of diagnosis of Ewing sarcoma was a predisposing factor for sterility in these patients, just as it was for the females with osteosarcoma. In all, 19 of these females became pregnant and delivered a total of 31 healthy children; only 1 premature delivery was reported. The median age at the time of the first pregnancy was 26 years (range, 20-34 years). There were 3 voluntary abortions. In all 4 subgroups, no increase in congenital malformations was observed in the offspring of any patient who had conceived after therapy.

Cardiotoxicity

Osteosarcoma cohort

Of the 883 patients with osteosarcoma, 18 (2%) experienced cardiomyopathy. Of these 18 patients, there were 11 females and 7 males, and their median age was 12 years. Cardiomyopathy was evidenced by a reduction in LVEF to <45%. The median interval from the cessation of chemotherapy to the onset of cardiomyopathy was 2 months (range, 0-234 months). Seven patients experienced early cardiotoxicity (0-2 months), and the other 11 patients had delayed symptoms onset (range, 3-234 months).

Of the 18 patients who experienced cardiomyopathy, 16 were symptomatic for congestive heart failure. Half of the 18 patients died—6 of congestive heart failure and 3 of metastatic cancer. Among the 9 patients who remained alive, 4 patients underwent heart transplantation and remained alive and free of disease, 3 were receiving medication for dilated cardiomyopathy, and 2 achieved normalization of their LVEF. The median total dose of doxorubicin in the 18 patients was 480 mg/m^2 (range, 300-480 mg/m^2).

Ewing sarcoma cohort

Cardiotoxicity was observed in 7 of the 543 patients with Ewing sarcoma (1.3%), including 4 males and 3 females (median age, 8 years). The median interval from the cessation of chemotherapy to the onset of cardiomyopathy was 3 months (range, 0-138 months). Early cardiotoxicity was observed in 3 patients at 0 months, 2 months, and 2 months; the others had delayed cardiotoxicity (range, 3-138 months).

At study's end, all 7 patients remained alive. Four of these patients were receiving medical treatment for dilated cardiomyopathy, 2 underwent heart transplantation, and 2 achieved normalization of their LVEF after 6 months and 12 months. The median total dose of doxorubicin received by the 7 patients was 400 mg/m^2 (range, 400-420 mg/m^2). A summary of the study findings is provided as a flow chart in Figure 3.

DISCUSSION

Osteosarcoma and Ewing sarcoma, along with Hodgkin lymphoma and central nervous system tumors, are associated with the greatest risks for adverse chronic health conditions in long-term survivors. These adverse conditions include SMNs, sterility, and cardiac impairment.[16]

Second Malignant Neoplasms

Our findings with regard to SMNs are in accordance with those of previous studies, in which the cumulative incidence of SMNs 20 years after a diagnosis of bone sarcoma ranged from 1.6% to 5.4%.[17,18] Compared with other analyses of patients with Ewing sarcoma who received both adjuvant and neoadjuvant chemotherapy on earlier protocols (1972-1999),[2] our study indicated a lower rate of radiotherapy-induced osteosarcoma; this lower rate probably was attributable to the smaller doses of radiotherapy that were used in the more recent protocols. Likewise, the low cumulative incidence of SMNs and

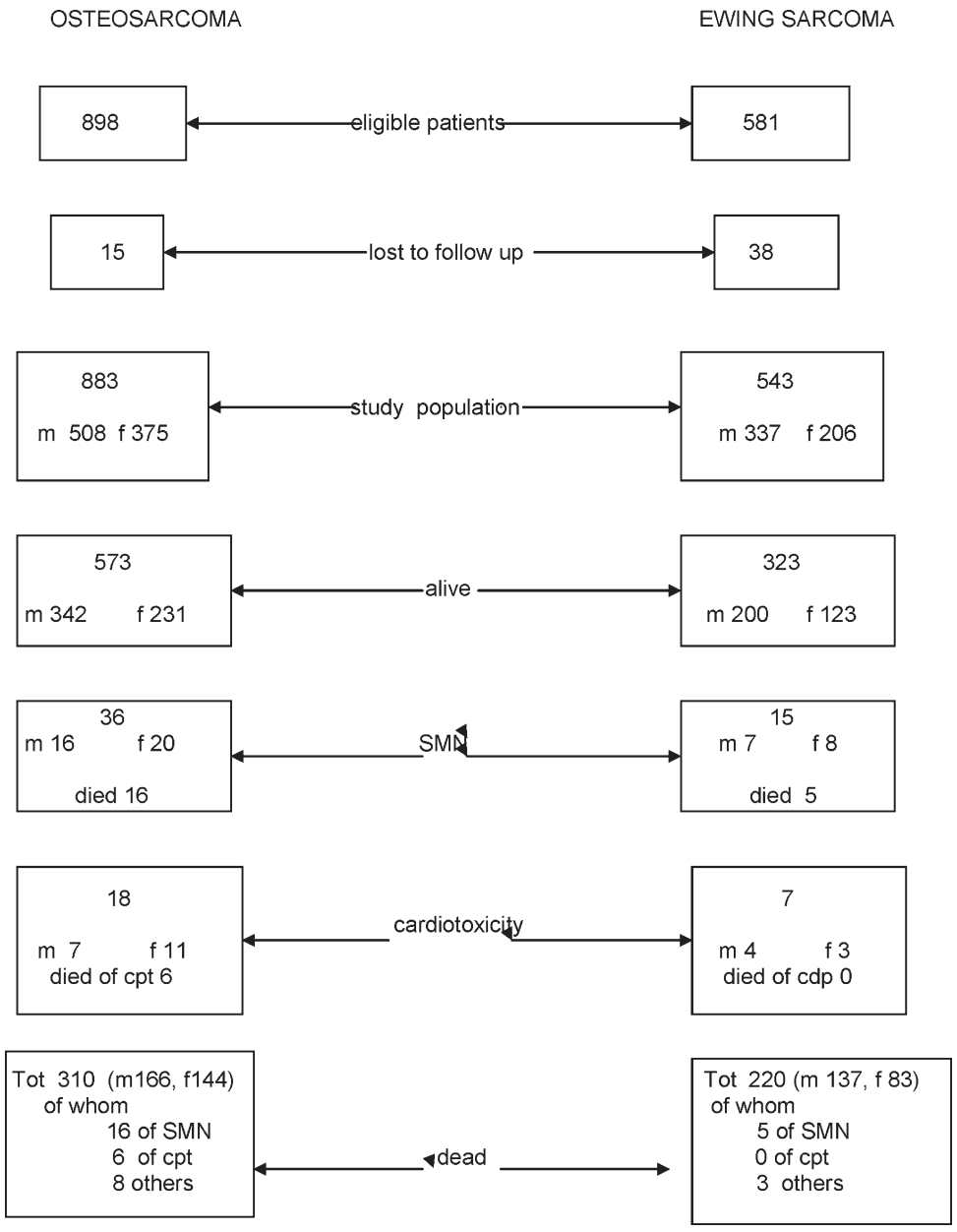

Figure 3. This is a flow chart of the study population. SNM indicates second malignant neoplasm; m, male; f, female; cpt, cardiomyopathy; Tot, total.

cardiotoxicity in our Ewing sarcoma patients can be attributed in part to the smaller doses of doxorubicin used in the more recent protocols. Another reason for the low incidence may be the relatively high proportion of patients with Ewing sarcoma who were lost to follow-up—38 of 581 patients (6.5%) with Ewing sarcoma compared with 15 of 898 patients (1.7%) with osteosarcoma. Finally, the median duration of follow-up was much shorter in the Ewing sarcoma cohort than in the osteosarcoma cohort (86 months and 126 months, respectively).

It is interesting to note that, 10 to 20 years after the diagnosis of a primary bone sarcoma, our patients were more likely to develop an SMN than a recurrent bone cancer. This finding is consistent with other reports.[19] In our study, breast cancer was the most common SMN among the osteosarcoma survivors, but only 1 breast cancer was diagnosed in our Ewing sarcoma survivors. Breast cancer also was the most common SMN in the Childhood Cancer Survivor Study.[20] For our study, we were not able to analyze standardized incidence ratios or the absolute

excess risk of SMN among the general pediatric population, because there is no national pediatric tumor registry in Italy, although there is a network (the Italian Association of Cancer Registries [AIRTum]) of several regional tumor registries that cover approximately 33% of the pediatric population. Only estimated data are available from the projection of data from 1 region (Piedmont) over the entire country.[21]

The median age at osteosarcoma diagnosis in those patients who developed breast cancer was younger than that of the osteosarcoma group as a whole (13 years and 15 years, respectively). Neglia et al[17] analyzed 13,581 long-term pediatric cancer survivors and observed that, in females, a younger age at diagnosis (range, 5-9 years) was a risk factor for breast cancer. This finding may be related to the exposure of growing breast tissue to carcinogens. Other studies have reported a high incidence of breast cancer as an SMN after chest radiotherapy in patients with Hodgkin lymphoma and after total lung irradiation in patients with Ewing sarcoma.[16,17] A greater risk of breast cancer as an SMN also was reported in females who did not receive any chest radiotherapy. A possible genetic predisposition has been proposed by Oeffinger and Bhatia[22] and by Ginsberg et al[23]; the latter group reported a 9% cumulative incidence of SMN at 25 years in patients with Ewing cancer, which was almost twice as high as our finding. However, their study was conducted from 1970 through 1986, whereas our study was conducted from 1983 through 2006. The use and dose of radiotherapy for local treatment have decreased over the years. In the study by Ginsberg et al, radiotherapy was received by 80% of patients compared with approximately 50% of patients in our study.

In our study, more SMNs occurred in females than in males (28 SMNs vs 23 SMNs, respectively). If we consider patients with multiple SMNs, the ratio is 30:24. Because our study included more males than females, the disparity according to sex was greater than what it appears to be based simply on the number of SMNs in each group. Other investigators have reported that female sex is an unfavorable prognostic factor for an SMN[17] as well as for cardiotoxicity.[24]

Infertility

We were able to evaluate male infertility with a sperm test in only 16% of our patients with osteosarcoma and in 11% of our patients with Ewing sarcoma. There appeared to be some reluctance among males to undergo a sperm test, and it was much easier to evaluate the females on the basis of their menstrual status. Our own group reported previously that sterility in patients with osteosarcoma affects males more than females and that the risk of sterility is directly proportional to the total dose of the alkylating agent.[25] The risk of sterility also is greater for patients of both sexes who receive HDCT followed by peripheral blood stem cell rescue. We also reported previously that the most important predictive factor for sterility in females is age.[26] In patients with Ewing sarcoma, the major cause of infertility in both males and females is HDCT and subsequent autologous stem cell transplantation. Nonetheless, the small number of patients evaluable for fertility in our study certainly is a bias and makes it difficult to draw conclusions At our institute, we have offered women the option of cryopreservation of their ovarian tissue since 2006; for men, sperm banking is a common practice. Obviously, sperm banking is not an option for prepubertal males, and long-term infertility is certainly a possibility. However, these patients still may benefit from testicular biopsy sperm extraction, intracytoplasmic sperm injection, and in vitro fertilization.[27,28] Thanks to progress in fertility preservation techniques, 3 patients in our study were able to father children by in vitro fertilization.

Cardiotoxicity

In our study, cardiotoxicity was more common in the osteosarcoma cohort (2%) than in the Ewing sarcoma cohort (1.3%); the latter group received lower doses of doxorubicin, and all of these doses were infused over 24 hours rather than over 8 hours (the shorter infusion was received by patients with osteosarcoma in the earlier protocols). In fact, the greatest incidence of cardiomyopathy (7 of 18 patients) occurred on the earlier IOR-2 protocol, in which patients received the highest dose of doxorubicin (480 mg/m^2) over the short 8-hour time frame. Prolonged infusion has been identified as a protective factor against anthracycline (eg, doxorubicin) cardiotoxicity.[29]

Patients in our investigation who developed cardiotoxicity generally were younger; also, there was a slight preponderance of females among the patients with osteosarcoma, but not among the patients with Ewing sarcoma. Younger age as a risk factor for cardiotoxicity was observed by Pratt et al.[30] Those authors also observed that anthracycline cardiotoxicity was more relevant in children aged <4 years, because it impairs the growth of myocardiocytes.

Several hypotheses to explain the female predilection for cardiotoxicity have been proposed. Lipshultz et al based their theory on the finding that females have more body fat than males and that anthracyclines are poorly

absorbed in fat tissue; thus, they reasoned that the cellular concentration of anthracyclines would be increased in nonadipose tissue, such as heart muscle, in patients who receive doses calculated on the basis of body surface area.[24] Another explanation was offered by Krischer et al, who cited sex-related difference in anthracycline pharmacokinetics; compared with males, females have a lower clearance and a lower area under the curve ratio.[31]

In conclusion, all efforts should be made to monitor patients who have survived bone sarcoma with prolonged follow-up and to inform patients of possible late complication of their previous medical treatment. Efforts also are needed to balance treatments while reducing late side effects.

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