Urol Res (2000) 28:52–56

ORIGINAL PAPER

D. Katušin · B. Užarević · M. Petrovečki
M. Mlinac-Lucijanić · M. Marušić · Z. Mareković

Clinical, histopathological and flow-cytometric properties of incidental renal cell carcinomas

Received: 21 July 1998 / Accepted: 1 July 1999

Abstract Medical records of 63 patients operated on for renal cell carcinoma (RCC) between 1986 and 1996 in the Karlovac General Hospital were studied retrospectively. In 23 (36.5%) patients, the tumor was incidentally detected. The median patient age was 62 in the incidental group and 64 years in the symptomatic group ($P > 0.05$). Ultrasonography was the leading technique for incidental detection of RCC. The median tumor diameter was 6 cm in the incidental group and 9 cm in the symptomatic group ($P < 0.001$). Incidental carcinomas had a lower stage ($P = 0.022$) and a lower nuclear grade ($P < 0.001$) than the symptomatic ones. The incidental cases were associated with a more favorable ploidy status ($P = 0.027$) and a lower proliferative activity ($P = 0.005$). The 5-year survival rate was significantly higher in incidental (81.4%) than in symptomatic cases (44.3%) ($P = 0.020$). Univariate analysis showed that tumor stage, ploidy status, and proliferative activity were good prognostic parameters, while patient age, tumor size, and nuclear grade were not. Tumor stage was the only independent prognostic parameter in multivariate analysis. In conclusion, the incidentally detected RCC show more favorable clinical, histopathological, and flow-cytometric characteristics and their prognosis is significantly better than in symptomatic cases.

Key words Renal cell carcinoma · Incidental renal tumors · Flow-cytometry · Prognostic factors

D. Katušin (✉)
Karlovac General Hospital, Department of Urology,
Andrije Štampara 3, 47000 Karlovac, Croatia
e-mail: davorin.katusin@ka.tel.hr
Tel.: 385 47 331 299, Fax: +385 47 336 090

B. Užarević · M. Marušić
Department of Clinical Laboratory Diagnosis,
Zagreb University Hospital Center and School of Medicine,
Zagreb, Croatia

M. Petrovečki
Department of Informatics,
Rijeka University School of Medicine
and Dubrava Clinical Hospital, Zagreb, Croatia

M. Mlinac-Lucijanić
Division of Pathology, Karlovac General Hospital,
Karlovac, Croatia

Z. Mareković
Department of Urology,
Zagreb University Hospital Center, Zagreb, Croatia

Introduction

A widespread use of ultrasonography (US), computed tomography (CT), and other powerful diagnostic techniques over the last decades has increased the number of incidentally detected renal cell carcinomas (RCC) [1, 22, 23, 26]. Renal cancers are diagnosed in patients with symptoms or diseases not related to RCC or even in asymptomatic patients at preventive checkups. According to recent studies, 25–53% RCC are detected incidentally [8, 22].

These incidentally detected tumors are usually smaller, of a lower stage than symptomatic ones, and have a more favorable survival rate [1, 2, 8, 15, 16, 18, 22, 23]. The reports on the grade of these lesions are controversial [15, 16, 22, 23]. Despite numerous studies on this issue there is still no final answer on several questions:

1. What is the biological potential of these tumors? Are they merely early detected RCC or new "less malignant" varieties of this tumor?
2. Should they be treated radically, as symptomatic RCC, or could some of them be submitted to partial nephrectomy or to watchful waiting [11, 13, 17]?

The aim of this study was to compare clinical, histopathological and flow-cytometric characteristics of incidental and symptomatic RCC surgically treated at the Karlovac General Hospital over the past 11 years. We performed a flow-cytometric analysis of paraffin-embedded specimens of all renal cancers removed at surgery. We believe that assessing the ploidy status and proliferative activity of incidental RCC in comparison

with corresponding characteristics of symptomatic RCC could contribute to our better understanding of their nature and biological potential. Finally, we assessed the prognostic value of an incidental tumor finding and compared it with the significance of other prognostic parameters.

Patients and methods

Medical records of 63 consecutive patients operated on for RCC between January 1986 and November 1996 at the Karlovac General Hospital were reviewed. Only the patients submitted to surgical treatment (radical nephrectomy in 60 cases, simplex nephrectomy in two cases and partial nephrectomy in one case) and patients with histopathologically confirmed RCC were included. In 51 cases the access to the kidney was made through an abdominal and in 12 cases through a lumbar incision. Regional lymphadenectomies were performed in 51 cases. Overall, median patient age was 63 years (range 33–88). There were 38 men and 25 women (ratio 2:1.4). The cancer was considered incidentally detected in all patients with no symptoms and in those with the symptoms not related to local or systemic signs of renal carcinoma. Patients with microhematuria detected during examinations for diseases other than RCC were defined as incidental. Patients with symptoms such as pain, flank mass, hematuria, weight loss or fever originating from RCC or suspected metastatic disease were considered symptomatic. The preoperative evaluation consisted of physical examination, routine laboratory tests, chest X-ray, abdominal US and CT scan, and excretory urography in all patients. Other diagnostic techniques, including angiography, inferior venacavography, and bone scintigraphy, were performed as indicated. Clinical and pathological (pTNM) tumor stages were assessed according to the fifth edition of the TNM classification of malignant tumors from 1997 [19]. The tumor size was measured at its longest diameter on a pathological gross specimen. The grading of malignancy was based on the nuclear morphology as described by Fuhrman [6].

Sixty-three paraffin-embedded specimens of renal cancers removed at surgery were available for flow-cytometric analysis. All specimens were processed according to the method by Hedley et al. [10]. Two 30-μm sections were cut from the paraffin tumor blocks and deparaffinized in xylene, rehydrated through a series of decreasing ethanol concentrations, and twice washed in distilled water. The tissue was resuspended in 0.5% pepsin (Sigma, St. Louis, Mo., USA) in 0.9% NaCl (pH 1.5) and incubated in a water bath at 37°C for 30 min, with intermittent vortexing. Extracted nuclei were stained for DNA content according to modified Vindelov's method [25]. Ribonuclease S (Sigma) was added to approximately 1–2×10^6 nuclei and incubated in a water bath at 37°C for 30 min, centrifuged, and resuspended in 50 mg/ml propidium iodide (Sigma) for 30 min at room temperature. Stained samples were measured on FACScan flow cytometer (Becton Dickinson, San Jose, Calif., USA) using argon ion laser at 488 nm. For each DNA analysis 10,000 nuclei were counted. DNA histograms were analyzed with ModFIT program. A histogram was interpretable if the coefficient of variation of G_0/G_1 peak was less than ten. The DNA histograms were interpreted independently of the clinical data. Histograms with one symmetrical G_0/G_1 peak were classified as diploid and those with more than one G_0/G_1 as aneuploid. Diploid tumors with more than 15% of the total cell count in G_2/M fraction were considered DNA aneuploid (tetraploid). Proliferative activity was defined as the percentage of S-phase cells. In aneuploid cases the proliferative activity was calculated for the aneuploid cells only.

Statistics

Fisher's exact probability test was used to analyze the statistical difference in patient distribution between the groups. Survival rate was calculated by the Kaplan-Meier method. Cox's proportional hazards regression was used to evaluate the effect of parameters to patients' survival. In this analysis binary-split variables were coded zero (low and/or unfavorable) and one (high and/or favorable).

Results

Among 63 patients with RCC, the diagnosis was made incidentally in 23 cases (36.5%), whereas 40 patients (63.5%) presented with one of the symptoms suggestive of RCC. Median patient age was 62 years (range 42–82) in the "incidental" group, and 64 years (range 33–88) in the symptomatic group ($P > 0.05$). There was no difference in the male/female distribution between the groups (Table 1).

In 20 cases (87.0%), the diagnosis of incidental RCC was made using US, in two cases (8.7%) excretory urography, and in one case (4.3%) using CT scanning. Excretory urography was suggestive of RCC in 12 patients (52.1%) in the incidental group but more frequently (32 cases, 80.0%) in the symptomatic group ($P = 0.014$). The tumor was palpable in three (13.0%) of the incidental cases and in 22 (55.0%) of the symptomatic cases ($P = 0.009$). Microscopic hematuria was present in seven (30.4%) patients with incidentally discovered RCC, while in the symptomatic group 24 (60.0%) patients had gross (18) or microscopic hematuria (6) ($P = 0.022$). Accelerated erythrocyte

Table 1 Clinical, histopathological and flow-cytometric differences between patients with incidental ($n = 23$) and symptomatic ($n = 40$) renal cell carcinoma

Parameter	No. of patients with RCC		*P*
	Incidental ($n = 23$)	Symptomatic ($n = 40$)	
Age (≤60/>60 yrs)	10/13	16/24	0.705
Male/female	14/9	24/16	0.581
Right/left kidney	14/9	18/22	0.171
Tumor size (≤7/>7 cm)	17/6	10/30	<0.001
pTNM stage 1 + 2/3 + 4	19/4	13/27	0.022
Nuclear grade 1 + 2/3 + 4	20/3	15/25	<0.001
Diploid/nondiploid	19/4	22/18	0.027
S-phase (≤4%/>4%)	16/7	13/27	0.005
Accelerated SE (yes/no)	5/18	27/13	<0.001
Hematuria (yes/no)	7/16	24/16	0.022
Hypertension (yes/no)	10/13	12/28	0.911

sedimentation rate was more frequent in symptomatic patients 27/40 (67.5%) than in those with incidentally detected RCC 5/23 (21.7%) ($P < 0.001$). Anemia, polycytemia, and Stauffer's syndrome were found in 3.7%, 7.4%, and 0%, respectively, of incidental cases, and in 18.6%, 11.6%, and 13.9% of symptomatic patients respectively.

The median tumor size was 6 cm (range 1.5–14 cm) in patients with incidentally detected disease, compared to 9 cm (range 3–20 cm) in patients with previously suspected RCC ($P < 0.001$). There was no side preference between the groups (Table 1) and there were no bilateral tumors. Incidentally found tumors tended to be located on the lower pole of the kidney (60.8%), while only 37.5% tumors in symptomatic group occurred in this location.

Of 23 incidentally detected RCC, 15 were pTNM stage I, four were stage II, and four were stage III. No patient had a stage IV RCC. In the symptomatic group six patients had stage I tumor, seven were classified as stage II, 16 as stage III and 11 as stage IV. At the time of diagnosis, distant metastases were found in nine (22.5%) of 40 patients with symptomatic RCC, while patients with incidentally detected renal cancer had no metastatic disease. Tumor infiltration of the renal fat (T_{3a}) was histopathologically confirmed in 14 (35.0%) of 40 symptomatic patients and in four (17.4%) of 23 incidentally diagnosed cases ($P = 0.114$). Nodal involvement was found in six (15.0%) of 40 patients in the symptomatic group and none of the 23 patients in the incidental group ($P = 0.056$). Eleven (27.5%) symptomatic patients and one patient with an incidentally detected tumor presented with tumor extension into the renal vein or the vena cava.

According to the Fuhrman [6] nuclear grade, three incidental tumors were classified as G1, 17 as G2, three as G3 and none as G4. In the symptomatic group no tumors were G1, 15 were G2, 17 were G3 and eight were G4.

The flow-cytometric study showed that of 23 incidental tumors 19 were diploid, three were aneuploid, and one tetraploid. In the symptomatic group there were 22 diploid tumors, 16 aneuploid, and two tetraploid. Two diploid tumors, one from each group, had a high percentage of S-phase cells (more than 30%). The overall percentage of the cells in S-phase was 4.4% (0.6–32.3%). In the symptomatic group, this percentage was 6.4% (0.6–32.3%) and in the incidental group 3.1% (1.1–32.2%). There was a significant difference in tumor distribution as to the ploidy status ($P = 0.027$) and tumor cell proliferative activity ($P = 0.005$) between the incidental and symptomatic cases (Table 1).

The median follow-up of 63 patients in the study was 24 months (range 3–120 months), i.e., 28 months (range 8–120) for 23 patients with incidental tumor and 19 months (range 3–109) for 40 patients in symptomatic group. During the study one patient in the incidental group and nine patients in the symptomatic group developed metastases. The 5-year survival rate estimated by the Kaplan-Meier method was 56.9% for all patients in the study. It was 81.4% in the incidental group and 44.3% in the symptomatic group (Fig. 1). The survival rate evaluated by means of univariate statistics was significantly higher in patients with incidentally detected tumors ($P = 0.020$), those with low pTNM stage ($P < 0.001$), diploid tumors ($P = 0.029$) and tumors with S-phase lower than or equal to 4% ($P = 0.035$; Table 2). Age, sex, nuclear grade, tumor size and erythrocyte sedimentation rate did not prove to be significant prognostic factors of patients' survival. Multivariate analysis presented only the pTNM stage as an independent prognostic parameter among those tested ($P < 0.001$; Table 2).

Discussion

Incidentally detected RCC has been intriguing urologists continually over the last decades. The nature, prognostic value and optimal therapeutic procedure in these lesions remain unclear. According to recent reports, the proportion of incidentally detected RCC reaches 25–53%. Our data (36.5% incidentally detected RCC) are in agreement with these observations.

As in most published studies [8, 22], ultrasonography was the leading technique in incidental detection of

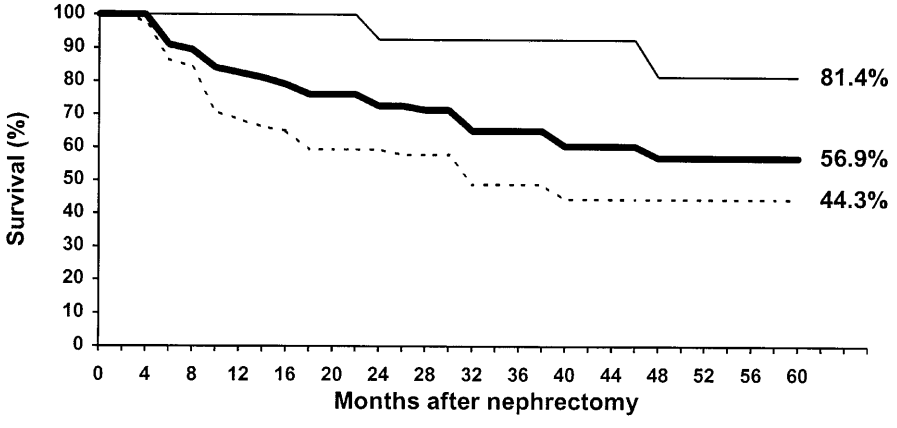

Fig. 1 Overall survival of patients with renal cell carcinoma (*heavy line*). The difference between the incidental (*full line*) and symptomatic (*dotted line*) groups was statistically significant ($P = 0.02$)

Table 2 Prognostic significance of the renal cell carcinoma parameters for a patient's survival ($n = 63$). Relative hazard ratio (RHR) was calculated as a ratio of the hazard function (exponent of regression coefficient) with 95% confidence interval limits (95% CIL) for all parameters with significant marginal (univariate) or partial (multivariate) effect on survival function

Parameter	Prognostic significance for survival (P)		Relative hazard ratio	
	Univariate	Multivariate	RHR	95% CIL
Incidental/symptomatic	0.020	0.133	0.38	1.02–1.63
Age (≤60/ > 60 years)	0.387	0.590		
Male/female	0.295	0.837		
Tumor size (≤7/ > 7 cm)	0.073	0.926		
pTNM stage (1 + 2/3 + 4)	< 0.001	< 0.001	8.56	2.51–29.13
Nuclear grade (1 + 2/3 + 4)	0.061	0.793		
Diploid/nondiploid	0.029	0.575	2.52	1.07–6.00
S-phase (≤4/ > 4%)	0.035	0.607	2.83	1.03–7.70
Accelerated SE (yes/no)	0.077	0.298		

RCC. We did not notice any higher efficiency in ultrasound detection of lesions on the right kidney as reported by Vallancian et al. [24] and Rousseau et al. [18].

The comparison of clinical characteristics of patients with incidental and symptomatic RCC revealed no statistically significant differences in age between these two groups. Only Guignard et al. [9] and Nakano et al. [15] reported older patients in the incidental group. This observation can be explained by the fact that older people are more frequently submitted to ultrasound and CT examinations due to other illnesses. This results in there being a greater chance of detecting the asymptomatic renal lesion.

In all published studies, the mean tumor mass measured through the tumor maximal diameter is significantly smaller in incidentally detected cases (3.6–6.1 cm) than in symptomatic cases (4.8–8.1 cm) [2, 13, 16, 18, 23]. The median tumor diameter in our study was longer than in previously mentioned series in both patient groups. It is interesting that some of the tumors in the incidental group, despite their great size (12–14 cm), remained asymptomatic. Although the tumor size had no prognostic value in univariate and multivariate analyses, it must be emphasized that none of our patients with tumors measuring 5 cm or less in the maximal diameter (11 patients) died of RCC or developed metastases during the follow-up.

Incidental RCC tended to be located in the lower pole of the kidney. This site allows the tumor to painlessly spread to the lower part of the retroperitoneum and may explain why these lesions remain asymptomatic for such a long time.

Tumor stage is recognized by many authors as the most important prognostic factor in RCC [7, 19]. In our study it was the only factor with an independent prognostic value. Like other authors [8, 13, 15, 16, 23], we found incidental tumors to have a significantly lower stage in comparison with the symptomatic ones. These differences between the two patient groups are probably the main cause of the higher survival rate in patients with incidentally detected renal cancers. Most, but not all studies have recognized the tumor grade as the second important prognostic parameter in RCC [3, 7]. While some of the series show more favorable grades in the incidental group, other authors do not find differences in grade distribution between the groups [15, 16, 23]. In our study, incidental tumors were associated with lower nuclear grades, but tumor grade had no significant prognostic value.

Flow-cytometric analysis of our material showed a more favorable ploidy status and a lower proliferative activity of incidentally detected tumors. This observation, as well as the lower grade of the incidentally found tumors, suggests that incidental RCC are a "less malignant" variety of this entity. Ploidy status has shown a prognostic value in most published studies on RCC [4, 5, 14], but Lanigan [12] and Tannapfel [21] failed to prove such a value. In our study ploidy status and proliferative activity had a prognostic value at univariate, but not at multivariate analysis. In some studies a higher percentage of aneuploid tumors has been found than in our study [5]. This difference can be explained by the fact that samples for our flow-cytometric study were obtained from only one site in a tumor. Tumor heterogeneity of DNA content is described in 30% of RCC [12]. Multiple samples can increase the probability for the detection the areas with aneuploid characteristics.

In conclusion, in comparison with symptomatic RCC, incidental RCC are usually smaller and associated with a lower stage, more favorable grade, and ploidy status. Although incidental cancers showed a significantly higher 5-year survival rate, detection (incidental or symptomatic) was not found to be an independent prognostic factor. The higher survival rate in the incidental group can be explained by their lower grade and favorable ploidy status, which imply their "lower malignant potential." However, we also confirmed the finding published by others [7, 20] that the main reason for their better prognosis is associated with their lower stage, i.e., that this is the most powerful prognostic factor in RCC. We confirmed this by observation that the tumor stage proved to be the only significant factor influencing survival in the multivariate analysis (Table 2). Altogether, this implies that incidental RCC are primarily "detected early", and probably are "a less malignant" form of this malignancy.

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