In this issue of Mayo Clinic Proceedings, Morrissey et al\(^1\) report the most promising proof of concept yet for the use of urine-based markers in the diagnosis of renal cell carcinoma. They show that 2 proteins that are highly expressed by renal cell carcinoma, the water-channel aquaporin-1 (AQP1) and the lipid droplet–associated protein adipophilin (ADFP), are significantly more abundant in urine samples from patients with renal cell carcinomas than in control participants.

Very few tumor markers are both organ and malignancy specific. For example, the carcinoembryonic antigen level can be elevated in patients with carcinomas from a variety of organs, including colon, pancreas, stomach, lung, and breast, in addition to inflammatory conditions that affect those organs, and may be increased in some healthy individuals. Markers that appear to be organ specific, such as prostate-specific antigen and thyroglobulin, can be elevated in both benign and malignant conditions. Thus, tumor markers have not gained widespread acceptance in screening. They appear to have the most utility as diagnostic adjuncts, as prognostic indicators, and in following up patients.

By these accounts, AQP1 and ADFP seem unlikely tumor marker candidates. Although both proteins are expressed at relatively high levels in the kidney, they are also expressed almost ubiquitously throughout the rest of the human body, albeit at lower levels, a finding that does not bode well for a high degree of organ specificity. Moreover, by the nature of their relatively high expression in proximal renal tubular epithelium, they seem ill-suited to distinguish normal tubular epithelium from its transformed cancerous counterpart.

However, the nature of the analyte, urine, and the biology of renal epithelium tilt the balance in favor of these markers. Urine contains very low concentrations of circulating proteins in the absence of renal disease. In addition, neither AQP1 nor ADFP is a secreted protein. Aquaporin-1 is a membrane protein, and ADFP is an intracellular protein associated with lipid droplets. Therefore, AQP1 or ADFP found in urine can be expected to originate from epithelium facing the filtrate side, rather than the blood side, of the nephron. Within the nephron, concentrations are highest in the proximal tubules. Thus, with urine testing, AQP1 and ADFP not only are organ specific for the kidney but also are localized to the structures that give rise to most renal cell carcinomas.\(^2-4\)

In addition, AQP1 and ADFP are greatly overexpressed in renal cell carcinomas compared with normal tubules, albeit more so in clear cell carcinomas than in the papillary morphotype.\(^5-7\) The diagnostic feature that has given rise to the term clear cell carcinoma is in fact caused by lipid droplet accumulation within the tumor cells, all of which contain copious amounts of ADFP. Cancer cell shedding alone might consequently be expected to elevate urinary AQP1 and ADFP concentrations, but many cells cast off significant numbers of exosomes during their life cycle. These 50- to 90-nm diameter vesicles allow cells to selectively shed certain membrane portions, which can serve a role in cell-to-cell nutrient transport, signaling, and even exchange of nucleic acids and pathogens, as well as allowing selective export of lipids, proteins, peptides, cytokines, and various cellular structures and components.\(^8\) Renal cell carcinomas have been found to shed particularly high numbers of exosomes.\(^9\) It seems plausible—and proven in the case of AQP1—that these exosomes contain AQP1 and ADFP.

Thus, AQP1 and ADFP in urine might make surprisingly good candidate tumor markers for the main renal
cell carcinoma subtypes, and the study by Morrissey et al seems to support this notion. Their article is, of course, inconclusive. Like most proof-of-principle studies, there are omissions, primarily inadequate control groups and lack of independent verification of the findings in a second patient cohort. The comparison groups consisted of healthy volunteers and patients undergoing surgery for a variety of reasons unrelated to any renal pathology, whereas the patient group was highly preselected and thereby enriched for patients with a high likelihood of having a renal cell carcinoma. Indeed, 33 of the 42 patients had a renal cell carcinoma. Such extreme contrasts between patients and controls often favor overoptimistic estimates of the diagnostic utility of a test. It is easy to imagine that a variety of nonmalignant tubular disorders might lead to excessive shedding of normal tubular epithelium, which could elevate urinary AQP1 and ADFP levels. Similarly, renal expression levels of these proteins might differ from their average healthy state in a variety of different physiologic and pathophysiologic states. For example, tubular expression and levels of urinary excretion of AQP2 vary 5- to 6-fold depending on the hydration state of a person.10

Grant, unlike AQP1, AQP2 is regulated by antidiuretic hormone, but this still illustrates the point that a good deal more work needs to be done, both in healthy volunteers under different conditions and in patients with a variety of illnesses of the kidneys, heart, and liver.

In contrast, it seems irrefutable that the patients with tumors had much higher urine concentrations of AQP1 and ADFP, and that these were most likely caused by the tumors, as levels declined profoundly after treatment. Thus, the proof of principle is there, and performing further studies seems worthwhile, and, ultimately, if such studies are promising, a prospective, blinded validation study should be performed. However, with that strategy, converting the assay to a different format will be necessary. Western blot analysis is too slow, costly, and technically demanding for large-scale studies or routine clinical diagnosis. Moreover, at best it is a semiquantitative method and cannot easily be standardized across different laboratories. Therefore, converting the assay to a solution-based, quantitative immunoassay seems prudent. Unfortunately, this task could be more difficult than one might imagine. Membrane proteins and lipid-associated proteins are often difficult to solubilize, which could impede antibody development, creation of control and calibration materials, and antigen retrieval from urine.

Morrissey et al raise the possibility of population screening for renal cell carcinoma—a bit of a stretch with a pilot study but an interesting idea. Would the potential reward, a test for screening and early detection, diagnosis, and follow-up of renal cell carcinoma, be worth the efforts and costs? Surgical intervention appears to be still needed because these markers, even in this small number of cases evaluated, identified only 2 types of renal cell carcinoma. Additionally, some benign renal tumors may need surgical intervention for reasons unrelated to malignancy. In contrast, if the test were to be applied as a tool for population screening, similar to prostate-specific antigen screening or fecal occult blood testing, there is a high likelihood this would result in earlier diagnosis of renal cell carcinoma. Incidence rates of renal cell carcinoma have risen during the past 40 years, paralleling the use of imaging modalities and incidental discoveries of kidney cancers. The size of tumors has decreased, and mortality from renal cell carcinoma increases at only one-third the rate that the number of new cases is growing.11 It remains unclear whether the increasing discrepancy between incidence rates and mortality rates in screened populations reflects an actual survival benefit for patients or an increasing proportion of biologically indolent cases that might never have caused clinical disease. Precedent for this has been suggested in thyroid cancer, prostate cancer, melanoma, and, to a lesser degree, breast cancer, all of which have seen increasing case numbers with no equal increase in relative mortality during the past few decades, as screening has been applied to their detection.12,13 The evidence in these tumors indicates that some of the tumors found by screening may be indolent, and some patients might not have benefited from early detection. The other major questions would be at which age screening should start and how frequently screening should be done to identify renal cell carcinomas at an early enough stage. What would the negative and positive predictive values of this test be when applied to population screening, given the low incidence rates of renal cell carcinoma, and how much would it cost? This type of testing may prove too expensive for population-based testing, but it may have utility in screening high-risk populations, such as those with familial kidney cancer predisposition, or as an adjunct in evaluation of an incidentally discovered renal mass.

Therefore, we hope that Morrissey et al or other investigators will extend their studies in the manner suggested in this editorial, to define whether this test can fulfill the early promise that it shows.

Stefan K. Grebe, MD
Lori A. Erickson, MD
Department of Laboratory Medicine and Pathology
Mayo Clinic,
Rochester, MN