Oncologic Impact of Renal Tissue Adjacent to Renal Cell Carcinoma

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Abstract. *Aim: The aim of the study was to investigate the clinical impact of the surgical margin width after nephron-sparing surgery (NSS) on the oncological course of renal cell carcinoma (RCC). Patients and Methods: The study comprised of 126 RCC patients with NSS between 2002 and 2009. Inclusion criteria were negative resection margins and a tumor diameter of ≤100 mm with the possibility of a complete circumferential histopathological reevaluation. The minimal benign margin width was correlated to the patients' clinical course. Results: Median safety margin width was revealed to be 1 mm. Nine of 126 patients (7.1%) developed recurrent disease (five local, four distant). All patients with local recurrence had safety margins ≤1 mm, whereas out of 49 patients with a margin >1 mm no one developed local recurrence (p=0.0245). Safety margin ≤1 mm showed associations with increased risk for overall recurrence in univariate and multivariate analysis (p=0.0531 and 0.0539, respectively). Conclusion: Tumor adjacent renal parenchyma may have oncological relevance, corroborating the need for further molecular investigation of tumor-adjacent tissue in RCC.*

In renal cell cancer nephron sparing surgery (NSS) techniques have always considered a significant safety margin to be mandatory to minimize the risk for local recurrence (1, 2). Nowadays simple enucleation of the renal mass with an intact capsule is generally considered to be sufficient for oncological control comparable to radical nephrectomy in renal cell carcinoma (RCC) (2, 3).

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However, the relevance of suspected premalignant conditions within healthy-looking renal tissue outside the pseudocapsule still remains unclear. Histopathological data showed cancer foci in 39% of T1b tumors beyond the pseudocapsule within 3 mm of the primary tumor (4) and the significance of these lesions cannot not be yet sufficiently determined. Tumor heterogeneity studies revealed higher tumor aggressiveness in peripheral tumor areas compared to the central zone (5) and even provide evidence for molecularly altered tumor-adjacent healthy tissue in several urological cancers (6, 7). The fact that histologically, renal parenchymal tissue may already possess molecular alterations associated with malignancy raises the question whether these areas may influence the clinicopathological course of RCC after partial resection. The aim of the present study was to investigate the hypothesis of a possible oncological impact of adjacent benign renal tissue by the assessment of the individual safety margin width and to compare the clinical course of RCC patients after NSS.

Patients and Methods

The study comprised of 126 patients with histopathologically confirmed RCC and NSS diagnosed between 2002 and 2009 with histologically-negative surgical margins and with the possibility of a complete circumferential histopathological reevaluation (Figure 1A-B). Patients with previous surgery for RCC, positive resection margins, tumor diameter >100 mm or incomplete follow-up were excluded from the analysis. Institutional review board approval had been obtained prior to data analysis (No. 123/2013 BO2). Table I shows patients' characteristics of the whole cohort as well as of sub-groups with margin widths ≤1 mm and >1 mm, respectively.

The width of the respective safety margin of renal parenchyma adjacent to the tumor was assessed histopathologically and measured circumferentially by means of a microscopic scale on H&E stained slides. As 'margin width' the minimal distance between areas showing RCC and the external border of the benign renal tissue was defined. These margins consisted of either the tumor capsule plus few cell layers or of additional renal parenchyma

of a varying extent (Figure 1C-F). First benign margin widths were re-evaluated and classified microscopically into sub-groups of margin ≤1 mm, >1 to ≤2 mm, >2 to ≤3 mm, >3 to ≤4 mm, >4 to ≤5 mm and >5mm. Afterwards the cohort was subdivided by the median margin width.

All patients were monitored postoperatively according to the European Association of Urology Guidelines by computed tomography or magnetic resonance imaging. Clinical course was assessed and time to recurrence, cancer specific (CCS) and overall survival (OS) were determined. Individual margin width was correlated to clinical data by Chi-square and Wilkoxon/Kruskal-Wallis-tests and by univariate and multivariate Cox proportional hazard analysis including T-stage and tumor grading. JMP 7.0® (SAS Inc., Cary, NC, USA) software was used for analysis.

Results

Patients' characteristics are summarized in Table I. The study included 34 female and 92 male patients (27.0% and 73.0%). Median age was 65 years (range 17-82 years). The surgical approach was open in 86 and laparoscopy in 40 cases (68.3% and 31.7%). Most common histology was clear cell (cc)RCC (84; 66.7%). No affected lymph nodes were known at time of surgery. In one patient (0.8%) a singular sternal metastasis was known at time of surgery and resected immediately after partial nephrectomy. The patient did not develop any tumor recurrence within the observation period. Median follow-up was 65.5 months (range=1-116 months).

All specimens were microscopically re-evaluated to exclude positive surgical margins and the pseudocapsule showed complete integrity in all included specimen.

The median histopathological parenchymal margin width was 1 mm (range <1-13 mm). Margin widths distributed as follows: ≤1 mm (77, 61.1%), >1 to ≤2 mm (20, 15.8%), >2 to ≤3 mm (13, 10.3%), >3 to ≤4 mm (5, 4.0%), >4 to ≤5 mm (5, 4.0%), >5mm (6, 4.8%).

The cohort was then subdivided by the median margin width. There were no statistical differences between these subgroups, 'margin ≤1 mm' and 'margin >1 mm' (Table I). Notably, there was no difference in subtype distribution between patients with safety margins of ≤1 and >1 mm (Table I). Nine of 126 patients had local or distant disease relapse (7.1%). In five patients (4.0%) the recurrent lesion was in the formerly treated kidney whereas it was systemic in four patients (3.2%). The patient with the initially diagnosed sternal metastasis did not develop local or distant recurrent disease within the observation period. Initial histology of the five patients with local recurrence revealed ccRCC in four and papillary (pap)RCC in one patient. Initial T-stages were pT1a in one patient, pT1b and pT3a in two patients, respectively. The median tumor diameter was 28 mm (range=8-63 mm) (Table III). In case of a secondary surgery due to tumor recurrence all histological reports revealed the same tumor entity as in the primarily performed NSS. All five patients with locally recurrent disease had a safety margin ≤1 mm whereas out of 49 patients with a margin >1 mm no one developed local recurrence (p=0.0245). Concerning overall recurrence, eight of 77 patients (10.4%) with a benign margin ≤1 mm were recurrent whereas only one of 49 patients (2.0%) with a safety margin >1 mm had RCC relapse (p=0.0540, Figure 2). Univariate and multivariate Cox proportional hazard analysis showed that a safety margin of ≤1 mm tends to result in an increased risk for overall recurrence (p=0.0531 and p=0.0539, Table II). There was no correlation between safety margin ≤1 mm and CSS or OS (p=0.16 and p=0.97, respectively).

Table I. *Patients' characteristics of the study cohort and the subgroups showing margin width ≤1 and >1 mm, respectively.*

Variables	All patients	Saftey margin ≤1 mm	Saftey margin >1 mm	*p*-Value
Patients - n (%)	126 (100)	77 (61.1)	49 (38.9)	
Gender - n (%)				0.5722
Female	34 (27.0)	20 (26.0)	14 (28.6)	
Column1				
Male	92 (73.0)	57 (74.0)	35 (71.4)	
Age, years				0.6704
Median (range)	65 (17-82)	65 (17-81)	65 (34-82)	
Approach - n (%)				0.7159
Laparoscopic	40 (31.7)	24 (31.2)	16 (32.7)	
Open	86 (68.3)	53 (68.8)	33 (67.3)	
Tumor-Diameter (mm)				0.5462
Minimum	5	5	9	
Maximum	95	80	95	
Median	29	29	29	
Final histology - n (%)				0.9902
ccRCC	84 (66.7)	52 (67.5)	32 (65.3)	
papRCC	33 (26.2)	20 (26.0)	13 (26.6)	
chrRCC	8 (6.3)	5 (6.5)	3 (6.1)	
unclassified RCC	1 (0.8)		1 (2.0)	
T-Stage - n (%)				0.8895
1a	92 (73.0)	56 (72.7)	36 (73.5)	
1b	28 (22.2)	17 (22.1)	11 (22.5)	
2	2 (1.6)	1 (1.3)	1 (2.0)	
3a	3 (2.4)	2 (2.6)	1 (2.0)	
3b	1 (0.8)	1 (1.3)	0	
Grading - n (%)				0.6088
1	43 (34.1)	26 (33.8)	17 (34.7)	
2	77 (61.1)	47 (61.0)	30 (61.2)	
3	1 (0.8)	1(1.3)	0	
4	0	0	0	
n.a.	5 (4.0)	3 (3.9)	2 (4.1)	
Tumor recurrence - n (%)				
Overall	9 (7.1)	8 (10.4)	1 (2.0)	0.0540
Local	5 (4.0)	5 (6.5)	0	0.0245

ccRCC, Clear cell renal cell carcinoma; papRCC, papillary renal cell carcinoma; chRCC, chromophobe renal cell carcinoma; n.a., not applicable.

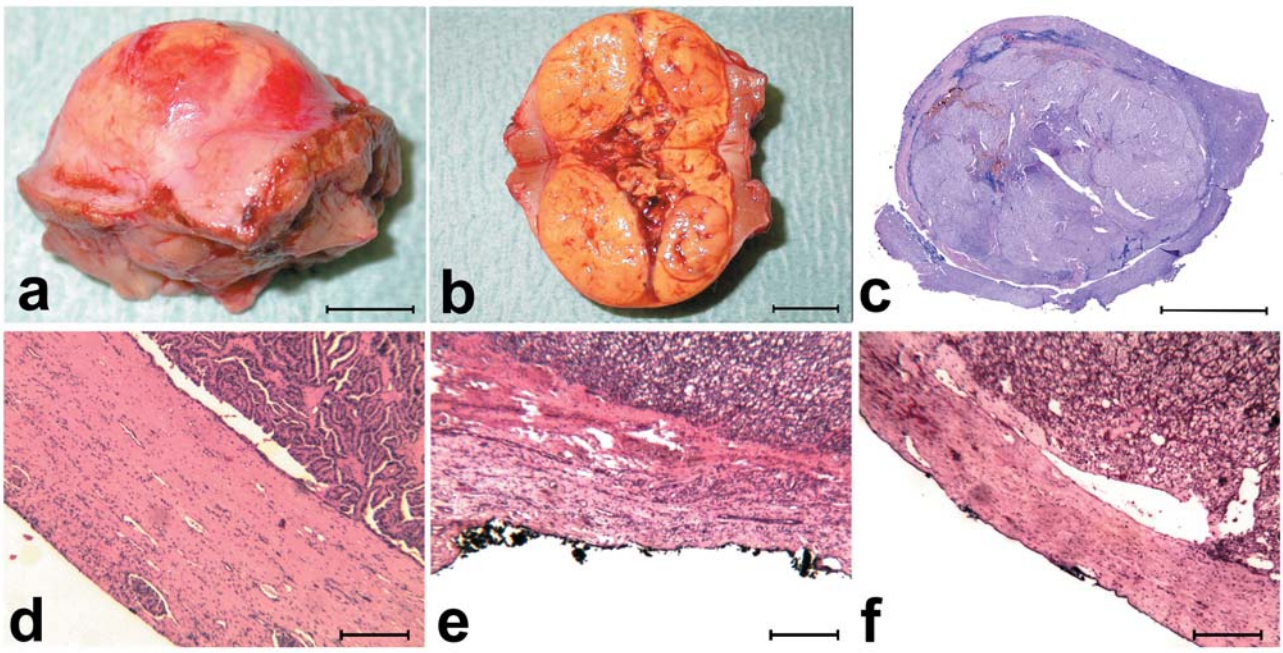

Figure 1. *A-F: Partial nephrectomy specimen, A: Macroscopic view. B: Macroscopic view open cut halves. C: Histological whole mount section. D-F: Histological demonstration of different margin widths. C-F: H&E staining, bar upper row=1 cm, bar lower row=200 μm.*

Table II. *Uni- and multivariate Cox regression analyses concerning local and overall recurrence dependent on margin width ≤1 or >1 mm.*

Parameters	Univariate analysis			Multivariate analysis		
	Recurrent disease			Recurrent disease		
	HR	95 % CI	*p*-Value	HR	95 % CI	*p*-Value
T-Stage, T<3 *vs*. T≥3	0.08	0.02-0.59	0.0177	0.05	0.009-0.44	0.0102
Grading, G1 *vs*. G2/3	0.65	0.17-2.62	0.5263	0.42	0.08-1.93	0.2609
Margin width: ≤1 *vs*. > 1 mm	0.18	0.01-1.01	0.0531	0.18	0.01-1.02	0.0539

Discussion

The relevance of suspected premalignant lesions within healthy-looking renal tissue outside the pseudocapsule still remains unclear (8-9). The aim of the present study was to investigate whether the tumor adjacent margin width may influence the clinical course of RCC and we found all patients from our collective with locally recurrent disease after NSS showing a margin width of ≤1 mm. Although negative resection margins and an intact pseudocapsule were reported in all investigated specimens, these findings may implicate a correlation of renal parenchymal margin and at least local tumor recurrence. In search for a histological pendant, some reports could give a hint: even in the case of an intact pseudocapsule transboundary positive cancer lesions have been reported to occur in RCC with an incidence of up to 17% (10). Zucchi *et al*. investigated tumor surrounding benign tissue in patients with RCC and nephron-sparing surgery and satellite lesions were identified at a mean of 5.3 mm within the surrounding benign tissue (11). Chen *et al*. were able to show that even in 39% of RCC specimens cancer lesions existed beyond the pseudocapsule within 3 mm of the primary tumor (4). Other authors also reported that a protection from local tumor recurrence cannot be warranted by negative surgical margins in NSS (12). Kwon *et al*. reported local tumor recurrence in four of 713, Permpongkosol *et al*. in 12 of 511 and Antic *et al*. in six of 406 patients even though negative margins were found in the histological examination (12-14). No recurrences were found in patients with low

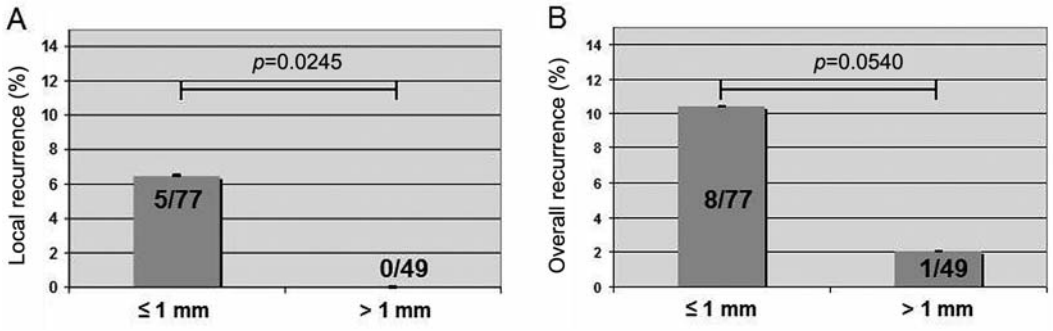

Figure 2. *A-B: Rates of local and overall recurrence in the subgroups with margin width ≤1 and >1 mm. A: Local recurrence, B: overall recurrence.*

Table III. *Characteristics of patients with local tumor recurrence.*

Age, years	Gender	Surgery	RCC typ	Stage	Grading	Tumor diameter, mm	Bening margin, mm	Time surgery to recurrence, mo
60	male	laparoscopic	papRCC	T1a	2	8	≤ 1 mm	41
66	male	open	ccRCC	T2b	1	50	≤ 1 mm	19
65	male	open	ccRCC	T3a	2	44	≤ 1 mm	17
64	male	open	ccRCC	T2b	1	50	≤ 1 mm	43
66	male	open	ccRCC	T3a	2	63	≤ 1 mm	37

malignant tumors in these studies (12-14). In our patients with local recurrence only, two had a higher stage in the final histological report (Table III). Especially Fuhrman nuclear grading was not higher than 2 in these patients. Therefore, based on our findings, local recurrence rates cannot only be explained by RCC with higher malignant potential. In the presented study the benign margin tissue itself was not investigated regarding molecular changes. However, recent data give evidence for molecular alterations in benign looking renal parenchyma leading to precancerous lesions of RCC (8). Arai *et al*. showed that benign tissue from the renal cortex in RCC-bearing kidneys was in a premalignant stage regarding the DNA methylation status (15). Kanai *et al*. were able to show that benign tissue in RCC patients is at a precancerous stage and shows DNA methylation alterations (16). Recently, Atschekzei *et al*. supported this hypothesis by demonstrating significant hypermethylation in benign renal tissue samples from RCC patients compared to benign kidney tissue form patients without RCC and moreover these processes seem to be associated with elevated risk for RCC and even with higher recurrence rates (9). Hence, histologically benign renal tissue in RCC patients may already have a premalignant potential interfering with local recurrence (8-9, 15). These findings are congruent to other malignancies: apart of RCC, in prostate cancer also, molecular alterations were found in peritumoral healthy tissue (6-7). Above this correlation between surgical margin width and recurrence there was no correlation seen to cancer specific or overall survival in the presented data. This might be due to limited number of cases - or this investigated factor is covered by stronger prognostic parameters: Previous studies showed cancer specific survival not to be influenced by the presence of malignant satellite lesions after nephron sparing surgery (11). It has been presumed that survival rates were not associated with resection margin width because of distant metastases following partial nephrectomy are more related to biological aggressiveness of the tumor rather than to the quality of the surrounding tissue (17-18). In this context one possible clinical bias could be excluded: different subtypes of RCC show a different prognosis and more aggressive RCC cancer subtypes are known to have worse clinical outcome than others (19-20). However, our data revealed no differences within the histopathological subtypes between the patients with safety margins of ≤1 and >1 mm.

There are inherent limitations to be stated, such as the limited number of cases and recurrence events, multiple surgeons as well as different surgical approaches performed possibly influencing the benign margin width and outcome of this study. To reduce the impact of these variables on the presented findings further prospective studies with higher number of patients and recurrence events as well as clear defined surgical techniques are need to reach more definitive results. In addition tumor adjacent benign-looking tissue should be investigated in R0-patients were local recurrence was found to assess whether premalignant lesions or processes have already been existent.

However, despite the small study population and the low number of local recurrences a significant result regarding the impact of surgical margins in RCC was observed.

Conclusion

The width of tumor adjacent renal parenchyma may have oncological relevance. Herein, the demonstrated data should point to further molecular studies to clarify the molecular status of this tumor adjacent tissue. These findings then have to be inserted into a topographic context to enable describing molecular conditions in dependence of the histological border of RCC. Finally these results may influence clinical strategies and improve treatment of RCC.

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