Evaluation of Urine Aquaporin-1 and Perilipin-2 Concentrations as Biomarkers to Screen for Renal Cell Carcinoma
A Prospective Cohort Study

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IMPORTANCE Historically, early detection of small asymptomatic kidney tumors presages better patient outcome. Screening for asymptomatic renal tumors by abdominal imaging is not cost-effective and cannot reliably distinguish benign from malignant tumors.

OBJECTIVE This investigation evaluated the clinical utility, sensitivity, and specificity of urine aquaporin-1 (AQP1) and perilipin-2 (PLIN2) concentrations as unique, noninvasive biomarkers to diagnose malignant clear cell or papillary renal cell carcinoma (RCC) in a screening paradigm.

DESIGN, SETTING, AND PARTICIPANTS From February through December 2012, urine samples were obtained from 720 patients undergoing routine abdominal computed tomography (CT) (screening population), 80 healthy controls, and 19 patients with pathologically confirmed RCC.

MAIN OUTCOMES AND MEASURES Urine AQP1 and PLIN2 concentrations were measured by sensitive and specific enzyme-linked immunosorbent assay and Western blot procedures, respectively, in all groups. In the otherwise asymptomatic screening population, the absence or presence of a renal mass and RCC were verified by abdominal CT and by postnephrectomy pathologic diagnosis, respectively.

RESULTS Urine AQP1 and PLIN2 concentrations were significantly higher (all \( P < .001 \)) in the 19 patients with known RCC (AQP1 median [95% CI], 225.0 [121.0-450.0] ng/mg urine creatinine; and PLIN2 median [95% CI], 37.8 [22.8-83.7] absorbance units/mg creatinine) than in the 80 healthy controls (AQP1 median [95% CI], 1.1 [0.9-1.3] ng/mg urine creatinine; and PLIN2 median [95% CI], 3.1 [2.4-3.7] absorbance units/mg creatinine) and the 720 patient screening population (AQP1 median [95% CI], 0.5 [0.0-1.0] ng/mg urine creatinine; and PLIN2 median [95% CI], 0 [0-0] absorbance units/mg creatinine). The area under the receiver operating characteristic curve for urine AQP1 and PLIN2 concentrations individually or in combination was 0.990 or greater, with 95% or greater sensitivity and 91% or greater specificity compared with controls or the screening population. Of the 720 screened patients, 3 had biomarker concentrations suggestive of RCC and were found to have an imaged renal mass by CT. Two of the patients had pathologically confirmed RCC in further evaluation.

CONCLUSIONS AND RELEVANCE These results demonstrate the clinical utility, specificity, and sensitivity of urine AQP1 and PLIN2 to diagnose RCC. These tumor-specific proteins have high clinical validity and substantial potential as specific diagnostic and screening biomarkers for clear cell or papillary RCC and in the differential diagnosis of imaged renal masses.

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caners of the kidney and renal pelvis are the most lethal urologic malignant neoplasms. There has been a steady rise in their incidence, and they now account for almost 4% of adult malignant neoplasms. The overall age-adjusted renal cancer incidence is 51.7 per 100,000 for individuals older than 50 years.5

Because of increased use of abdominal imaging, there has been a consequent increase in incidental discovery of occult small renal masses. Historically, incidental rather than symptomatic discovery has resulted in stage migration toward smaller, intrarenally confined, less malignant tumors and consequently better outcomes.3-6 Patients with smaller nonsymptomatic, incidentally detected tumors have a 5-year disease-free survival of 85%, while patients with larger tumors detected symptomatically have a 5-year disease-free survival of only 62%.6,7 The prognosis for metastatic renal cell carcinoma (RCC) is even worse; the 5-year RCC-specific survival ranges from approximately 40% with nodal metastases to approximately 20% with distant metastases.8

Early detection confers other benefits. This includes minimally invasive laparoscopic and partial vs total nephrectomy or percutaneous radiofrequency and cryoablation techniques, which offer shorter hospitalization, faster recovery, less pain and disability, fewer complications, and lower costs compared with open nephrectomy.2,9,10 Nephron-sparing partial nephrectomy preserves renal mass and long-term renal function and minimizes future chronic kidney disease.9,10 Thus, early diagnosis of asymptomatic RCC portends identification of smaller, earlier-stage tumors, with a targeted and less morbid intervention and better outcomes.

Population screening is required to achieve more widespread early diagnosis and test the notion that such screening-based detection would further reduce RCC morbidity and mortality.4 Nevertheless, the only currently available modalities for population screening for RCC (more precisely, renal masses) are expensive computed tomography (CT) and magnetic resonance imaging (MRI) and would not be cost-effective. Furthermore, although CT and MRI are generally accurate for detecting RCC, certain benign masses are radiologically indistinguishable from RCC.14-19

An alternative to radiologic screening would use sensitive and specific tumor marker(s). Nevertheless, there are no readily available or clinically validated RCC screening biomarkers.18 Our previous studies showed that urine aquaporin-1 (AQP1) and perilipin-2 (PLIN2, formerly called ADFP) concentrations are sensitive and specific biomarkers for the early noninvasive detection of clear cell or papillary subtypes of kidney cancer.20-23 These biomarkers were significantly higher in patients with clear cell and papillary cancers compared with controls, correlated with tumor size and stage (but not grade),20,21 and decreased by over 83% following tumor excision.20,21 Urine AQP1 or PLIN2 concentrations were not increased in patients with common noncancerous kidney diseases such as diabetic nephropathy, glomerulonephritis, and urinary tract infections;20 noncancerous kidney tumors (oncocytomas, angiomylipomas)21; or bladder or prostate cancer.23 Thus, common kidney disease and nonrenal urologic cancers do not confound the ability of AQP1 and PLIN2 concentration to detect clear cell and papillary cancers, suggesting they have potential for population screening and/or differential diagnosis of imaged renal masses. These studies20-23 are consistent with the first 2 phases of diagnostic cancer biomarker development24 by identifying promising biomarkers (phase 1) and establishing that the biomarkers identify clinical disease from potential confounding diseases (phase 2). Nevertheless, investigations to date have evaluated only the assay and early clinical validity, with small patient numbers in discovery and validation cohorts known a priori to have or not have RCC, and sensitivity and specificity have not been established in larger clinical validation cohorts and in a prospective, blinded fashion.

This investigation tested the hypothesis that urine concentrations of AQP1 and PLIN2 are prospectively diagnostic of RCC in a screening paradigm, using a convenience sample of 720 patients undergoing abdominal CT for routine clinical evaluation. Urine AQP1 and PLIN2 concentrations were prospectively measured and subsequently compared with CT results, which radiologically established the presence or absence of a renal mass. The presence or absence of kidney cancer was surgically confirmed to see if the biomarkers were predictive of RCC as validated by postsurgical pathologic diagnosis. This determined whether the biomarkers were able to diagnose early preclinical disease and establish a “screen positive rule,” thus satisfying phase 3 of diagnostic cancer biomarker development.24

Methods

Patients

Study approval was obtained from the institutional review board of Washington University in St Louis, and written informed consent was obtained from all patients. From February through December 2012, urine samples were obtained from 720 patients undergoing abdominal CT with contrast for a variety of benign and malignant diseases (screening population); 19 a priori patients preoperatively on the day of surgery, who were undergoing partial or radical nephrectomy with...
a presumptive diagnosis of RCC based on a CT-imaged renal mass (and whose postoperative pathologic diagnosis established clear cell or papillary kidney cancer); and 80 self-defined healthy controls. Owing to the prominent cancer focus of our outpatient clinics, many patients having CT examinations were current or former patients with cancer. The CT cohort was subsequently designated as (1) no history of cancer (n = 334) or (2) history of cancer, either current or remote (n = 386). All 720 CT study findings and the preoperative CT images of the 19 a priori patients were reviewed by a subspecialty trained abdominal radiologist (V.M.M.) to identify or exclude a renal tumor blinded to the patient condition and biomarker results. Further patient details are given in eTable 1 in the Supplement.

**AQP1 and PLIN2 Measurement**

Urine AQP1 concentration was determined by an enzyme-linked immunosorbent assay (ELISA) using a proprietary monoclonal capture antibody and a commercially available antibody (H-55; Santa Cruz Biotechnology) as a detector. The methodology of the AQP1 ELISA is described in detail in the eMethods in the Supplement. The urine PLIN2 concentration was determined by a sensitive and specific Western blot procedure as previously described.20,21,23

**Statistical Analysis**

Statistical analysis was performed using the R statistical software (http://www.r-project.org) and Analyze-it for Excel 2010 (Analyze-it Software, Ltd). Receiver operating characteristic (ROC) analysis was performed for each biomarker individually using a nonparametric method.25 Further details are given in the eMethods in the Supplement.

**Results**

**Patient Characteristics**

The 4 groups (healthy controls, a priori patients with RCC, and patients who underwent CT screening with or without a history of cancer) were statistically homogeneous based on age (P = .20, analysis of variance [ANOVA] with post hoc Tukey test [eTable 1 in the Supplement]). The sex of the healthy controls and CT screening patients with or without cancer history was statistically indistinguishable (P = .40, \( \chi^2 \) test for proportions). The sex distribution of all 4 groups was significantly different (P = .006) owing to more men among the patients with RCC. These patients were slightly older than the healthy controls (P = .02) and those CT screening patients without (P = .008) or with (P = .009) a history of cancer (all by post hoc Tukey HSD [honest significant difference] test following ANOVA), eTable 2 in the Supplement summarizes the tumor stage, grade, node involvement, and incidence of distant metastases of the 19 a priori patients following pathologically based confirmation of RCC.

**Urine Biomarker Concentrations**

Urine AQP1 concentrations (median [95% CI]) in the 19 a priori patients with RCC (225.0 [121.0–450.0] ng/mg urine creatinine) were significantly higher than in the healthy controls (1.1 [0.9–1.3] ng/mg urine creatinine), the CT screening patients without history of cancer (0 [0–1.0] ng/mg urine creatinine), and the CT screening patients with a history of cancer (1.0 [0.1–1.0] ng/mg urine creatinine) (all \( P < .001 \)) (Figure 1A). Urine AQP1 concentrations in CT screening patients with a history of cancer was not different from the healthy controls (1.0 [0.1–1.0] vs 1.1 [0.9–1.3] ng/mg urine creatinine, respectively; \( P = .08 \)).
AQP1 concentrations in the CT screening patients with or without cancer history were not significantly different (1.0 [0-1.0] vs 0 [0-1.0] ng/mg urine creatinine, respectively; \( P = .43 \)). Urine AQP1 concentrations in patients with confirmed RCC and controls did not overlap. However, AQP1 concentrations in 35 CT screening patients without cancer history and 56 CT screening patients with a cancer history did overlap with that of patients with RCC (Figure 1A).

The median (95% CI) urine PLIN2 concentration in patients with documented RCC cancer was 37.8 (22.8-83.7) absorbance units/mg creatinine, which was significantly higher than the healthy controls (3.1 [2.4-3.7] absorbance units/mg creatinine), CT screening patients without cancer history (0 [0-0] absorbance units/mg creatinine), and CT screening patients with a cancer history (0 [0-0] absorbance units/mg creatinine) (all \( P < .001 \)) (Figure 1B). The median (95% CI) urine PLIN2 concentration in the CT screening patients with or without a cancer history was not significantly different (1.0 [0-1.0] vs 0 [0-1.0] absorbance units/mg creatinine; \( P = .43 \)).

Urine biomarker concentrations in the 19 patients enrolled a priori with proven RCC were proportional to tumor size (Spearman correlation coefficients for AQP1 and PLIN2, 0.78 and 0.72, respectively; both \( P < .001 \)) (Figure 2). If biomarker concentrations were simply expressed per milliliter of urine rather than normalized to urine creatinine concentration, the correlation coefficients decreased to 0.67 for AQP1 and 0.63 for PLIN2 (data not shown).

A comparison was also made between the 19 a priori patients with RCC and those with various nonkidney cancer subgroups (Figure 3). Significant differences, or lack thereof, in biomarker concentrations between subgroups are summarized in eTable 4 in the Supplement. AQP1 and PLIN2 concentrations in patients with RCC were significantly higher than every other cancer subgroup (lung, prostate, colorectal, gastrointestinal, uterine, ovarian, pancreatic, lymphoma, and breast). A few patients with active lymphoma, lung, ovarian, breast, and other cancers had urine AQP1 or PLIN2 levels that overlapped with that of patients with RCC (Figure 3).

The area under the receiver operating characteristic (AUROC) sensitivity and specificity of AQP1 and PLIN2 (normalized to urine creatinine) at the optimal cutoff to identify patients with RCC from others was determined using receiver operating characteristic (ROC) analysis (Figure 4). Compared with healthy controls, urine AQP1 concentration had 100% sensitivity, 100% specificity, and an AUROC of 0.991 (95% CI, 0.985-0.997) with a cutoff of 96 ng/mg creatinine, maximizing the Youden Index \(^2\) (Figure 4C). Similarly, urine PLIN2 concentration had 100% sensitivity, 98% specificity, and an AUROC of 0.996 (95% CI, 0.992-1.000), with a cutoff of 9.8 absorbance units/mg creatinine (Figure 4D). If the ROC analysis was confined to the 13 a priori patients with T1a tumors, the ROC values were not significantly different (data not shown).

An internal validation of the biomarkers by means of resampling was also conducted because the number of patients with kidney cancer was much smaller than the screening population. Data for all 720 screened patients were randomly sampled in cohorts of 19 patients and compared with that of the 19 a priori patients with confirmed RCC. This was repetitively resampled 200 times. The resulting AUROC was 0.990 (95% CI, 0.952-1.000) for AQP1 and 0.997 (95% CI, 0.972-1.000) for PLIN2 (data not shown).

Additional statistical analyses were performed to assess whether both biomarkers together provided improved sensitivity and/or specificity. A logistic regression model using the averaged numerical values of each patient’s AQP1 and PLIN2 concentrations (after median centering) resulted in 95% sensitivity, 98% specificity, and an AUROC of 0.990 (95% CI, 0.990-1.000) (Figure 4E) at the cutoff maximizing...
the Youden index. The sensitivities and specificities of the combination are not significantly different from those of the individual markers.

Individual biomarker concentrations of the CT screening patients and the 19 a priori patients with RCC were evaluated next (Figure 5). Most CT screening patients had low AQPI and PLIN2 concentrations that were below the cutoff values (derived from the ROC analysis in Figure 4C and D), while those with a known renal mass presenting for nephrectomy and found to have RCC had AQPI and PLIN2 concentrations above the cutoff values. When the same biomarker data were expressed per milliliter of urine (without normalization to urine creatinine, eFigure 1A and B in the Supplement), the same relative relationships seen in Figure 5 remained.

Of notable interest were the 3 patients among the 720 screened patients, not known a priori to have a renal mass or RCC, who had urine AQPI and PLIN2 concentrations that clustered with those of the 19 patients with documented RCC.

Figure 3. Dot and Box Representation of Urine Aquaporin-1 (AQPI) and Perilipin-2 (PLIN2) Concentrations

A) Urine AQPI concentrations

B) Urine PLIN2 concentrations

Depicted are the median (bars), first, second, third, and fourth quartiles. The boxes and vertical lines represent the 95% CI and range, respectively. Values of individuals within the first through fourth quartile are represented by "O"; values of individuals over 1.5 times but under 3 times the interquartile range are represented by "+"; and individuals exceeding 3 times the interquartile range are represented by "X." The median urine AQPI concentration for 19 patients enrolled a priori with confirmed kidney cancer (clear cell or papillary subtypes) was significantly different from that of 386 patients with a history of cancer in various nonkidney tissue and organs, including lung (n = 89), prostate (n = 12), colorectal (n = 25), gastrointestinal (GI) (n = 11), uterine (n = 16), ovarian (n = 26), pancreatic (n = 13), lymphoma (n = 38), breast (n = 44), and various other organs or tissues (n = 95) (P < .001). Significant differences, or lack thereof, in urine AQPI and PLIN2 concentrations between all these different tissues and organs are summarized in eTable 4 in the Supplement.
Biomarkers to Screen for Renal Cell Carcinoma

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Figure 4. Receiver Operating Characteristic Analysis of Urine Aquaporin-1 (AQP1) and Perilipin-2 (PLIN2) Concentrations

A and B, AQP1 and PLIN2 concentrations, respectively, in 19 enrolled a priori patients with renal cell carcinoma (RCC) compared with 80 healthy controls. C and D, AQP1 and PLIN2 concentrations, respectively, in 19 a priori patients with RCC compared with 720 screened patients. E, Average AQP1 and PLIN2 concentrations in 19 a priori patients with RCC were compared with the average in the 720 screened patients.

(Figure 5). In a screening paradigm, based on the cutoff values, these elevations would have been flagged as suggestive of RCC and patients would be recommended for further evaluation. Indeed, when their CT scans were evaluated, all were found to have an imaged renal mass. Two patients subsequently underwent partial nephrectomy and were postsurgically diagnosed as having grade 2, stage T1a clear cell carcinomas. The third patient died before any further evaluation and diagnosis were available. Median (interquartile range [IQR]) urine biomarker concentrations for these 3 incidentally discovered patients were 145 (135-253) ng/mg urine creatinine for AQP1 and 29 (27-56) absorbance units/mg creatinine for PLIN2. Adding the urine biomarker concentrations of these 3 patients to the regression analysis (open circles, eFigure 2A and B in the Supplement) did not change the correlations.

Also noteworthy were biomarker concentrations of 16 patients initially identified with indeterminate renal lesions on CT (neither clearly normal nor clearly suggestive of RCC) based on single-phase screening CT. Their median (IQR) AQP1 and PLIN2 concentrations were 1 (0-48) ng/mg urine creatinine and 0 (0.0-0.3) absorbance units/mg creatinine, respectively. These were significantly less than the median concentration of the 3 patients with incidentally discovered RCC (145 [135-253] ng/mg urine creatinine [P = .006] for AQP1 and 29 [27-56] absorbance units/mg creatinine [P < .001] for PLIN2) and statistically indistinguishable from the screening patients without cancer history (0 [0-0] ng/mg urine creatinine [P = .37] for AQP1 and 0 [0-0] absorbance units/mg creatinine [P = .31] for PLIN2) or with a cancer history (0 [0-0] ng/mg urine creatinine [P = .54] for AQP1 and 0 [0-0] absorbance units/mg creatinine [P = .22] for PLIN2). In a differential diagnosis paradigm, based on AQP1 and PLIN2, the 16 patients with indeterminate renal lesions would have been considered not to have RCC because both biomarkers were not elevated. They were subsequently diagnosed as having noncancerous hemorrhagic cysts on further analysis.

Discussion

This investigation evaluated the potential clinical utility of urine AQP1 and PLIN2 concentration to diagnose clear cell and papillary RCC in patients, thus satisfying phase 3 of cancer biomarker development.24 The major findings indicate that both biomarkers had favorable sensitivity and specificity. In patients with RCC compared with normal controls, sensitivity was 100% and 100%, specificity was 100% and 91%, and AUROC was 1.000 and 0.990 for AQP1 and PLIN2, respectively, at optimum cutoffs. In patients with RCC compared with a heterogeneous population of patients, both with and without a cancer history, sensitivity was 100% and 100% for AQP1 and PLIN2, respectively; specificity was 96% and 98%, respectively; and AUROC was 0.991 and 0.996, respectively. While not an independent validation cohort, internal validation by Monte Carlo resampling found AUROCs of 0.990 and 0.997 for AQP1 and PLIN2, respectively. Therefore the overall high degree of sensitivity and specificity establishes the clinical validity of these biomarkers.
AQP1 and PLIN2 were normalized to creatinine excretion, a common approach to standardize urine analyte reporting. No significant differences in results or conclusions were observed between normalized and nonnormalized results, although normalization to creatinine concentration is the standard means of expressing excretion of analytes in urine to minimize the impact of hydration status from patient to patient or in the same patient over time.

A major difference between this and our previous investigations was the prospective nature of the present design. In previous studies (encompassing phases 1 and 2 of cancer biomarker development) that retrospectively studied populations known to have specific diseases (although all samples were analyzed in a blinded and coded fashion), the sensitivity of urine AQP1 concentration to differentiate RCC from various common renal diseases, nonrenal urologic cancers, and non-RCC renal masses was 93% to 100%, and specificity was 94% to 100%, with AUROCs of 0.960 to 1.000. The sensitivity of urine PLIN2 concentration to differentiate RCC from various common renal diseases, nonrenal urologic cancers, and non-RCC renal masses was 91% to 100%, specificity was 88% to 100%, and AUROCs were 0.910 to 1.000. Another major difference between investigations was the smaller comparator cohorts (18-47 patients) in the previous studies. This present prospective, larger-scale investigation nevertheless replicated the sensitivities and specificities observed previously in retrospective, smaller cohort studies.

The specificity of urine AQP1 and PLIN2 concentration for RCC, seen previously, is further expanded herein. This investigation showed that urine AQP1 and PLIN2 concentrations in patients with RCC were together significantly higher than that in patients with lung, prostate, colorectal, gastrointestinal, uterine, ovarian, pancreatic, lymphoma, breast, or a variety of other cancers.

One major implication of this investigation, owing to its prospective nature, is the predictive value of AQP1 and PLIN2. On the basis of a historical institutional incidental discovery rate of 2 per 1000 patients with an imaged renal mass, 1 to 2 patients with an incidental renal mass subsequently diagnosed as having RCC were anticipated in the screening protocol. The investigation did identify 3 of 720 patients who had abnormal renal cancer biomarkers. These 3 patients were subsequently found to have an imaged renal mass suggestive of RCC on CT. Two of these patients were found to have pathologically confirmed clear cell carcinoma after undergoing nephrectomy (the third died before further evaluation). This prospective use of AQP1 and PLIN2 to screen populations for and identify RCC presages a potential clinical use of these biomarkers. Both comparator populations of healthy volunteers and patients were representative of populations that might be screened for RCC using these biomarkers. Thus a major implication is that AQP1 and PLIN2 might be suitable for population screening for RCC.

Tempering this consideration is that while earlier incidental detection of RCC portends better patient outcomes, the value of population screening to achieve greater detection and theoretically better outcomes remains unproven. Renal cell carcinoma has a low prevalence. Individually, AQP1 had a positive predictive value of approximately 3% (3 of 91 patients with elevated AQP1 concentrations had an incidentally discovered renal mass) and that of PLIN2 was approximately 19% (3 of 16 patients) (eTable 3 in the Supplement). However, both markers together had a positive predictive value of 100% because only the same 3 patients had a significant elevation of both biomarkers and had a renal mass. Thus, although ROC analysis did not suggest improved detection using both biomarkers, results from the 3 CT screening patients incidentally discovered to have a renal mass suggest value in measuring both biomarkers for accurate RCC diagnosis. In addition, the paradigm of early detection leading to a mortality benefit has not been realized for all cancers. For example, the prostate, lung, colorectal, and ovarian randomized screening trial comparing annual to opportunistic screening for these cancers over a 13-year period encompassing over 148 000 patients showed little to no cause-specific mortality benefit.
A second major implication of this investigation, supported by the clinical validation of AQP1 and PLIN2, is their potential use in differential diagnosis of imaged renal masses, specifically to differentiate clear cell or papillary RCC from other imaged renal masses such as nonmalignant oncocytomas, angiomylipomas, or radiologically indeterminate lesions. 21 Although approximately two-thirds of imaged renal masses are clear cell RCC, approximately 15% to 20% are benign. 30-32 Imaging alone may struggle to distinguish benign lesions such as oncocytomas and relatively lipid-poor angiomylipomas from RCC. 14-19,30-32 Because the common clinical approach to an imaged renal mass is partial or total nephrectomy, 8,12,14,15,21,23 this may result in the unfortunate removal of a normal kidney. 5,15-17,22 Indeed, in a study, approximately one-third of patients with a benign tumor underwent a radical nephrectomy. 21 One approach to differential diagnosis of imaged renal masses is biopsy. 16,19,30,32 However, only approximately 80% are of diagnostic value, while the remaining 20% are nondiagnostic. 16,31 Moreover, renal biopsy is invasive with potential for complications. In contrast, the sensitivity and specificity of measuring AQP1 and PLIN2 for RCC vs other renal masses demonstrates their analytical and clinical validity, potential superiority compared with renal biopsy, and potential application in the differential diagnosis and further evaluation of inadvertently identified imaged renal masses. As exemplified in this investigation, AQP1 and PLIN2 measurement might obviate the need for follow-up multiphase CT, MRI, or ultrasonography, or even surgical removal, of indeterminate renal CT lesions. In addition, AQP1 and PLIN2 measurement may be useful in differentiating RCC from other diseases causing hematuria. Thus, AQP1 and PLIN2 have potential application in differential diagnosis of imaged renal masses, further differentiation of radiologically indeterminate lesions, and the potential to obviate removal of a noncancerous kidney.

There are some caveats to this investigation. While AQP1 measurement is by ELISA and presently applicable for screening and diagnosis, the cumbersome nature of Western blotting precludes application of PLIN2 measurement to large-scale investigations or RCC screening. Development of a sensitive and specific ELISA for PLIN2 concentration in urine will increase assay efficiency and enable widespread implementation. Second, urine AQP1 and PLIN2 concentrations detect clear cell and papillary RCC (90% for RCC) but not the chromophobe subtype of RCC. 20-24,21,23 However, this subtype accounts for only approximately 5% of RCC cases. Third, the CT screening population was heavily skewed (386 of 720 patients) toward those with various (nonrenal) cancers. This may account for the higher background levels of urine AQP1 (Figure 3A, Figure 4A, and eFigure 1A in the Supplement) compared with smaller previous comparator groups (common noncancerous kidney diseases, bladder and prostate cancer, and noncancerous imaged renal masses [18-44 patients]). 20,21,23 Further AQP1 ELISA improvements may potentially reduce this background.

Conclusions

This investigation supports the ability of urine AQP1 and PLIN2 concentrations to diagnose patients as having occult RCC in a screening protocol. At present, it appears optimal to measure both AQP1 and PLIN2 for identifying patients with RCC. Overall, it validates the clinical utility of urine AQP1 and PLIN2 as biomarkers with applicability for early and noninvasive detection and screening for RCC, satisfying phase 3 of cancer biomarker discovery. In addition, urine AQP1 and PLIN2 have potential application for differential diagnosis of imaged renal masses.

References

Urinary Biomarkers for the Detection and Management of Localized Renal Cell Carcinoma

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The incidence of renal masses is increasing because of the widespread use of abdominal imaging. As a result, urologists are often faced with a dilemma in the diagnostic and therapeutic approach to these masses, which are typically small (diameter <4 cm), asymptomatic, and often seen in patients with advanced age, comorbidities, or compromised renal function. Radiologic features can sometimes, but not always, differentiate benign from malignant tumors. Renal mass biopsy has recently been pursued more frequently with the hope of differentiating a benign or less aggressive mass, such as a chromophobe tumor or a low-grade clear cell tumor, from a malignant tumor. Indeed, there is a growing body of evidence supporting acceptable patient outcomes with active surveillance of such small renal masses (SRMs).

In this context, Morrissey et al present data building on their prior publications regarding the potential clinical utility of urine markers, aquaporin 1 (AQP1) and perilipin 2 (PLIN2), as biomarkers to screen for renal cell carcinoma (RCC). AQP1 is a water-transport protein found in the glomerular capillary endothelium and apical membrane of the proximal tubule in normal kidneys. PLIN2 is an adipocyte differentiation-related protein whose transcriptional activation is mediated by hypoxia-inducible factor and is up-regulated in clear cell RCC. The authors measured AQP1 and PLIN2 levels in 3 distinct populations: 720 patients undergoing routine abdominal computed tomography (the screening population), 80

Related article page 204

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