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Prognostic significance of spontaneous tumour necrosis in osteosarcoma

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Abstract. Preoperative chemotherapy is an integral part of the management of osteosarcoma, and the extent of tumour necrosis found at operation is an important prognostic variable. Knowledge about spontaneous, pretherapy necrosis is difficult to obtain but provides important quantitative information about the necrotic effect of chemotherapy. Using three different methods, we studied spontaneous tumour necrosis in 20 localized intramedullary osteosarcomas of the distal femur diagnosed between 1963 and 1972. All patients received surgical treatment only. All six patients with spontaneous necrosis involving more than 20% of tumour died. Five of 14 patients with necrosis amounting to less than 20% were long-term, disease-free survivors. The extent of necrosis was independent of tumour size. Two semiquantitative methods of evaluation were easily applied and reproducible. Spontaneous necrosis in untreated osteosarcomas occurs frequently; extensive necrosis may indicate a rapid clinical course. Tumour necrosis can be quantified reliably in clinical work.

Key words: Chemotherapy – Morphometry – Necrosis – Osteosarcoma – Prognosis

Introduction

Tumour necrosis resulting from preoperative chemotherapy is a recognized prognostic determinant in osteosarcoma. A statistically significant relationship exists between the extent of postchemotherapy necrosis, measured in percentage of necrotic tumour mass, and postoperative survival (Raymond et al. 1987; Rosen et al. 1979). Sequential angiography and magnetic resonance imaging before and during preoperative chemotherapy, by providing a record of changes in neoplastic angiogenesis, have proved useful, if indirect, measures of the response of the tumour to preoperative chemotherapy (Kumpan et al. 1986; Pan et al. 1990). The underlying assumption is that untreated primary osteosarcoma is, to a great extent, composed of viable tumour cells. In malignant tumours in general, the relative volume of spontaneous necrosis is considered to be an expression of a given tumour's growth rate and thus its biological aggressiveness. A highly necrotic osteosarcoma would be expected to spread earlier, independent of other clinicopathological variables, than a largely viable tumour. Knowledge about the extent of pretreatment necrosis in a given tumour would influence the appraisal of the effects of chemotherapy. Data on prechemotherapy necrosis can clearly be obtained only by indirect means, not by a thorough histopathological evaluation in any way comparable to the work-up of resection specimens.

We studied 20 preoperatively untreated high-grade intramedullary osteosarcomas of the distal femur seen at the Mayo Clinic between 1963 and 1972 in order to map the extent of spontaneous necrosis and analyse the relationship between the extent of necrosis and clinical outcome. We compared methods for the evaluation of tumour necrosis.

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Materials and methods

Twenty patients with high-grade intramedullary osteosarcoma localized to the distal femur at the time of diagnosis were included in the study. Their ages ranged from 10 to 51 years (mean 16 years). No patient received preoperative or postoperative radiation or chemotherapy. With the exception of sections taken at the time of initial diagnosis, the entire tumour tissue from all patients was preserved in formalin and available for analysis.

The gross specimens were processed as follows: a 3 mm slice of the entire specimen parallel to the initial plane of sectioning was obtained with a band saw, every attempt being made to include the largest diameter of tumour as well as the greater of the two minor axes. This slice was placed in a transparent plastic envelope and photocopied. This photocopy was used as a template to identify the location of each section of tumour submitted for routine tissue processing. Between seven and 20 (mean 13) slides stained with haematoxylin and eosin were made from each tumour.

During microscopic review, areas of viable and nonviable tumour were outlined with a marking pen on the glass slide, the slide was photographed, and the print was enlarged threefold. Three methods of evaluation were used. Method 1 used an XY-plotter (HI-PAD Digitizer; Houston Instruments, Austin, Tex.) and an IBM PC-XT microcomputer to evaluate and calculate areas of viable and necrotic tumour on each photograph. In method 2, a stereological assessment of viable and necrotic areas was obtained by placing a grid over the enlarged prints and using point counting with a manual haematology cell counter. In method 3, microscopical estimate of the relative area of necrosis on each slide was made, without the aid of other techniques. The results of methods 1 and 2 were expressed as percentages, by area, of necrotic tumour. The microscopic estimation of necrosis (method 3) was expressed in two ways: as grades of necrosis, slightly modified from other authors (Rosen et al. 1979; Salzer-Kuntschik et al. 1983a, b) – grade 1, little or no necrosis; grade 2A, less than 50% necrosis by area: grade 2B, 50–95% necrosis; grade 3, more than 95%; grade 4, no viable tumour identified – and as a composite percentage. Method 3 was applied at the time of microscopic review and without knowledge of the results from the other two methods.

Necrosis was defined as the absence of viable tumour cells. Loss of viability denoted the failure of tumour cell nuclei to take up the haematoxylin stain. Thus defined, necrosis may be represented by eosinophilic coagulative necrosis of the tumour (Fig. 1). Haemorrhagic necrosis (Fig. 2) constituted another pattern of viability loss. A third pattern showed preservation of architecture, including tumour cell boundaries. Here, cytoplasm and, in particular, nuclei were discerned as eosinophilic silhouettes or "ghosts". Mere smudging or distortion of nuclei (Fig. 3) did not constitute necrosis. The tumours were staged according to the Musculoskeletal Tumor Society (Enneking 1988). Follow-up information was obtained from the patients' Mayo Clinic charts.

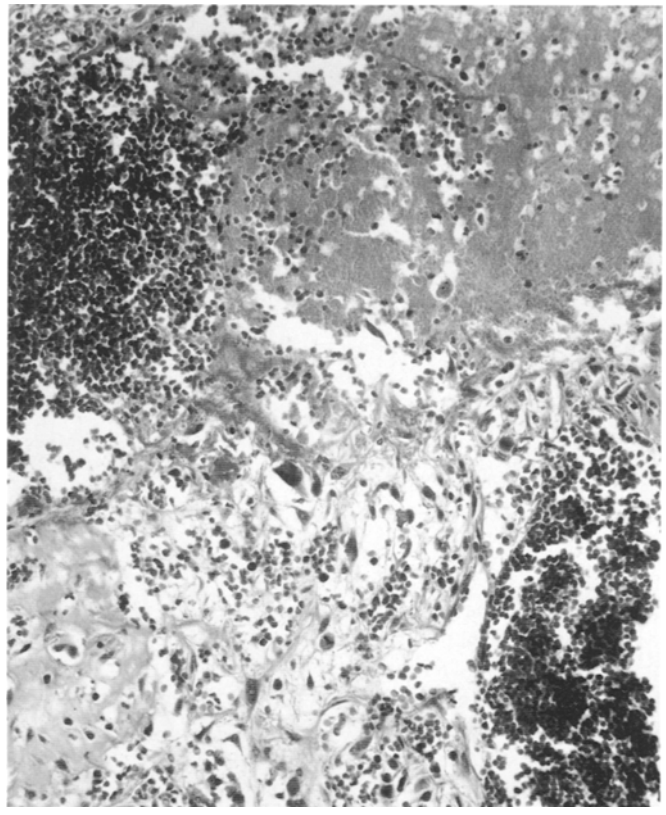

Fig. 2. Haemorrhagic necrosis. Viable tumour cells in lower half of photomicrograph. (Haematoxylin and eosin, ×250)

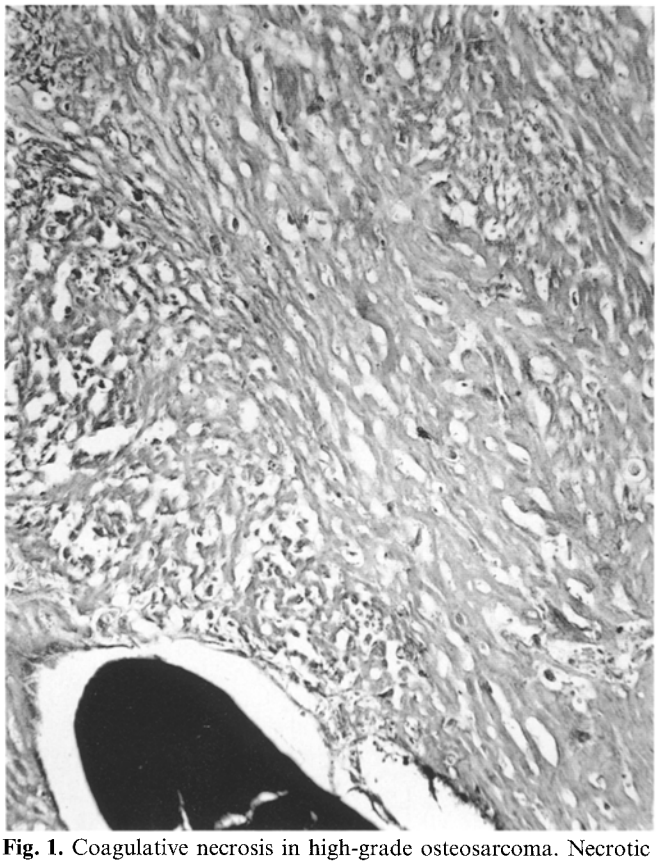

Fig. 1. Coagulative necrosis in high-grade osteosarcoma. Necrotic tumour surrounding a bony trabecula. (Haematoxylin and eosin, ×250)

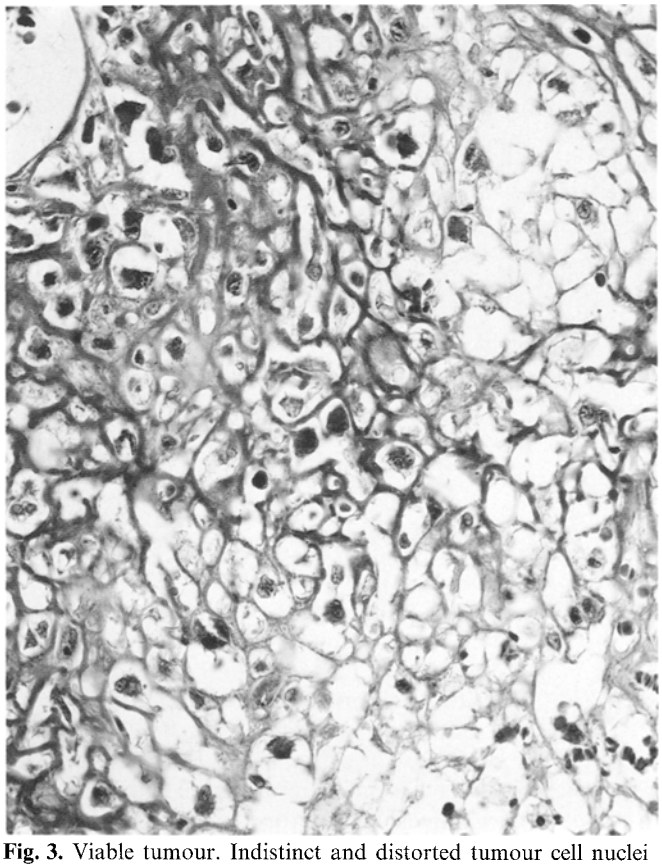

Fig. 3. Viable tumour. Indistinct and distorted tumour cell nuclei with smudged chromatin. An identical histological appearance may result from chemotherapy. (Haematoxylin and eosin, ×250)

Table 1. Clinical and pathological characteristics of 20 patients with osteosarcoma of the distal femur

Patient no.	Age (years/sex)	Tumour size (cm)	Osteosarcoma type[a]	Stage[b]	Follow up	(months)[c]	Necrosis (%)			Necrosis grade (microscopic estimate)[d]
							Digitizing pad	Point counting	Microscopic estimate	
1	10/M	11	O	IIB	DOD	28	1	1	<1	2A
2	20/F	12	O	IIB	DOD	17	36	33	22	2A
3	11/M	6	O	IIA	DOD	9	38	39	31	2A
4	13/F	8.5	O	IIB	NED	122	2	2	2	2A
5	14/F	8.5	O	IIA	DOD	11	2	2	2	2A
6	11/M	12	O	IIB	NED	150	5	5	10	2A
7	16/M	10	T	IIB	DOD	11	12	12	10	2A
8	16/F	6.5	O	IIA	NED	117	1	1	<1	2A
9	17/F	10	O	IIB	DOD	6	42	41	18	2A
10	14/M	8	F	IIB	DOD	7	13	12	11	2A
11	17/F	10.5	C	IIB	NED	183	0	0	0	1
12	12/M	8	O	IIB	DOD	8	69	67	41	2B
13	14/F	9	O	IIB	DOD	5	0	0	0	1
14	16/M	9	O	IIB	DOD	6	3	2	<11	2A
15	11/F	16	O	IIB	NED	159	19	19	1	2A
16	18/M	8	O	IIB	DOD	45	2	2	1	2A
17	51/F	8	O	IIB	DOD	44	25	21	10	2A
18	15/M	14.5	T	IIB	DOD	6	49	50	62	2B
19	10/F	11	C	IIB	DOD	17	0	0	0	1
20	10/M	8.5	O	IIB	DOD	14	3	3	0	1

No patient received chemotherapy or radiation

[a] C, Chondroblastic; F, fibroblastic; O, osteoblastic; T, telangiectatic

[b] Stage IIA, Localized high-grade tumour without cortical breakthrough; stage IIB, localized high-grade tumour with cortical breakthrough

[c] DOD, Died of osteosarcoma; NED, no evidence of disease

[d] Grade 1, Little or no necrosis; grade 2A, necrosis <50%; grade 2B, 50–95%; grade 3, >95%; grade 4, no viable tumour

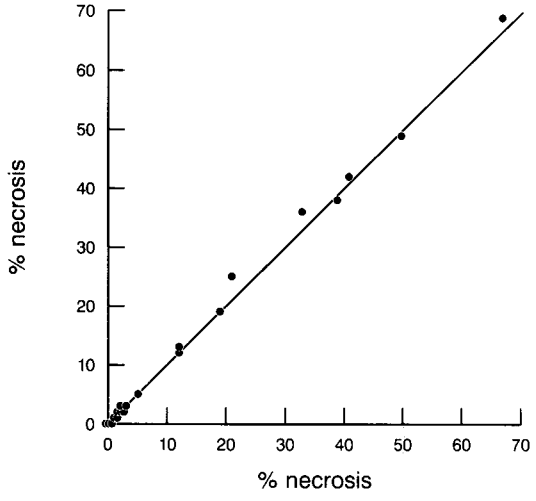

Fig. 4. Necrosis (%) in osteosarcoma tissue measured by method 1 (digitizing pad), *y*-axis, and method 2 (point counting), *x*-axis. $r = 0.998$, $P < 0.0001$

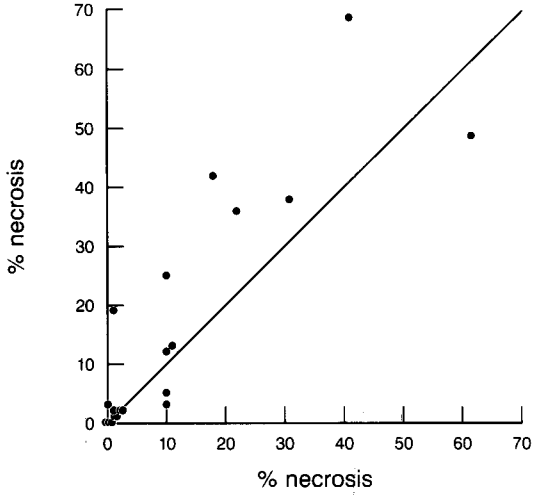

Fig. 5. Necrosis (%) in osteosarcoma tissue measured by method 1 (digitizing pad), *y*-axis, and method 3 (microscopic estimate), *x*-axis. $r = 0.88$, $P < 0.001$. Values from method 3 were significantly lower than from method 1, $P < 0.05$

Results

Table 1 shows the clinical variables for each of the 20 patients as well as results of measurement of tumour necrosis. There was a close correlation between values for necrosis derived by methods 1 and 2 (Fig. 4). The results of microscopic estimation of necrosis, although significantly correlated with the results of the other two methods (e.g. method 1), did not correlate as clearly as methods 1 and 2 did with each other (Fig. 5). Values from method 3 were generally lower than from method 1. There was no relationship between the extent of necrosis, as determined by any of the methods, and tumour diameter (Fig. 6).

Six tumours showed greater than 20% necrosis (range 25–67%; mean 43%). All six patients died of their dis-

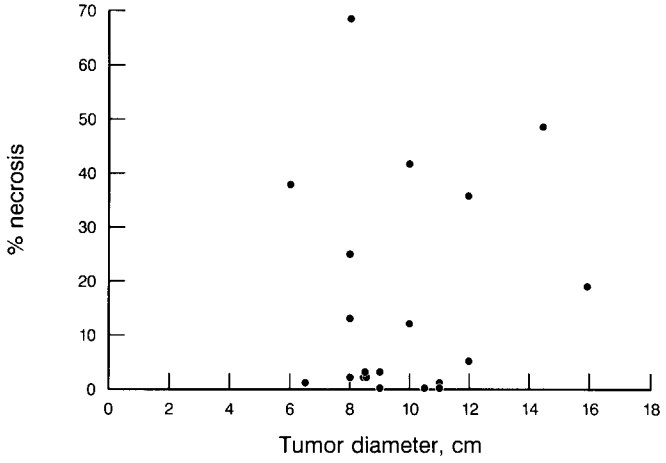

Fig. 6. Necrosis (%) in osteosarcoma tissue measured by method 1 (digitizing pad), y-axis, and tumor size, x-axis. $r=0.12$, $P>0.05$

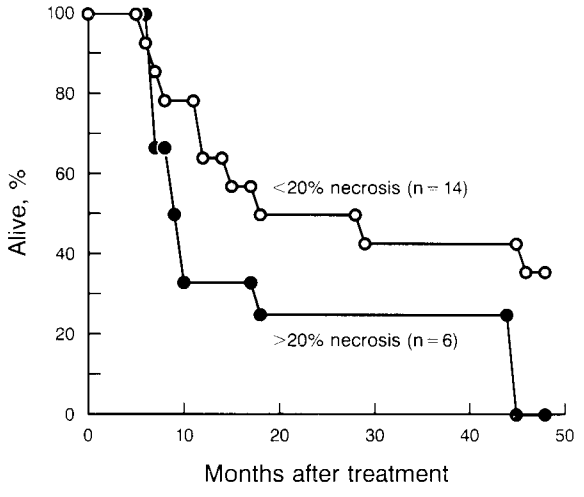

Fig. 7. Percentage alive by time (months) after treatment for patients with <20% necrosis and with >20% necrosis. Rank-sum test, $P=0.07$

ease 6–44 months (mean 15 months) postdiagnosis. Fourteen patients had less than 20% necrosis in their tumours (range 0–19%; mean 4%). Nine of these 14 patients (64%) died of disease 5–45 months (mean 16 months) postdiagnosis. The remaining five living patients had a mean observation period of 146 months (range 117–183 months). The overall difference in survival (Fig. 7) was marked: at 2 years, 17% of the high-necrosis and 50% of the low-necrosis patients were alive. Statistical testing by the rank-sum test, however, resulted in a P value of only 0.07.

Discussion

Postchemotherapy necrosis in conventional high-grade osteosarcomas is an important prognosticator of biological behaviour. The extent of postchemotherapy necrosis in a surgically removed primary tumour is considered to be indicative of a particular patient's responsiveness to a particular chemotherapeutic regimen (Rosen et al. 1982, 1984). Raymond and colleagues (1987) found that, of several contributing clinicopathological determinants, tissue necrosis was the single most significant predictor of prognosis in a given patient. This interdependence of necrosis in the primary tumour and prognosis appears to hold true even if metastases show less necrosis than, or a cellular composition different from, the primary tumour (Nachman et al. 1987; Roessner et al. 1984).

Little attention has been paid to the presence, extent, and biological significance of spontaneous, pretreatment necrosis in osteosarcomas. A positive correlation between spontaneous necrosis and survival would tend to diminish the favourable prognostic impact of postchemotherapy necrosis, thus weakening the arguments in favour of presurgical chemotherapy. However, if spontaneous necrosis adversely affected the prognosis, then that would strengthen the arguments in support of preoperative chemotherapy, indicating that chemotherapy not only overcomes the unfavourable effect of necrosis but also possesses an additional definitive therapeutic effect. This is what we found in this study.

Three recent histopathological studies have addressed spontaneous necrosis in osteosarcoma. Misdorp and colleagues (1988) analysed 44 patients with osteosarcomas of various bones. Their findings in untreated patients differed from ours in that only 12 out of 22 untreated patients (55%) had less than 50% necrosis, a figure significantly lower than ours, even if their methodology was somewhat different. Their study, however, differed from ours because half of their patients had tumours in sites other than the distal femur. Von Hochstetter (1990) investigated 15 high-grade and low-grade osteosarcomas of various bones and reported results comparable to ours, including the observation that the extent of necrosis was independent of tumour size. Springfield and co-workers (1991) reported their findings of spontaneous necrosis in 76 osteosarcomas of various sites. They included all histological subtypes and grades except the telangiectatic variety. Almost half (45%) of the lesions did not show evidence of spontaneous necrosis, a markedly higher proportion of non-necrotic tumours than we and the two other studies found. Variations in subtypes, sites, and grades of their tumours probably account for this difference. Sixty-two of their patients received postoperative chemotherapy. Thus, none of these three studies specifically addressed the biological significance of spontaneous necrosis.

Our results indicate that spontaneous necrosis in high-grade osteosarcomas portends a rapid clinical course. By selecting a group of patients with tumours of the distal femur only and by including only those with "conventional" osteosarcomas in the Dahlin classification (Dahlin and Unni 1986) in stages IIA and IIB (Enneking 1988), we tried to exclude as many confounding variables as possible. The fact that tumour diameter did not correlate with extent of necrosis indicates that necrosis operates as an independent variable in prognosis. The biological predictive value of necrosis, irrespective of tumour size, also holds true for sarcomas of soft tissue (Costa et al. 1984). Detailed histopathological ex-

amination of tumours before chemotherapy is impossible. Even if the histological appearances in pre- and postchemotherapy tumours differ somewhat (Misdorp 1986; Picci et al. 1985; Raymond et al. 1987), these differences are nonspecific and permit no reliable quantification of, or discrimination between, the two types of necrosis, even by experienced bone pathologists (Fig. 3). Other, and indirect, modalities, including angiography (Carrasco et al. 1987; Kumpan et al. 1986) and magnetic resonance imaging (Pan et al. 1990; Sanchez et al. 1990), are needed.

There was good general agreement between our two semiquantitative and reproducible methods of mapping necrosis. Either point counting or digital pad methodology is well suited to pathological study. It appears that the third approach, a simple histological estimate of the relative area of necrosis, is less reliable, even at an institution with considerable experience in the diagnosis and treatment of malignant bone tumours.

We conclude that spontaneous necrosis in intramedullary high-grade osteosarcomas occurs frequently, is independent of tumour size, and is significantly less extensive than necrosis found in tumours subjected to preoperative chemotherapy. Osteosarcomas with significant spontaneous tumour necrosis may be expected to grow faster and disseminate sooner than osteosarcomas with small areas of spontaneous tumour necrosis. Spontaneous tumour necrosis may be rapidly and reproducibly assessed by either point counting or digitized computer pad; simple histological assessment based on slide reviews alone are less reproducible and less predictive of biological behavior than the first two methods.

References

Carrasco CH, Chawla SP, Benjamin RS, Raymond AK, Ayala AG, Murray JA, Wallace S (1987) Arteriographic prediction of tumor necrosis after primary treatment of osteosarcoma in adults (abstract). Proc Annu Meet Am Soc Clin Oncol 6:129

Costa J, Wesley RA, Glatstein E, Rosenberg SA (1984) The grading of soft tissue sarcomas: results of a clinicohistopathologic correlation in a series of 163 cases. Cancer 53:530–541

Dahlin DC, Unni KK (1986) Bone tumors: general aspects and data on 8,542 cases. Thomas, Springfield, Ill., pp 269–321

Enneking WF (1988) Musculoskeletal tumor staging – 1987 update. In: Ryan JR, Baker LH (eds) Recent concepts in sarcoma treatment. Kluwer Academic, Dordrecht, pp 30–39

Hochstetter AR von (1990) Spontaneous necrosis in osteosarcomas. Virchows Arch [A] 417:5–8

Kumpan W, Lechner G, Wittich GR, Salzer-Kuntschik M, Delling G, Kotz R, Hajek P, Sekera J (1986) The angiographic response of osteosarcoma following pre-operative chemotherapy. Skeletal Radiol 15:96–102

Misdorp W (1986) The assessment of necrosis in osteosarcomas. In: Oosterom AT van, Unnik JAM van (eds) Management of soft tissue and bone sarcomas. Raven, New York, pp 241–243

Misdorp W, Hart G, Delemarre JFM, Voute PA, Eijken JW van der (1988) An analysis of spontaneous and chemotherapy-associated changes in skeletal osteosarcomas. J Pathol 156:119–128

Nachman J, Simon MA, Dean L, Shermeta D, Dawson P, Vogelzang NJ (1987) Disparate histologic responses in simultaneously resected primary and metastatic osteosarcoma following intravenous neoadjuvant chemotherapy. J Clin Oncol 5:1185–1190

Pan G, Raymond AK, Carrasco CH, Wallace S, Kim EE, Schirkhoda A, Jaffe N, Murray JA, Benjamin RS (1990) Osteosarcoma: MR imaging after preoperative chemotherapy. Radiology 174:517–526

Picci P, Bacci G, Campanacci M, Gasparini M, Pilotti S, Cerasoli S, Bertoni F, Guerra A, Capanna R, Albisinni U, Galletti S, Gherlinzoni F, Calderoni P, Sudanese A, Baldini N, Bernini M, Jaffe N (1985) Histologic evaluation of necrosis in osteosarcoma induced by chemotherapy: regional mapping of viable and nonviable tumor. Cancer 56:1515–1521

Raymond AK, Chawla SP, Carrasco CH, Ayala AG, Fanning CV, Grice B, Armen T, Plager C, Papadopoulos NEJ, Edeiken J, Wallace S, Jaffe N, Murray JA, Benjamin RS (1987) Osteosarcoma chemotherapy effect: a prognostic factor. Semin Diagn Pathol 4:212–236

Roessner A, Müller KM, Vollhaber HH, Grundman E (1984) Operierte Lungenmetastasen hochmaligner Osteosarkome nach Zytostatikatherapie. (abstract) Verh Dtsch Ges Pathol 68:406

Rosen G, Marcove RC, Caparros B, Nirenberg A, Kosloff C, Huvos AG (1979) Primary osteogenic sarcoma: the rationale for preoperative chemotherapy and delayed surgery. Cancer 43:2163–2177

Rosen G, Caparros B, Huvos AG, Kosloff C, Nirenberg A, Cacavio A, Marcove RC, Lane JM, Mehta B, Urban C (1982) Preoperative chemotherapy for osteogenic sarcoma: selection of postoperative adjuvant chemotherapy based on the response of the primary tumor to preoperative chemotherapy. Cancer 49:1221–1230

Rosen G, Caparros B, Groshen S, Nirenberg A, Cacavio A, Marcove RC, Lane JM, Huvos AG (1984) Primary osteogenic sarcoma of the femur: a model for the use of preoperative chemotherapy in high risk malignant tumors. Cancer Invest 2:181–192

Salzer-Kuntschik M, Brand G, Delling G (1983a) Bestimmung des morphologischen Regressionsgrades nach Chemotherapie bei malignen Knochentumoren. Pathologe 4:135–141

Salzer-Kuntschik M, Delling G, Beron G, Sigmund R (1983b) Morphological grades of regression in osteosarcoma after polychemotherapy – study COSS 80. J Cancer Res Clin Oncol 106 [Suppl]:21–24

Sanchez RB, Quinn SF, Walling A, Estrada J, Greenberg H (1990) Musculoskeletal neoplasms after intraarterial chemotherapy: correlation of MR images with pathologic specimens. Radiology 174:237–240

Springfield DS, Schakel ME Jr, Spanier SS (1991) Spontaneous necrosis in osteosarcoma. Clin Orthop 263:233–237