Virchows Arch [Pathol Anat] (1985) 405:215–223

Virchows Archiv A

Nuclear polymorphism in osteosarcomas as a prognostic factor for the effect of chemotherapy

A quantitative study

R. Apel, G. Delling, H. Krumme, K. Winkler[1], M. Salzer-Kuntschik[2]

Institut of Pathology (Head: Prof. Dr. G. Seifert)
[1] Clinic of Pediatrics, Division for Haematology and Oncology (Head: Prof. Dr. G. Landbeck)
University of Hamburg, Martinstrasse 52, D-2000 Hamburg 20
[2] Institute of Pathology, University of Vienna, Austria

Summary. A current strategy for osteosarcoma treatment is neo-adjuvant chemotherapy prior to the resection of the tumour. It appears that some tumours respond very well to the cytostatic therapy, while others show little or no effect. It is desirable to be able predict the response of the tumour before starting chemotherapy.

16 biopsy specimens from patients with osteosarcoma who had been treated according to the protocol of the study COSS-80 and COSS-82 were examined. 100 tumour cells from each biopsy have been measured by an electronic interactive image analysis system (IBAS II; Kontron/ZEISS). After completion of chemotherapy en bloc resection of the tumour was performed. The entire surgical specimen was completely examined at two levels by means of undecalcified sections, and assigned a grade for the effect of chemotherapy analogous to the grading of Salzer-Kuntschik et al. (1983). The quantitative analysis of tumour cell nuclei revealed two different patterns of nuclear sizes, which were correlated significantly with the chemotherapy response ($P < 0.002$).

Tumour cell nuclei of well responders were significantly larger and showed a greater variance in size (mean value $66 + 41$ μm^2), than those of poor responders (mean value $38 + 18$ μm^2).

We conclude from our results that quantitative analysis and classification of nuclear size of osteosarcoma cells may be useful for predicting chemotherapy response in patients with osteosarcoma.

Key words: Osteosarcoma – Chemotherapy – Quantitative Caryometry – Morphometry – Tumour regression

In the past ten years the therapeutic concepts in the treatment of osteosarcoma have changed considerably. In former years amputation was per-

Offprint requests to: G. Delling at the above address

Table 1. Age, sex and localisation of 16 investigated osteosarcomas

Case	Histo number	Age	Sex	Grade of regression	Localisation
1	0563/83	15	m	I	prox. Tibia
2	0632/81	16	m	I	Radius
3	2330/81	12	m	I	Femurdiaphy.
4	1312/80	24	m	I	dist. Femur
5	1747/81	15	f	I	dist. Femur
6	1598/81	7	m	II	prox. Tibia
7	1342/82	19	m	II	prox. Tibia
8	1469/82	18	m	II	dist. Femur
9	1616/80	10	f	III	prox. Tibia
10	0047/83	15	f	III	prox. Tibia
11	2281/81	14	m	IV	dist. Femur
12	2085/80	16	f	IV	dist. Femur
13	2116/82	17	m	IV	dist. Femur
14	1117/82	15	m	V	dist. Femur
15	2283/81	13	f	V	dist. Femur
16	1205/82	47	m	V	prox. Femur

formed immediately after diagnosis, while today a so-called neo-adjuvant chemotherapy precedes surgery. The main reason for this strategy was to prepare for limb sparing procedures. However, as the degree of tumour cell destruction after preoperative chemotherapy proved highly predictive for the disease free survival interval of patients, the chemotherapy could be amended depending on the response of the tumour to chemotherapy (Rosen et al. 1979; Rosen et al. 1982; Winkler et al. 1983). To date, the response of the tumour to chemotherapy could only be assessed by intensive histological examination of surgical specimens.

It is desirable to forecast the response of the tumour before starting chemotherapy in order to decide on the appropriate chemotherapeutic protocol at the moment of biopsy. In this context Rosen et al. (1982) state that undifferentiated osteosarcomas showed better response to chemotherapy.

The aim of the work presented here is to determine the grade of osteosarcoma differentiation using quantitative data.

Patients and methods

16 case records and specimens of patients with osteosarcoma treated according to the protocol of the COSS-80[1] or, the COSS-82-study, were examined. The patients were between 7 and 19 years old. 11 were males and 5 females. The osteosarcoma was located in the proximal tibia (5), distal femur (8), proximal femur (1), femur diaphysis (1) and radius (1). After chemotherapy 10 cases showed more than 90% necrotic tumour tissue and 6 cases showed between 10 and 80% viable tumour areas.

The age, sex and localisation of the tumours are listed in Table 1.

After completion of chemotherapy en bloc resection or amputation of the tumour was done. The entire surgical specimen was completely examined in two levels by means of undecal-

1 COSS 80 = Cooperative Osteogenic Sarcoma Study

Table 2. Histological grades of regression in bone tumours after chemotherapy (Salzer-Kuntschik et al. 1983).

Grades of regression	Morphological Changes
Responder	
I	no viable tumour cells
II	some tumour cells or tumour isle < 0.5 mm
III	some areas of viable tumour less than 5 mm in diameter. The viable tumour is less than 10% of total tumour area.
Non-responder	
IV	10–50% viable tumour area
V	50–80% viable tumour area
VI	no effect of chemotherapy

cified sections. The carefully reconstructed tumour was assigned a grade of regression for the effect of chemotherapy according to the grading of Salzer-Kuntschik et al. (1983).

The morphological criteria of grades of regression are shown in table 2.

To reduce bias during the selection of cell nuclei, all measurements were carried out without knowledge of the grade of regression.

16 biopsies from patients with the definitive diagnosis of osteosarcoma were examined. The usual size of the biopsy was $1 \times 1 \times 1$ cm. In order to reduce shrinkage of tumour cells due to decalcification undecalcified biopsies were embedded in methyl-metacrylate (Delling 1972). Of each biopsy 5 µm thick sections were cut on a rotating microtome. They were stained by Goldner-stain, v. Kossa-stain and toluidin blue reaction.

The biopsies were taken from the periphery of the tumours showing high cellularity.

From each biopsy 100 unselected and undamaged tumour cell nuclei were measured with an electronic interactive image analysis system (IBAS II; Kontron/ZEISS). The magnification for examination used was 1000×.

Manually, using a cursor, the nuclei of these cells were outlined as displayed on a video monitor. The IBAS II then computed the nuclear area along with the standard deviation and the mean values.

Repeated measurements of the same section (reproducibility) showed no significant differences in terms of nuclear size. The deviation from the mean value for responder cases was 7% and for non-responder cases 4%. The measurements of the same section by different investigators produced comparable results (the deviation from the mean value for responder cases was 9.2% and for nonresponder cases 4.5%.

Statistical analysis

The measured nuclei were classified by size in steps of 10 μm^2. Simultaneously the mean value, standard deviation, median and mode were calculated.

We compared the "responder group" (grade I–III) – which showed a good effect to chemotherapy – with the "nonresponder group" (grade IV–VI) with little or no effect to preoperative chemotherapy.

Statistical evaluation included unpaired Student's-t-test and Wilcoxon's *u*-test.

Results

The 16 osteosarcomas were located centrally. 10 (62%) of the 16 examined showed a good response to preoperative chemotherapy (grade of regression:

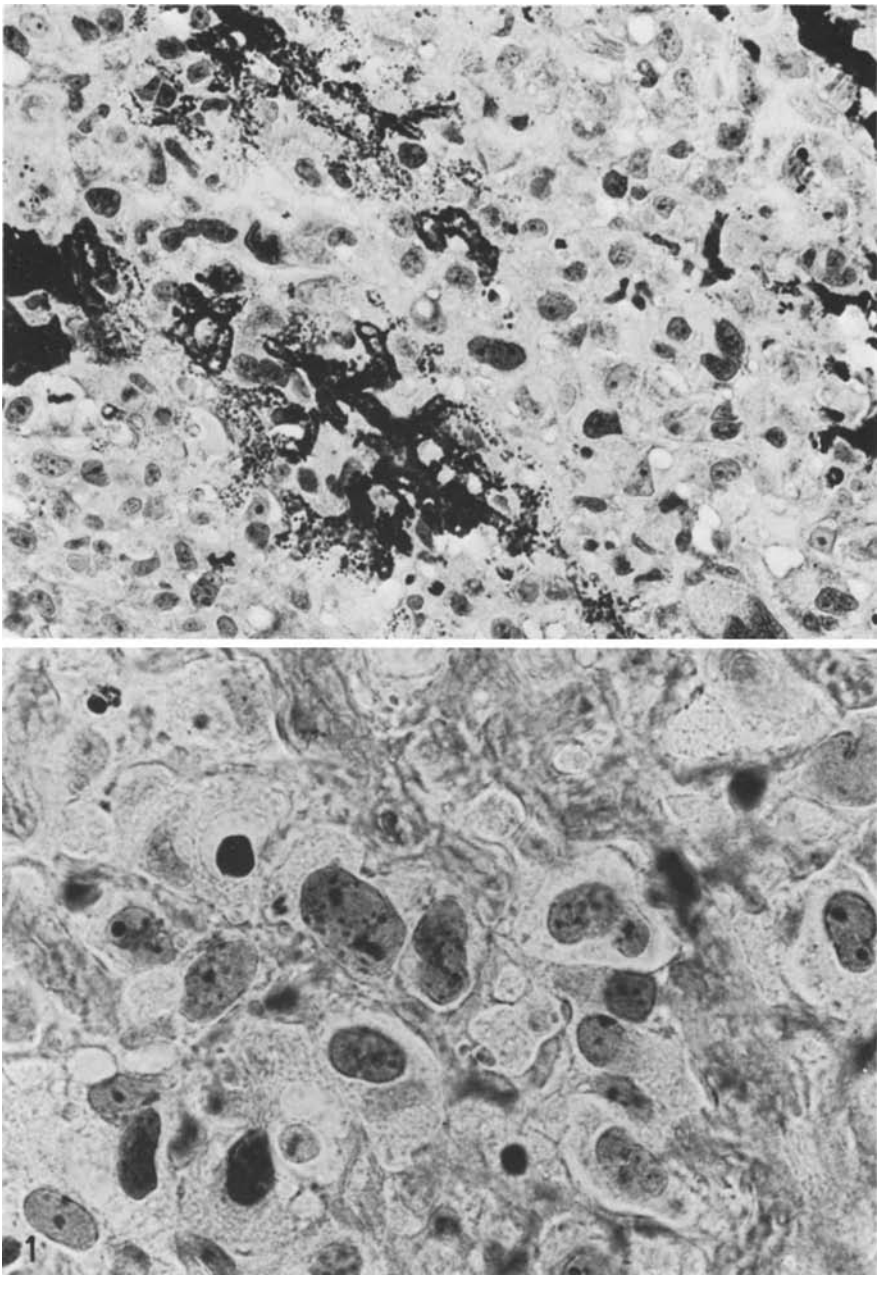

Fig. 1. Osteosarcoma (responder) with nuclear polymorphism, formation of osteoid and mineralised bone (*black areas*). Undecalcified preparation, toluidin blue reaction, ×250 (*top*), ×850 (*below*)

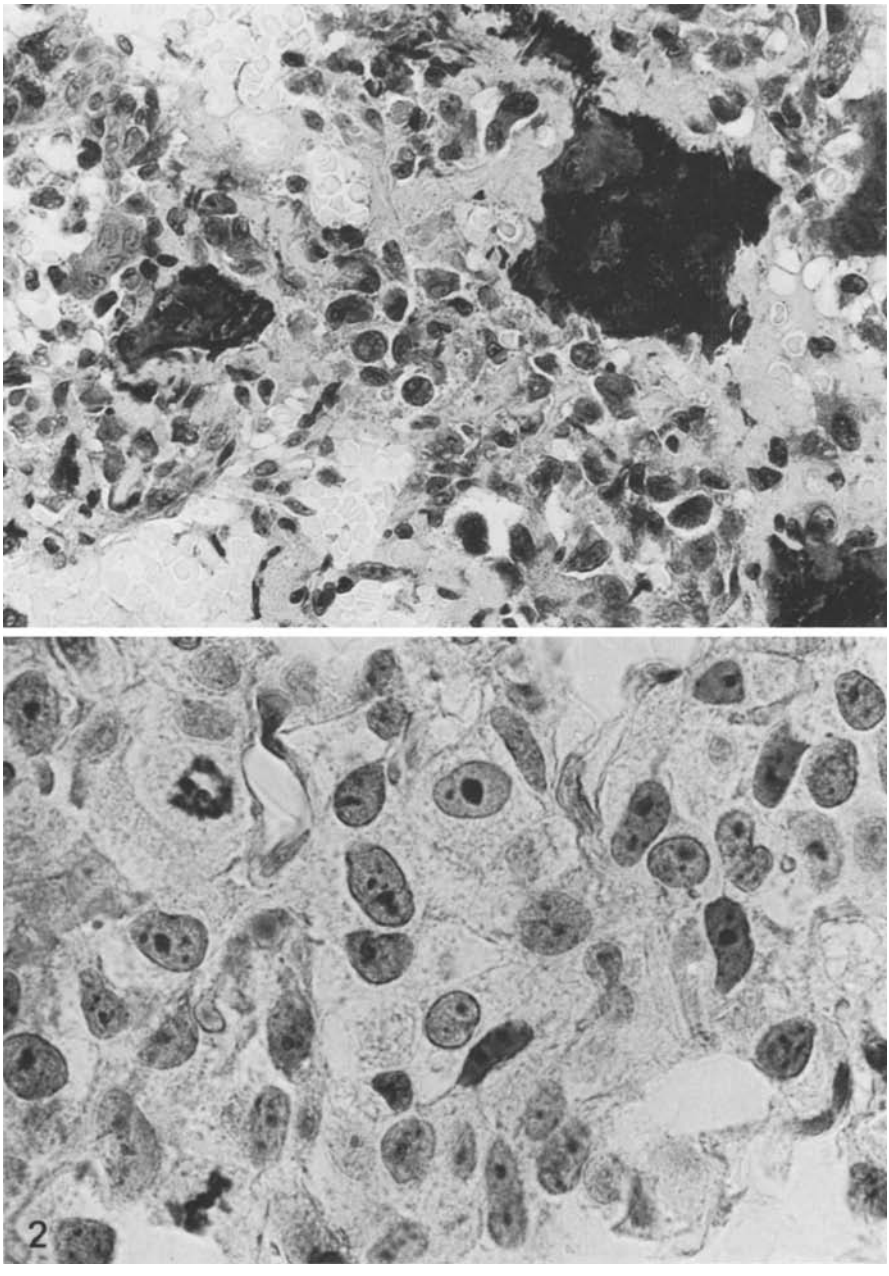

Fig. 2. Osteosarcoma (non-responder) with smaller degree of nuclear polymorphism, measured by computerised image analysis, formation of osteoid and mineralised bone (*black areas*). Undecalcified preparation, toluidin blue reaction, × 250 (*top*), × 850 (*below*)

Table 3. Mean nuclear size (μm^2) of 16 osteosarcomas

	Case	Mean nuclear size[a]	Mean value of the group
Responder	1	66.5±27.6	66±41*
	2	62.4±40.3	
	3	57.9±27.0	
	4	63.5±50.0	
	5	58.7±21.0	
	6	74.1±41.5	
	7	73.5±24.7	
	8	68.3±42.5	
	9	61.1±46.4	
	10	62.5±28.4	
Nonresponder	11	34.6±17.4	38±18*
	12	40.8±17.7	
	13	24.5±12.2	
	14	42.1±18.7	
	15	41.8±19.0	
	16	40.5±19.4	

[a] mean ± standard deviation

* $p > 0.002$ (Wilcoxon's u-test)

I–III). In these cases, the primary sites were the tibia in 5 patients (50%), the femur in 4 patients (40%) and the radius in 1 patient (10%).

With 6 patients (38%) in whom chemotherapy showed little effect, the tumour originated in the femur (100%).

The cells of the osteosarcomas which responded well showed an appreciable nuclear and cytoplasmatic pleomorphism and a number of exceptionally large cells with hyperchromatic giant or multiple nuclei. Between multiple nucleolated nuclei, nuclei with prominent nucleoli could also be found (Fig. 1).

The nonresponding tumour cells showed less nuclear polymorphism with a mixture of rounded, ovoid and fusiform cells with coarsely stipped chromatin network or with clear nucleoli (Fig. 2).

Because the microscopical view is a subjective one, we could not differentiate between responding and nonresponding tumours using this method, in contrast to karyometric data. The 16 investigated osteogenic sarcomas revealed a statistically significant difference in size of nuclei ($P < 0.002$) that correlated with the classification of the response to the chemotherapy (Table 3).

Well responding tumour cells were significantly larger and obviously deviant from the average (mean value 66±41 μm^2).

In the nonresponder group, nuclei with a size of more than 100 μm^2 were very rare (<2%). The standard deviation was significantly lower (mean value 38±18 μm^2). Thus, the responder showed a statistically confirmed nuclear polymorphism, caused by cells with a nuclear size of more than 100 μm^2 (Fig. 4).

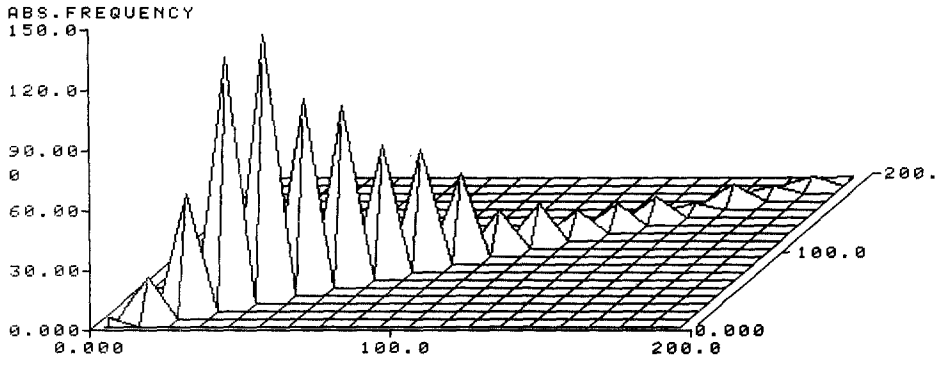

Fig. 3. Nuclear size (μm^2) distribution of osteosarcoma cells in 10 responder cases

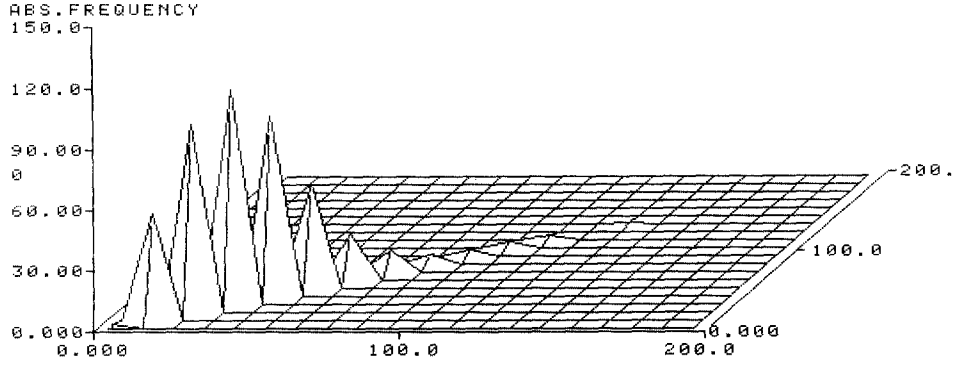

Fig. 4. Nuclear size (μm^2) distribution of osteosarcoma cells in 6 non-responder cases

Discussion

From these quantitative examinations it can be concluded that the grade of differentiation of osteosarcoma is an excellent index for predicting the effect of chemotherapy. After histological examination of the surgical specimen it can be demonstrated, retrospectively, that the chemotherapy has a good effect on the polymorphcellular osteosarcoma, the tumour was either completely devitalized or reduced to less than 10% of viable tumour cells. Generally the small cell variety of osteosarcoma responded badly to chemotherapy.

Taking the distribution of nuclear size in a biopsy as a criterion, a classification into responder or non-responder was always possible in this study. In agreement with these findings, Rosen et al. (1982) found that the small cell variety of osteosarcoma showed less responsiveness to chemotherapy, particularly to high-dose-methotrexate therapy. This statement was based on qualitative observations only.

Campanacci et al. (1981), Salzer-Kuntschik et al. (1983) showed that

age and sex of the patients had no significant influence on the prognosis of osteosarcoma, while the localisation of the tumour in the femur had a poorer prognosis than in the tibia or in another site. But in the individual case, one cannot estimate the prognosis from these variables.

Changes in tumour or patient metabolism, variations in cell kinetics and/or mutation-selection mechanisms are probably of importance for the origin of drug resistance of osteosarcoma, but have not been investigated in detail (Claryssee et al. 1976). There might also be instances of relative drug resistance, not related to the intrinsic qualities of the tumour cells, but due to clinical condition of the host and the advanced stage of the disease. For example the tumour volume, vascularisation and bone marrow reserve could limit the effect of chemotherapy.

Different distribution of ground substances were found in the biopsies, there were fibroplastic osteosarcomas and osteoblastic sarcomas with chondroid areas. The biopsies from the 16 osteosarcomas were always taken from the tumour periphery, so we could not estimate the influence of ground substance formation.

The method used proved to be precise and easy to handle for a routine measurement. The acceptable range of intra- and interobserver error utilizing our semi-automatic technique permits a reasonable interpretation of data. Gamel et al. (1982) investigating uveal melanoma came to comparable results using a similar technique. They showed that the standard deviation of the nuclear circumference correlates highly with patient mortality. In addition, using computer-assisted measurements of different cell nuclear variables Stenkuist et al. (1978) and Baak et al. (1982) predicted the prognosis of human breast cancer more exactly than it was possible with the TMN-System.

We conclude from our results that the statistical classification of nuclear size of osteosarcoma cells enables a differentiation between "responding" and "non-responding" tumours prior the chemotherapy.

References

Baak JBA, Kurver PHJ, Boon ME (1982) Quantitative Image. Analyses For The Prognosis Of Breast Cancer. Anal Quant Cytol 4:142

Campanacci M, Bacci G, Bertoni F, Picci P, Minutillo A, Franceschi C (1981) The Treatment of Osteosarcoma of the Extremities. Cancer 48:1569–1581

Claryssee A, Kanis Y, Mathe G (1976) Cancer Chemotherapy. Springer, Berlin Heidelberg New York, pp 36–70, 125–126

Delling G (1972) Über eine vereinfachte Methylmetacrylateinbettung für unentkalkte Knochenschnitte. Beitr Pathol 145:100–105

Delling G, Salzer-Kuntschik M (1983) Morphological Changes in Osteosarcoma. J Canc Res Clin Onc 106:32–37

Gamel JW, McLean IW, Greenberg RA, Zimmerman LE, Lichtenstein SJ (1982) Computerized Histologic Assessment of Malignant Potential. Hum Pathol 13:893–897

Rosen G, Marcove R, Caparros B, Nirenberg A, Kosloff C, Huvos A (1979) Primary Osteogenic Sarcoma. Cancer 43:2163–2177

Rosen G, Caparros B, Huvos A, Kosloff C, Nirenberg A, Cacavio A, Marcove RC, Lane JM, Mehta B, Urban C (1982) Preoperative Chemotherapy for Osteogenic Sarcoma. Cancer 49:1221–1230

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

Stenkvist B, Westman-Naeser S, Holmquist J, Nordin B, Bengtsson E, Erikson O, Fox C (1978) Computerized Nuclear Morphometry as an Objective Method for Characterizing Human Cancer Cell Populations. Canc Res 38:4688–4697

Weibel ER (1979) Stereology Methods, Vol 1. Academic Press London New York

Winkler K, Beron G, Kotz R, Salzer-Kuntschik M, Beck J, Brandeis W, Ebell W, Erttmann R, Göbel U, Havers W, Henze G, Hinderfeld L, Höcker P, Jobke A, Jürgens H, Kabisch H, Landbeck G, Preusser P, Prindull G, Ramach W, Ritter J, Sekera J, Treuner J, Wüst G (1983) Adjuvant Chemotherapy in Osteosarcoma-Effects of Cisplatinum, BCD, and Fibroblast Interferon in Sequential Combination with HD-MTX and Adriamycin. J Cancer Res Clin Oncol 106:1–7

Accepted September 24, 1984