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Histological grading and morphometric analysis of cartilaginous tumours

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Summary. Morphometric analysis of cartilaginous tumours was performed on 25 chondrosarcomas, 9 cases of enchondroma (ENCH), and 2 chondroblastic osteosarcomas (CBOS). The chondrosarcomas were classified into three grades of malignancy according to Evans' histological classification and were further divided into low and high grades of malignancy. Cellularity, nuclear area, binucleate cells and mitotic figures were examined using formalin-fixed and paraffin-embedded specimens. The cellularity was significantly higher in high-grade chondrosarcoma (HGCS) than in low-grade chondrosarcoma (LGCS) ($P<0.005$). The nuclear area was larger in more malignant lesions. Significant differences in the nuclear area were found between ENCH and LGCS ($P<0.005$) and between LGCS and HGCS ($P<0.01$). Binucleate cells were found more frequently in LGCS than in ENCH ($P<0.005$). Although a few mitotic figures were found in HGCS, they were extremely rare in chondrosarcomas. Mitotic figures, however, were easily found in CBOS when compared with HGCS ($P<0.05$). These results suggest that nuclear area and binucleate cells are useful for differentiation between benign and malignant cartilaginous lesions and that easily detectable mitotic figures are a reliable marker for neoplastic cartilage in osteosarcoma.

Key words: Chondrosarcoma – Morphometry – Histological grading – Mitotic figure

Introduction

Chondrosarcoma was first recognized as a separate entity from osteosarcoma by Lichtenstein and Jaffe in 1943. Their criteria of chondrosarcoma have been generally accepted and several grading systems of chondrosarcoma have been proposed by O'Neal and Ackerman (1951, 1952), Campanacci et al. (1975) and Evans et al. (1977). These grading systems, however, are still controversial (O'Neal and Ackerman 1952; Dahlin and Henderson 1956; Schiller 1985). Differential diagnosis between benign and malignant chondroid tumours is also very difficult and sometimes impossible especially in biopsy specimens (Mirra et al. 1985; Schiller 1985; Dahlin and Unni 1986).

There have been reports on the cyto- or histomorphometric or quantitative analysis of cartilaginous lesions in recent years (Kreicberg et al. 1981; Zeppa et al. 1989). In these papers nuclear area was generally considered to be the most significant factor for histological grading of chondrosarcomas.

We have performed a histomorphometric study on low- and high-grade chondrosarcomas (LGCS and HGCS, respectively), enchondroma (ENCH) and neoplastic cartilage in chondroblastic osteosarcoma (CBOS) in order to clarify the histological variables for grading chondrosarcoma. Cellularity, nuclear area, binucleate cells and mitotic figures were examined in these cartilaginous lesions. We have shown in this study that mitotic figures in chondrosarcoma are less frequent than previously described (Cuvelier and Roels 1979; Kreicberg et al. 1981; Alho et al. 1983) and that easily detectable mitotic figures are the most important cytological finding for the diagnosis of neoplastic cartilage in CBOS. Our study also showed that nuclear area and binucleate cells were useful for differential diagnosis between ENCH and LGCS, and that cellularity was the main difference between LGCS and HGCS.

Materials and methods

We examined material obtained during surgery from 24 cases (25 tumours) of chondrosarcoma at the University of Tokyo Hospital from 1969 to 1989. Nine ENCH cases and the neoplastic cartilage of 2 CBOS cases were also studied and the findings were compared with those for chondrosarcoma. All the materials were fixed with 10% formalin and decalcified according to Plank-Rychlo's method, if necessary, and embedded in paraffin. The specimens were cut

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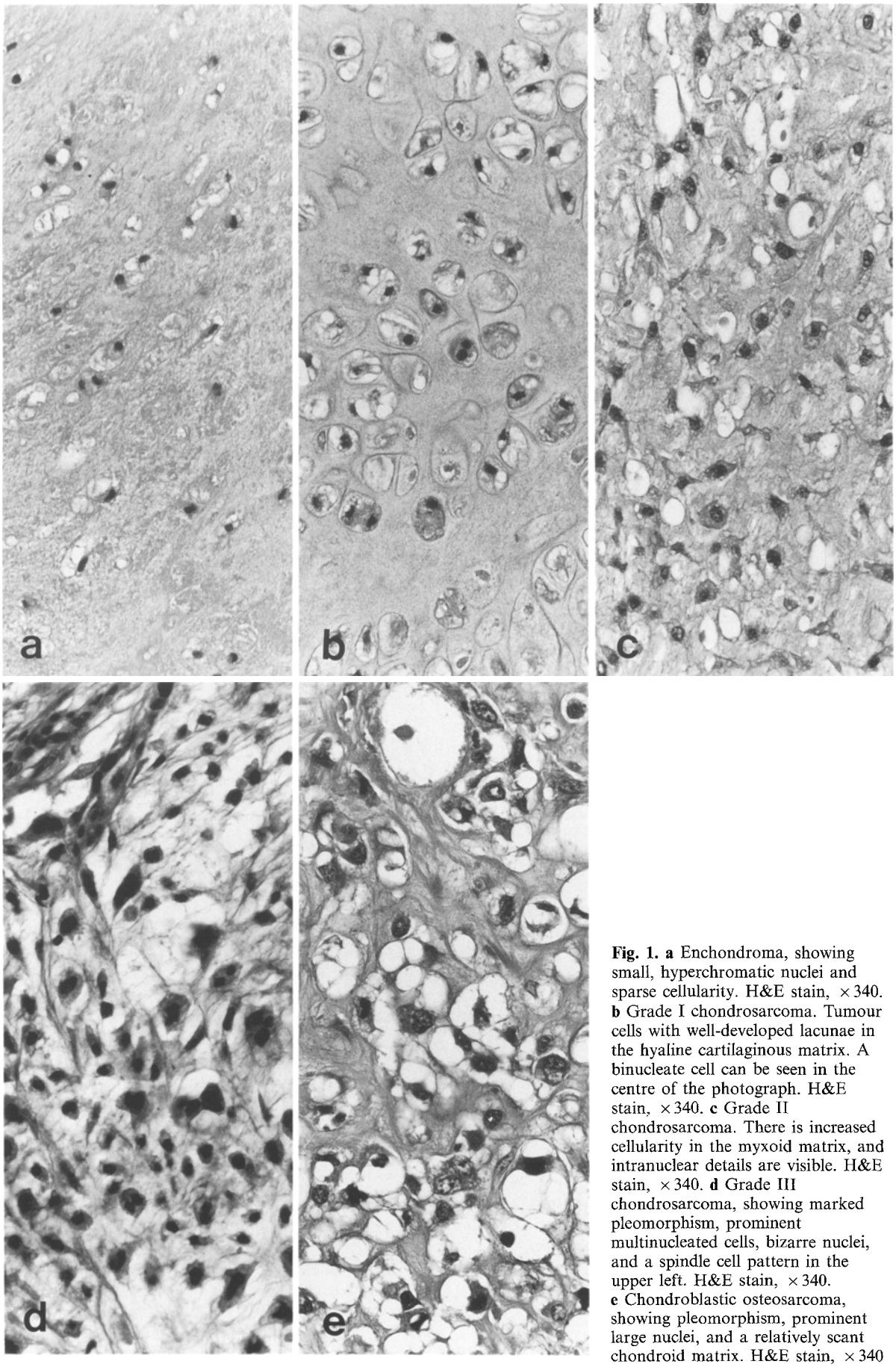

Fig. 1. a Enchondroma, showing small, hyperchromatic nuclei and sparse cellularity. H&E stain, ×340. **b** Grade I chondrosarcoma. Tumour cells with well-developed lacunae in the hyaline cartilaginous matrix. A binucleate cell can be seen in the centre of the photograph. H&E stain, ×340. **c** Grade II chondrosarcoma. There is increased cellularity in the myxoid matrix, and intranuclear details are visible. H&E stain, ×340. **d** Grade III chondrosarcoma, showing marked pleomorphism, prominent multinucleated cells, bizarre nuclei, and a spindle cell pattern in the upper left. H&E stain, ×340. **e** Chondroblastic osteosarcoma, showing pleomorphism, prominent large nuclei, and a relatively scant chondroid matrix. H&E stain, ×340

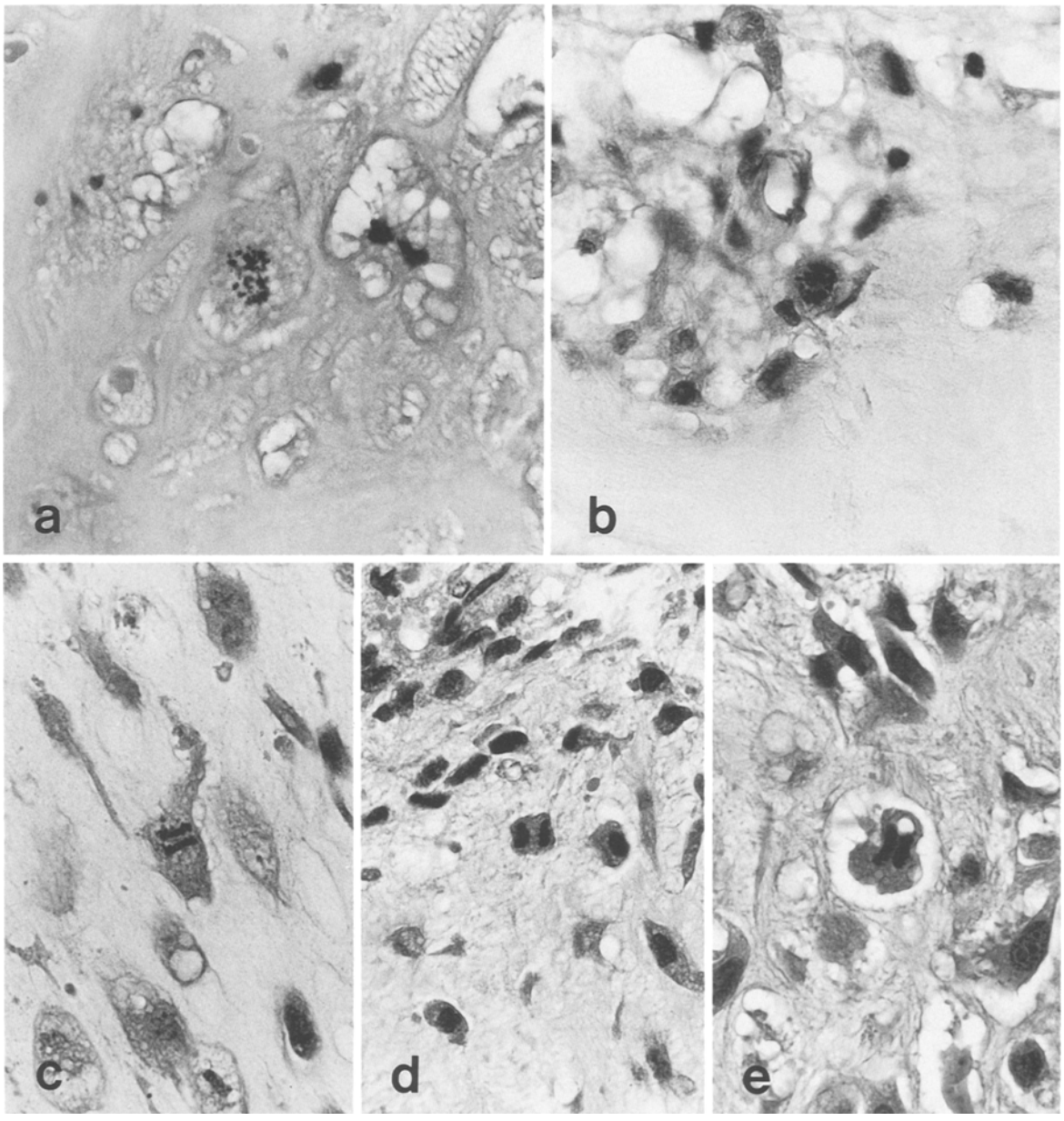

Fig. 2. Mitotic figures: **a** prometaphase in grade I chondrosarcoma. H&E stain, ×450. **b** Metaphase in grade I chondrosarcoma. H&E stain, ×680. **c** Anaphase in grade II chondrosarcoma. H&E stain, ×680. **d** Late anaphase in grade II chondrosarcoma. H&E stain, ×680. **e** Anaphase in chondroblastic osteosarcoma. H&E stain, ×680

into sections 4 μm in thickness and stained with haematoxylin and eosin. Chondrosarcoma was classified into three groups (grade I, II, III) according to the histological grading system described by Evans et al. (1977). They were further grouped into low and high grades of malignancy. Grade I chondrosarcoma was classified as LGCS and grades II and III as HGCS (Fig. 1).

The cellularity of the tumours was determined by direct counting of 16 high-power fields (400×) using a Weibel's multipurpose reticle (Weibel et al. 1966) in the eyepiece of the microscope. Viable cell number per square millimetre was measured in most of the cellular areas. Measurement of nuclear area was performed using a Zeiss Kontron IBAS-2000 system coupled with a personal computer and a Nikon microscope at a high magnification (400×). Cellular images were reproduced on a monitor. Nuclear boundaries were traced with a cursor on a digitized tablet connected to the video-monitor on which the tracings were controlled. Two hundred nuclei were measured for each tumour studied. Binucleate chondrocytes were estimated by direct counting among 1000 tumour cells and expressed as a percentage. Nuclear area and binucleate cells were examined within the same areas which were used for the determination of cellularity.

Generally, mitotic figures were seldom seen in cartilaginous tumours. They were sometimes difficult to identify due to condensation or agglutination of the tumour cell nuclei caused by fixation artefact (Schiller 1985). Therefore, only the unequivocal mitotic figures which were clearly determined as prometaphase, metaphase, anaphase and telophase were counted (Fig. 2). All the slides obtained from the maximum cut surface of the tumours were examined for mitotic figures. The real numbers of mitotic figures were counted in each tumour and they were expressed as a value per square centimetre in order to avoid bias of the tumour size. Statistical analysis between the four groups (ENCH, LGCS, HGCS and CBOS) was performed for each variable with non-parametric Wilcoxon test.

Table 1. Clinicopathological and morphometric data

	Case no.	Age/sex	Location	Diagnosis	Cellularity (cells/mm^2)	Nuclear area (μm^2)	Binucleated cells (%)	Mitotic figures (/cm^2) (*N*)
Enchondroma	1	44/F	Hand	ENCH	297	19.22± 5.69	2.2	(0)
	2	16/F	Hand	ENCH	225	15.35± 5.11	1.3	(0)
	3	53/F	Hand	ENCH	412	15.23± 5.72	1.9	(0)
	4	35/F	Hand	ENCH	274	15.04± 4.95	0.4	(0)
	5	9/M	Hand	ENCH	283	14.05± 5.11	0.8	(0)
	6	21/F	Hand	ENCH	241	13.35± 6.94	0.8	(0)
	7	24/F	Hand	ENCH	341	14.10± 5.26	1.1	(0)
Low-grade chondrosarcoma	8	38/M	Humerus	CS, G I	742	20.01± 5.76	6.8	(0)
	9	59/M	Sacrum	CS, G I	155	21.62± 8.88	2.4	(0)
	10	52/F	Femur	CS, G I	279	24.17± 9.30	3.9	(0)
	11	42/M	Scapula	CS, G I	444	25.63± 8.76	2.5	(0)
	12	46/F	Pelvis	CS, G I	268	20.86± 6.49	2.2	(0)
	13	35/M	Scapula	CS, G I	360	21.25± 7.13	2.0	(0)
	14	48/M	Rib	CS, G I	357	21.99± 6.54	3.6	0.05(1)
	15	57/M	Rib	CS, G I	226	21.84± 7.19	2.4	(0)
	16	28/M	Pelvis	CS, G I	250	21.55± 6.16	4.3	0.01(1)
	17	56/M	Pelvis	CS, G I	321	28.58±11.04	1.2	(0)
	18	35/F	Spine	CS, G I	265	25.08± 9.86	2.7	0.04(1)
	19	53/M	Sternum	CS, G I	401	22.35± 9.04	3.9	(0)
	20	43/M	Sternum	CS, G I	163	20.90± 5.77	2.3	(0)
	21	22/F	Pelvis	CS, G I	237	22.66± 8.90	1.5	(0)
	22	49/F	Femur	CS, G I	372	24.15± 8.49	4.7	(0)
High-grade chondrosarcoma	23	68/F	Femur	CS, G II	1090	36.15±11.33	3.4	1.10(10)
	24	14/M	Femur	CS, G II	616	25.34± 7.67	1.0	0.82(2)
	25	45/M	Pelvis	CS, G II	990	25.12± 7.75	3.6	0.08(6)
	26	38/M	Humerus	CS, G II	731	20.21± 5.97	0.9	(0)
	27	65/F	Rib	CS, G II	334	25.93±10.10	5.2	(0)
	28	66/F	Pelvis	CS, G II	1548	24.30± 8.04	2.3	(0)
	29	54/F	Femur	CS, G III	1483	41.71±34.31	3.5	1.25(25)
Chondromatosis	30[a]	68/M	Foot	CS, G I	282	25.56± 9.55	4.2	(0)
			Fibula	CS, G II	1527	32.42±10.14	0.6	(0)
	31[b]	54/M	Humerus	CS, G II	974	26.14± 8.94	1.3	(0)
			Scapula	ENCH	182	18.14± 7.52	1.1	(0)
			Hand	ENCH	300	16.91± 6.97	1.2	(0)
Chondroblastic Osteosarcoma	32	12/M	Femur	OS	1586	33.69±10.58	4.5	12.14(164)
	33	20/M	Jaw	OS	830	45.84±18.80	3.3	5.78(49)

ENCH, Enchondroma; CS, chondrosarcoma; G I, grade I; G II, grade II; G III, grade III; OS, osteosarcoma
[a] Maffucci's syndrome; [b] Ollier's disease
The actual number of mitotic figures is showed in parenthesis (*N*)

Table 2. Summary and statistical analysis of morphometric data

	ENCH (*n*=9)	LGCS (*n*=16)	HGCS (*n*=9)	CBOS (*n*=2)
Cellularity (cells/mm^2)	283.9±70.0	320.1±138.3	1032.6±429.2	1208.0±534.6
	NS	$P<0.005$	NS	
Nuclear area (μm^2)	15.71±1.98	23.01±2.30	28.59±6.78	39.77±8.59
	$P<0.005$	$P<0.01$	NS	
Binucleate cells (%)	1.20±0.56	3.16±1.43	2.42±1.59	3.90±0.39
	$P<0.005$	NS	NS	
Mitotic figures (/cm^2)	0	0.006±0.015	0.361±0.534	8.960±4.497
	NS	NS	$P<0.05$	

Values are mean ± standard deviation; NS, not significant; ENCH, enchondroma; LGCS, low-grade chondrosarcoma; HGCS, high-grade chondrosarcoma; CBOS, chondroblastic osteosarcoma

Results

All the clinicopathological and morphometric data are shown in Tables 1 and 2.

The cellularity tended to increase according with the histological grade of the tumours, especially in LGCS and HGCS, with a statistical significance of $P<0.005$.

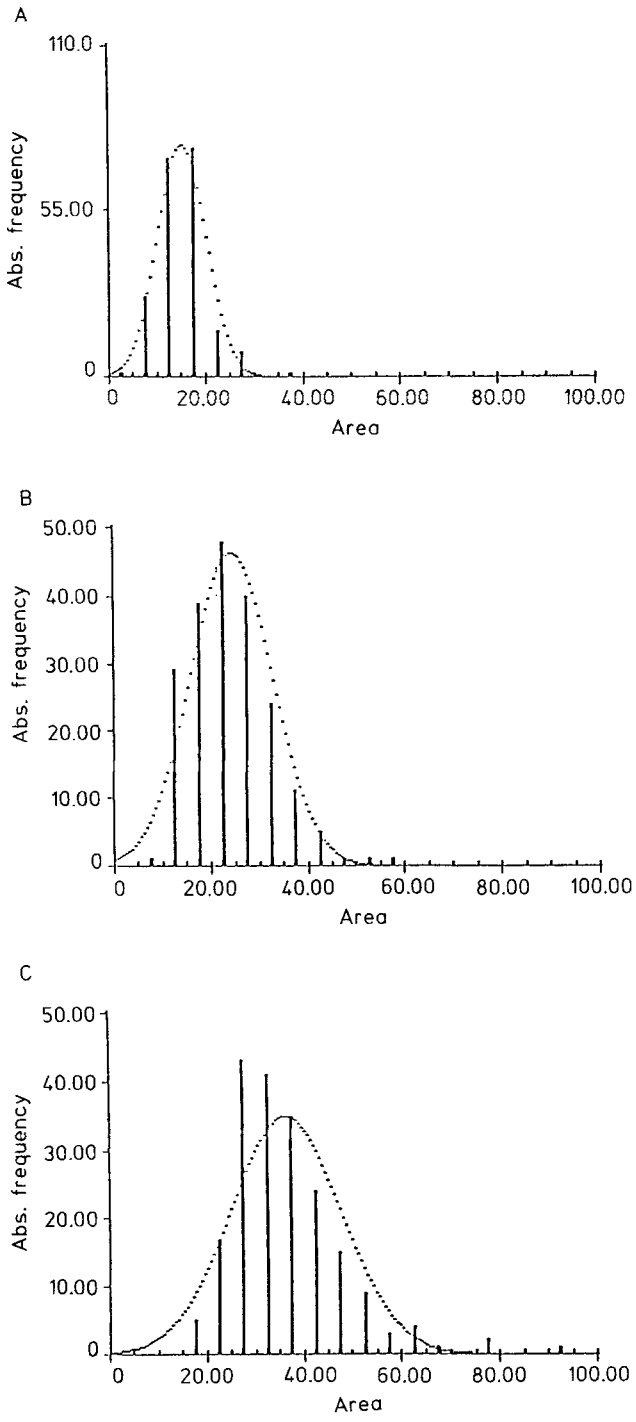

Fig. 3. Histogram of the nuclear area in representative cases of enchondroma (**a**), low-grade chondrosarcoma (**b**), and high-grade chondrosarcoma (**c**). Nuclear area increased according to the histological grade. *Abs.frequency* in the *ordinate* shows the actual number of cells and *Area* in the *abscissa* shows nuclear area (μm^2)

There were no significant differences between ENCH and LGCS or between HGCS and CBOS.

The mean nuclear area tended to increase in higher histological grades. The nuclear areas in LGCS and HGCS were approximately 1.5 times and 1.8 times larger, respectively than those of ENCH (Fig. 3). Statistical significances were demonstrated between the nuclear areas in ENCH and LGCS ($P<0.005$) and between LGCS and HGCS ($P<0.01$). There was no significant difference between the nuclear areas in HGCS and CBOS.

Binucleate cells were less frequent in ENCH than in LGCS with a significant difference of $P<0.005$. HGCS showed a lower frequency of binucleate cells in comparison not only with CBOS but also with LGCS, although there was no significant difference.

There were no mitotic figures in ENCH in the material we studied. In 3 of 16 LGCS cases (cases 14, 16, 18) 1 mitotic figure only was found. Mitotic figures were

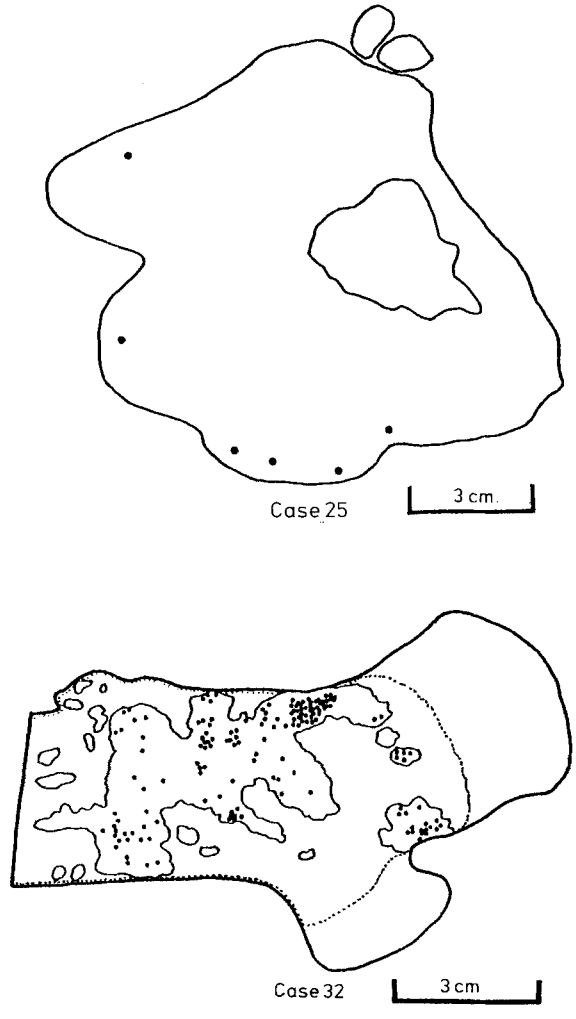

Fig. 4. Schematic drawing of the maximum cut surfaces of the tumours with plotting of mitotic figures. One dot corresponds to 1 mitotic figure. In case 25 (grade II chondrosarcoma of the pelvis), six mitotic figures were observed. In case 32 (chondroblastic osteosarcoma of the femoral head), many mitotic figures were found. In case 32, the whole tumour is enclosed by *broken lines*, while the chondroid areas are enclosed by *solid lines*

observed in 3 out of 8 cases of grade II chondrosarcoma. Ten mitotic figures were found in case 23, 2 in case 24 and 6 in case 25 (Fig. 4). No mitotic figures could be found in the other 5 grade II chondrosarcoma cases (cases 26–28, 30 and 31). One grade III chondrosarcoma case (case 29) showed 25 mitotic figures. Furthermore, fibrosarcoma-like lesions which occupied a small area of the periphery of the tumour lobule showed higher mitotic activity (34 mitotic figures, 54.84 mitotic figures/cm^2) than chondroid or myxoid areas (1.25 mitotic figures/cm^2) in the grade III chondrosarcoma case. High mitotic activity was found in the chondroid area of CBOS: 164 mitotic figures in case 32 (Fig. 4) and 49 mitotic figures in case 33. There was a significant difference of mitotic activity between HGCS and CBOS ($P < 0.05$).

Discussion

The differential diagnosis between benign and malignant chondroid tumours is often very difficult in histological material (Mirra et al. 1985; Schiller 1985; Dahlin and Unni 1986). Some benign chondroid tumours show more distinct cytological atypia than well-differentiated chondrosarcoma. This morphometric study showed that nuclear area and binucleate cells were useful markers for distinguishing LGCS from ENCH. However, cellularity was not important in the differential diagnosis between ENCH and LGCS. Although mitotic figures could not be detected in ENCH by our observation, it was not helpful for differentiation between LGCS and ENCH, because mitotic figures were very rare in LGCS.

Some histological criteria of grading systems for chondrosarcoma have been proposed in the literature (O'Neal and Ackerman 1951, 1952; Campanacci et al. 1975; Evans et al. 1977). It is, however, not easy to evaluate the grade of chondrosarcoma, because these criteria are not completely quantitative. The mean cellularity of HGCS was 3.2 times higher than that of LGCS and our study shows that cellularity is the most important determinant for differentiation between grade I and II chondrosarcomas. Nuclear area could also be a significant factor for grading, but the difference between grade I and II chondrosarcomas was not large enough.

According to a few reports in which cartilaginous lesions were studied by cytomorphometry (Mitchell and Sokoloff 1987; Zeppa et al. 1989), the mean nuclear area in benign chondroid lesions was about 37 μm^2. It was about 55 μm^2 in well-differentiated chondrosarcoma, while the value in moderately differentiated chondrosarcoma was 73 μm^2. Our data for nuclear area were smaller than those previously reported. Smear slides were used in the previous studies, while 4-μm-thick histological sections from paraffin blocks were used in this morphometric study. The difference in the materials might have influenced the morphometric data. In fact, Mitchell and Sokoloff (1987) described intranuclear details such as nucleoli and chromatin patterns in the chondrocytes of ENCH in cytological specimens. In contrast, the nuclei of ENCH were hyperchromatic and condensed in histological specimens, and the nuclear details were usually not visible. It is considered that the nuclei of chondrocytes and their cytoplasm might shrink during the process of tissue preparation for paraffin embedding.

Mitotic figures were rare in chondrosarcoma, even in HGCS. Evans et al. (1977) reported that the criteria for grade III chondrosarcoma were 2 or more mitoses per 10 high-power fields in the most active areas of the tumours or areas exhibiting a spindle cell pattern. In fact, the mitotic rate tended to increase in this study according to the histological grade. In our experience, however, mitotic figures in chondrosarcoma seem to be less frequent than those previously described (Cuvelier and Roels 1979; Kreicberg et al. 1981; Alho et al. 1983). The fibrosarcoma-like spindle cell area in grade III chondrosarcoma showed a higher mitotic rate than the chondroid area in the same tumour. This might suggest a tendency for de-differentiation (Dahlin and Beabout 1971; Mirra and Marcove 1974) at the periphery of the tumour lobule in comparison with its central portion.

It is often difficult to distinguish chondrosarcoma from CBOS with limited specimens such as biopsied specimens. The present study revealed that it was relatively easy to find mitotic figures in CBOS compared with HGCS and the difference was statistically significant. The existence and quantity of mitotic figures are important histological findings for differentiating malignant chondroid tumours, including chondrosarcoma and CBOS.

It was shown in this study that the most significant determinants for differential diagnosis between ENCH and LGCS are nuclear area and binucleate cells. Cellularity and mitotic rate are important for differentiation between LGCS and HGCS, whereas mitotic rate is only useful for differentiating the cartilaginous tissues of osteosarcoma from chondrosarcoma.

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