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Stage pT3a of renal clear cell carcinoma: do tumors with sinus fat involvement behave the same as those with perinephric fat involvement?

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Abstract

Introduction: In this report, we review our series of patients with pT3a clear cell renal carcinoma (CCRC) and comment on their outcome. *Materials and Methods*: We have reviewed 260 cases of CCRC operated in the Móstoles General Hospital, Madrid, between 2000 and 2004. We have found 30 cases with pT3a tumors. Eleven of them were invading the perinephric fat, nine were invading the renal sinus fat and ten were pT3a locally but showed metastasis at the moment of diagnosis (cM1, TNM stage IV). We have analyzed the prognostic influence of histopathological parameters (vascular invasion, size, Fuhrman grade) and also immunohistochemical ones (p53, cyclin D1, proliferation index with Ki67, bcl-2 and vascular density with CD34). *Results*: Only six of 10 patients with perinephric fat involvement died of disease compared with all the patients with sinus fat involvement, suggesting a worse prognosis for the latter. However, this difference did not reach statistical significance, probably due to the small number of cases. Of all the clinical, histological and immunohistochemical factors analyzed, only cyclin D1 was a strong indicator of worse prognosis in pT3a CCRC (p=0.02). We could not show any statistically significant relation between vascular density and prognosis. Vascular invasion was the only histological parameter that showed a trend toward significance (p=0.09). *Conclusions*: Sinus fat involvement might be underestimated in some series. A protocol for nephrectomy specimen handling could improve the detection rate of sinus fat involvement and allow the performance of randomized prospective studies to determine whether these tumors behave similarly.

Keywords: renal clear cell carcinoma, prognosis, TNM staging, cyclin D1, specimen sampling.

⊡ Introduction

Since the 50's the tumor node metastasis (TNM) [1] staging system is widely used to stage the tumors of almost every organ. This staging system has recently been reviewed for most tumors and the pT3a stage for clear renal cell carcinoma has been significantly changed. In the 2010 staging system, adrenal invasion has been considered as a pT4 stage with a clearly shown worse prognosis, separating it from stage pT3 lesions [2]. However and despite several reports on this issue published in the last decade [3–5], the present modification has not changed the other two categories of stage pT3a lesions, namely perinephric fat and renal sinus fat involvement and they are still considered together as the same group. Bonsib SM [6] was the first author to describe the different behavior of tumors invading the renal sinus. In 2000, he reported a short series of 31 cases (22 of clear cell type), which he subsequently enlarged to reach 100 cases [7] and analyzed the prognostic significance of sinus fat invasion. His contribution led to the introduction of this new category in the 2002 *TNM Classification*. Bonsib SM considered that tumors invading the sinus fat could have a worse prognosis for they had direct access to the rich lympho-vascular network in this anatomic area and did not have any fibrous barrier (the renal capsule), as opposed to tumors invading the perinephric fat.

The aim of the present study is to analyze the biological behavior of our pT3a stage patients and to determine which factors can influence prognosis of these patients.

⊡ Materials and Methods

This retrospective study has been designed and performed at the Departments of Urology and Surgical Pathology of Móstoles Hospital, Madrid. We have reviewed all the cases of CCRC diagnosed between 2000 and 2004. We have chosen this time period to have a long enough follow-up time and also because in 2000 the Surgical Department started using a protocol for nephrectomy specimens handling in which the renal sinus fat was sectioned and sampled widely (at least 10 paraffin blocks). With these inclusion criteria, we have found 30 patients with a diagnosis of CCRC stage $pT_{3a}N_{0-1}M_{0-1}$ treated with a non-laparoscopic radical nephrectomy, from which we had paraffin-embedded archival tissue. Two urologists (DSA and JJG) have reviewed the clinical data, including outcome. All the

histological samples have been reviewed by two pathologists (MJFA and SCP). To overcome problems of inter-observer variability in the evaluation of the histopathological and immunohistochemical results, two pathologists reviewed the slides and saw together the discordant cases to find agreement.

These 30 cases could be divided in 11 patients with perinephric fat involvement ($PT_{3a}N_0M_0$-PF), nine with sinus fat involvement ($PT_{3a}N_0M_0$-SF) and 10 with pT_{3a} tumors that had already metastasized, five of which had renal sinus involvement ($pT_{3a}N_xM_1$) (Figure 1, A and B).
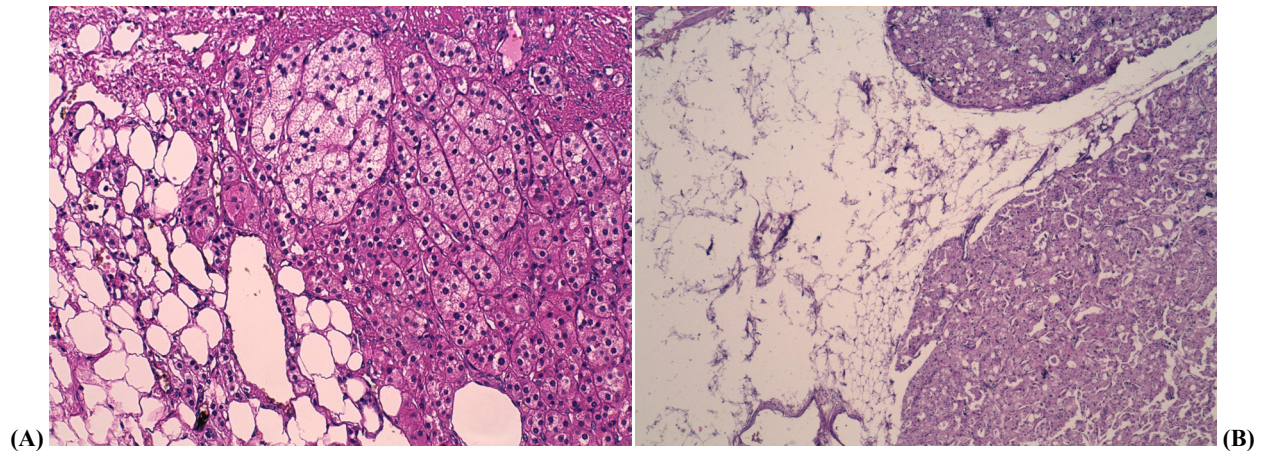

Figure 1 – *(A) Invasion of the renal sinus fat (HE stained section, ×200). (B) Invasion of the perinephric fat (HE stained section, ×200).*

In these cases we have collected clinical data (age at diagnosis, sex, Charlson's comorbidity score and long-term outcome), determined general pathological prognosticators (tumor size, grade, presence of necrosis and vascular invasion) and also performed immuno-histochemistry on archival blocks representative of the tumors, including proliferation markers (Ki67 and cyclin D1), vascular markers (CD34 to measure vascular density, an allegedly prognosticator in many neoplasms), tumor-suppressor genes (p53) and apoptosis markers (bcl-2). The characteristics of the reagents are summarized in Table 1.

Table 1 – *Features of the immunohistochemical markers employed in the study. All the reagents are prediluted*

Reagent	Species	Clone	Manufacturer
Ki67		MIB-1	
Cyclin D1		SP4	
CD34	Mouse	Qbend10	Dako
P53		DO7	
Bcl-2		124	

The immunohistochemical panel has been performed following a standardized methodology. In short, we made 6-μm sections of the paraffin blocks and stained them in the Autostainer PlusLink (Dako, Denmark). Antigen demasking was obtained with the Target retrieval solution and the work system was EnVision, also manufactured by Dako. The slides were counter-stained with Hematoxylin. Both positive and negative external controls were included for all the reagents. The pathologists participating in the study have counted 400 cells in representative areas of the tumor and recorded the percentage of cells with expression of the marker (considering valuable the nuclear staining for cyclin D1, p53 and Ki67 and cytoplasmic staining for bcl-2 and CD34). Subsequently the cases were classified as negative or positive using a cut-off point established with the mean value of the whole series. The vascular density was measured in the three most vascular high power fields and expressed as number of vessels/mm^2 [8]. Figure 2 (A to D) shows representative areas of the slides stained with immunohistochemistry.
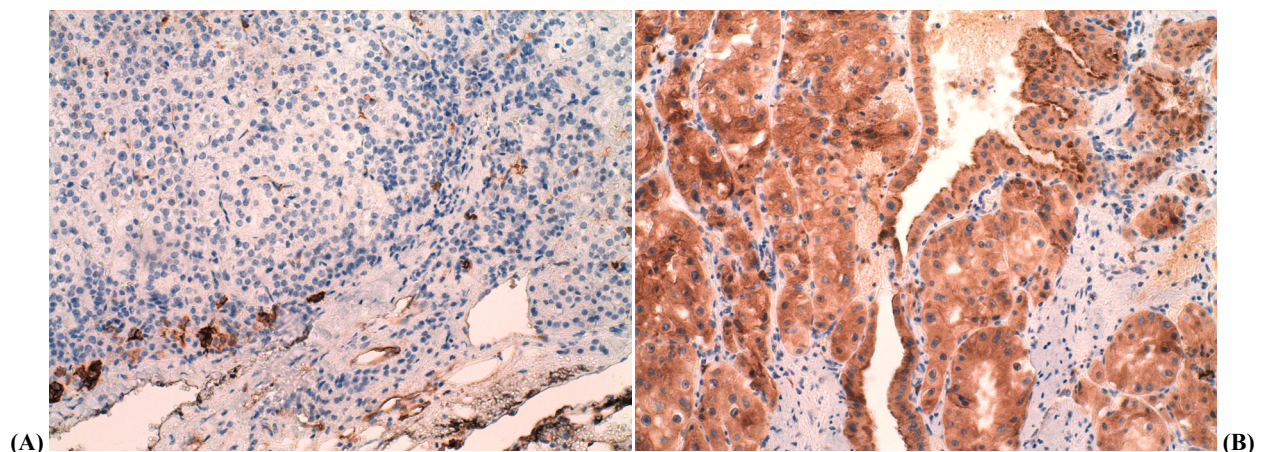

Figure 2 – *Immunohistochemistry for: CD34 (note vascular lumens, mainly at the tumor periphery – A); bcl-2 (B).*
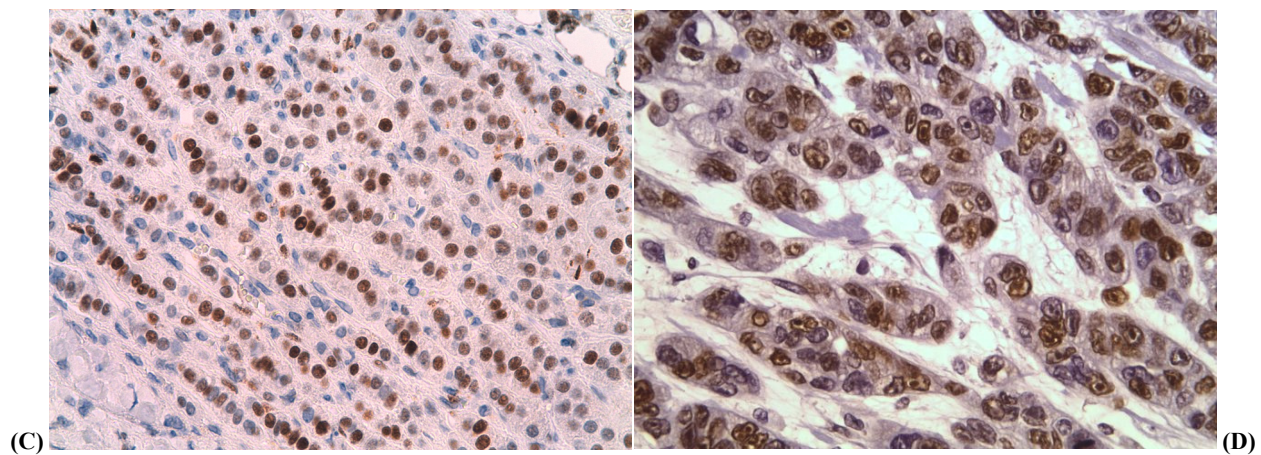
(C) **(D)**

Figure 2 (continued) – *Immunohistochemistry for: p53 (C); cyclin D1 (D).*

The statistical analysis was performed with the software SPSS® 13.0 (SPSS Inc, Chicago, Illinois, USA) for Windows®, considering as significant a *p*-value <0.05 for the null hypothesis. Quantitative variables were represented with the mean and the 95% confidence interval and qualitative ones with percentages. The differences between groups for the quantitative variables were analyzed with the Student' *t*-test or Analysis of Variance (ANOVA) and the possible association between qualitative variables with chi-square test. The overall survival was estimated with Kaplan–Meier curves and the log-rank test was used to measure significance of the inter-group differences.

Results

We have established three homogeneous groups of patients: 11 cases with perinephric fat involvement ($pT_{3a}N_0M_0$-PF), nine with renal sinus fat invasion ($pT_{3a}N_0M_0$-SF) and 10 with local pT3a tumors that had already caused distant metastasis ($pT_{3a}N_xM_1$). We did not find any statistically significant differences between the three groups for age (*p*=0.83), nuclear grade (*p*=0.72) or tumor size (*p*=0.75). We found statistically significant differences for gender distribution and mortality between the three groups, as shown in Table 2. The median follow-up time for all the groups was 931 days.

Figure 3 shows the Kaplan–Meier survival curves. Despite the differences between the groups did not achieve statistical significance (*p*=0.1), it can be noted that the three curves run an almost parallel course and the prognosis of patients with sinus fat involvement seems intermediate between metastatic patients and cases with perinephric fat involvement. In fact in the subgroup analysis we found significant differences in survival between perinephric fat involvement and metastatic disease (*p*=0.02 for the log-rank test), but not between sinus fat and metastatic disease (*p*=0.62). This suggests renal sinus fat shares a worse prognosis with metastatic tumors and behaves differently from perinephric fat involving tumors.

None of the histological variables included in the study had a significant influence on overall survival. The *p*-values obtained in the log-rank test for these variables were 0.361 for the tumor size, 0.167 for the presence of necrosis and 0.84 for the vascular density within the tumor. The presence of vascular invasion by tumor cells showed a trend toward significance (*p*=0.09) (Figure 4).

Table 2 – *General features of the three groups included in the study. PF: Perinephric fat involvement; SF: Sinus fat involvement; DM: Distant metastasis, p: Alpha error*

	PF*	SF	DM	*p*
No. of cases	11	9	10	–
Age [years]	60.9 50.39–71.42	57.62 48–67.25	58.79 53.97–63.6	0.83
Gender (M/F)	2/9	6/3	10/0	<0.01
Follow-up time [days]	1755.9 803.5–2708.2	1621.7 431–2812.5	1251 153–2655.6	0.77
Fuhrman grade* I–II III–IV	6 (54%) 5	5 (55%) 3	4 (40%) 6	0.72
Outcome (death)	6 (54%)	9 (100%)	9 (90%)	0.02
Charlson's index* 0–2 ≥3	5 (45%) 6	6 (66%) 3	5 (50%) 5	0.66
Tumor size [cm]	7.68 5.2–10.1	6.78 4.7–8.7	7.9 5.5–10.3	0.75

*See references [25] and [26];

As for the immunohistochemical variables analyzed in the present study, only cyclin D1 overexpression (Table 3) was significantly associated with a worse prognosis (*p*=0.02) (Figures 5 and 2D).

Table 3 – *Summary of the results of the immuno-histochemical analysis. The p-value reflects the verosimility of the null hypothesis when comparing survival between the two groups generated for each immunohistochemical variable according to the calculated cut-off point*

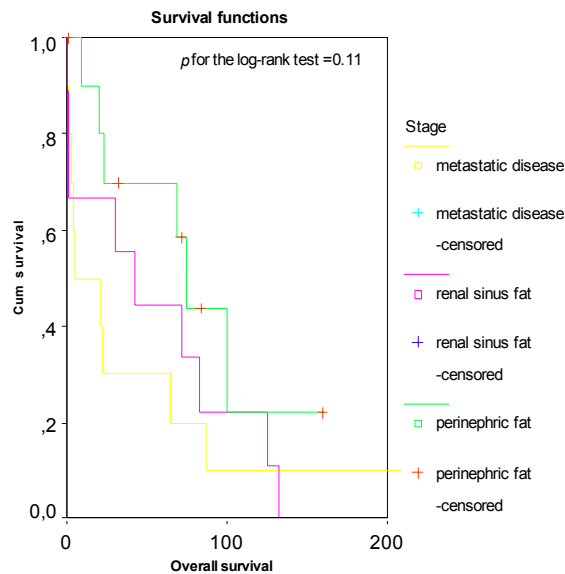| Immunohistochemistry	Cut-off point (% positivity)	*p*-value
P53	0	0.85
Bcl-2	20	0.88
Ki67	10	0.44
Cyclin D1	6	0.02

Figure 3 – *Kaplan–Meier curves correlating overall survival of CCRC stage pT3a with the involvement of the sinus or the perinephric fat.*
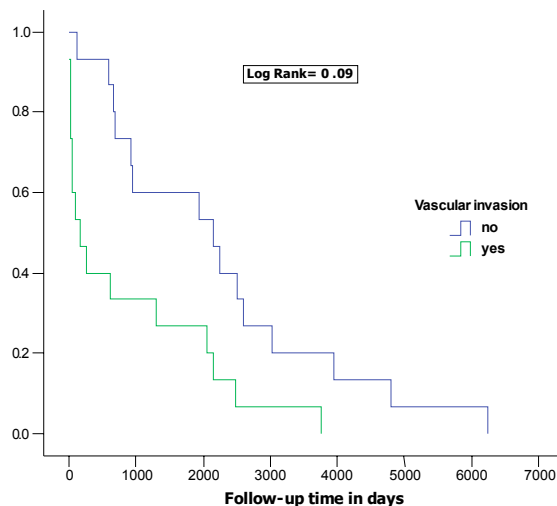

Figure 4 – *Kaplan–Meier curves correlating overall survival of CCRC stage pT3a with the presence or absence of vascular invasion.*
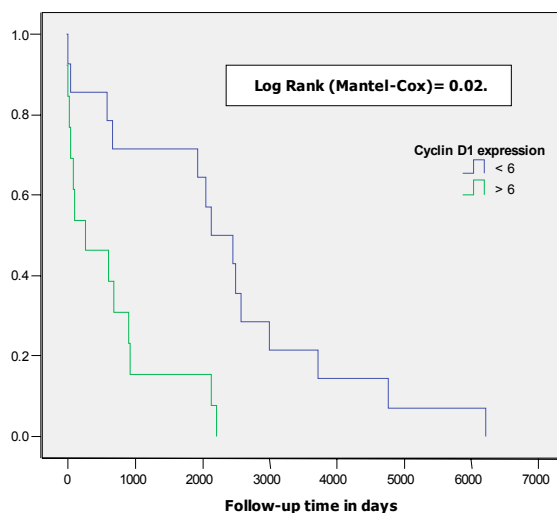

Figure 5 – *Kaplan–Meier curves correlating overall survival of CCRC stage pT3a with the expression of cyclin D1.*

Discussion

Our study tries to define the biological behavior of pT3a tumors and find whether the invasion of renal sinus fat and perinephric fat change prognosis. The recent large series from several groups have proposed changes in the TNM classification that have only been partially taken into account in the last modification. In a large multi-center European study, Ficarra V *et al.* [5] proposed to change the present classification system. They showed that T3 and T4 patients can be divided in three groups with different behavior, but unluckily they did not differentiate the involvement of the renal sinus fat and the perinephric fat and based their groups in the presence or absence of caval invasion and perinephric fat involvement. In another large review by Terrone C *et al.* [3] the authors analyzed 513 patients from three different Italian hospitals and they found 260 pT3a tumors, 35 of which had exclusive involvement of the renal sinus fat. These authors concluded that the presence of isolated fat invasion, either perinephic or sinusal, was associated with a better prognosis and that only vascular invasion associated with fat invasion significantly worsened prognosis. We found it peculiar that there were so few cases with renal sinus fat involvement. In a recent report by Griffiths DFR *et al.* [9] they proposed to extensively sample the interface between renal sinus and tumor. If the area was too large to be completely embedded, they recommended at least 10 paraffin blocks from this area. With this approach, they showed a significant increase in the percentage of vessel invasion and also of direct renal sinus fat involvement compared with previous routinely handled specimens. They concluded that the worse prognosis of some supposedly localized tumors could be explained by the lack of detection of vascular or renal sinus involvement. In the original series from Bonsib SM after a thorough sampling of the renal sinus fat 55% of 74 CCRC invaded the sinus and this contrasts with the bare 13.4% in the series by Terrone C and is more in accordance with our 46% (14/30 tumors). We feel the insufficient sampling of the renal sinus fat adds complexity to the analysis of the prognostic influence of this pattern involvement and that it is necessary to perform prospective studies with properly sampled specimens, perhaps following Griffiths' protocol, if we are to compare these two stages confidently.

The main prognosticators in CCRC are the presence of distant metastases at diagnosis, the pathological stage, the tumor grade and the comorbidities associated to the tumor [10, 11]. In recent years, several groups have analyzed a possible relation between some biological and immunohistochemical factors and the tumor response to different adjuvant therapies, even proposing the incorporation of these factors to survival models for CCRC that would also incorporate stage, grade and Charlson's index [12, 13]. There have been reports showing a significant association between CCRC prognosis and p53 mutations [14, 15] or proliferative activity measured with Ki67 [16]. Our study has not been powered enough to show the possible prognostic influence of these factors.

Vascular density has behaved as a significant prognosticator for many tumors and has even led to the development of new-targeted therapies with anti-angiogenic drugs [17, 18]. However, in CCRC most authors seem to discard a possible prognostic influence of vascular density within the tumor. In our series, vascular density has not behaved as a significant prognosticator. However, as expected, vascular invasion tends to worsen prognosis in our series. We have also shown that tumor invading the renal sinus fat have significantly more vascular invasions as perinephric fat invading ones (7/9 *vs.* 3/11, *p*=0.02). This finding seems to confirm the hypothesis by Bonsib SM in the sense that a lack of a fibrous capsule between the tumor and the vascular networks makes it easier for renal sinus tumors to invade the vessels and give rise to distant metastasis. This can be one of the reasons for the worse prognosis of tumors in this location.

Lee CT *et al.* have compared the expression of bcl-2 in pT3 or pT4 CCRC and have shown a higher expression in metastatic tumors, but this difference has not changed survival according to their report [19]. In a recent review of 119 CCRC by Phuoc NB *et al.* [20] they have analyzed the possible prognostic influence of seven different immunohistochemical markers on prognosis, showing that Ki67 and p53 high expression are associated with a worse prognosis in terms of disease free survival, while bcl-2 and cyclin D1 expression seemed to increase the mortality risk, mainly in low grade tumors. This possible prognostic influence of cyclin D1 has been confirmed in our series for pT3a tumors and these results are giving way to the development of drugs that inhibit cyclin and seem to induce an arrest of the cell cycle in several tumor cell lines, mainly resistant to cisplatin [12]. Nevertheless, these results obtained in retrospective studies should be confirmed in randomized prospective clinical trials before these new therapeutic alternatives could be considered as alternatives to current drugs in locally-advanced or metastatic CCRC.

Our study has several drawbacks. The first is the low number of patients, which can bias the conclusions, mainly of the survival analysis. Besides, the median follow-up time is relatively short (approximately three years). However, we find this topic is far from settled in the literature and several recent reports continue to argue about it. Conclusions are varied and some confirm the worse prognosis of sinus fat involvement [21], other limit it to certain subgroups of patients [22, 23] and other disregard the prognostic utility of this factor [24]. This confirms furthermore studies are necessary to settle this matter.

⊟ Conclusions

We herein report the results of our series of pT3a CCRC patients and analyze the factors that can influence prognosis. We feel insufficient sampling of the transition between the tumor and the renal sinus fat can confound the results of previous study comparing the prognostic significance of renal sinus and perinephric fat and that prospective studies with a similar protocol for specimen handling are necessary to settle this matter.

References

[1] Denoix PF, *Note on the possible role of the International Union against Cancer in nomenclature, classification, analytical index, bibliography and documentation*, Acta Unio Int Contra Cancrum, 1952, 8(Special No):92–96.

[2] Thompson RH, Leibovich BC, Cheville JC, Lohse CM, Frank I, Kwon ED, Zincke H, Blute ML, *Should direct ipsilateral invasion from renal cell carcinoma be classified as pT3a?*, J Urol, 2005, 173(3):918–921.

[3] Terrone C, Gontero P, Volpe A, Porpiglia F, Bollito E, Zattoni F, Frea B, Tizzani A, Fontana D, Scarpa RM, Rosetti SR, *Proposal of an improved prognostic classification for pT3 renal cell carcinoma*, J Urol, 2008, 180(1):72–78.

[4] Jung SJ, Ro JY, Truong LD, Ayala AG, Shen SS, *Reappraisal of T3N0/NxM0 renal cell carcinoma: significance of extent of fat invasion, renal vein invasion, and adrenal invasion*, Hum Pathol, 2008, 39(11):1689–1694.

[5] Ficarra V, Galfano A, Guillé F, Schips L, Tostain J, Mejean A, Lang H, Mulders P, De La Taille A, Chautard D, Descotes JL, Cindolo L, Novara G, Rioux-Leclercq N, Zattoni F, Artibani W, Patard JJ, *A new staging system for locally advanced (pT3-4) renal cell carcinoma: a multicenter European study including 2,000 patients*, J Urol, 2007, 178(2):418–424; discussion 423–424.

[6] Bonsib SM, Gibson D, Mhoon M, Greene GF, *Renal sinus involvement in renal cell carcinomas*, Am J Surg Pathol, 2000, 24(3):451–458.

[7] Bonsib SM, *The renal sinus is the principal invasive pathway: a prospective study of 100 renal cell carcinomas*, Am J Surg Pathol, 2004, 28(12):1594–1600.

[8] Fernandez-Aceñero MJ, González JF, Gallego MG, Ballesteros PA, *Vascular enumeration as a significant prognosticator for invasive breast carcinoma*, J Clin Oncol, 1998, 16(5):1684–1688.

[9] Griffiths DFR, Nind N, O'Brien CJ, Rashid M, Verghese A, *Evaluation of a protocol examining nephrectomy specimens with renal cell carcinoma*, J Clin Pathol, 2003, 56(5):374–377.

[10] Kim HL, Seligson D, Liu X, Janzen N, Bui MH, Yu H, Shi T, Belldegrun AS, Horvath S, Figlin RA, *Using tumor markers to predict the survival of patients with metastatic renal cell carcinoma*, J Urol, 2005, 173(5):1496–1501.

[11] Santos Arrontes D, Fernández Aceñero MJ, García González JI, Martín Muñoz MP, Paniagua Andrés P, *Survival analysis of clear cell renal carcinoma according to the Charlson comorbidity index*, J Urol, 2008, 179(3):857–861.

[12] Ficarra V, Martignoni G, Lohse C, Novara G, Pea M, Cavalleri S, Artibani W, *External validation of the Mayo Clinic Stage, Size, Grade and Necrosis (SSIGN) score to predict cancer specific survival using a European series of conventional renal cell carcinoma*, J Urol, 2006, 175(4):1235–1239.

[13] Dudderidge TJ, Stoeber K, Loddo M, Atkinson G, Fanshawe T, Griffiths DF, Williams GH, *Mcm2, Geminin, and Ki67 define proliferative state and are prognostic markers in renal cell carcinoma*, Clin Cancer Res, 2005, 11(7):2510–2517.

[14] Girgin C, Tarhan H, Hekimgil M, Sezer A, Gürel G, *P53 mutations and other prognostic factors of renal cell carcinoma*, Urol Int, 2001, 66(2):78–83.

[15] Ljungberg B, *Prognostic markers in renal cell carcinoma*, Curr Opin Urol, 2007, 17(5):303–308.

[16] Yildiz E, Ayan S, Goze F, Gokce G, Gultekin EY, *Relation of microvessel density with microvascular invasion, metastasis and prognosis in renal cell carcinoma*, BJU Int, 2008, 101(6):758–764.

[17] Iwata T, Miyata Y, Kanda S, Nishikido M, Hayashi T, Sakai H, Kanetake H, *Lymphangiogenesis and angiogenesis in conventional renal cell carcinoma: association with vascular endothelial growth factors A to D immunohistochemistry*, Urology, 2008, 71(4):749–754.

[18] Joshi KS, Rathos MJ, Mahajan P, Wagh V, Shenoy S, Bhatia D, Chile S, Sivakumar M, Maier A, Fiebig HH, Sharma S, *P276-00, a novel cyclin-dependent inhibitor induces G1-G2 arrest, shows antitumor activity on cisplatin-resistant cells and significant in vivo efficacy in tumor models*, Mol Cancer Ther, 2007, 6(3):926–934.

[19] Lee CT, Genega EM, Hutchinson B, Fearn PA, Kattan MW, Russo P, Reuter VE, *Conventional (clear cell) renal carcinoma metastases have greater bcl-2 expression than high-risk primary tumors*, Urol Oncol, 2003, 21(3):179–184.

[20] Phuoc NB, Ehara H, Gotoh T, Nakano M, Yokoi S, Deguchi T, Hirose Y, *Immunohistochemical analysis with multiple antibodies in search of prognostic markers for clear cell renal cell carcinoma*, Urology, 2007, 69(5):843–848.

[21] Bedke J, Buse S, Pritsch M, Macher-Goeppinger S, Schirmacher P, Haferkamp A, Hohenfellner M, *Perinephric and renal sinus fat infiltration in pT3a renal cell carcinoma: possible prognostic differences*, BJU Int, 2009, 103(10):1349–1354.

[22] Jeon HG, Jeong IG, Kwak C, Kim HH, Lee SE, Lee E, *Reevaluation of renal cell carcinoma and perirenal fat invasion only*, J Urol, 2009, 182(5):2137–2143.

[23] Bertini R, Roscigno M, Freschi M, Strada E, Petralia G, Pasta A, Matloob R, Sozzi F, Da Pozzo L, Colombo R, Guazzoni G, Doglioni C, Montorsi F, Rigatti P, *Renal sinus fat invasion in pT3a clear cell renal cell carcinoma affects outcomes of patients without nodal involvement or distant metastases*, J Urol, 2009, 181(5):2027–2032.

[24] Margulis V, Tamboli P, Matin SF, Meisner M, Swanson DA, Wood CG, *Location of extrarenal tumor extension does not impact survival of patients with pT3a renal cell carcinoma*, J Urol, 2007, 178(5):1878–1882.

[25] Fuhrman SA, Lasky LC, Limas C, *Prognostic significance of morphologic parameters in renal cell carcinoma*, Am J Surg Pathol, 1982, 6(7):655–663.

[26] Charlson ME, Pompei P, Ales KL, MacKenzie CR, *A new method of classifying prognostic comorbidity in longitudinal studies: development and validation*, J Chronic Dis, 1987, 40(5):373–383.

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