Virchows Arch. A Path. Anat. and Histol. 373, 311 – 325 (1977)

Virchows Archiv A

Aneurysmal Bone Cyst and Telangiectatic Osteosarcoma

A Histopathological and Morphometric Study

D.J. Ruiter[1], C.J. Cornelisse[1], Th.G. van Rijssel[1], and E.A. van der Velde[2]

[1] Department of Pathology,
[2] Department of Medical Statistics, Leiden University Medical Centre, Leiden, The Netherlands

Summary. In a series of 105 cases of aneurysmal bone cyst, 18 showed an unusually high level of mitotic activity and/or increased nuclear pleomorphism which complicated the differential diagnosis with respect to telangiectatic osteosarcoma. An attempt was made to use semi-automatized morphometric and histophotometric techniques to establish objective morphological differences between these unusual cases of aneurysmal bone cyst and 16 cases of telangiectatic osteosarcoma. Three cases (two of aneurysmal bone cyst and one of telangiectatic osteosarcoma) proved unsuitable for analysis. In 24 of the remaining 31 cases (77%) a computerized discriminant analysis permitted correct discrimination with a high degree of certainty on the basis of quantitative nuclear characteristics determined in paraffin sections. In the other 7 cases the diagnosis was less certain (3), doubtful (2) or erroneous (2). The relevant nuclear characteristics were (in ascending sequence of discrimination): the largest nuclear surface area, the mitotic index, and the percentage of nuclear sections exceeding an arbitrarily chosen limit of 60 μ^2.

The criterion of nuclear size for discrimination between these benign and malignant lesions could be applied for two reasons: firstly, because a group of extremely large nuclei occur in malignant cases, and secondly, because the average nuclear size is larger in malignant than in benign lesions. The extremely large nuclei occur as only a small percentage of the total nuclear population. The other variables investigated, i.e., cellularity and nuclear contour ratio, did not contribute greatly to the differentiation. In 11 cases, the average nuclear Feulgen extinction was estimated as an additional variable.

Key words: Aneurysmal bone cyst – Telangiectatic osteosarcoma – Morphometry – DNA histophotometry – Computerised discriminant analysis.

For offprints contact: Dirk J. Ruiter, M.D., Pathologisch Laboratorium, Boerhaavekwartier, Wassenaarseweg 62, Leiden, The Netherlands

Introduction

Aneurysmal bone cyst is a solitary benign lesion of the skeleton consisting of thinwalled cavities usually filled with liquid blood. These cavities have a smooth surface, partially lined by a flattened cell layer and multinuclear giant cells, and are separated by septa which, as the solid elements of the cystic lesion, consist of cellular fibroblastic tissue containing fibrohyalin and osteoid substance. In combination with the tissue rich in intercellular substance, the more cellular tissue can show a typical layered architecture (Ruiter et al., accepted for publication).

The same kind of tissue, i.e., with multinuclear giant cells, osteoid substance, and vascular spaces, can be found in osteosarcoma of the telangiectatic type. According to some authors the differentiation between telangiectatic osteosarcoma and aneurysmal bone cyst can only be made by evaluation of nuclear details (Clough et al., 1968; Tillman et al., 1968) such as hyperchromasia, pleomorphism and mitotic activity, but detailed data are not given. Because in our experience these characteristics of osteosarcoma also can be variably present in some cases of aneurysmal bone cyst, discrimination can be very difficult. In practice, the differentiation is based mainly on qualitative evaluation of histopathological characteristics, which involves a certain degree of subjectivity (Van der Heul, 1962), and requires very extensive experience. To minimalize subjectivity, we investigated the possibility of discriminating between known cases of both conditions on the basis of objective quantifiable histopathological characteristics in the hope of obtaining a better understanding of the components on which reliable discrimination should be based and to contribute to improved differentiation in difficult cases. This made it necessary to quantitate histopathological characteristics that could be important for discrimination between these benign and malignant lesions.

It is known from many studies (Fossa, 1975; Nodskov-Pedersen, 1971) that various malignant tumors show larger nuclei, often in association with a higher nuclear DNA content, than their benign counterparts. Investigation of giant cell tumors of the skeleton has shown that the number of mitoses is higher and the nuclear pleomorphism greater in the malignant and the locally aggressive variants than in the benign variants (Jaffe, 1958; Netherlands Committee on Bone Tumours, 1966). We therefore chose as main discrimination variables the nuclear size, mitotic index, cellularity, and nuclear DNA content. These variables were applied in a computerized discriminant analaysis to establish their contribution to adequate discrimination, as recently done with urothelial cell material by Koss et al. (1975).

Material and Methods

Patient Population. Eighteen cases of aneurysmal bone cyst were compared with 16 cases of telangiectatic osteosarcoma, all diagnosed by the Netherlands Committee on Bone Tumours. The former cases were selected from a series of 105 cases reported elsewhere (Ruiter et al., accepted for publication), on the basis of a high mitotic index (13 or more in 50 fields $\times 750$) (15 cases) and/or

nuclear pleomorphism (3 cases). The 16 cases of telangiectatic osteosarcoma constituted all of the cases collected by the Committee between 1953 and the middle of 1974, when the total osteosarcoma series amounted about 500 cases. In our cases the diagnosis telangiectatic osteosarcoma had been made if the neoplastisc tissue showed formation of irregular osteoid by tumor cells and contained many blood-filled spaces, lined mainly by flattenend tumor cells and multinuclear giant cells of the osteoclastic type (Farr et al., 1975).

Layered architecture was found in 18 out of the 19 cases of aneurysmal bone cyst and in 9 of the 16 cases of telangiectatic osteosarcoma (Fig. 1). In the former group two cases showed both fibrin and necrosis whereas in the latter group fibrin was found in 9 cases and necrosis in 12. Two cases of aneurysmal bone cyst and one case of telangiectatic osteosarcoma could not be adequately investigated because of marked nuclear pyknosis and/or excessive thickness of the histological sections. This left 31 cases for analysis.

Clinical Data and Follow Up (of all 34 Cases). Twelve of the 18 cases of *aneurysmal bone cyst* recurred between 2 and 21 months after treatment. Three of these cases recurred twice. The duration of the follow-up period after the last treatment was 5 years or longer in 7 cases, between 4 and 5 years in 4 cases, between 2 and 4 years in 6 cases and in one case in a year and a half. In all but one case in which no follow up data could be obtained the patients are free of disease.

Age distribution, sex, and localization were in agreement with the series of 105 cases reported elsewhere (Ruiter et al., accepted for publication). The age and sex distribution of the cases of *telangiectatic osteosarcoma* are shown in Figure 2, where the distinct male preponderance is evident. Localization is shown in Figure 3. Follow up data for the 13 fatal cases are shown in Figure 4. Three patients are free of disease 3, 5 and 6 years after the definitive treatment. The form of treatment varied widely: primary ablative surgery in 6 cases, ablative surgery after curettage in 3, ablative surgery after irradiation in 2, excision followed by irradiation (fibula) in 1, excision (skull) in 1 and only irradiation in 2; for 1 case no data were available. In all fatal cases lung metastases were demonstrated by the thorax X-ray or at autopsy, with the exception of one case with the primary localization in the skull, where local growth lead to death.

Histopathology and Morphometry

Mitotic Index. All paraffin slides (HE) were reviewed, and the mitotic index was determined as described in detail elsewhere (Ruiter et al., accepted for publication). Counts were made blindly in cellular areas and in 4 different parts of the specimen to reduce sample error.

Nuclear Area and Cellularity Measurements. The area of about 300 sectioned nuclei was determined in each case. Three microscopical fields ($\times 250$) with high cellularity and always showing mitoses, were photographed with a Zeiss photomicroscope, and 2 prints (8×10 inch) were made from each negative, each representing half of the selected field. The total enlargement factor was 1000. The morphometric measurements were carried out with a system developed by Ploem et al., consisting essentially of a graphic tablet interfaced to a PDP-12 computer (Digital Equipment Corporation, Maynard, Mass., USA), which can be used for both direct morphometry on microscopical images and morphometry on photographic prints or transparencies. For the latter purpose, a pen containing a capacitive sensor is used to trace the contours, the electronic signals from the pen being converted to coordinates by the specially constructed interface. The coordinates are fed into the computer, and the area and perimetry are calculated with a specially developed computer program. To test the reproducibility, five nuclear contours were traced 40 times each. The variation coefficient varied with nuclear size, ranging from 3.5 to 7%, the largest nuclei showing relatively the smallest deviation. The accuracy was tested by comparing the calculated area of two circles with different radii with the area measured five times. No differences amounting to more than 2.4% of the calculated value were found.

The photographic print to be measured was laid on the tablet and covered by a transparent film provided with longitudinal lines 2 cm apart. All nuclei which touched or cut these lines were traced. Nuclei not in focus and those of lymphocytes, multinuclear giant cells and capillary endothelium, were not included. The nuclear contours were then displayed on a 611 Tectronix storage

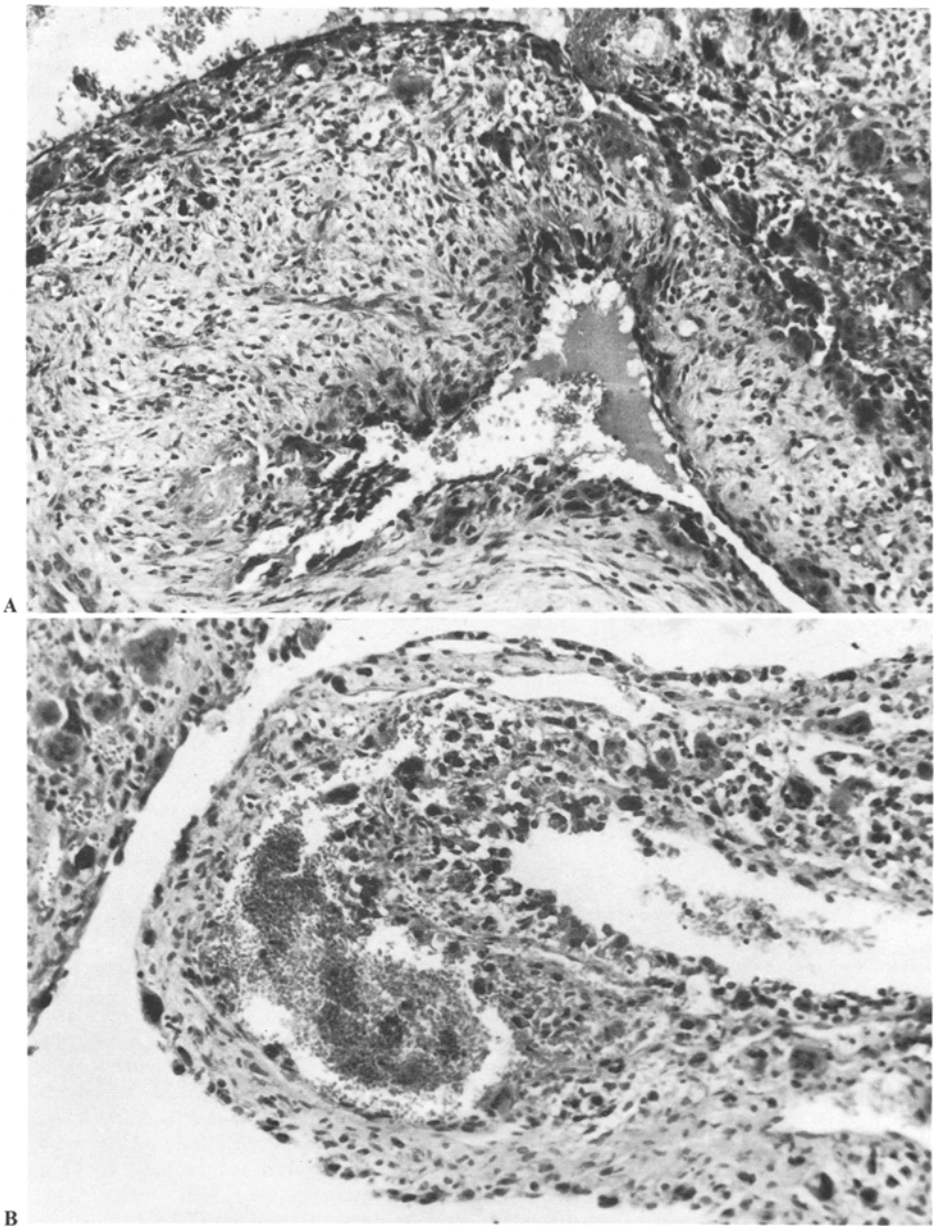

Fig. 1A and B. HE, original magnification ×100. **A** Septum of aneurysmal bone cyst. Note the endothelial lining covering the cellular tissue containing multinuclear giant cells, and situated adjacent to the more fibrous tissue in the central part of the septum. **B** Septum of telangiectatic osteosarcoma. Note the resemblance to the architecture in A, but also some scattered large hyperchromatic nuclei

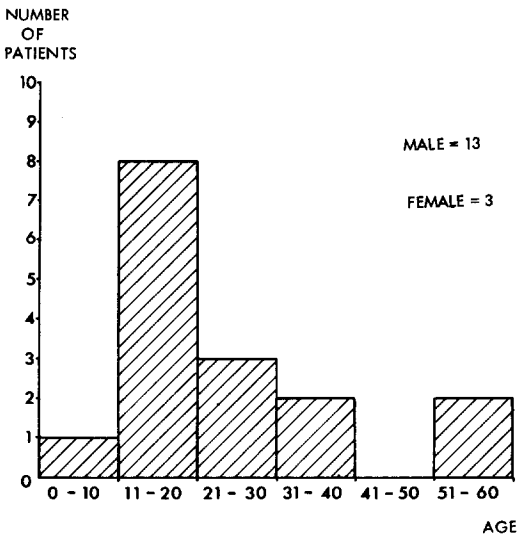

Fig. 2. Age distribution in 16 cases of telangiectatic osteosarcoma

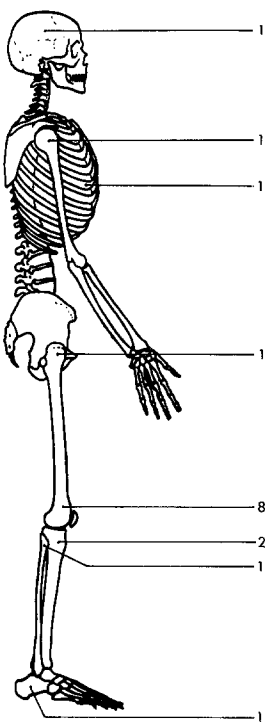

Fig. 3. Localisation of the cases of telangiectatic osteosarcoma. For the long tubular bones a distinction is made according to proximal or distal end

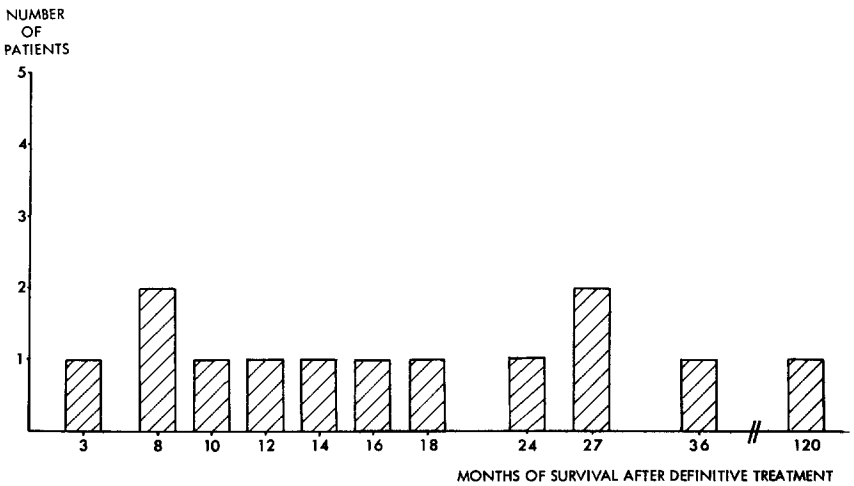

Fig. 4. Outcome of the 13 fatal cases of telangiectatic osteosarcoma

oscilloscope (Portland, Oregon, USA) to see whether the contour had been completely traced. When all nuclei of a single print were traced and a number of about 300 was reached, no further measurements were made.

Usually, 4 prints were needed to complete the quota. The mean cellularity was defined as the number of nuclei measured divided by the number of prints used. The computer gave rescaled values of the nuclear areas, expressed in squared microns. These values were used to make histograms with a standard class width of 5 μ^2, starting with zero, from which the 50th, 80th and 90th percentiles, the mean value, the highest value, and the percentage of traced nuclear sections exceeding an arbitrarily chosen limit of 60 μ^2 were derived.

Measurement of the Nuclear Contour Ratio. A shape factor was calculated for the largest 50 nuclei of each case from the outline to area ratio according to the formula area^2/outline 4, with which the minimal value for this ratio is one. The mean value and frequency distribution were then determined in each case. The histograms were plotted with a fixed class width of 1.

Discriminant Analysis. In addition to the six variables derived from the histogram, the mean cellularity per photographic print and the mitotic index were used as discriminants in the statistical analysis. Because the frequency distributions of the two groups of cases appeared to be non-normal and the variance covariance matrices were unequal, the usual linear discriminant analysis was not appropriate.

The discriminant analysis was therefore done by a method based on estimates of the frequency density function (Meisel, 1972). Details of the application of this method can be found in the manual of the program ALLOC (Hermans et al., 1975). Here it will suffice it to mention that the criterion for adding a new discriminating variable to those already selected is an increase in the number of individuals, which can be correctly allocated. The allocation is based on posterior probabilities, which are calculated from density estimates. In this process the density estimates are calculated without regard to the individual who is to be allocated.

Measurement of DNA Concentration in Nuclear Sections. For the 11 cases (5 of aneurysmal bone cyst and 6 of telangiectatic osteosarcoma) for which the paraffin blocks were still available, 3 μ thick sections were cut and stained according to Feulgen as described by Duijndam and van Duijn (1973). The thickness of the sections was estimated by adjusting the micrometer just beyond the level of focus in both directions at 20 different places per section. The places to be measured were selected by following a fixed route with the mechanical stage, as indicated below. At each

place the thickness was measured four times, and the average was taken as representative value. The vertical displacement of the stage was registered by a clock micrometer with an accuracy of one micron.

Scanning cytophotmetry was used for a quantitative estimation of the average nuclear staining density (mean local extinction) in the section. For this purpose, one section of each case was examined. Ten fields per section ($\times 750$) were selected by describing a fixed route with the scan table between two fields to be measured. This route consisted of two fields to the right followed by two fields downward ($\times 100$).

The measurements were done with a Leitz MPV-II microphotometer equipped with a Zeiss 0.5 micron scanning stage connected to a PDP-12 computer. The oil immersion objective was an NA 1.3 Leitz ($\times 63$). The total scan magnification was 256. The condensor was an NA 0.6 Zeiss objective ($\times 40$). Scanning was performed with a step size of 2 micron and a spot size of 2 micron. Each scanned field measured 100×100 squared microns and contained on average about 30 nuclei. Scanning occurred under control of the Zeiss APAMOS program, version II.

For each field, the mean local extinction (total extinction divided by scanned area) was determined and a histogram of the local extinction distribution was made. The background between the nuclei was eliminated by considering only extinction values above a certain minimal level.

The average of the mean extinction for the ten measured fields was taken as the variable for the average nuclear DNA concentration.

Results

Histopathology (34 Cases). The mitotic indices of aneurysmal bone cyst and telangiectatic osteosarcoma are shown in Figure 5. In the former the highest mitotic index was 26, but in 5 cases of telangiectatic osteosarcoma the index lay below this value; in 10 other cases of telangiectatic osteosarcoma it ranged between 30 and 70, and one case showed a mitotic index of 216.

Morphometry (31 Cases). Representative histograms of the nuclear areas are shown in Figure 6. In general a unimodal distribution with a tail at the higher values was found in both conditions, but two cases of telangiectatic osteosarcoma showed a bimodal distribution, the peaks lying at about 30 and 50 μ^2. The topological distribution of these two populations of nuclei in the photographic prints was diffuse. The shape of the histograms was generally broader and flatter for telangiectatic osteosarcoma than for aneurysmal bone cyst.

Table 1 shows the mean values and standard deviations of the parameters investigated in this study. As a general trend, the values for the sarcoma group are higher than those of the cyst group.

Discriminant Analysis (31 Cases). The difference between the two groups was substantiated by the discriminant analysis which yielded the contribution of each variable to the discrimination. On the basis of the eight variables (see Table 1), the computer program ALLOC chose the greatest nuclear area as the best discriminant. Discrimination improved when the mitotic index was added (as illustrated by Fig. 7) and improved further when the percentage of nuclear sections with an area exceeding 60 μ^2 was included as well (see Table 2). The other variables did not contribute very much to the classification. The ALLOC classification was correct with a high degree of certainty in 24 cases, and with a relatively high degree in 3 cases (see Table 2). Doubtful discrimination was made in two cases of aneurysmal bone cyst, and two cases of telangiectatic osteosarcoma were incorrectly assigned to the bone cyst group.

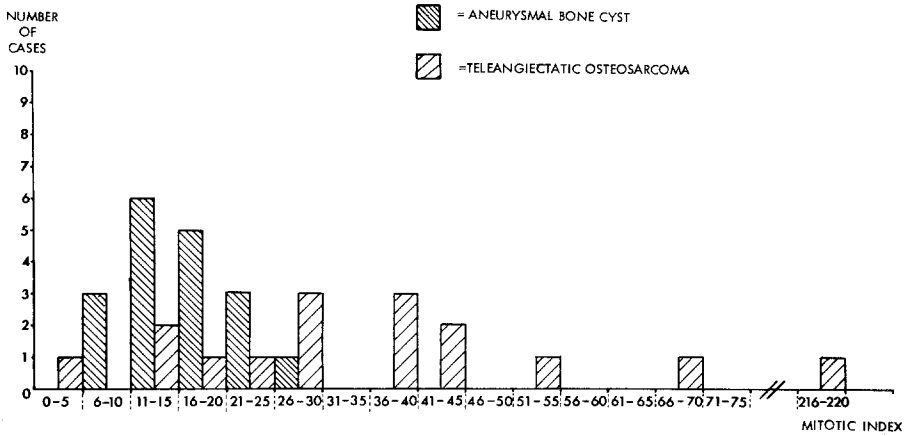

Fig. 5. Mitotic index in 18 cases of aneurysmal bone cyst and 16 cases of telangiectatic osteosarcoma

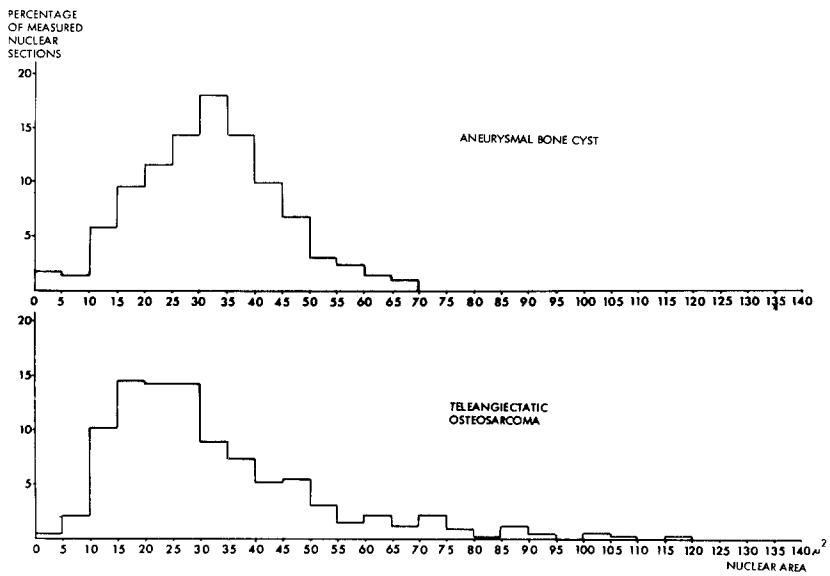

Fig. 6. Histogram showing the results obtained for the nuclear surface area in a representative case of aneurysmal bone cyst and one of telangiectatic osteosarcoma

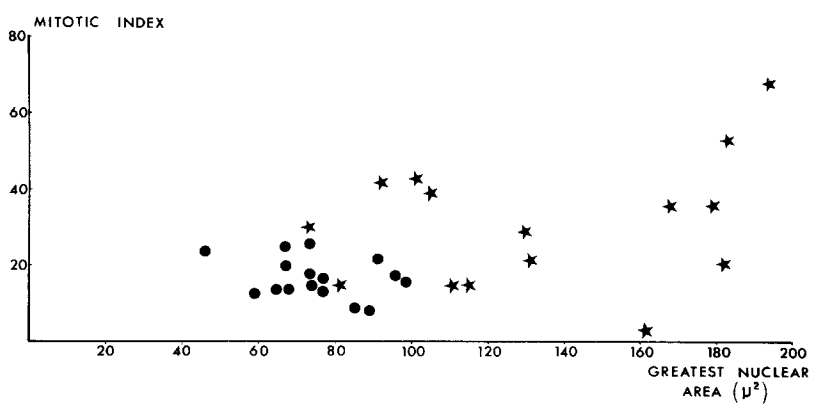

Fig. 7. Discrimination between 16 cases of aneurysmal bone cyst (dots) and 15 cases of telangiectatic osteosarcoma (stars) on the basis of two variables in the discriminant analysis

Table 1. Variables investigated

Variables	ABC ($n=16$)		TOS ($n=15$)	
	mean	SD	mean	SD
Mean cellularity	79.31	17.01	89.80	22.41
Mean nuclear area (μ^2)	63.54	12.33	83.64	13.89
Largest nuclear area (μ^2)	75.31	13.92	133.73	40.84
P50 (μ^2)	24.38	5.30	31.38	6.40
P80 (μ^2)	36.24	7.06	48.09	7.60
P90 (μ^2)	43.40	7.92	58.86	8.52
Percentage exceeding 60 μ^2	2.18	1.90	10.05	6.28
Mitotic index	16.94	5.35	31.73	16.41

Table 2. Classification according to ALLOC

Correct diagnosis	Number of patients with posterior probability[a]					Discriminating variables
	0–10%	10–20%	20–80%	80–90%	90–100%	
ABC	0	0	6	10	0	Largest nuclear area
TOS	0	2	4	1	8	
ABC	0	0	4	4	8	Largest nuclear area
TOS	1	1	2	0	11	Mitotic index
ABC	0	0	2	2	12	Largest nuclear area
TOS	2	0	0	1	12	Mitotic index. Percentage exceeding 60 μ^2

[a] Posterior probability as to assignment to the correct diagnostic group. The a priori probability was 50%

Table 3. Results of remeasurement in two cases of teleangiectatic osteosarcoma incorrectly assigned in the discriminant analysis

	Case BA 2485		Case BA 2666	
	measurement		measurement	
	First	Second	First	Second
Mean cellularity	157	171	74	99
Mean nuclear area (μ^2)	27	24	26	24
Largest nuclear area (μ^2)	81	97	73	147
P 50 (μ^2)	23	19	23	19
P 80 (μ^2)	40	34	38	35
P 90 (μ^2)	51	47	47	43
Percentage exceeding 60 μ^2	4.5	4.4	1.4	3.7

For the latter two cases photographs were taken of other cellular parts of the section in which mitoses and relatively large nuclei were present. The nuclear-size histograms were in agreement with the original measurements, but the largest nuclear area was much larger particularly for case BA2666 (see Table 3). These new values led to correct assignment to the true diagnosis with a high degree of certainty in both cases. When new variables derived from the histograms of

Table 4. Average nuclear DNA concentration of 11 investigated cases

	Bone tumor registry number	Mean local extinction	Mean section thickness (μ)	Mean local extinction corrected for section thickness	Mean nuclear area (μ^2)	Mean nuclear integrated extinction (arbitrary units)
Aneurysmal bone cyst	BA 1049	0.089	3.20	0.027	30.1	0.813
	BA 1080	0.200	5.70	0.035	28.3	0.993
	BA 2306	0.134	3.40	0.039	25.8	1.006
	BA 2991	0.225	4.75	0.047	24.6	1.155
	BA 3038	0.100	5.10	0.020	22.3	0.445
Teleangiectatic osteosarcoma	BA 1463	0.114	4.35	0.026	38.3	0.987
	BA 1715	0.235	3.21	0.073	44.4	3.238
	BA 2666	0.238	4.80	0.050	26.5	1.322
	BA 2769	0.085	3.10	0.027	34.3	0.922
	BA 3362	0.135	2.73	0.049	37.7	1.851
	BA 3807	0.063	3.70	0.017	28.5	0.484

the nuclear area (i.e., the percentages of nuclear sections exceeding 50, 70 and 80 μ^2 and the average of the three highest values) were included in the discriminant analysis, the results of the discrimination were less satisfactory than those of the original analysis. Here, the single best variable was the percentage of nuclear sections exceeding 70 μ^2, followed by the average of the three highest values and the 90th percentile, in that order. The average of the three highest values showed a very high correlation coefficient with the greatest value, i.e., 0.97 and 0.93 for the cases of aneurysmal bone cyst and telangiectatic osteosarcoma, respectively.

Nuclear Contour Ratio (31 Cases). Regarding the parameter concerning the nuclear contour for aneurysmal bone cyst and telangiectatic osteosarcoma, the mean area to outline ratios were quite similar: 2.20 (SD $\pm$ 0.28), 2.07 (SD $\pm$ 0.28), respectively. In most of the cases in both groups the peak frequency in the histogram was in the class from one to two, in the others between two and three.

Approach to Average Nuclear DNA Concentration (11 Cases). As can be seen in Table 4, the mean local extinction for the ten measured fields shows the same range for the cases of both aneurysmal bone cyst and telangiectatic osteosarcoma. Two cases of aneurysmal bone cyst have relatively high values, as do two cases of telangiectatic osteosarcoma, and one other case in the latter group shows a relatively low value. If the mean local extinction is corrected for the mean section thickness, the scattering of the values decreases for the cases of aneurysmal bone cyst but remains the same for those of telangiectatic osteosarcoma. To obtain the mean nuclear integrated extinction, the corrected mean local extinction was multiplied by the mean nuclear area of the particular case. As can be seen, there is tendency for the mean nuclear integrated extinction to lie slightly higher for the cases of telangiectatic osteosarcoma than for those of aneurysmal bone cyst, whereas the range of the values of the former is

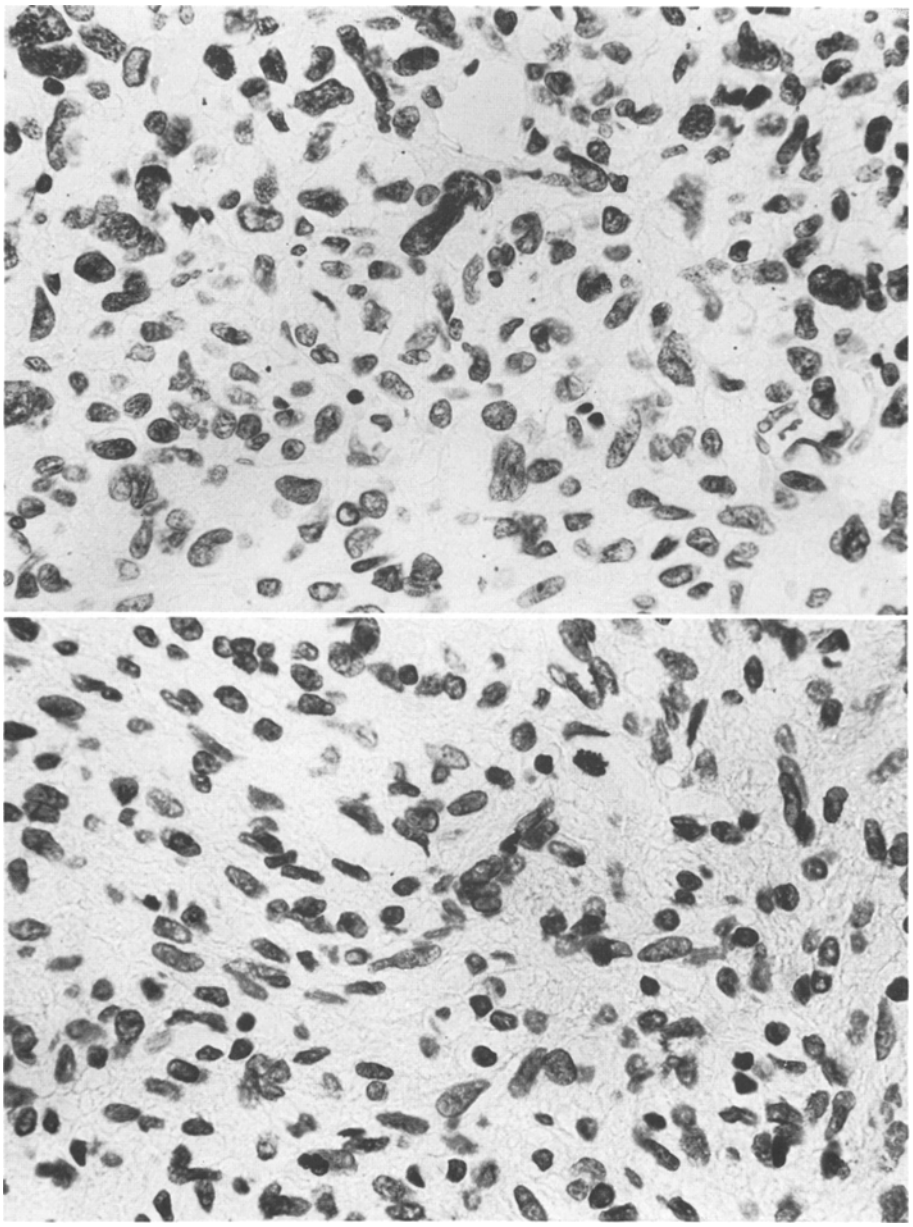

Fig. 8A and B. Feulgen, original magnification ×400. **A** Telangiectatic osteosarcoma (BA 2666). The nuclear pleomorphism is more marked here than in B. Note some large hyperchromatic nuclei. **B** Aneurysmal bone cyst (BA 2991). Moderate degree of nuclear pleomorphism

considerably wider than that of the latter. The mean values do not statistically significant differ between the two groups ($T=1.28$). One of the false-negative cases of telangiectatic osteosarcoma in the discriminant analysis (BA2666) showed a rather high mean nuclear staining density. This case is illustrated in Figure 8A (compare with Fig. 8B).

Discussion

In cases of mesenchymal proliferation with marked growth activity and nuclear pleomorphism, the exclusion of malignancy may be difficult. This is sometimes the case in aneurysmal bone cysts, and leads to confusion with telangiectatic osteosarcoma (Clough et al., 1968; Reed et al., 1964; Tillman et al., 1968). To evaluate the methods used in discriminating between these two diseases and to contribute to improved differentiation in difficult cases, a group of 16 selected cases of aneurysmal bone cyst with unusually high mitotic activity and/or nuclear pleomorphism was compared with a group of 15 cases of telangiectatic osteosarcoma. Nuclear characteristics were chosen as basis for this comparison, because previous studies had shown that many malignant tumors in man have larger nuclei with a higher DNA content than are seen in non-malignant tissue (Fossa, 1975; Nodskov-Pedersen, 1971).

The results of the present study show that it is possible to make a reasonably reliable discrimination between the two groups of patients on the basis of only a very small number of nuclear characteristics. The best discrimination with a high degree of certainty was obtained with a *combination of three quantitative variables, i.e., the largest nuclear area, the mitotic index, and the percentage of nuclei with an area exceeding* 60 μ^2 (in ascending order of discriminatary value). Both of the nuclear-size characteristics, which proved to be the most important for discrimination, were derived from the tail of the histogram. This means that on the basis of nuclear size, the differential diagnosis between these benign and malignant lesions is possible in the first place because the malignant cases show a small number (i.e., only a few percent of the total population) of extremely large nuclei, and in the second place because in these cases the average nuclear size is larger. This was further substantiated by the results of the discriminant analysis with four additional variables derived from the histograms of the nuclear area. The importance of extreme variable values for the discrimination between benign and malignant lesions is also demonstrated by the findings of Koss et al. (1975) who showed that the maximal extinction value in cell images was the single best discriminating variable between urothelial carcinoma and normal cells, the other variables being the cell area and the total extinction. However, we agree with Koss et al. (1975) that the individual variables are not the best discriminators and that a combination of variables deserves serious consideration.

The *erroneous classification* of two cases of telangiectatic osteosarcoma might be explained in several ways. Firstly, the frequency of large nuclei in these cases was rather low in comparison with the other cases of telangiectatic osteosarcoma and this led to sample error at the first measurement. Secondly, anomalous

nuclear shrinkage could have occurred as a result of sub-optimal fixation. Thirdly, only part of a very lobulated large nucleus might have been in focus in the photographic print, giving the impression of a single nucleus.

As could be expected the contour ratio of the 50 largest nuclei per case did not contribute to the discrimination, since increased values occur in both round nuclei with an irregular nuclear membrane and elongated nuclei. Probably, more sophisticated algorithmes will to be developed to take nuclear-membrane irregularities into account adequately.

Measurement of *Feulgen DNA concentrations* in nuclear sections encounters several difficulties. Firstly, a nuclear section may not be representative of a whole nucleus, because both the population of nuclei and the chromatin distribution are not homogenous and therefore mathematical correction of the volumes would be not reliable. Secondly, the measurements must be made in isolated, uncut nuclei (Tavares, 1968); this is not difficult in tissue with nuclear dispersion, such as heart muscle (Kompmann et al., 1966) but in highly cellular tissue like that of tumors, nuclei overlap very frequently, as Tavares (1968) reported for 10 μ sections of mammary carcinoma tissue. With these considerations in mind, we decided not to measure individual nuclear sections in the present study but to establish the mean nuclear extinction per microscopical field, which could serve as an objective criterion for the nuclear (hyper)chromasia as evaluated by the histopathologist. This mean local extinction was determined by dividing the total extinction by the number of picture points above a certain level of extinction, thereby avoiding the distribution error. The histophotometric measurements indicated a trend, in the sense that the mean Feulgen staining density per histological section was higher in the 6 cases of telangiectatic osteosarcoma than in the 5 cases of aneurysmal bone cyst. Since the mean nuclear area found for telangiectatic osteosarcoma is not smaller but larger than that of aneurysmal bone cyst, the histophotometric measurements indicate a higher nuclear DNA content in the former group (see Table 4), which could be expected (Fossa, 1975; Nodskov-Pedersen, 1971).

The fact that this difference between the two groups is not more marked may be related to the global approach to histological sections. However, one of the cases (BA 2666) which the discrimination analysis incorrectly classified as aneurysmal bone cyst, showed a rather high average DNA value (see Table 4), which would have supported the assignment to the telangiectatic osteosarcoma group.

With respect to the relationship between nuclear size and nuclear staining density, the histograms of the nuclear area of most of the cases of telangiectatic osteosarcoma in the present study show the same distribution and scattering as those of the optical density values of urothelial carcinomas in the study done by Koss et al. (1975). However, these authors used Papanicolaou not Feulgen stained preparations. A high degree of scattering was also found in the DNA histograms of two cases of "polymorphous osteosarcoma" reported by Födisch et al. (1974) and one of osteosarcoma (not further defined) reported by Stich and Steele (1962). These findings suggest a similar increase of both nuclear size and nuclear Feulgen DNA content in osteosarcoma, but for uterine cervical carcinoma Valeri et al. (1967) described an increase of Feulgen DNA values without a proportional increase in nuclear volume.

Because the probability of selection induced errors is high in procedures which are not fully automated, we decided to define strict criteria for selection. In addition, in tumors with a complex structure selection is necessary for the study of variables associated with biological behavior, since—as is generally accepted—mitoses should be counted in cellular parts of specimen where there is no appreciable amount of intercellular substance and no signs of regressive change (Van der Heul, 1962; Price, 1961).

The primary aim of the present study was to provide some objective variables to assist the histopathologist who is confronted with a difficult differential diagnostic problem, and not, of course, to offer a new method for histopathological investigation. The findings of this retrospective study should be applied with some caution, because negative assignment amounted to about 10 percent and because the value of the findings (which must be considered as training set (Koss et al., 1975) could not be substantiated in a test set since the lesions in question are rare.

Acknowledgements. The authors wish to thank the Department of Histo-Cytochemistry of the Medical Faculty of the University of Leiden for facilities and technical advice, Miss M.D.M. van Oort and Miss K. Kleiverda for technical assistance, Mr. K. van der Ham and Mr. W. van Kleef for preparing the photographs and figures and Mrs. M.W.J. Broekhuizen-Dubbelaar and Miss I.A. Kruyff for typing the manuscript.

References

Clough, J.R., Price, C.H.: Aneurysmal bone cysts. A review of twelve cases. J. Bone Jt Surg. **50-B**, 116–127 (1968)

Duijndam, W.A.L., Van Duijn, P.: The dependance of the absorbance of the final chromophore formed in the feulgen-schiff reaction on the pH of the medium. Histochemie **35**, 373–375 (1973)

Farr, G.H., Huvos, A.F., Marcove, R.C., Higinbotham, N.L., Foote, F.W., Jr.: Telangiectatic osteosarcoma. A review of twenty-eight cases. Cancer (Philad.) **34**, 1150–1158 (1974)

Födisch, H.J., Mikuz, G., Walter, N.: Cytophotometrische Untersuchungen und Knochengeschwülste. Verhandlungen der deutschen Gesellschaft für Pathologie. 58 Tagung. Editor G. Seifert, S. 425–429. Stuttgart: Fischer 1974

Fossa, S.D.: Feulgen-DNA-values in transitional cell carcinoma of the human urinary bladder. Beitr. Path. **155**, 44–55 (1975)

Van der Heul, R.O.: Het periostale ossificerende fibrosarcoom en de gradering van osteosarcomen. Thesis Leiden, 1962. Batteljee and Terpstra, Leiden

Hermans, J., Habbema, J.D.F.: Manual for the ALLOC discriminant analysis programs. Technical report of the Department of Medical Statistics, University of Leyden, The Netherlands (1975)

Jaffe, H.L.: Tumors and tumorous conditions of the bones and joints. Philadelphia: Lea and Febiger 1958

Kompmann, M., Paddags, I., Sandritter, W.: Feulgen cytophometric DNA determinations in human hearts. Arch. Path. **82**, 303–308 (1966)

Koss, L.G., Bartels, P.H., Bibbo, M., Freed, S.Z., Taylor, J., Wied, G.L.: Computer discrimination between benign and malignant urothelial cells. Acta cytol. (Philad.) **19**, 378–391 (1975)

Meisel, W.S.: Computer oriented approaches to pattern recognition, Chap. VI. New York: Academic Press 1972

Netherlands Committee on Bone Tumours: Radiologic atlas on bone tumours. Vol. I. The Hague: Mouton 1966, 17–58 and Vol. II, 1973, 427–453

Nodskov-Pedersen, S.: Degree of malignancy of cancer involving the cervix uteri on the basis of clinical stage, histology, size and nuclei and content of DNA. Acta path. microbiol. scand. Sect. A, **79**, 617–628 (1971)

Ploem, J.S., Comelisse, C.J.: The use of a graphic tablet for direct morphometric analysis of microscopic images. (Submitted for publication)

Price, C.H.G.: Osteogenic sarcoma. An analysis of survival and its relationship to histological grading and structure. J. Bone Jt Surg. **43-B**, 300–313 (1961)

Reed, R.J., Rothenberg, M.: Lesions of bone that may be confused with aneurysmal bone cyst. Clin. Orthop. Rel. Res. **35**, 150–162 (1964)

Ruiter, D.J., Van Rijssel, Th.G., Van der Velde, E.A.: Aneurysmal bone cysts. A clinicopathological study of 105 cases. Cancer (Philad.) (accepted for publication)

Stich, H.F., Steele, H.D.: DNA content of tumor cells. III. Mosaic composition of sarcomas and carcinomas in man. J. Nat. Cancer Inst. **28**, 1207–1218 (1962)

Tavares, A.S.: Ploidy and histological types of mammary carcinomas. Europ. J. Cancer **3**, 449–455 (1968)

Tillman, B.P., Dahlin, D.C., Lipscomb, P.R., Stewart, J.R.: Aneurysmal bone cyst: An analysis of ninety-five cases. Mayo Clin. Proc. **43**, 478–495 (1968)

Valeri, V., Cruz, A.R., Brandao, H.J.S., Lison, L.: Relationship between cell nuclear volume and deoxyribonucleic acid of cells of normal epithelium, of carcinoma in situ and of invasive carcinoma of the uterine cervix. Acta cytol. (Philad.) **11**, 488–496 (1967)

Received October 11, 1976