

# Prognostic Significance of Preoperative Molecular Serum Analysis in Renal Cancer

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## ABSTRACT

**Purpose:** We evaluated the postoperative clinical course of patients with renal cancer identified preoperatively by microsatellite analysis to examine the correlation between microsatellite alterations and risk of disease recurrence and patient mortality 2 years after nephrectomy.

**Experimental Design:** A panel of 28 microsatellite markers was used previously to assess loss of heterozygosity and microsatellite instability in urine, serum, and tumor DNA of 30 patients with clinically organ-confined renal masses who underwent partial or radical nephrectomy. The clinical reports and imaging data in the medical records of patients with a minimum follow-up of 2 years were retrospectively reviewed to determine their postoperative course.

**Results:** Two-year follow-up was available for the 30 patients (100%) who entered the study. Mean age was  $61.6 \pm 12.9$  years (range, 21–77 years). Tumor stage was associated with patient mortality ( $P = 0.03$ ). Tumor grade was associated with mortality ( $P = 0.03$ ) and disease recurrence ( $P < 0.01$ ). The frequency of microsatellite alterations (loss of heterozygosity) found in the preoperative serum of patients with renal masses served as a prognostic indicator for disease recurrence ( $P < 0.01$ ).

**Conclusions:** Analysis of microsatellite alterations found in preoperative blood samples is a promising method for the detection of renal cancer. The presence of frequent molecular changes in preoperative serum was associated with disease recurrence. These findings suggest a role for microsatellite analysis in future studies attempting to stratify patients with clinically organ-confined renal cancer into

low- and high-risk prognostic groups. Larger prospective randomized trials are needed to validate the clinical utility of this observation.

## INTRODUCTION

Approximately 30,000 new cases of renal cancer are reported each year in the United States, with about 12,000 individuals dying annually from this disease (1). Nearly 50% of patients have organ-confined tumors at the time of diagnosis. Approximately 25% of patients harbor locally invasive cancer, and the remaining 25% of individuals have metastatic disease on initial presentation (2, 3). Identification of patients with organ-confined renal carcinoma may be of importance for long-term disease-free survival after radical or partial nephrectomy (4). Reports of clinical symptoms such as the “classic triad” of pain, hematuria, and palpable flank mass are limited to approximately 10% of patients (5). Relatively few diagnostic tools are available for the early detection of renal tumors, although the increased use of radiographic imaging modalities such as computed tomography and ultrasound has aided in disease diagnosis. The development of novel techniques for early detection of renal carcinoma and prognostic application for disease stratification is therefore critical for improving clinical outcomes.

Aberrations in repetitive genomic elements known as microsatellite sequences have been increasingly used as diagnostic markers for the detection of cancers including breast, bladder, and lung cancer (6–8). Microsatellite alterations are tumor-specific changes and have been used to detect cancer cells in a variety of human bodily fluids including sputum, serum, and urine (9, 10). Genetic alterations including LOH<sup>2</sup> are also specific to tumor cells and have been found in the blood of approximately 60% of patients with renal cancer (2, 9). We have previously demonstrated that microsatellite alterations found in the serum of patients with renal cancer are identical to those found in the primary tumor (2). Preoperative serum microsatellite analysis may therefore serve as a highly specific test not only for disease detection but also for disease prognostication.

Variables including tumor stage and grade have been used previously as prognostic indicators for renal cell cancer (3). Few clinically useful tumor markers currently exist for this type of cancer, and to date, no preoperative molecular markers have been used for disease prognostication. Molecular markers including Ki-67 expression and proliferating cell nuclear antigen have been useful for predicting renal cancer prognosis; however, these studies have relied predominantly on immunohistochemical analysis of tumor specimens obtained at the time of surgery (11, 12). We report for the first time the use of preoperative

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<sup>2</sup> The abbreviations used are: LOH, loss of heterozygosity; MI, microsatellite instability; RCC, renal cell carcinoma; TCC, transitional cell carcinoma.

Table 1 Clinical characteristics and microsatellite status in the tumor, urine, and serum of patients with recurrent disease

Patient no.	Pathology	Age (yrs)	pTNM <sup>a</sup>	Grade <sup>b</sup>	Symptoms	LOH, tumor/urine/serum <sup>c</sup>	MI, tumor/urine/serum <sup>c</sup>	Recurrence	Death
1345	RCC, clear cell	65	T <sub>3</sub> bN <sub>0</sub> M <sub>1</sub>	III	Metastasis (lung, s.c.)	2/0/0	0/0/0	Yes	Yes
1353	RCC, clear cell	72	T <sub>3</sub> aN <sub>x</sub> M <sub>x</sub>	III	None	2/1/1	0/0/0	Yes	Yes
1523	RCC, clear cell	21	T <sub>2</sub> N <sub>0</sub> M <sub>x</sub>	II	None	6/1/1	0/0/0	Yes	No
1552	RCC, hypernephroid	62	T <sub>3</sub> N <sub>x</sub> M <sub>x</sub>	III	None	5/3/2	0/0/0	Yes	Yes
1290	TCC, renal pelvis	77	T <sub>1</sub> N <sub>x</sub> M <sub>x</sub>	III	Hematuria/positive cytologic specimen	13/8/3	1/1/0	Yes	No
1550	RCC, papillary	54	T <sub>2</sub> N <sub>0</sub> M <sub>x</sub>	II	None	11/3/2	0/0/0	Yes	Yes

<sup>a</sup> American Joint Committee on Cancer staging. pTNM: p, pathologic stage; T, tumor size; N, node status; M, metastatic status.

<sup>b</sup> American Joint Committee on Cancer.

<sup>c</sup> Numbers under LOH and MI columns indicate positive microsatellite markers in tumor, urine, and serum DNA.

serum-based microsatellite analysis as a potential prognostic marker for renal carcinoma.

## MATERIALS AND METHODS

Microsatellite analysis of urine, serum, and tumor DNA from 30 patients with clinically organ-confined solid renal masses was performed previously (2). Of the 30 patients enrolled in the original study, 25 had malignant tumors (RCC or TCC) and 5 patients had tumors of low malignant potential (angiomyolipoma, metanephric nephroma, or oncocytoma). Urine and serum specimens were obtained from each patient before surgery. A total of 28 microsatellite markers were used to assess LOH and MI in each sample.

The records of all 30 patients who underwent partial or radical nephrectomy were retrospectively reviewed. Patients had a minimum follow-up of 2 years with radiological evaluation and/or physical examination to monitor for disease recurrence and vital status. Fisher's exact test was performed to evaluate the association between microsatellite alterations found in preoperative urine and serum specimens and disease recurrence and patient mortality 2 years after nephrectomy. Reported *P*s are two-sided.

## RESULTS

Two-year follow up was available for all 30 patients (100%) who entered the study. Mean age was  $61.6 \pm 12.9$  years (range, 21–77 years). A total of 6 of 30 patients (20%) had evidence of recurrent disease 2 years after nephrectomy. The clinical characteristics and microsatellite status in the tumor, urine, and serum of patients with recurrent disease are shown in Table 1. Complications from metastatic disease resulted in mortality in four of six cases (67%).

Table 2 demonstrates clinical outcome (recurrence or death) for 25 patients with malignant kidney tumors 2 years after nephrectomy according to tumor stage. A correlation between increasing tumor stage and disease recurrence was observed ( $P = 0.07$ ). Tumor stage was significantly associated with patient mortality at 2 years after surgery ( $P = 0.03$ ). The clinical outcome (recurrence or death) for the 25 patients with malignant kidney tumors 2 years after nephrectomy according to tumor grade is shown in Table 3. Tumor grade was associated with disease recurrence ( $P < 0.01$ ) and patient mortality ( $P = 0.03$ ).

Molecular analysis for LOH was performed previously on

Table 2 Clinical outcome 2 years after nephrectomy according to tumor stage

	Tumor stage			<i>P</i> <sup>a</sup>
	T <sub>1</sub> ( <i>n</i> = 4)	T <sub>2</sub> ( <i>n</i> = 16)	T <sub>3</sub> ( <i>n</i> = 5)	
Recurrence	1 (25)	2 (13)	3 (60)	0.069
Death	0 (0)	1 (6)	3 (60)	0.028

<sup>a</sup> Two-sided, Fisher's exact test.

<sup>b</sup> Number (percentage) of patients within each group.

preoperative serum and urine DNA as well as the operative specimens of all 30 patients, including those with tumors of low malignant potential. Table 4 demonstrates the clinical outcome 2 years after nephrectomy in these 30 patients according to the number of observed serum LOH. Only 1 of 15 patients (7%) with no detectable serum LOH was found to have recurrent disease, whereas 2 of 12 patients (17%) with 1 detectable serum LOH were found to have disease recurrence 2 years after nephrectomy. Interestingly, all 3 patients (100%) who had 2 or 3 detectable serum LOH changes were found to have recurrent disease 2 years after surgery. The frequency of microsatellite alterations (LOH) found in the preoperative serum of patients with renal masses was significantly associated with disease recurrence ( $P < 0.01$ ). There was also a correlation between a higher number of serum LOH changes and patient mortality at 2 years after nephrectomy ( $P = 0.06$ ). Urine LOH was not associated with disease recurrence ( $P = 0.33$ ) or patient mortality ( $P = 1.0$ ). There was also no significant association between LOH changes found in the tumor specimens and disease recurrence ( $P = 0.16$ ) or death ( $P = 0.76$ ).

There was no correlation between serum ( $P = 0.48$ ) or urine ( $P = 0.24$ ) LOH and tumor stage. Microsatellite alterations (LOH) detected in preoperative serum or urine were also not associated with tumor grade ( $P = 0.36$  and  $P = 0.88$ , respectively). No significant differences were observed between serum or urine microsatellite alterations (LOH) found in clear cell *versus* papillary RCCs. There was no association between serum MI and disease recurrence ( $P = 1.0$ ) or death ( $P = 1.0$ ). There was also no correlation between urine MI and disease recurrence ( $P = 1.0$ ) or urine MI and death ( $P = 1.0$ ). MI detected in operative tumor specimens demonstrated no association with disease recurrence ( $P = 0.63$ ) or death ( $P = 0.27$ ).

**Table 3** Clinical outcome 2 years after nephrectomy according to tumor grade

	Tumor grade			<i>P</i> <sup>a</sup>
	1	2	3	
	( <i>n</i> = 6)	( <i>n</i> = 14)	( <i>n</i> = 5)	
Recurrence	0 (0)	2 (14)	4 (80)	0.008
Death	0 (0)	1 (7)	3 (60)	0.025

<sup>a</sup> Two-sided, Fisher's exact test.<sup>b</sup> Number (percentage) of patients within each group.

A total of 28 microsatellite markers were used for analysis of serum, urine, and tumor DNA (2). Microsatellite alterations (LOH) detected in the preoperative serum DNA of six patients with recurrent disease were observed at seven loci (chromosomal location) as follows: *D3S1038* (3p), *D8S307* (8p), *IFN-α* (9p), *THO* (11p), *MJD* (14q), *D17S654* (17p), and *MBP* (18q). No particular microsatellite marker among this group was more significantly altered in patients who died. Serum LOH alterations unique to three of six (50%) patients with disease recurrence involved at least one of four loci (*D3S1038*, *IFN-α*, *D17S654*, and *MBP*). None of the microsatellite alterations observed at these four loci were detected in the serum DNA of the remaining 24 patients without recurrent disease. In every case, microsatellite changes found in serum DNA were identical to those found in the primary tumor. There was no correlation between urine microsatellite changes (LOH) and disease recurrence ( $P = 0.33$ ). These alterations were distributed over a total of 11 loci (chromosomal location) and included *DS1560* (3p), *D3S1038* (3p), *D4S243* (4pq), *D8S348* (8q), *IFN-α* (9p), *THO* (11p), *D13S802* (13q), *D17S695* (17p), *D17S654* (17p), *D18S51* (18q), and *MBP* (18q).

## DISCUSSION

The use of serum microsatellite alterations for disease prognosis has been reported previously for patients with head and neck cancers (13). We have demonstrated for the first time the utility of preoperative serum microsatellite analysis for prognostic application in renal cancer. The presence of frequent molecular changes (LOH) in preoperative serum was associated with disease recurrence at 2 years after nephrectomy ( $P < 0.01$ ). Preoperative microsatellite analysis could potentially be used for stratification of patients into low- and high-risk groups for disease recurrence. This information could then be used for treatment decision-making and experimental neoadjuvant or adjuvant therapies. The patterns of microsatellite alterations may also potentially be used to identify patients who would benefit most from chemotherapy, immunotherapy, or gene therapy. The application of such adjuvant therapies may prove to be most beneficial to those patients identified to have a high risk for early disease recurrence.

Tumor stage and grade were found to be significantly associated with disease recurrence and patient mortality, consistent with previous reports in the literature (3). We did not find a significant association between disease recurrence and urinary microsatellite alterations (LOH or MI). Cancer cells derived from renal tumors may spread via the urinary tract or lymphatic

**Table 4** Clinical outcome 2 years after nephrectomy according to serum LOH

	Serum LOH <sup>a</sup>			<i>P</i> <sup>b</sup>
	0	1	2-3	
	( <i>n</i> = 15)	( <i>n</i> = 12)	( <i>n</i> = 3)	
Recurrence	1 (7) <sup>c</sup>	2 (17)	3 (100)	0.004
Death	1 (7)	1 (8)	2 (67)	0.057

<sup>a</sup> Numbers under serum LOH indicate the number microsatellite markers demonstrating loss of heterozygosity.<sup>b</sup> Two-sided, Fisher's exact test.<sup>c</sup> Number (percentage) of patients within each group.

or hematogenous routes. The prognostic utility of serum microsatellite alterations in renal cancer may reflect the increased biological potential of tumor cells that have metastasized via the bloodstream as compared with the urinary tract. Microsatellite alterations (LOH) were found in the serum DNA of five of six (83%) patients with recurrent disease. In each case, the identical genetic alteration was also found in DNA from the corresponding tumor sample. These findings confirm the presence of cells in the circulation that contain the same LOH changes as the primary tumor and suggest hematogenous spread from the original population of tumor cells. The prognostic significance of microsatellite alterations (LOH) in preoperative serum DNA may therefore represent established micrometastatic disease at the time of operative treatment. Indeed, tumor cells have been detected in the blood of patients with various stages of renal cancer, and serum microsatellite alterations have been observed in nearly 60% of patients with renal tumors (2, 9). These findings, in addition to the high specificity of microsatellite analysis, make this test particularly attractive for screening larger populations.

At the present time, it remains unclear whether early detection of renal cancer and prompt surgical intervention will lead to a reduction in long-term disease recurrence or patient mortality (14). Nevertheless, it remains plausible that preoperative information such as serum microsatellite status could be used in future treatment algorithms. Our findings suggest a role for microsatellite analysis in studies attempting to stratify patients with clinically organ-confined renal cancer into low- and high-risk prognostic groups. Larger prospective randomized trials are needed to validate the clinical utility of our observation.

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