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Original Articles

The Prognostic Significance of Different Histomorphologic Features in Chondrosarcoma

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Summary. Cellularity, mitotic rate and nuclear size were determined objectively in 47 chondrosarcomas. Grading was also performed, according to the systems of O'Neal and Ackerman and of Evans. The survival rate and incidence of metastases 10 years after diagnosis were calculated.

The prognostic significance of the objectively determined cytological parameters was investigated. The accuracy of determination of these cytological parameters by grading (Evans) was also analyzed.

Nuclear size seemed to be the best prognostic parameter, while cellularity appeared to be the strongest determinant for grading.

Gross differences of nuclear size can be assessed visually, while more subtle, although prognostically significant, differences can only be determined by objective means.

The results of this study indicate that the mere determination of nuclear size by objective means may give significant prognostic information, which is equal to, or even better than, conventional grading of chondrosarcoma.

Key words: Chondrosarcoma – prognosis – nuclear size – mitotic rate – cellularity.

Introduction

The basic diagnostic criteria for chondrosarcoma of bone, as proposed by Jaffe and Lichtenstein in 1943, are still widely accepted [16]. Since then the introduction of a grading system by O'Neal and Ackerman (19), and the recognition of histopathological subgroups such as dedifferentiated, mesenchymal and clear-cell chondrosarcoma, has further improved the diagnosis of this bone tumor [4, 5, 7, 18, 20, 22]. However, the histopathological distinction between benign and malignant cartilaginous bone tumors may be very taxing. Furthermore, the grading of malignancy in chondrosarcomas is still considered very difficult

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and sometimes unreliable [6, 13, 15, 17, 19]. Several studies have shown that high grade chondrosarcomas are associated with a lower survival rate than low grade chondrosarcomas, but most lack statistical proof of these observations. Although there is general agreement on most of the histopathological parameters of importance for grading chondrosarcomas, there seems to be a certain discrepancy between histopathological grading and clinical course. This can only be explained to some extent by factors such as tumor localization, tumor size and treatment.

A correlation between grading and clinical course in chondrosarcoma was reported in 1977 by Evans et al., who used a modification of the conventional grading system [10]. In addition to the evaluation of intercellular background and frequency of lacuane containing multiple nuclei, cytological criteria such as nuclear size, cellularity and mitotic rate were found to be of decisive prognostic importance. Although this grading system may seem better defined than previous systems, it is still based on a highly subjective evaluation of different cytological parameters.

The present study was aimed at investigating the prognostic significance of nuclear size, cellularity and mitotic rate in chondrosarcoma. It was also considered important of investigating how well the histopathological grading (according to Evans) corresponded with an objective determination of these parameters.

Material and Methods

Material. This material was originally selected from the Swedish Cancer Registry for a retrospective prognostic DNA study of chondrosarcoma to be published later. Forty-seven cases fulfilling the requirements for cytophotometrical DNA measurements also met the conditions for this particular study. Since specimens suitable for cytophotometrical measurements proved to be limited, this material cannot be considered fully representative. Nevertheless, it permits a comparative study of different prognostic parameters.

Survival rates and the incidence of metastases 10 years after diagnosis could be determined for all 47 patients in this study. Forty-four patients had been treated surgically, while three patients, considered inoperable, although free of metastases, received no treatment.

The age and sex distribution is presented in Fig. 1. Tumor localization is presented in Fig. 2.

Histopathology. The 47 original surgical or biopsy specimens were studied. New sections were cut and stained with hematoxylin and eosin. The slides were reviewed by two pathologists, independently and without any knowledge of the corresponding clinical data. One pathologist graded according to O'Neal and Ackerman, the other according to Evans (Table 1). Dedifferentiated chondrosarcomas were evaluated as Grade III.

Cellularity, Mitotic Rate and Nuclear Size. The same slides that were reviewed histopathologically in this study were also used for the objective determination of cellularity and mitotic rate.

The most cellular areas in each slide were located with 10 × objective. Cellularity was determined by counting the number of cells in 5 to 10 high power fields (40 ×). The average cell number per high power field was calculated and used as a measure of cellularity.

The mitotic rate was determined in each slide within the same tissue areas that were used for the determination of cellularity. The number of mitotic figures in 5 to 20 high power fields (40 ×) was counted and the mitotic rate (A) was expressed as the number of mitotic figures per ten high power fields. Furthermore, the number of mitotic figures per thousand cells (mitotic rate B) was calculated using the results from the determination of cellularity. Occasionally there were difficulties in identifying mitotic figures with certainty. However, only unequivocal mitotic figures were counted.

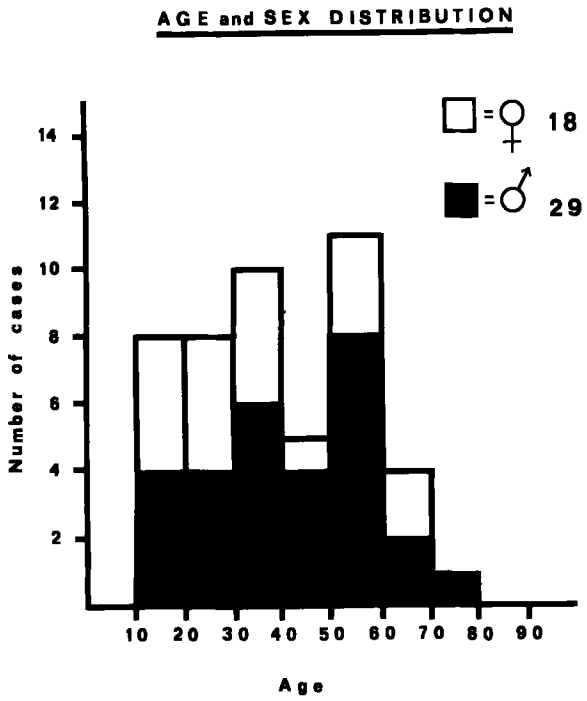

Fig. 1. Distribution of 47 cases of chondrosarcoma according to age and sex

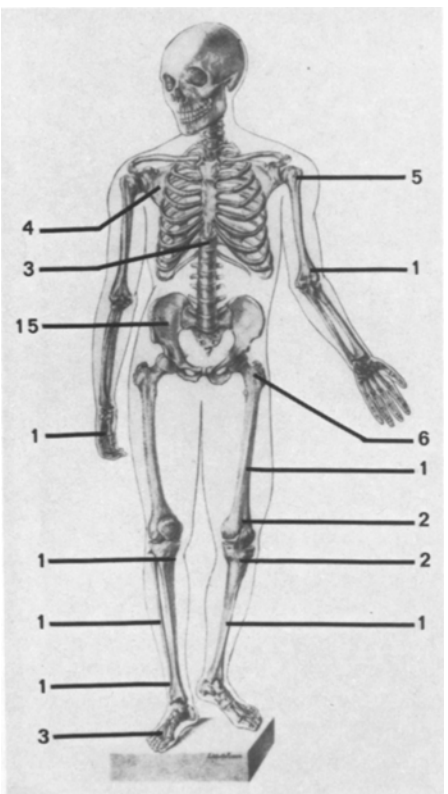

Fig. 2. Skeletal distribution of 47 chondrosarcomas

TOTAL S=180 A= 65 TI= 2789 MI= 0.4291 TE= 26.331
ME= 0.405 DE= 9

			85	84			
		61	45	43	43		
	65	33	29	32	30	42	
	34	26	27	26	27	31	84
81	30	27	36	45	26	28	76
76	36	27	30	38	30	28	83
74	38	35	32	29	30	27	
84	44	32	30	26	29	35	
	55	29	23	23	26	47	
	70	37	29	26	32	69	
		68	54	45	67		

Fig. 3. Computerized transcription from a cytophotometrical DNA measurement of a Feulgen stained chondrosarcoma cell nucleus. Each transmission value corresponds to an area of 1 μm^2. The total sum of the transmission values (A) denotes the nuclear size (projected area) of the cell in square micron

Accurate determinations of nuclear size (μm^2) were obtained by cytophotometrical measurements in Feulgen-stained tissue sections [14] which were used primarily for a DNA analysis to be published later. The sections were obtained from the same paraffin blocks, from which sections had been taken for histopathological grading and determination of cellularity and mitotic rate. Computerized transcription from these DNA measurements provided topographical representations of each nuclear size and form (Fig. 3). The cell nuclei were chosen at random for measurement. The sizes of 80 to 120 nuclei were determined in each slide. The 50th and 90th percentile (P50 and P90) for the nuclear size were determined as measures of median and extreme nuclear size in each cell population.

Statistics

To minimize the influence of extreme values, the logarithmic values of the objectively determined parameters were used for the calculation of mean values.

Geometric means and confidence limits (95%) were calculated from the logarithmic values.

Pearson correlation coefficients were determined between the different parameters in this study. Student's *t*-test was used to investigate whether there were any significant differences between the different parameters in various respects. *P*-values >0.05 were considered not to be significant (n.s.).

Multiple regression analysis [9] was applied to investigate whether a combination of several objectively determined cytological parameters would increase the correlation with grading and whether such a combination would give additional prognostic information.

Results

The overall 10 year survival in this series was 49%.

The distribution of the 47 chondrosarcomas by grade according to O'Neal and Ackerman and to Evans are presented in Table 1. There was a tendency to classify more cases as grade II instead of grade I when using the O'Neal and Ackerman grading. A closer comparison of grading according to O'Neal and Ackerman and to Evans showed agreement in 25 cases (53%), a one-grade difference in 20 cases (43%) and a two-grade difference in only two cases (4%) (Table 2).

Table 1. Distribution of 47 chondrosarcomas with respect to grading

Grade	O'Neal-Ackerman	Evans
I	13 (28%)	21 (45%)
II	23 (49%)	15 (32%)
III	11 (23%)	11 (23%)

Number and percentage of 47 chondrosarcomas of different grades according to O'Neal and Ackerman and to Evans

Table 2. Comparative grading of chondrosarcoma

		Evans		
		I	II	III
O'Neal-Ackerman	I	9	2	2
	II	12	9	2
	III	0	4	7

Grading of 47 chondrosarcomas according to O'Neal and Ackerman and to Evans. Complete agreement (underlined) was obtained in 25 cases

Table 3. Grading and clinical course

	10 year Survival		Metastasis	
Grade	O'Neal-Ackerman	Evans	O'Neal-Ackerman	Evans
I	61%	62%	31%	35%
II	56%	40%	45%	53%
III	18%	36%	64%	55%

Percentage of surviving and metastasizing cases of each grade according to O'Neal Ackerman and to Evans

Table 4. The prognostic significance of grading

	10 year Survival		Metastasis	
Grade	O'Neal-Ackerman	Evans	O'Neal-Ackerman	Evans
I–II	$P=0.777$ n.s.	$P=0.205$ n.s.	$P=0.467$ n.s.	$P=0.242$ n.s.
II–III	$P=0.036$	$P=0.858$ n.s.	$P=0.285$ n.s.	$P=0.954$ n.s.
I–III	$P=0.032$	$P=0.180$ n.s.	$P=0.117$ n.s.	$P=0.260$ n.s.

Statistically tested differences in survival and metastatic rate between tumours of different grades according to O'Neal and Ackerman and to Evans

The survival rate and incidence of metastases 10 years after diagnosis in relation to grading (O'Neal and Ackerman/Evans) is presented in Table 3. Differences in 10 year survival and the incidence of metastases between tumors of different grades were analyzed using Student's *t*-test (Table 4).

There was a significant correlation between grading according to Evans and the objectively determined cytological parameters, although some overlapping of these parameters between tumors of different grades was noted (Table

Table 5. Grading and objective cytology

1. Cellularity	$r=0.72$	$P<0.001$
2. Mitotic rate A	$r=0.70$	$P<0.001$
3. Mitotic rate B	$r=0.63$	$P<0.001$
4. Median nuclear size	$r=0.59$	$P<0.001$
5. Extreme nuclear size	$r=0.58$	$P<0.001$

Correlation coefficients between grading according to Evans and the objectively determined cytological parameters

Table 6. Intercorrelation between different cytological parameters

Cellularity – mitotic rate (A)	$r=0.65$	$P<0.001$
Cellularity – median nuclear size (P50)	$r=0.54$	$P<0.001$
Median nuclear size (P50) – mitotic rate (A)	$r=0.44$	$P=0.002$

Table 7. Relation between morphological parameters and clinical course

10 year					
Survival			Metastasis		
1. Median nuclear size	$r=0.40$	$P=0.006$	1. Median nuclear size	$r=0.33$	$P=0.021$
2. Extreme nuclear size	$r=0.32$	$P=0.028$	2. Cellularity	$r=0.30$	$P=0.037$
3. Grading (O-A)	$r=0.30$	$P=0.041$	3. Extreme nuclear size	$r=0.29$	$P=0.051$
4. Cellularity	$r=0.26$	$P=0.083$	4. Grading (O-A)	$r=0.23$	$P=0.114$
5. Grading (E)	$r=0.22$	$P=0.139$	5. Grading (E)	$r=0.19$	$P=0.210$
6. Mitotic rate (A)	$r=0.11$	$P=0.441$	6. Mitotic rate (A)	$r=0.11$	$P=0.455$
7. Mitotic rate (B)	$r=0.07$	$P=0.615$	7. Mitotic rate (B)	$r=0.06$	$P=0.706$

Correlations(r) between different morphological parameters and the clinical course (10 year). Significant correlations (Stutent's *t*-test) are underlined. O-A = O'Neal-Ackerman. E = Evans

5). Statistical analysis also showed that there was a strong intercorrelation between these parameters (Table 6).

Nuclear size was found to correlate both with the survival and the incidence of metastases (Table 7). Cellularity was significantly correlated with the occurence of metastases but not with survival after 10 years (Table 7). No correlation was found between mitotic rate and the clinical course (Table 7).

Since nuclear size (median value, P50) and cellularity seemed to be the strongest prognostic factors these parameters were further analyzed. Comparisons of tumor cellularity and mean nuclear size were made between 10-year survivors and non-survivors and between patients with and without metastases within 10 years after diagnosis using Student's *t*-test. This test was also used to compare chondrosarcomas of different grades with respect to mean nuclear size and cellularity.

Significant differences were found between the different morphological types of chondrosarcoma, both with respect to nuclear size and cellularity (Table 8). These differences in relation to grading were more pronounced for cellularity

Table 8. Nuclear size and cellularity in relation to grading

Chondrosarcoma	*n*	Nuclear size			Cellularity		
		Mean (μm^2)	C.L.	*P*	Mean	C.L.	*P*
Grade I	21	32	29–35		45	35– 57	
				0.032			0.001
Grade II	15	38	33–44		85	69–111	
				0.018			0.001
Grade III	11	47	41–54		217	135–347	

Mean nuclear size and cellularity of 47 chondrosarcomas of different grades. Student's *t*-test (*P*-values) was used to compare grade I and II, and grade II and III chondrosarcomas with respect to nuclear size and cellularity

n=number of tumours of each grade. Mean (μm^2)=the mean of the median (P50) nuclear sizes in square micron. Mean=the mean of the average number of cells per high power microscopic field (40×). C.L.=confidence limits (95%). *P*=*P*-values (Student's *t*-test)

Table 9. Nuclear size and cellularity in relation to clinical course

Cases	*n*	Nuclear size			Cellularity		
		Mean (μm^2)	C.L.	*P*	Mean	C.L.	*P*
Surviving	23	33	31–36		64	47– 85	
				0.006			0.083
Non-surviving	24	41	36–46		98	66–144	
Non-metastasizing	26	34	31–37		63	48– 84	
				0.025			0.037
Metastasizing	21	41	36–47		106	70–160	

Mean nuclear size and cellularity of 47 chondrosarcomas characterized by different clinical courses (10 year). Student's *t*-test (*P*-value) was used to compare surviving and non-surviving as well as non metastasizing and metastasizing cases with respect to nuclear size and cellularity

n=number of patients. Mean (μm^2)=the mean of the median (P50) nuclear sizes in square micron. Mean=the mean of the average number of cells per high power microscopic field (40×). C.L.=confidence limits (95%). *P*=*P*-values (Student's *t*-test)

than nuclear size. Of greater interest however, is the fact that chondrosarcomas characterized by different clinical courses showed significant differences of nuclear size (Table 9).

Multiple regression analysis showed that *combinations* of cytological parameters did not correlate better with grading (Evans) than *individual* cytological parameters, confirming the observed intercorrelation between these parameters. Nor did such combinations correlate better with survival rate or the incidence of metastases.

In summary there was a fairly good agreement between grading according to O'Neal and Ackerman and to Evans. Cellularity seemed to be the strongest

determinant for grading (Evans). Nuclear size, on the other hand, appeared to be the most important factor related to prognosis.

Discussion

The histopathological grading of chondrosarcoma is based on various qualitative and quantitative morphological findings. This study was an attempt to analyze more closely the accuracy of determination of different well defined morphological features in chondrosarcoma as well as their prognostic relevance.

Nuclear size was found to be a most significant predictor of prognosis. Cellularity appeared to be the strongest determinant for grading. The data from this study also indicate that cellularity, mitotic rate and nuclear size, on which grading is based to a great extent, can be evaluated subjectively with a fair degree of accuracy. Closer analysis, however, showed that these parameters were strongly intercorrelated. This is what could be expected since these cytological features are closely related to each other. Increased cellularity, mitotic rate and nuclear size may to a certain extent be seen as three different expressions of increased proliferative activity. The small differences (6–9 μm^2) in nuclear size, however, that were noted between chondrosarcomas of different grades can probably not be detected by eye and are therefore unlikely by themselves to be an important factor in the subjective categorization of a tumor to a particular grade. The fact that nuclear size differed in tumors of various grades rather reflect the influence of other parameters such as cellularity and mitotic rate, which probably are of greater significance for the subjective grading. Since differences in cellularity are more conspicious and easier to evaluate visually than differences in the other cytological parameters investigated, it seems reasonably to assume that grading was based mainly on cellularity. Statistical data indicate that this is probably true.

This observation does not conflict with the fact that the pathologist who graded according to Evans succeeded very well in following the criteria for this system. Nevertheless, this "adequately" performed grading was not correlated significantly with the clinical course.

Although grading according to Evans seemed to be of limited prognostic value, two of the cytological parameters of this system were individually correlated with the clinical course. Nuclear size and cellularity were thus found to provide significant prognostic information. The fact that mitotic rate gave no prognostic information may be attributed to the difficulties of identifying mitotic figures with certainty in this old archival material.

Combinations of the cytological parameters did not improve the prognostic information, since these parameters were strongly intercorrelated. For prognostic purposes it seems sufficient to limit the cytological determinations to nuclear size.

This parameter, however, cannot be determined as accurately by the pathologist as by objective means. Extreme differences of nuclear size may be assessed visually, but more subtle differences cannot. Chondrosarcomas characterized by different clinical courses showed small differences of nuclear size. In general,

these differences, although statistically and prognostically significant, can probably not be distinguished visually.

The results from this study also indicate that other factors than those investigated are involved in grading and may be of importance for prognosis. It is a well established fact that the clinical course in chondrosarcoma is influenced by factors such as tumor localization, tumor size and treatment [3, 6, 8, 13, 15, 17, 19]. The overall evaluation of chondrosarcoma thus includes a wide variety of parameters. This study was merely an attempt to determine the *relative* prognostic significance of different histomorphological features in *identical specimens of chondrosarcoma.*

This study shows that it is possible to obtain significant prognostic information in chondrosarcoma by the mere determination of nuclear size. This implies not only that nuclear size is an important prognostic factor, but also that an objective parameter may be used as a basis for more relevant comparisons between different series of chondrosarcoma.

The prognostic significance of nuclear size may be explained by the fact that this parameter is related to proliferative activity [2, 21]. Proliferating cells have been shown to contain an increased amount of nuclear protein [2, 21]. Furthermore, aneuploidy in malignant cells may also contribute to increased nuclear size [1, 11, 12, 14]. This calls for further investigations of different factors related to nuclear size.

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