Published in final edited form as:

J Urol. 2013 May ; 189(5): 1913–1920. doi:10.1016/j.juro.2012.11.034.

The Specificity of Urinary Aquaporin-1 and Adipophilin to Screen for Renal Cell Carcinoma

Jeremiah J. Morrissey, Ph.D.^{1,2} and Evan D. Kharasch, M.D., Ph.D.^{1,2,3}

¹Department of Anesthesiology, Division of Clinical and Translational Research, Washington University in St. Louis, St. Louis, Missouri.

²Siteman Cancer Center, Washington University in St. Louis, St. Louis, Missouri.

³Department of Biochemistry and Molecular Biophysics, Washington University in St. Louis, St. Louis, Missouri.

Abstract

Purpose—Renal cancer is frequently asymptomatic until late stages of disease and has poor prognosis when not discovered early. Aquaporin-1 (AQP-1) and adipophilin (ADFP) are recently discovered sensitive urine biomarkers of clear cell and papillary kidney cancer. This investigation sought to validate these biomarkers in a second cohort of patients and determine the effect of common kidney diseases on their specificity.

Materials and Methods—Urine samples were obtained from 36 patients with clear cell or papillary kidney cancer, 43 controls, 44 patients with documented urinary tract infection, 24 patients diagnosed with diabetic nephropathy, and 18 patients diagnosed with glomerulonephritis. Urinary concentrations of AQP-1 and ADFP (normalized to urine creatinine) were determined by a sensitive and specific Western blot procedure.

Results—Urine AQP-1 and ADFP concentrations in patients with kidney cancer were 23- and 4-fold greater than controls and decreased 83-84% after tumor excision. There was a linear correlation between urinary AQP-1 and ADFP concentrations and tumor size (each $P < 0.001$). Urine AQP-1 and ADFP concentrations of patients with kidney cancer were 11- to 23-fold and 17- to 25-fold greater, respectively, than in patients with the common kidney diseases.

Conclusions—The ability of urinary AQP-1 and ADFP to identify patients with kidney cancer compared to controls was validated in a second cohort of patients. Common kidney diseases do not adversely increase urinary AQP-1 and ADFP concentrations or reduce their specificity to screen for renal cancer.

Keywords

Urine Biomarkers; Kidney Cancer; Aquaporin-1; Adipophilin

Corresponding Author: Jeremiah J. Morrissey, Department of Anesthesiology, Division of Clinical and Translational Research, Campus Box 8054, Washington University in St. Louis, 660 S. Euclid Ave., St. Louis, Missouri 63110-1093, USA. morrisse@wustl.edu Phone: 314-362-8625 Fax: 314-362-8334; Second author: kharasch@wustl.edu.

Trial Registration: clinical trials.gov Identifier: NCT00851994

Portions of this study were presented as a poster for the American Society of Nephrology annual meeting November 10-13, 2011 in Philadelphia, PA.

Introduction

Renal cancer is generally silent and frequently fatal. About 80% of kidney tumors are discovered incidentally during abdominal imaging (computer-assisted tomography, ultrasound or magnetic resonance) performed for unrelated diagnostic reasons.¹⁻⁷ When symptomatically diagnosed; renal cancer has frequently metastasized to lymph nodes or other organs in 30-40% of patients.^{5,8} Renal cancer is resistant to chemotherapy, and metastatic disease portends a poor prognosis, with 2 year survival of 18% (9) and 5 year survival of 5% or less.² More than 58,000 cases of kidney cancer were diagnosed, and over 13,000 deaths occurred in the United States in 2010⁹. One in 67 adults in the United States will develop kidney cancer during their lifetime.^{9,10} Clear cell and papillary cancers comprise nearly 90% of patients afflicted with kidney cancer.^{2, 11, 12}

There is presently no diagnostic modality for the early detection of renal cancer, other than incidental radiologic discovery, and no method for surveillance of recurrence or response to chemotherapy. Population screening would require higher through-put and lower cost than imaging techniques and haphazard discovery. Periodic imaging as a screening tool for early detection of renal cancer is unfeasible and routine imaging for surveillance, recurrence or monitoring treatment carries a radiation risk.^{12, 14} Thus, kidney cancer is a major, and under recognized, public health problem. Unfortunately, there is no existing biomarker for kidney cancer diagnosis, and to enable population screening. Currently emerging biomarkers of renal disease or injury, such as neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1)¹⁵⁻¹⁸ do not appear applicable to renal cancer because they lack specificity.¹⁹ Thus, an unmet need and barrier to progress in the field is a specific diagnostic test for the early detection of kidney cancer, particularly at a curative stage.

Our laboratory made the seminal discovery that urinary concentrations of the exosomal proteins aquaporin-1 (AQP-1) and adipophilin (ADFP) are sensitive diagnostic markers of clear cell and papillary kidney cancer.²⁰ Since these subtypes account for almost 90% of kidney cancers,^{2, 11, 12} potential application for renal cancer screening is extensive. Nonetheless, biomarker utility is determined by specificity as well as sensitivity.

This investigation first sought to validate these markers in another cohort of patients with kidney cancer. Second, to test the hypothesis that common non-cancerous kidney diseases such as urinary tract infections, diabetic nephropathy or glomerulonephritis do not increase urine concentrations of AQP-1 and ADFP, and thus interfere with the ability of these two biomarkers to specifically detect kidney cancer. Confirmation of the hypothesis would strengthen the specificity of the biomarkers and their value in screening patient populations for renal cancer.

Patients and Methods

Patients

The protocol was approved by the Washington University Institutional Review Board, and all patients gave written informed consent to participate. In brief, from November 2009 to June 2010 pre-nephrectomy urine was obtained from 36 patients (age range 44 to 86 years) with clear cell carcinoma (n=33) or papillary carcinoma (n=3), based on histologic analysis of excised tumor specimens. Within this group, 23 (age range 44 to 78 years) had stage T1a and 4 had T1b disease without nodal or metastatic involvement, 4 had stage T2, and 5 had stage T3 tumors. There were no noted metastases or node involvement. Twenty seven patients provided a post-nephrectomy urine sample one month later. An age (34 to 88 years) and sex-matched group of patients (n=43) undergoing non-renal surgery with no known renal diseases, was recruited as a control group.

The priority in selecting the surgical control patients was to mirror as closely as possible the age distribution of the kidney cancer cohort. There was a bi-modal age distribution in the patients who were referred for surgery due to an imaged renal mass.¹⁹ Within age bins, surgical controls were then enrolled to match sex, weight, smoking history and pre-operative serum creatinine to determine estimated GFR (eGFR) to mirror approximate kidney function. Urine samples, after centrifugation at 1800g for 10 minutes to remove debris, were stored at -80°C until analysis.

Anonymized urine samples from 44 patients (18-101 yr) with known urinary tract infections were obtained from a hospital clinical laboratory. Urine samples from 24 patients with diabetic nephropathy (18-75 yr) and 18 patients with glomerulonephritis (3-76 yr) of various etiologies, primarily lupus nephritis and IgA nephropathy, were obtained from an archived sample repository that was stored at -80°C until analysis.

Sample size calculations were based on results of the surgical control group using the mean and standard deviation of the urinary AQP1 and ADFP concentrations. To detect a 2-fold (conservative assumption) increase in biomarker concentrations in the kidney disease patients in the present study, based on a two-sided t-test and 80% power, would require 16 patients for AQP1 and 6 for ADFP (0.05 significance).

Urine Analysis

Urinary AQP-1 and ADFP concentrations were determined as previously described.²⁰ Briefly, thawed urine was centrifuged (1800g for 10 minutes) to remove debris before processing for Western blot analysis. The urinary creatinine concentration was quantified by the Jaffe reaction.²¹ Protein from 100 μL of spun urine was precipitated with 1.5 mL of ice-cold acetone methanol (1:1), centrifuged, and washed with fresh acetone-methanol (1.5 mL). This had been shown in preliminary studies to quantitatively precipitate both markers from urine. Precipitated proteins were dissolved in an amount of sodium dodecyl sulfate (SDS) sample buffer such that the 5 μL of sample applied to the gel reflected the amount of urine containing 10 μg of creatinine for patients with kidney cancer or 20 μg creatinine for the control and confounder groups. Urine samples, processed for Western blot in SDS-sample buffer, were stored at 4°C before analysis. The blocked membranes were incubated with 1:500 dilution of anti-AQP1 (H-55) antibody or a 1:200 dilution of anti-ADFP (H-80) antibody (both from Santa Cruz Biotechnology Inc, Santa Cruz, CA) in blocking buffer that contained 0.1% Tween-20 overnight. After washing, the membranes were incubated with a 1:2000 dilution of donkey anti-rabbit IgG IRDye 680 (LICOR Biosciences, Lincoln, NE) in blocking buffer with 0.1% Tween-20 for 1 hour. Both AQP1 and ADFP were visualized and quantified using an infrared imager (Odyssey Infrared Imager; LI-COR) and proprietary software. Preliminary studies had shown that the response of both biomarkers to this analysis was linear over the range of concentrations found in patient urine. Both AQP1 and ADFP were quantified using arbitrary absorbance units. On each gel, samples from 2 different patients with clear cell carcinoma were analyzed and used to normalize the signal response across all gels run within the same or different days.

Statistical Methods

The difference of markers and marker change in the diagnosis groups were first assessed for normality and then tested by Wilcoxon rank sum test. ROC curves were calculated to determine the sensitivity and specificity of the markers to identify patients with renal cancer. Analyses were implemented in Analyse-It for Microsoft Excel. The results are reported as the mean $\pm$ 1 standard deviation or the median with 1st and 3rd quartiles as appropriate.

Results

Within the validation cohort, the 36 nephrectomy patients with clear cell and papillary carcinoma were statistically indistinguishable from the 43 control patients undergoing non-renal surgery. There were no differences in age (Wilcoxon rank sum test, $P=0.941$), sex (Chi-square, $P=0.492$), weight (Wilcoxon rank sum test, $P=0.752$), and a smoking (a risk factor for kidney cancer) history exceeding 20 years (Chi-square test, $P=0.635$).

In patients with kidney cancer the urine concentrations [median(1st, 3rd quartile)] of AQP-1 34(20, 76) and ADFP 25(17, 65), were significantly greater than the control patients 1.5(1.0, 2.0) and 6(4, 8), respectively (both $P<0.001$, Wilcoxon rank sum test) (Table 1 and Figures 1A and 1B). There was no overlap in the AQP-1 concentration ranges of control and kidney cancer patients, although 5 patients with kidney cancer had ADFP concentrations that were in the range found for the control patients (Figure 1B). Seven control patients had urine ADFP concentrations that overlapped with those of the kidney cancer patients. The urine concentrations of AQP-1 and ADFP in the patients with renal cancer were significantly correlated with tumor size, based on maximum tumor dimension (Figures 2A and 2B, respectively). The Spearman correlation coefficient for AQP-1 was 0.89 (95% confidence interval, 0.79 to 0.94, $P<0.001$) and that for ADFP was 0.92 (95% confidence interval 0.86 to 0.96, $P<0.001$). The pre-nephrectomy urine AQP-1 and ADFP concentrations of the 27 patients providing a post-nephrectomy urine sample were 27(20, 73) and 35(17, 48), respectively. Both biomarkers were significantly decreased by 83-84% ($P<0.001$ for both, Wilcoxon signed rank test) by tumor removal to 8(2, 11) for AQP-1 and 6(2, 7) for ADFP (Figure 3).

Figure 4 depicts a Western blot of urinary AQP-1 and ADFP in patients with diabetic nephropathy (upper) or urinary tract infections (lower), and in comparison to two patients with clear cell carcinoma. The range of urinary AQP-1 concentrations of the different patient groups and their relation to each other is shown in Figure 1A. The patients with diabetic nephropathy had a median AQP-1 concentration of 1.5 (1.0, 4.0) (Table 1). One patient had a relative AQP-1 concentration of 47. Patients with kidney cancer had significantly more urinary AQP-1 compared to the patients with diabetic nephropathy ($P<0.001$, Wilcoxon rank sum test). Apart from the outlier, seven patients with kidney cancer had urine AQP-1 concentrations that slightly overlapped with those of patients with diabetic nephropathy and seven patients with diabetic nephropathy had AQP-1 concentrations that overlapped those of patients with kidney cancer (Figure 1A). The patients with glomerulonephritis had a median AQP-1 concentration of 3.0(1.0, 6.0). One patient with glomerulonephritis, due to IgA nephropathy, had an AQP-1 concentration of 23. Patients with kidney cancer had significantly higher urinary AQP-concentrations than patients with glomerulonephritis ($P<0.001$, Wilcoxon rank sum test). Apart from the outlier, eight patients with kidney cancer had urine AQP-1 concentrations that slightly overlapped with those of patients with glomerulonephritis while six patients with glomerulonephritis had urine AQP-1 concentrations that overlapped with those of the cancer patients (Figure 1A). The 44 patients with a documented urinary tract infection had a median concentration of 1.5 (1.0, 3.6) of urinary AQP-1 with a molecular weight of 28kDa by Western blot (Figure 4, lower). This was significantly lower ($P<0.001$, Wilcoxon rank-sum test) than the level seen in the kidney cancer patients (Table 1 and Figure 1A). AQP-1 is normally expressed in the proximal tubule as a 28kDa protein, some of which normally undergoes glycosylation²², and is a constituent of urinary exosomes.²³⁻²⁵ It is not known if the higher weight Western blot bands present in patient R with an infection of the urinary tract represent alternative glycosylated forms of AQP-1. The identity of the reactive proteins was not determined. Apart from two outliers, six patients with kidney cancer had urine AQP-1

concentrations that slightly overlapped with those of patients with urinary tract infections (Figure 1A).

As was found for AQP-1, urinary ADFP concentrations in patients with clear cell or papillary subtypes of kidney cancer 25 (17, 65) were significantly higher compared to those with diabetic nephropathy 1.5 (1.0, 2.0) or glomerulonephritis 1.5 (1.0, 2.1) ($P < 0.001$, Table 1 and Figure 1B). ADFP concentrations in three patients with diabetic nephropathy and two with glomerulonephritis, all outliers, slightly overlapped with the range of ADFP concentrations found in the kidney cancer patients (Figure 1B). Patients with urinary tract infections had low levels of urinary ADFP, with a median concentration of 1.0 (0, 1.0), similar to that seen with diabetic nephropathy or glomerulonephritis. There was a significant difference between the increased urinary AQP-1 and ADFP concentrations in patients with clear cell and papillary kidney cancer compared to those with urinary tract infections with no overlap in the concentration ranges ($P < 0.001$ for each) (Figure 1B).

A measure of biomarker screening sensitivity and specificity is derived from receiver operating characteristic curve (ROC) analysis. When the data of the 23 patients with stage T1a kidney cancer (tumors ≤ 4 cm, a more stringent test of sensitivity and specificity compared with all renal cancer patients) are compared to the 43 control patients for urinary AQP-1 concentrations, the area under the ROC curve was 0.99 ($P < 0.001$) (Figure 5A). The median AQP-1 concentration of these 23 patients was 22 (10, 32) relative units. The ROC values for AQP-1 comparing patients with stage T1a kidney cancer to patients with diabetic nephropathy, glomerulonephritis and urinary tract infections was 0.96 ($P < 0.001$) (Figure 5B). The corresponding area under the ROC curve for urinary ADFP concentrations with the control and renal cancer patients was 0.94 ($P < 0.001$) (Figure 5C). The median urinary ADFP concentration of the 23 patients with T1a tumors was 18 (13, 25). The ROC values for ADFP comparing patients with stage T1a kidney cancer to patients with diabetic nephropathy, glomerulonephritis and urinary tract infections was 0.98 ($P < 0.001$) (Figure 5D).

Discussion

Previously, our laboratory made the seminal discovery that urinary AQP-1 and ADFP represent the first non-invasive assay to potentially diagnose kidney cancer at a treatable stage of the disease and to preserve post-nephrectomy kidney function through early detection and partial nephrectomy.²⁰ This previous investigation demonstrated multiple log-order increases of urine AQP-1 and ADFP concentrations in renal cancer patients compared to both young healthy controls and age-matched controls, with no overlap in the concentration ranges for cancer patients and healthy controls, thereby demonstrating excellent biomarker sensitivity.²⁰ Increased urinary ADFP concentrations are consistent with the formation of intracellular lipid droplets, an underlying feature of clear cell and papillary renal tumors.²⁶ Neoplastic growth and the angiogenesis associated with this growth seem to require increased AQP-1 expression.²² These pathophysiologic considerations, linked together with the proximal tubule origin of clear cell and papillary kidney cancer, can account for the presence of AQP-1 and ADFP in the urine of patients with these two renal cancer subtypes.

Our present study, based upon a new cohort of patients, validates our original study.²⁰ This investigation also focused more on kidney cancer patients with small tumor sizes (up to about 2 cm). With this focus, there was some overlap in urine ADFP concentrations between the renal cancer patients and the control patients. This may be due to technical considerations (twice the urine equivalent was used for the control patients in this than the previous study), more patients with kidney cancer with small T1a tumors (a more stringent

test of sensitivity and specificity compared with all renal cancer patients), a larger control group (43 at present vs. 15 previously), and recruiting a control patient group reflecting more stringent demographics to the kidney cancer group. The urine concentration of both biomarkers was significantly decreased by tumor excision (Figure 3). This is in agreement with our original study²⁰ and further supports their clinical utility to detect the presence and absence of tumor. The ROC curves for both biomarkers in our original study²⁰ were both 1.0 and in the present study were 0.99 for AQP-1 and 0.94 for ADFP. The slight decrease in ROC value for ADFP was undoubtedly due to the modest overlap of values between the control and the cancer patients; however, a value of 0.94 indicates substantial sensitivity and specificity.

Since the specificity of these biomarkers vis a vis common kidney disease remained unknown, this investigation tested the hypothesis that common kidney diseases do not increase urine concentrations of AQP-1 and ADFP and adversely interfere with the screening ability of these biomarkers. It is estimated that over 13% of adults in developed countries have some form of chronic kidney disease (defined as an estimated glomerular filtration rate of less than 60 ml/min/1.73m²) due in large part to diabetes, glomerulonephritis and urinary tract infections.²⁷ Post-infectious glomerulonephritis is becoming an increasing problem of elderly patients.²⁸ Thus the patient groups with urinary tract infections, diabetic nephropathy and glomerulonephritis reflect common kidney diseases which could potentially confound the detection of patients with kidney cancer, especially those with undiagnosed early chronic kidney disease. Urine from patients with urinary tract infections, diabetic nephropathy or glomerulonephritis was therefore evaluated.. It was unknown whether any of these patients may have had renal cancer. The cohorts of common kidney disease patients who were tested reflected the age range of patients with kidney cancer.

Urine AQP-1 concentrations in the patients with diabetic nephropathy, glomerulonephritis or urinary tract infection were statistically significantly albeit modestly increased above those of similarly-aged controls (Table 1). Mechanisms contributing to the modest rise in urinary AQP-1 excretion in these 3 non-cancerous renal diseases are not presently known. Despite these modest increases, median AQP-1 concentrations in the urine of patients with clear cell and papillary kidney cancer were still nevertheless significantly 11- to 23-fold higher than in patients with diabetic nephropathy, glomerulonephritis or urinary tract infections (Table 1 and Figure 1A), and 23-fold higher than in age-matched control patients. Therefore, urinary tract infections, diabetic nephropathy or glomerulonephritis, do not appear to interfere with the ability of AQP-1 to screen for kidney cancer.

ADFP excretion in the patients with urinary tract infections, diabetic nephropathy or glomerulonephritis was not significantly different from that of the control group (Table 1). Median urinary ADFP concentrations of patients with clear cell and papillary kidney cancer were 17- to 25-fold higher than in patients with these common renal diseases (Table 1 and Figure 1B), and 4-fold higher than in age-matched control patients. Therefore, because ADFP is not increased in urinary tract infections, diabetic nephropathy or glomerulonephritis, these common renal diseases do not interfere with the ability of ADFP to screen for kidney cancer.

Thus, the important, novel finding of this investigation is that the common kidney diseases of diabetic nephropathy, glomerulonephritis or urinary tract infections did not confound the ability of either AQP-1 or ADFP to screen for renal cancer. These results support the hypothesis that the common kidney diseases of urinary tract infection, diabetic nephropathy and glomerulonephritis do not increase urine concentrations of AQP-1 and ADFP and adversely interfere with the screening ability of these biomarkers to detect clear cell and

papillary kidney cancer, and strengthen their specificity and potential value in screening patient populations.

Conclusions

This investigation provides initial validation that measuring urinary AQP-1 and ADFP concentrations can provide a means of identifying patients with clear cell and papillary kidney cancer without interference from underlying common kidney diseases. The current sensitive and specific form of screening by Western blot, however, is cumbersome. A more efficient means of screening awaits the development of sensitive and specific ELISAs for each protein. Further study by others in a multi-institutional setting with a larger cohort is necessary to truly validate the screening value of these markers.

Acknowledgments

The authors wish to thank members of the Department of Urologic Surgery, Washington University in St. Louis and members of the Department of Orthopedic Surgery for their cooperation, and recognize Karen Frey, Research Patient Coordinator for obtaining patient consent, history and specimens used in the study.

Support for this study was provided by the Department of Anesthesiology, Washington University in St. Louis School of Medicine, a grant from the Bear Cub Fund of Washington University, a grant from the Barnes-Jewish Hospital Foundation, and a grant from the National Institutes of Health R01CA141521 each to JJM, and the P30 Kidney Translational Research Core of the Washington University George M. O'Brien Center for Kidney Disease Research (DK079333).

References

1. Ball W, LeFevre S, Jarup L, et al. Comparison of different methods for spatial analysis of cancer data in Utah. *Environ Health Perspect.* 2008; 116:1120. [PubMed: 18709141]
2. Cohen HT, McGovern FJ. Renal-cell carcinoma. *N Engl J Med.* 2005; 353:2477. [PubMed: 16339096]
3. Feldstein MS, Rhodes DJ, Parker AS, et al. The haphazard approach to the early detection of asymptomatic renal cancer: results from a contemporary executive health programme. *BJU Int.* 2009; 104:53. [PubMed: 19154476]
4. Figlin RA. Renal cell carcinoma: management of advanced disease. *J Urol.* 1999; 161:381. [PubMed: 9915408]
5. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. *CA Cancer J Clin.* 2008; 58:7. [PubMed: 18096864]
6. Lopez-Abente G, Aragonés N, Perez-Gomez B, et al. Kidney cancer mortality in Spain: geographic patterns and possible hypotheses. *BMC Cancer.* 2008; 8:293. [PubMed: 18844980]
7. Nogueira M, Kim HL. Molecular markers for predicting prognosis of renal cell carcinoma. *Urol Oncol.* 2008; 26:113. [PubMed: 18312928]
8. Levy DA, Slaton JW, Swanson DA, et al. Stage specific guidelines for surveillance after radical nephrectomy for local renal cell carcinoma. *J Urol.* 1998; 159:1163. [PubMed: 9507823]
9. NCI. National Cancer Institute. SEER.; 2011. <http://seer.cancer.gov/statfacts/html/kidrp.html>
10. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2009. *CA Cancer J Clin.* 2009; 59:225. [PubMed: 19474385]
11. Pantuck AJ, Zisman A, Belldgrun AS. The changing natural history of renal cell carcinoma. *J Urol.* 2001; 166:1611. [PubMed: 11586189]
12. Rini BI, Campbell SC, Escudier B. Renal cell carcinoma. *Lancet.* 2009; 37:1119. [PubMed: 19269025]
13. De Mauri A, Brambilla M, Chiarinotti D, et al. Estimated radiation exposure from medical imaging in hemodialysis patients. *J Am Soc Nephrol.* 2011; 22:571. [PubMed: 21355057]
14. Kinsella SM, Coyle JP, Long EB, et al. Maintenance hemodialysis patients have high cumulative radiation exposure. *Kidney Int.* 2010; 78:789. [PubMed: 20592715]

15. Han WK, Alinani A, Wu CL, et al. Human kidney injury molecule-1 is a tissue and urinary tumor marker of renal cell carcinoma. *J Am Soc Nephrol.* 2005; 16:1126. [PubMed: 15744000]
16. Lin F, Zhang PL, Yang XJ, et al. Human kidney injury molecule-1 (hKIM-1): a useful immunohistochemical marker for diagnosing renal cell carcinoma and ovarian clear cell carcinoma. *Am J Surg Pathol.* 2007; 31:371. [PubMed: 17325478]
17. Vila MR, Kaplan GG, Feigelsstock D, et al. Hepatitis A virus receptor blocks cell differentiation and is overexpressed in clear cell renal cell carcinoma. *Kidney Int.* 2004; 65:1761. [PubMed: 15086915]
18. Bonventre JV. Kidney Injury Molecule-1 (KIM-1): a specific and sensitive biomarker of kidney injury. *Scand J Clin Lab Invest Suppl.* 2008; 241:78. [PubMed: 18569971]
19. Morrissey JJ, London AN, Lambert MC, Kharasch ED. Sensitivity and specificity of urinary neutrophil gelatinase-associated lipocalin and kidney injury molecule -1 for the diagnosis of renal cell carcinoma. *Am J Nephrol.* 2011; 34:391. [PubMed: 21912102]
20. Morrissey JJ, London AN, Luo J, Kharasch ED. Mayo Clin Proc.: Urinary biomarkers for the early diagnosis of kidney cancer. *Mayo Clinic Proc.* 2010; 85:413.
21. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron.* 1976; 16:31. [PubMed: 1244564]
22. Hu J, Verkman AS. Increased migration and metastatic potential of tumor cells expressing aquaporin water channels. *Faseb J.* 2006; 20:1892. [PubMed: 16818469]
23. Cheruvanky A, Zhou H, Pisitkun T, et al. Rapid isolation of urinary exosomal biomarkers using a nanomembrane ultrafiltration concentrator. *Am J Physiol Renal Physiol.* 2007; 292:F1657. [PubMed: 17229675]
24. Gonzales PA, Pisitkun T, Hoffert JD, et al. Large-scale proteomics and phosphoproteomics of urinary exosomes. *J Am Soc Nephrol.* 2009; 20:363. [PubMed: 19056867]
25. Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. *Proc Natl Acad Sci U S A.* 2004; 101(36):13368–13373. [PubMed: 15326289]
26. Tickoo SK, Gopalan A. Pathologic features of renal cortical tumors. *Urol Clin North Am.* 2008; 35:551. [PubMed: 18992609]
27. Coresh J, Selvin E, Stevens LA, et al. Prevalence of chronic kidney disease in the United States. *JAMA.* 2007; 298:2038. [PubMed: 17986697]
28. Nasr SH, Fidler ME, Valeri AM, et al. Postinfectious glomerulonephritis in the elderly. *J Am Soc Nephrol.* 2011; 22:187. [PubMed: 21051737]

Figure 1.

Dot- and box-plot of urinary biomarker concentrations in the 43 control, 44 patients with urinary tract infections (UTI), 24 patients with diabetic nephropathy (DN), 18 patients with glomerulonephritis (GN) and 36 patients with renal cancer. (A) AQP-1 (B) ADFP. (+) outliers > 1.5 and < 3 interquartile range, (x) outliers > 3 interquartile range. For statistical significance see Table 1.

Figure 2.

Urinary biomarker concentrations as a function of tumor size. (A) AQP-1 Spearman correlation coefficient of the 36 patients with clear cell and papillary kidney cancer is 0.89 (95% Confidence Interval, 0.79-0.94, $P < 0.0001$). (B) ADFP Spearman correlation coefficient is 0.92 (95% Confidence Interval, 0.86-0.96, $P < 0.0001$). Also depicted are the 43 control patients with presumed tumor size of zero not factored into the Spearman calculation.

Figure 3. Pre- and post-nephrectomy urine AQP-1 and ADFP concentrations. Results are shown for only the twenty seven patients who provided both a pre- and post-nephrectomy urine sample. Each line represents an individual patient. The biomarker concentrations were corrected for the urinary creatinine excretion.

Figure 4. Urinary AQP-1 and ADFP levels in patients with diabetic nephropathy (DN) (upper) or urinary tract infections (UTI) (lower). Two patients with renal cancer (RCC) are shown for comparison.

Figure 5.

Receiver Operating Characteristic Curve analysis of the sensitivity and specificity of urinary biomarkers to detect kidney cancer. (A) AQP-1 application to diagnose T1a kidney cancer (clear cell and papillary) compared to control patients (ROC area 0.99, 95% confidence interval 0.97 to 1.00, $P < 0.0001$). (B) AQP-1 application to diagnose T1a kidney cancer (clear cell and papillary) compared to patients with kidney disease (ROC area 0.96, 95% confidence interval 0.93 to 0.99, $P < 0.0001$). (C) ADFP application to diagnose T1a kidney cancer (clear cell and papillary) compared to control patients (ROC area 0.94, 95% confidence interval 0.88 to 0.99, $P < 0.0001$). (D) Analysis of ADFP to diagnose T1a kidney cancer (clear cell and papillary) compared to patients with kidney disease (ROC area 0.98, 95% confidence interval 0.96 to 1.00, $P < 0.0001$).

Table 1

Urinary AQP-1 and ADFP Concentrations by Kidney Disease

Disease	N	Urine AQP-1 (relative/mg UCr)	Urine ADFP (relative/mg UCr)
Controls	43	1.5 (1, 2) ^a	6 (4, 8) ^f
Kidney Cancer	36	34 (20, 76) ^b	25 (17, 65) ^g
Diabetic Nephropathy	24	1.5 (1.0, 4.0) ^c	1.5 (1.0, 2.0) ^h
Glomerulonephritis	18	3.0 (1.0, 6.0) ^d	1.5 (1.0, 2.1) ⁱ
Urinary Tract Infection	44	1.5 (1.0, 3.6) ^e	1.0 (0, 1.0) ^j

Results are presented as the median (1st quartile, 3rd quartile).

Biomarker concentrations were normalized to urine creatinine concentrations.

Statistical significance by Wilcoxon rank sum test for AQP-1.

^aP<0.001 to Renal Cancer, P=0.948 to Diabetic Nephropathy, P=0.102 to Glomerulonephritis, and P=0.599 to Urinary Tract Infection.

^bP<0.001 to Control, Diabetic Nephropathy, Glomerulonephritis, and Urinary Tract Infection.

^cP=0.948 to Control, P<0.001 to Renal Cancer, P=0.274 to Glomerulonephritis, and P=0.732 to Urinary Tract Infection.

^dP=0.102 to Control, P<0.001 to Renal Cancer, P=0.274 to Diabetic Nephropathy, and P=0.115 to Urinary Tract Infection.

^eP=0.559 to Control, P<0.001 to Renal Cancer, P=0.732 to Diabetic Nephropathy, and P=0.115 to Glomerulonephritis.

Statistical significance by Wilcoxon rank sum test for ADFP.

^fP<0.001 to Renal Cancer, Diabetic Nephropathy, Glomerulonephritis and Urinary tract Infection.

^gP<0.001 to Control, Diabetic Nephropathy, Glomerulonephritis, and Urinary Tract Infection.

^hP<0.001 to Control and Renal Cancer, P=0.882 to Glomerulonephritis, and P=0.732 to Urinary Tract Infection.

ⁱP<0.001 to Control, Renal Cancer and Urinary Tract Infection, and P=0.882 to Diabetic Nephropathy.

^jP<0.001 to Control, Renal Cancer, Diabetic Nephropathy, and Glomerulonephritis.