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Selective nuclear morphometry as a prognostic factor of survival in renal cell carcinoma

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Summary. In the present study, we sought to determine the predictive value of selective nuclear morphometry (SNM) for patient outcome in renal cell carcinoma (RCC). Tumor samples of 140 renal adenocarcinomas diagnosed and treated with radical nephrectomy and hilar lymphadenectomy between 1970 and 1988 with a minimum follow up of 5 years in all the cases were studied by SNM. The morphometric analysis was performed in the most malignant tumor selected zone. Selection was based on cytological criteria including nuclear grade. Nuclear morphometric features analyzed were: area, perimeter, major diameter, major and minor diameter of the equivalent ellipse, volume of the equivalent ellipse and sphere, circumference diameter, and shape factors. The results showed that in the selected zone tumor nuclei were larger than in the zones selected at random. There was an inverse correlation between morphometric parameters and survival and a direct one between tumoral grade and stage. Tumors of the long-term survival group of patients presented nuclei with smaller morphometric measurements than tumors of short term survival group, with significant differences between them ($p<0.05$). In the survival analysis carried out by the Kaplan-Meier method significant differences existed between different groups formed from break point for: area, perimeter, major diameter, major and minor diameter of the ellipse, volume of the ellipse and sphere, circumference diameter and perimeter shape factor. In the multivariate analysis carried out by the Cox method, the feature with the most predictable value related to survival, was the tumor stage. Morphometric value with the highest punctuation in the test was major nuclear diameter. The rest of the morphometric values (except elliptic shape factor and elongation factor) were also significant but they did not improve prognostic information of the major nuclear diameter. SNM offers a useful aid in a more objective grading of RCC. Multivariate Cox analysis revealed additional value of karyometry to tumor stage. SNM can be a useful tool for stratification of patients with RCC.

Key words: Renal cell carcinoma, Morphometry, Prognosis

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Introduction

Renal cell carcinoma (RCC) is the most frequent tumor of renal parenchyma, where it constitutes greater than 90% of primary malignant neoplasms (Medeiros and Weiss, 1990), and it accounts for 3% of all adult malignancies. Patients with RCC generally have a relatively poor prognosis, the 5-year survival rate being close to 50% (Guinan et al., 1995). RCC is well known for its unpredictable behavior and tendency to recur and metastasize years after diagnosis. The basic factors estimating the prognosis of these patients are the pathological stage and the nuclear grade (Fuhrman et al., 1982; Medeiros and Weiss, 1990). However, although nuclear grade predicts survival it does not have a uniform application and it is a subjective method. Therefore, there is a need for karyometric methods which can provide more objective evaluation. In recent years, several morphometric studies have demonstrated the value of karyometry as a prognostic factor in RCC (Gilchrist et al., 1984; Tosi et al., 1986; Bibbo et al., 1987; Murphy et al., 1990; Gutierrez et al., 1992; Eskelinen et al., 1993; van der Poel et al., 1993). These studies were done on randomly-selected zones of renal carcinoma. Nevertheless, RCC is very heterogeneous and cytological tumoral characteristics vary in different zones (van der Poel et al., 1993). In order to avoid this bias, we carried out a karyometric study of RCC selecting the tumor zone with higher malignancy, on the basis of nuclear grade and pleomorphism, which could reflect more precisely the aggressiveness of the neoplasm.

Materials and methods

A retrospective study was made of the 140 patients with RCC treated between 1970 and 1988 at the

Table 1. Clinical data of the patients

No. of patients		140
Mean age (range)		59 (11-86)
Sex (Males/Females)		92/48
Site of tumor (Right/Left)		74/66
Stage Robson	UICC	
I	T1-2N0M0	41
II	T3aN0M0	32
IIIA	T3bN0M0	32
IIIB	T1-3N1-3M0	6
IIIC	T3bN1-3M0	6
IVA	T4N0-3M0-1	4
IVB	T1-3N0-3M1	19

Marqués de Valdecilla University Hospital. All the patients underwent radical nephrectomy and hilar lymphadenectomy. The minimum follow up time was five years or until death, in all the cases. Postsurgical clinical staging of the tumors was carried out according to Robson et al. (1969) and by the TNM classification system of the UICC of 1987 (Schröeder et al., 1988) (Table 1).

The patients were divided into two groups: a) long-term survival group, if they lived five years from the beginning of the treatment; and b) short-time survival group when they died before the above mentioned period. Analysis included only deaths due to RCC. Histological studies were performed using histological preparations obtained from representative samples of the tumors fixed for 24 to 48 hours in 10% buffered neutral formalin and embedded in paraffin. At least one sample was taken for each one cm of the tumor in every case. Sections, 5 μm thick, were stained with hematoxylin and eosin. Histological classification using Fleming's system was performed (Fleming, 1993). Collecting duct carcinoma cases were excluded. The tumors were also classified based on cytoplasmic characteristics as clear, granular, chromophobe, mixed, or spindle cell (Murphy et al., 1994). A nuclear grade was assigned to each neoplasm using Fuhrman's grading system (Fuhrman et al., 1982). Morphological assessment was performed in a blind manner. The zone with the highest grade of malignancy was selected within every preparation by the following criteria: a) higher nuclear grade according to Fuhrman et al. (1982); b) higher cellular pleomorphism; and c) higher presence of mitoses and atypias. All the investigations were performed in the selected zone. Selective nuclear morphometry was performed with the MOP-Videoplan semi-automatic image analyzer (Kontron, Eching, Germany). This system is equipped with an Olympus BH-2 microscope (Olympus, Tokyo, Japan), a Sony DXC-101P video camera (Sony Corporation, Tokyo, Japan) attached to the microscope, a graphic tablet and a computer. The software used was the standard 5.41 version (Kontron, 1983). In every case, 100 consecutive tumoral nuclei from the most malignant selective zone were measured. The nuclear images observed with a x100 objective were projected on the monitor screen and were outlined with an electromagnetic pen. The following parameters were evaluated for each nucleus: area, perimeter, major diameter, major and minor diameter of the equivalent ellipse, volume of the ellipse, volume of the sphere, circumference diameter, and the following shape factors: 1) perimeter shape factor (For $Pe=[4\pi \times area]/perimeter^2$). Its value is =1 for circle, and <1 for ellipse and irregular structures; 2) elliptic shape factor (For $Ar=area/[4\pi \times major\ diameter \times minor\ diameter]$). Its value for regular structures, circle and ellipse is =1, and <1 for irregular structures; 3) elongation factor (For Ell=minor diameter/major diameter). Its value is =1 for circle, and <1 for elliptical structures.

To assess the intraobserver accuracy and reproducibility of the measurements, the coefficient of variation (CV) was used which was calculated in six series of twenty measurements each as described by Fleege et al. (1988). A value of CV between 1 and 2 is considered acceptable (Collan et al., 1986). In our study the global value was 1.8%.

Statistical analysis was performed with the S.A.S. statistical package (SAS Institute Inc., Cary, NC, USA). For each prognostic factor survival curves were made according to the Kaplan-Meier method (Kaplan and Meier, 1958). They were tested by the log-rank test (Peto et al., 1977). The association of various prognostic factors was analyzed using Student's t test, chi-square test, and Pearson's correlation test. Multivariate analysis of the variables that were significant for survival was carried out by the Cox proportional hazards model (Cox, 1972). The stepwise method was used to select the model, with use of the likelihood-ratio test. A significance level of 0.05 was required for entering and removing covariates. The effect of the prognostic factors that contributed significantly to the model was calculated in terms of relative risk.

Results

The results of nuclear morphometric measurements are shown in Table 2. Comparing the morphometric measurements of long-term survival and short-term survival group, using the Student's t test, significant differences were observed between mean measurements of both groups for the following nuclear features: area, perimeter, major diameter, circumference diameter, major and minor diameter of the ellipse, volume of the ellipse, volume of the sphere and For Pe. There was a inverse correlation between the morphometric variables and survival: short-term survival patients showed cells with larger nuclei than those of the long-term survival group (Table 2). Overall five-year survival of the 140 patients with RCC was 46%. Analyzing the different morphometric variables a statistically significant correlation was observed among all of them with exception of shape factors. The distribution of the cases according to nuclear grade, Robson stage, overall survival at 5 years, histopathological and cytological

Table 2. Histoquantitative data related to long-term and short-term survival (Student's t test)

	MEAN	SURVIVAL Long-term	Short-term	p
Area	76±23.9	68.9±18.8	82.1±26.2	0.001
Perimeter	31.5±4.8	30.0±3.9	32.7±5.1	0.0006
Major diameter	10.8±1.7	10.3±1.4	11.3±1.8	0.0003
Circumference diameter	9.6±1.4	9.2±1.1	10.0±1.5	0.001
Ellipse major diameter	11.2±1.8	10.6±1.4	11.7±1.9	0.0006
Ellipse minor diameter	8.4±1.2	8.1±1.0	8.7±1.3	0.003
Ellipse volume	462.8±224.8	397.1±165.3	519.8±250.4	0.001
Sphere volume	395.5±195.5	337.8±143.0	445.6±220.6	0.001
For Pe	0.93±0.02	0.93±0.01	0.92±0.02	0.005
For Ar	0.99±0.03	0.99±0.01	0.99±0.003	0.1
For Ell	0.76±0.08	0.76±0.04	0.76±0.11	0.9

Table 4. Relationships among survival and nuclear area with Fleming classification and cellular type of tumors.

	No. PATIENTS	SURVIVAL 5 YEARS (%)	NUCLEAR AREA *
Fleming classification			
No esp/Nonpap	123	46.3	75±22.9
Papillary	14	50	80.8±25.1
Chromophobe	1	100	78
Sarcomatoid	2	0	105.5±70.2
Cellular type			
Clear	65	58.4	64.4±16.3
Granular	56	41	85.7±24.8
Mixed	17	23.5	84.9±20.2
Spindle	2	0	105.5±70.2

*: nuclear area: mean ± SD.

classification, and nuclear area are shown in Tables 3 and 4. As the nuclear grade and the stage increased, the positive direct correlation with nuclear area and the inverse correlation with the five-year survival are observed. Clear cell tumors were more frequent, had a

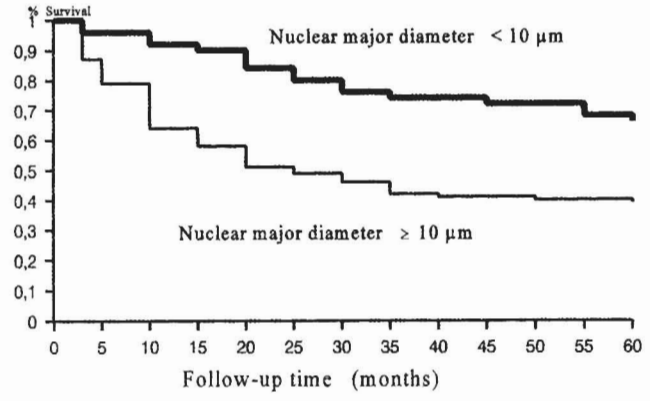

Fig. 1. Survival of patients categorized according to nuclear major diameter. The difference between the curves is highly significant. (p<0.0006).

Table 3. Relationships among nuclear grade and stage and survival and mean nuclear area.

	No. PATIENTS	SURVIVAL 5 YEARS (%)	NUCLEAR AREA (μm^2)*
Nuclear grade			
I	7	71.4	51.2±5.5
II	86	56.9	69.6±19.4
III	42	23.8	87.8±21
IV	5	20	122.1±34.4
		p<0.05 (#)	
Robson stage			
I	41	70.7	62.2±20.1
II	32	54.5	73.7±24.7
IIIA	32	46.8	73.5±18.8
IIIB	6	16.6	84.5±29.4
IIIC	6	33.3	88.2±14.7
IVA	4	0	84.1±25.4
IVB	19	0	94.7±28.1
		p<0.05 (#)	

*: nuclear area: mean±SD; #: Kaplan-Meier (Log-Rank analysis)

better prognosis and their nuclei were smaller, with a significant difference regarding the granular and mixed tumors. There were no significant differences regarding the survival between papillary and no special/non-papillary tumors, neither regarding the histoquantitative measurements.

In Table 5 the relationship between karyometry data and survival can be seen. Significant differences among different groups are clearly shown. Patients with tumors whose cells were formed by larger size nuclei (larger morphometric measurements) showed lower survival. In the multivariate analysis carried out by the Cox multiple regression method, all the morphometric variables proved to be significant (Table 6). However, major nuclear diameter showed the highest predictable value (Fig. 1) and in the second place the major diameter of ellipse. Taking into account all the parameters, the tumor stage by Robson classification, was the factor of the highest reliability to determine the survival of the patients (Table 6).

Discussion

Numerous pathological parameters have been studied to help predict survival in RCC. Previous investigations have shown that tumor stage and nuclear grade are the most reliable of these parameters. In our study clear cell tumors were the most frequent type and these patients had a better outcome than those with granular cell or mixed tumors. Tumors containing spindle cells, or sarcomatoid RCC, carried the worst prognosis. Many studies confirm these data (Bertoni et al., 1987; Ro et al., 1987). Nuclear grade II tumors were the most frequent type. According to survival two groups of patients can be formed: a) those with a favorable prognosis (grade I and II); and b) those with a dismal prognosis (grade III and IV). Fuhrman et al.

Table 5. Histoquantitative variables and survival analysis.

		No. PATIENTS	SURVIVAL (months)	SURVIVAL AT 5 YEARS (%)	LOG-RANK	p
Area	≥60μm^2	103	34.8	43	5.5	0.01
	<60μm^2	37	47.5	64		
Perimeter	≥30μm	80	32.3	41	6.6	0.01
	<30μm	60	45.1	60		
Major diameter	≥10μm	89	31.7	39	11.6	0.0006
	<10μm	51	48.3	66		
Ellipse major diameter	≥11μm	67	29.9	37	10.6	0.001
	<11μm	73	45	60		
Ellipse minor diameter	≥8μm	60	30.5	38	7.5	0.006
	<8μm	80	43.2	57		
Ellipse volume	≥400μm^3	76	31.9	35	6.8	0.009
	<400μm^3	64	44.7	59		
Circumference diameter	≥8.5μm	111	35.3	41	3.93	0.04
	<8.5μm	29	47	65		
Sphere volume	≥300μm^3	86	33	37	6.3	0.01
	<300μm^3	54	45.3	61		
For Pe	≥0.92	101	41.3	51	7.0	0.008
	<0.92	39	28.6	33		

Table 6. Independent predictors of survival in Cox's multivariate analysis.

	SCORE	SIGNIFICANCE (p)
Nuclear morphometry		
Area	17.39	<0.0000
Perimeter	18.23	<0.0000
Major diameter	21.09	<0.0000
Ellipse major diameter	18.54	<0.0000
Ellipse minor diameter	12.96	0.0003
Ellipse volume	18.18	<0.0000
Circumference diameter	15.20	0.0001
Sphere volume	18.03	<0.0000
For PE	12.73	0.0004
Robson stage	91.88	<0.0000

(1982) classified RCC into three distinct groups: favorable prognosis (grade I); dismal prognosis (grade IV); and a large group with an intermediate survival (grades II and III). Patients with stage I tumors had a significantly improved prognosis compared with those individuals with stage III or IV neoplasms. Survival is inversely correlated with increasing pathological stage. Many studies have shown the same conclusion (Robson et al., 1969; Guinan et al., 1995).

Because of the known reproducibility problems of the subjective grading methods, more objective criteria are needed. The principal advantages of nuclear morphometry in tumoral pathology are objectivity, reproducibility and accuracy for the same or different observers. To date, relatively few studies have addressed nuclear morphometry in RCC.

Several morphometric studies in random tumoral zones have been published in the literature. Gilchrist et al. (1984) assessed nuclear size subjectively and concluded that larger nuclei predicted shorter survival. Tosi et al. (1986) in a study of 47 cases, and Bibbo et al. (1987) in 19 cases found a correlation between nuclear morphometry and survival in patients with stage I RCC. Murphy et al. (1990) in a study of several nuclear morphometric features in 10 patients found that nuclear shape analysis allowed the correct assignment of outcome of localized carcinoma. Gutierrez et al. (1992) studied the prognostic significance of morphometry in 95 RCC. Nuclear area was the factor which showed the greatest statistical significance for prognosis. Taking a mean nuclear area of 35μm^2 allowed two prognostic groups to be established regardless of stage. Patients below the threshold had a good prognosis and those above it had a poor prognosis. Thus, 97 percent of patients of the first group survived after 5 years compared with 17 percent of those of the second group. Eskelinen et al. (1993) in a series of 135 RCCs asserted that clinical, histological and morphometric factors were significantly interrelated in that the metastatic high-grade tumors had larger nuclei, larger variation in nuclear size and shape and were also rapidly proliferating. In their study the results indicated that although an accurate prognostic evaluation of RCC can be based on subjective nuclear grading and karyometry, the simple assessment of mitotic index supplies most of the prognostic data, particularly in local tumors. Van der Poel et al. (1993) in 121 patients observed that quantification of anisokaryosis offered additional prognostic information to tumor stage.

Our morphometric analysis carried out in the most malignant tumoral zone shows greater nuclear morphometric measurements. All morphometric parameters (except For Ar and For Ell) were significantly related to patient survival and these quantitative variables were significant predictors of survival in a univariate analysis. In the Cox multivariate analysis, major nuclear diameter

was the most important morphometric parameter determinant for survival. However, pathological stage was the most important single prognostic indicator in RCC. A direct correlation was found between stage and nuclear morphometric parameters. Thus, karyometric analysis by SNM revealed additional value to tumor stage.

RCC is a heterogeneous and variable tumor, as different cytological characters are found in manifold intratumoral zones studied. An attempt has been made to avoid such a problem in the present morphometric analysis. The study has been carried out in the tumoral zone with the most malignant cytological characters (i.e., selective morphometry). We believe the parameters obtained will help predict individual tumor aggressiveness.

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