MAYO CLINIC

ORIGINAL ARTICLE

Urine Aquaporin 1 and Perilipin 2 Differentiate Renal Carcinomas From Other Imaged Renal Masses and Bladder and Prostate Cancer

Jeremiah J. Morrissey, PhD; Jonathan Mobley, MD; R. Sherburne Figenshau, MD; Joel Vetter, MS; Sam Bhayani, MD; and Evan D. Kharasch, MD, PhD

Abstract

Objective: To evaluate the sensitivity and specificity of urine aquaporin 1 (AQP1) and perilipin 2 (PLIN2) concentrations to diagnose clear cell or papillary renal cell carcinoma (RCC) by comparing urine concentrations of these unique biomarkers in patients with RCC, noncancer renal masses, bladder cancer, and prostate cancer.

Methods: From February 1, 2012, through October 31, 2012, preoperative urine samples were obtained from patients with a presumptive diagnosis of RCC based on an imaged renal mass, prostate cancer, or transitional cell bladder cancer. Imaged renal masses were diagnosed postnephrectomy—as malignant or benign—by histology. Urine AQP1 and PLIN2 concentrations were measured by using a sensitive and specific Western blot and normalized to urine creatinine concentration.

Results: Median concentrations of urine AQP1 and PLIN2 in patients with clear cell and papillary RCC (n=47) were 29 and 36 relative absorbance units/mg urine creatinine, respectively. In contrast, median concentrations in patients with bladder cancer (n=22) and prostate cancer (n=27), patients with chromophobe tumors (n=7), and patients with benign renal oncocytomas (n=9) and angiomyolipomas (n=7) were all less than 10 relative absorbance units/mg urine creatinine (Kruskal-Wallis test, $P<.001$ vs RCC for both biomarkers) and comparable with those in healthy controls. The area under the receiver operating characteristic curve ranged from 0.99 to 1.00 for both biomarkers.

Conclusion: These results support the specificity and sensitivity of urine AQP1 and PLIN2 concentrations for RCC. These novel tumor-specific proteins have high clinical validity and high potential as specific screening biomarkers for clear cell and papillary RCC as well as in the differential diagnosis of imaged renal masses.

Trial Registration: clinicaltrials.gov Identifier: NCT00851994

 ■ Mayo Clin Proc. 2015;90(1):35-42

Cancer of the kidney and renal pelvis accounts for approximately 4% of all malignant tumors in adults. The American Cancer Society anticipated 65,120 new cases and 13,680 deaths related to renal malignancies for 2013.[1] There has been an increase in the diagnosis of smaller, lower stage renal cell carcinoma (RCC) likely owing to a greater use of abdominal imaging and consequently incidental detection. Thus, the fraction of incidentally detected RCCs compared with all diagnosed RCCs increased from approximately 10% in 1970 to at least 60% by 1998.[2]

Pathological stage is one of the most important prognostic indicators for the survival of RCC.[3,4] Patients with presymptomatic, incidentally detected tumors have a 5-year disease-free survival rate of 85%, whereas patients with cancer detected symptomatically have a 5-year disease-free survival rate of only 62%.[2,5] The prognosis for metastatic RCC is even worse; the 5-year RCC-specific survival rate ranges from approximately 40% with nodal metastases to approximately 20% with distant metastases.[6,7] This result clearly establishes that early detection is beneficial and improves outcomes. Nevertheless, no noninvasive method is currently available to enable early diagnosis or screening for RCC.

An initial investigation in 2010 found higher urine aquaporin 1 (AQP1) and adipophilin (since renamed as perilipin 2 [PLIN2]) concentrations in patients with clear cell and papillary

From the Department of Anesthesiology, Division of Clinical and Translational Research (J.J.M., E.D.K.), Siteman Cancer Center (J.J.M., R.S.F., S.B., E.D.K.), Department of Urology (J.M., R.S.F., J.V., S.B.), and Department of Biochemistry and Molecular Biophysics (E.D.K.), Washington University in St Louis, St Louis, MO.

RCC than in controls.[8] These biomarker elevations were normalized after tumor removal.[8] To determine the specificity of AQP1 and PLIN2 for renal cancer vs common renal diseases, a second investigation compared urine AQP1 and PLIN2 concentrations in patients with RCC with those in patients with common noncancer kidney disease (diabetic nephropathy, glomerulonephritis, and urinary tract infection). That investigation found markedly higher median concentrations of urine AQP1 and PLIN2 in patients with RCC than in patients with noncancer kidney disease or patients without any renal disease. This second investigation also reaffirmed that AQP1 and PLIN2 concentrations were correlated with tumor size and were decreased by 83% to 84% after tumor removal.[9] This result suggests that urine concentrations of AQP1 and PLIN2 are not confounded by common noncancer kidney diseases but do indicate tumor burden. More specifically, these urine biomarkers reflected clear cell or papillary tumor size and stage, but not grade.[8-10]

Our previous studies provide some degree of analytical and clinical validity to the ability of urine AQP1 and PLIN2 to identify patients with clear cell or papillary subtypes of kidney cancer.[8-10] However, the ability of AQP1 and PLIN2 to differentiate patients with clear cell or papillary RCC from patients with other urinary tract cancers is unknown. In addition, a greater use of abdominal imaging has led to increased incidental detection of renal masses. Nevertheless, radiologic imaging cannot definitively differentiate all cancerous renal masses from benign ones.[11-21] Thus, the typical clinical approach is partial or radical nephrectomy of an imaged renal mass along with postoperative pathological analysis. Unfortunately, this results in the partial or total removal of otherwise normal kidneys in almost 20% of cases.[11-21] Therefore, an additional unmet clinical need is a biomarker for unambiguous differentiation of clear cell or papillary RCC from other, particularly benign, imaged renal masses. Thus, there is a need for further clinical validation of AQP1 and PLIN2 as biomarkers for RCC.

To address these questions, this investigation compared prenephrectomy (or preoperative) urine AQP1 and PLIN2 concentrations in patients with clear cell or papillary RCC with those in patients with other (noncancer) imaged renal masses and patients with prostate or bladder cancer to better understand the specificity and sensitivity of these 2 biomarkers for renal cancer.

METHODS

Patients

Approval was obtained from the Washington University Institutional Review Board (IRB ID 201202051), and written informed consent was obtained from all patients. From February 1, 2012, through October 31, 2012, preoperative urine samples were obtained on the day of operation from (1) consecutive patients with a presumptive diagnosis of kidney cancer based on an imaged renal mass, (2) 27 patients with prostate cancer, or (3) 22 patients with bladder cancer. Table 1 lists the demographic characteristics of the 47 patients with a preoperative imaged renal mass and a postsurgical histologically proven diagnosis of clear cell or papillary RCC, as well as a composite of 26 control patients undergoing operation for nonurologic issues spanning the ages of all patient groups. Table 1 also lists the demographic characteristics of the 7 patients with a postsurgically diagnosed chromophobe tumor or an angiomyolipoma and the 9 patients with a diagnosed oncocytoma who consented between November 2009 and October 2012. The composite control cohort consisted of 9 patients (mean age, 61 years) who matched the ages of the patients with RCC, chromophobe tumors, oncocytomas, angiomyolipomas, and prostate cancer (1-way analysis of variance [ANOVA], $P=.65$), with mean ages ranging from 56 to 64 years. An older control patient subgroup of 17 individuals had a mean age of 73 years, which closely matched the mean age of 75 years of the patients with bladder cancer (1-way ANOVA, $P=.47$). Table 2 summarizes the RCC tumor stage, grade, node involvement, and incidence of distant metastases of the 47 patients with RCC. Supplemental Table 1 (available online at http://www.mayoclinicproceedings.org) lists the prostate-specific antigen values, pathological stage, and grade of the 27 patients with prostate cancer. Supplemental Table 2 (available online at http://www.mayoclinicproceedings.org) lists pathological characteristics of the 22 patients with bladder cancer.

Sample size calculations were based on the results of the control group, which were