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Nuclear grading of renal cell carcinomas – is morphometry necessary?

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Summary. Comparative investigations of subjective with objective nuclear grading methods of renal cell carcinomas are almost completely lacking. Therefore, we graded 94 cases of this carcinomas by a simple, subjective microscopical estimation as well as by a morphogenetic measurement of nuclear area. Both procedures proved prognostically useful, but the best results were achieved by morphometry. By this method three prognostic groups of renal cell carcinoma were found, provided that the borderlines were drawn at 28 μm^2 and 60 μm^2, respectively. Particularly favourable and unfavourable cases could be separated from average ones, if the means and standard deviations of both the nuclear areas and the diameters were evaluated. Overall, morphometric nuclear analyses are highly desirable, if, for example, morphological data are to be used in the context of prognostic or therapeutic studies on renal cell carcinoma. However, there is a broad distribution of the values for individual cases so that, tumour-biologically, no exact demarcation of prognostically different groups can be expected.

Key words: Renal cell carcinomas – grading – nuclear area – morphometry

Introduction

Nuclear grading systems for renal cell carcinoma involve a variety of criteria, such as size, shape, and polymorphism of nuclei, structure and density of the chromatin, number and sizes of nucleoli, and mitotic figures (Fuhrmann et al. 1982; Skinner et al. 1971). However, none of these procedures is exactly defined and generally accepted, so that the above-mentioned criteria may be differently assessed and rated by various investigators resulting in differing results and poor reproducibility. For this reason Tosi et al. (1986) proposed the use of only one criterion for the grading procedure, namely the nuclear area measured morphometrically.

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Such measurements are hardly practicable in the daily diagnostic routine, in contrast to a subjective estimation of the nuclear area, which is quickly done in conventional microscopic analyses. We therefore compared the efficacy of a nuclear grading of renal cell carcinomas by morphometry with that by subjective estimation on the basis of survival data for 94 cases.

Materials and methods

Patients

A total of 102 consecutive cases of renal cell carcinoma, subjected to nephrectomy between 1975 and 1980, were examined. Of these, 94 patients could be followed-up for up to 9 years, with a minimum of 4 years. Patients who died within the first postoperative month or for reasons independent of tumour disease were excluded. At the time of the study 60 patients had died. The study involved 60 men and 34 woman with a mean age of 59 (range 32–77) years.

Stage of cases

The cases included 1 at stage T1, 32 at stage T2, 58 at stage T3, and 3 at stage T4 (TNM classification 1987). At the time of surgery, 24 cases were known to have either lymph node or distant metastases, or both. Renal vein invasion was seen in 31 cases. Of the 33 T1 and T2 carcinomas, 8 had metastasized, i.e. 25 tumours were in accordance with stage I defined by Robson et al. 1969.

Grading methods

Two methods were used in each case on the same 5-μm-thick paraffin section, stained with haematoxylin and eosin.

Morphometry of nuclei. From each section, 100 randomly chosen tumour cell nuclei were measured within those regions contained the largest nuclei. Each nucleus was carefully focused and incomplete nuclei were ignored. For comparison, 500 nuclei of normal proximal kidney tubules were studied. The nuclear areas and the maximal diameters were evaluated with a Zeiss-Kontron MOP-

AMO3 microprocessor-assisted planimeter on a digitizer tablet with projection of microscopic features, magnified 2000 × (for details, see Schulz 1988). In order to control the reproducibility of morphometric results, 46 renal cell carcinomas were measured in an identical manner by a second investigator without previous knowledge of the selected tumor area. Possible errors due to the measuring system (Fleege et al. 1988) were avoided.

Estimation of nuclear area. The nuclear area was estimated at medium power (objective 25 ×). The cases were divided into three groups of small (group 1), medium-sized (group 2), and large nuclei (group 3), the largest nuclei determining the grouping. Medium-sized nuclei were defined as corresponding to those of normal tubular epithelia. The reproducibility was assessed by comparing the grouping of 46 renal cell carcinomas by two independent investigators.

Statistics

For each chase, the means and standard deviations of the nuclear areas and diameters were calculated. The frequency distribution was tested for bimodality by the Haldanetest (1951). Survival curves were calculated by the Kaplan-Meier method (1958). Statistical significance of survival-curve differences was calculated by a generalized Wilcoxon-Breslow test. A stepwise proportional-hazards general linear model procedure (Cox-model modified by Harell 1986) was used to assess the interdependence of several prognostic factors with respect to their significance. Reproducibility of the results was tested using weighted κ statistics (Kramer and Feinstein 1981).

Results

The morphometric data for the nuclei varied considerably from case to case (Fig. 1). Thus, the mean nuclear areas ranged from 17.1 μm^2 up to 126.9 μm^2, and the mean diameters varied between 4.9 µm and 15.2 µm. Between these extremes, all transitions could be found, with an accumulation of the means resulting in a distribution to the left, suggesting bimodality; this however, could not be confirmed on applying the Haldanetest (1951). The spread of the intraindividual nuclear data increased continuously with increasing mean values (correlation coefficient, r, for mean values and SD of nuclear areas: $r = 0,.73$; for diameters: $r = 0.74$). The nuclear diameters and areas were closely correlated with each other ($r = 0.94$).

In view of the continuity of the morphometric data from low to high, arbitrary thresholds had to be drawn in order to form groups and to analyse their prognostic significance. In this respect, several possibilities were tested. A subdivision of the cases into three groups, based

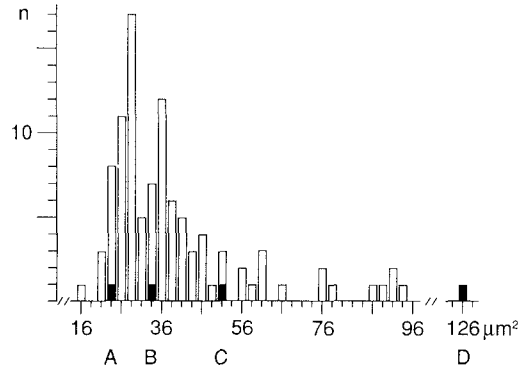
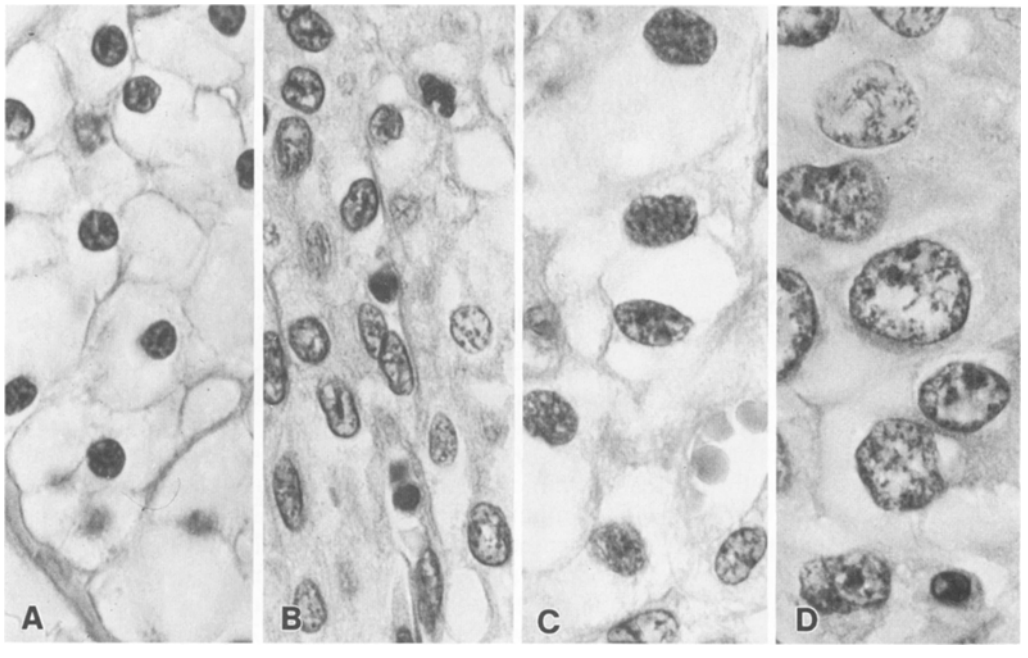


Fig. 1. Distribution of the mean values for nuclear areas of 102 renal cell carcinomas. There is a continuous transition from low to high values. *A-D* and the *black columns* indicate the position of the examples depicted below (H&E, × 850).



on the data for the mean nuclear areas, rendered significant differences between the three survival curves. The best prognostic discrimination was achieved by using thresholds of 28 μm^2 and 60 μm^2 (Fig. 2a). Similar results could also be reached by grouping only the latter 46 of the 94 consecutive cases of renal cell carcinoma.

In a further step, we selected those cases with very small mean nuclear areas, very small mean nuclear diameters and very small respective standard deviations, as well as cases that showed very high corresponding data (see legend to Fig. 2b). Seven cases fell into the first group and displayed particularly low data. In contrast, 8 cases belonged to the second group and revealed extremely high data. The first group showed an exceptionally good prognosis, while the second comprised cases with an extremely poor prognosis.

When the nuclear area was subjectively estimated, 27 cases were found to have homogeneous small nuclei (low grade), 53 had medium-sized nuclei (intermediate grade), and 14 had large nuclei (high grade).

The corresponding survival curves for these groups behaved differently at a high level of significance for the highly malignant group versus the group with intermediate malignancy, but this was not the case for the comparison of group of low versus that of intermediate grades of malignancy (Fig. 3).

Four-year survival rates were found to correlate with the data on nuclear areas obtained by measurements and estimations as well as those for all cases free from metastases (Table 1).

Within the T2 und T3 tumour groups free of metastases (defined by TNM classification 1987) both estimated and measured nuclear areas tended to correlate with the 4-year survival rates but without showing significant P-values between the small subgroups (Table 1). The same result was seen in a subdivision of the 25 cases of stage I disease (defined by Robson et al. 1969) with respect to the nuclear area.

Among the prognostic parameters, the presence of metastases was a highly significant criterion ($P<0.00001$) when the unadjusted χ^2 test was used. A high prognostic significance was also observed in the data obtained by both nuclear grading methods (Table 2). However, the morphometric data for the nuclear areas showed a lower P value ($P=0.0001$) than did those for the estimation of nuclear areas ($P=0.02$).

In contrast, renal vein invasion and T stage were of minor significance. This, however, may be due to the fact that our material contained only a few T1 and T4 cases.

If the prognostically meaningful parameters were adjusted stepwise, only the presence of metastases and the morphometric data for the nuclear areas displayed an outstanding, independent and significant rank (Table 2).

The reproducibility was tested by comparative investigations of 46 renal cell carcinomas: 74% of the cases were identically grouped by morphometry and 78% by estimation of nuclear area, as found by two independent observers. The κ values 0.55 (morphometry) and 0.69 (estimation) were comparable and underlined the substantial strength of agreement.

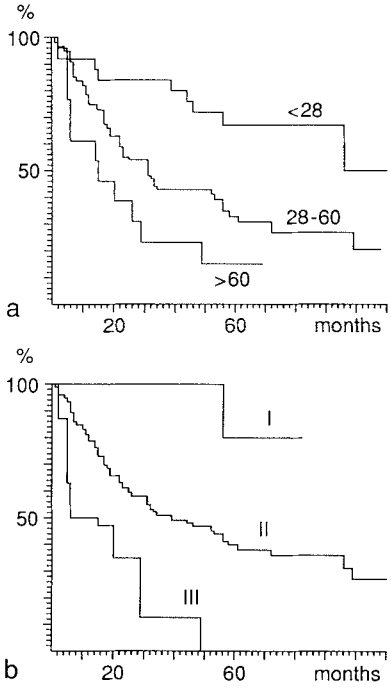


Fig. 2a, b. Morphometric grouping of 94 renal cell carcinomas. **a** Subdivision using nuclear area values of 28 μm^2 and 60 μm^2 as thresholds. The survival curves differ significantly (all cases $P=0.009$; 25 cases with nuclear areas <28 μm^2 vs 56 cases 28–60 μm^2 $P=0.006$; 13 cases with nuclear areas >60 μm^2 vs 56 cases 28–60 μm^2 $P=0.03$). **b** Renal cell carcinomas with particularly favourable or unfavourable prognosis selected on the basis of a combined evaluation of particularly low (group I) and high (group III) mean nuclear areas, mean nuclear diameters and low and high standard deviations. Group I = 7 cases (area <28 μm^2, SD <6.5 μm^2, diameter <7.2 μm, SD <0.9 μm). Group II = 79 intermediate cases. Group III = 8 cases (area >60 μm^2, SD >14.8 μm^2, diameter >9.6 μm, SD >2.2 μm). (Survival curves for all cases $P=0.0002$; group I vs II $P=0.028$; II vs III $P=0.001$)

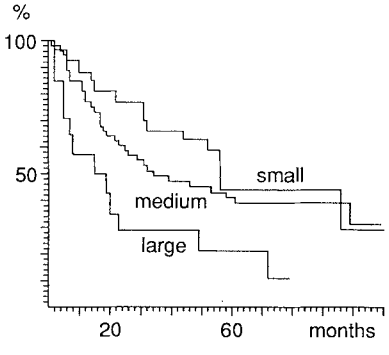


Fig. 3. Grouping by estimation of nuclear area. (Survival curves differed for all cases $P=0.01$; 27 cases of small nuclear areas vs 53 medium $P=0.24$; 14 cases of large nuclear areas vs 53 medium $P=0.24$; 14 Cases of large nuclear areas vs 53 medium $P=0.03$)

Discussion

Our results show that useful prognostic data for renal cell carcinoma may be gained when a simple estimation of the nuclear area is used. Moreover, such an estimation is not in the least time-consuming and is highly appropriate for routine purposes. However, the definition of

Table 1. Nuclear area measurement/estimation and 4-year survival rate (4-yrs)

Tumours		All cases			Cases without metastases		
		4-yrs	*n*	*P*	4-yrs	*n*	*P*
All tumours							
Measurement	$<28\ \mu m^2$	72	25		78	23	
	$28–60\ \mu m^2$	43	56	0.0009	56	39	0.0135
	$>60\ \mu m^2$	23	13		38	8	
Estimation	Small	63	27		73	22	
	Medium	45	53	0.0134	59	39	0.0911
	Large	29	14		44	9	
T2-tumours							
Measurement	$<28\ \mu m^2$	75	8		86	7	
	$28–60\ \mu m^2$	43	21	0.1186	60	15	0.2013
	$>60\ \mu m^2$	33	3		50	2	
Estimation	Small	64	11		78	9	
	Medium	39	18	0.6375	58	12	0.5017
	Large	67	3		67	3	
T3 tumours							
Measurement	$<28\ \mu m^2$	69	16		74	15	
	$29–60\ \mu m^2$	47	32	0.0121	54	24	0.0957
	$>60\ \mu m^2$	20	10		33	6	
Estimation	Small	60	15		67	12	
	Medium	50	34	0.0548	59	27	0.2328
	Large	22	9		33	6	

Table 2. Prognostic factors and survival rate. Cox model, modified by Harrel 1986

Variables	*P*				
	Unadjusted[a]	Step 1		Step 2	
		Metastases entered (Cox model)[c]	Adjusted for metastases[b]	Nuclear area measurement entered (Cox model)[c]	Adjusted for metastases and nuclear area measurement[b]
Metastases	<0.00001	<0.00001		<0.00001	
Nuclear area measured	0.0001		<0.01	0.003	
Nuclear area estimated	0.02		0.03		0.33
Vein involvement	0.08		0.53		0.61
T stage	0.14		0.38		0.54

[a] Simple unadjusted χ^2 *q* statistics
[b] χ^2 *q* statistics adjusted only for variables in the model
[c] Wald-χ^2 statistics for variables in the model

thresholds between groups of prognostically differing behaviour still remains a problem (Barry and Sharkey 1985; Colpaert et al. 1987), largely because of its subjective nature.

This disadvantage may be partly overcome by morphometric methods, which yield better reproducible data with higher precision than do nuclear grading systems (Baak and Oort 1983; Gundersen et al. 1981; Haapasalo et al. 1990). Among these data, those of nuclear areas are more informative and more reproducible than are nuclear circumferences or form factors (Barry and Sharkey 1985; Tosi et al. 1986). And indeed, the few data hitherto available for renal cell carcinoma (Tosi et al. 1986; Bibbo et al. 1987) show a particularly close inverse correlation between nuclear area and survival data, which is in accordance with our own results. Similar experiences have been reported earlier for other neoplasms (Baak and Oort 1983; Blomjous et al. 1990; Dardick et al. 1988; Schnürch et al. 1989; Donhuijsen and Leder 1985; Nomori et al. 1988; Ooms et al. 1981).

The question remains: how many prognostic groups should be cut off from morphometric data and where should the boundaries between the groups be set in order to achieve meaningful prognostic cohorts? In our hands, the best compromise was obtained by forming three groups in accordance with conventional grading pro-

cedures (Boxer et al. 1979; Ekfors et al. 1987; Mostofi 1981; Thoenes et al. 1986).

Published morphometric data on the prognosis of renal cell carcinoma are rare. Tosi et al. (1986) investigated 41 cases at stage I and found a distinct two-peak distribution of nuclear areas below 32 μm^2 for long-term survivors and values above 32 μm^2 for short-term survivors. Our 25 stage I cases (T1 and T2 carcinomas without metastases), subdivided with respect to this threshold (32 μm^2), revealed 11 patients with a 4-year survival rate of 82% in the group with small nuclei and 14 patients with a 57% 4-year survival rate in the group with large nuclei. Their survival curves did not differ significantly ($P = 0.2$). Furthermore, we found a considerable overlap of the morphometric data for long- and short-term survivors. Our results for all 94 cases did not detect the existence of a distinct two-peak distribution. The prognostic relevance of nuclear area for stage I renal cell carcinoma also was confirmed by the measurements of Bibbo et al. (1987) in 19 cases.

The existence of numerous cases of renal cell carcinoma on the borderline between low, intermediate and high grades of malignancy could not only be detected by morphometry but has also been substantiated by flow cytometry and autoradiography (Chin et al. 1985; Grignon et al. 1989; Kagawa et al. 1985; Rabes et al. 1979). This implies that even when the mean values for the groups differ clearly and significantly from each other, the allocation of an individual case to a given group may still be problematic insofar as the individual prognosis may not match the prognosis of the group. This is due to the existence of morphological and prognostic spectra within each of the defined groups, which mirror the individual behaviour of malignant neoplasms as a result of the genetic instability of their cells (Leder 1986; Ljungberg et al. 1985; Norton 1985). The histological inhomogeneity of renal cell carcinoma (Schulz 1988) has probably caused the limited reproducibility of the tumour grouping by morphometry.

Notwithstanding these objections, it should not be forgotten that the grade of malignancy is only one of the criteria that determine the prognosis of a given case. For example, in our investigation, the existence of metastases is the most important prognostic factor.

Doubtless nuclear grading by estimation of the nuclear sizes, which is easy to carry out, may render fairly relevant prognostic data. However, morphometric data are more objective and have more discriminative power than subjective grouping (van der Poel et al. 1990). Therefore, we strongly advise the use of morphometry when such data are to be used as a basis for prognostic and/or therapeutic scientific studies.

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