

Applications of Urine Protein Measurement

SCREENING FOR KIDNEY DISEASE

Although urine protein measurement can be used to assist in the diagnosis of kidney disease and to assess disease progression and response to therapy (discussed later), it is most commonly used as a screening test. Because screening tests are generally applied to relatively large numbers of patients, convenience and cost are major considerations. To make screening more convenient, a number of methods have been developed to measure urine protein in a single-voided, or "spot," urine sample, so that timed urine collections can be avoided.

In 1982, Viberti and co-workers^[431] reported that clinical (Albustix-positive) proteinuria subsequently developed in patients with insulin-dependent diabetes in whom albumin excretion rates were 30 to 140 $\mu\text{g}/\text{min}$ as measured by radioimmunoassay in timed overnight urine collections. In contrast, patients with excretion rates less than 30 $\mu\text{g}/\text{min}$ did not demonstrate overt proteinuria.^[431] These investigators coined the term "microalbuminuria" to indicate increased urine albumin excretion rates in patients with normal urine total protein.^[431] A follow-up of the original cohort confirmed that the patients with microalbuminuria had not only a higher risk of development of overt proteinuria but also a greater risk of dying from cardiovascular disease.^[432] Similar findings have been reported by others in patients with insulin-dependent and non-insulin-dependent diabetes.^{[433] [434] [435] [436] [437]} Some investigators have used levels of 15 to 150 $\mu\text{g}/\text{min}$ to define microalbuminuria,^[437] and others have used 20 to 200 $\mu\text{g}/\text{min}$.^{[433] [438]} Microalbuminuria has also been defined as urine albumin excretion of 30 to 300 mg/day.^[359] Whatever definition is used, microalbuminuria appears to be an important risk factor for end-organ damage in patients with diabetes. Similarly, in patients with essential hypertension, increased urine albumin excretion ratio (>30 mg/day) is associated with higher cardiovascular mortality.

Most studies showing a relationship between microalbuminuria and end-organ damage have used quantitative techniques to measure urine albumin excretion. Although few studies have examined whether other screening techniques predict outcome, there is no reason to believe that the results cannot be extrapolated to other screening tests, with differences in sensitivity and specificity taken into account. Indeed, albumin-to-creatinine ratios have been shown to predict the subsequent development of overt kidney disease. In a population of diabetic southwestern Native Americans, albumin-to-creatinine ratios of 0.03:0.30 mg/mg (micro-albuminuria range) were a strong predictor of diabetic nephropathy.^[406]

The recognition that microalbuminuria identifies diabetic patients at risk for subsequent kidney and cardiovascular disease complications has given great impetus to the development of effective screening tools. Borch-Johnsen and associates,^[438] using

published data, carried out a critical appraisal of screening for microalbuminuria in patients with diabetes. Making a number of assumptions, they performed a cost-benefit analysis of the impact of screening and antihypertensive treatment and concluded that screening and intervention programs are likely to lead to considerable reductions in cost and mortality.^[438] Ultimately, the effects of such screening and intervention programs must be evaluated in actual clinical trials.

The use of dipstick tests for total protein excretion and microalbuminuria to screen for kidney disease has not been rigorously examined in nondiabetic patient populations. Epidemiologic data suggest that even in nondiabetics, proteinuria is a risk factor for cardiovascular disease,^{[439] [440] [441] [442] [443]} perhaps because proteinuria is a sensitive indicator of kidney damage. However strong these correlations are statistically (low P value), the amount of unexplained variability (low r value) is great, suggesting that the sensitivity and specificity for proteinuria detection of kidney injury in the general population could be too low to make this a useful screening tool in individual patients. Nevertheless, data to assess this issue are generally not available for individuals who are not diabetic. Regardless of whether measuring urine protein excretion in the general population is a cost-effective approach to the early detection of kidney disease, such screening may be useful when combined with other clinical parameters in estimating vascular disease risk. However, the prospective data needed to assess the utility of this application of urine protein excretion are also incomplete.

The appropriate manner in which to use various tests to screen for kidney disease has not been extensively investigated. Because the number of false-positive results of dipstick tests for protein excretion is high, a positive result should probably be followed by tests designed to more accurately quantitate urine protein excretion. However, in some clinical circumstances, the likelihood that a positive dipstick test result for urine protein excretion indicates CKD is so low that the screening test should be repeated at a later date before more costly quantitation procedures are undertaken. A positive dipstick test result for protein in a patient with a urinary tract infection, for example, could be dismissed if results of subsequent post-treatment tests are negative. Fever can cause tubular and glomerular proteinuria that most often disappears when the fever resolves.^{[444] [445] [446]} Congestive heart failure and seizures can also cause transient proteinuria.^[447] Light or strenuous exercise is often associated with urine protein excretion that resolves spontaneously.^{[448] [449]}

It seems clear that, even in the absence of identifiable causes of transient proteinuria, some individuals have increases in urine protein excretion that are not associated with kidney disease.^[450] This proteinuria can be classified in two categories, intermittent or persistent and postural. Several dipstick measurements of urine protein over time can be made to determine whether proteinuria follows either of these two distinct patterns. Intermittent proteinuria is less well characterized than postural proteinuria, but it appears to be relatively benign in otherwise normal individuals. It has been shown, for example, that mortality after more than 40 years of follow-up of college students with intermittently positive urine protein screen results was no different from that of normal

individuals.^[451] However, few histologic studies including sufficiently large numbers of patients have been carried out to precisely characterize intermittent proteinuria.^[452]

Posture can cause a rise in urine protein excretion in otherwise normal individuals.^{[450] [453]} This postural proteinuria should be distinguished from the increase in proteinuria seen in patients with kidney disease who assume an upright posture. Postural proteinuria usually does not exceed 1 g/24 hours. It is usually diagnosed through detection of protein excretion during the day that is absent at night while the patient is recumbent. Kidney histology in patients with postural proteinuria is generally normal or nonspecific.^{[454] [455] [456]} Patients with postural proteinuria have been shown to have an excellent long-term prognosis.^[457] Indeed, six patients diagnosed by Thomas Addis had no evidence of kidney disease after 42 to 50 years of follow-up.^[458] Even in individuals without postural proteinuria or kidney disease, levels of urine protein excretion are lower at night than during the day.^[459] Thus, the timing of urine collection is likely to influence the sensitivity and specificity of screening tests for urine protein excretion.

DIAGNOSIS AND PROGNOSIS

Once proteinuria has been detected by screening, the clinician must not only confirm the results of screening but also precisely quantitate the amount of protein excretion in a timed urine collection. Quantifying urine protein excretion can help to distinguish glomerular from tubular proteinuria. If, for example, a patient's protein excretion is in the nephrotic range (e.g., >3 g/24 hours), a glomerular source is almost certain. Quantitation of urine protein excretion can also provide useful prognostic information and assist in monitoring the response to therapy.

After detection and quantification, determination of the composition of urine protein may provide diagnostic information. Higher amounts of albumin and HMW proteins suggest glomerular proteinuria, whereas isolated increases in LMW protein fractions are more suggestive of tubular proteinuria. It is unusual for tubular proteinuria to exceed 1 to 2 g/day, and only a small fraction of protein excretion due to tubular damage should be albumin. Tubular proteins are heterogeneous, although α_2 -microglobulin is often a major constituent.

β_2 -Microglobulin is an LMW (11.8 kD) protein that has been identified as the light chain of class I major histocompatibility antigens (e.g., human leukocyte antigens [HLAs] A, B, and C).^[460] β_2 -Microglobulin is most commonly measured in urine with radioimmunoassay or ELISA. It is freely filtered at the glomerulus and is avidly taken up and catabolized by the proximal tubule. Not surprisingly, therefore, detectable urinary levels of β_2 -microglobulin have been associated with many pathologic conditions involving the proximal tubule, including aminoglycoside toxicity, Balkan endemic nephropathy, heavy metal nephropathies, radiocontrast nephropathy, and kidney transplant rejection.^{[461] [462] [463] [464] [465] [466]} β_2 -Microglobulin has been found to be useful in distinguishing upper from lower urinary tract infection.^[467] Because urine β_2 -microglobulin is a nonspecific marker of kidney tubular injury, it is not useful in differentiating

causes of kidney disease. However, when the likely cause is already known, measurement of β_2 -microglobulin may be useful in detecting and monitoring injury. Nevertheless, the sensitivity and specificity of this test of tubular injury have generally not been established in different clinical situations in which prior probabilities of various kidney disorders may strongly influence its usefulness. Thus, the test may be useful in monitoring factory workers exposed to heavy metals in whom other causes of tubular injury could be expected to be uncommon. On the other hand, measuring β_2 -microglobulin may be of limited value in diagnosing kidney transplant rejection, because other causes of tubular injury are common in transplant recipients.

Glomerular proteinuria can be further characterized as selective or nonselective.^{[468] [469]} Patients with a clearance ratio of IgG (an HMW protein) to albumin that is less than 0.10 are said to have a selective glomerular proteinuria, whereas those with IgG-to-albumin clearance ratios of greater than 0.50 have a nonselective pattern. In general, selective proteinuria is more often seen in patients with minimal change disease and predicts a good response to treatment with corticosteroids.^{[468] [469]} The sensitivity and specificity of determining the selectivity of glomerular proteinuria have not been systematically examined in large numbers of patients with different kidney diseases. Moreover, the cost of the protein separation procedures has limited their widespread clinical use.

Plasma cell dyscrasias can produce monoclonal proteins, immunoglobulin, free light chains, and combinations of these. Light chains are filtered at the glomerulus and may appear in the urine as Bence Jones protein. The detection of urine immunoglobulin light chains can be the first clue to a number of important clinical syndromes associated with plasma cell dyscrasias that involve the kidney.^{[470] [471] [472]} Unfortunately, urine immunoglobulin light chains may not be detected by reagent strip tests for protein. However, plasma cell dyscrasias may also manifest as proteinuria or albuminuria when the glomerular deposition of light chains causes disruption of the normally impermeable capillary wall.^{[473] [474] [475] [476]} The diagnosis of a plasma cell dyscrasia can be entertained when a tall, narrow band on electrophoresis suggests the presence of a monoclonal γ -globulin or immunoglobulin light chain.^[477] However, monoclonal proteins are best detected with serum and urine immunoelectrophoresis.^[477]

Once patients have been screened and a diagnosis of kidney disease has been established, measuring the amount of urine protein can provide additional prognostic information and can be used to monitor the response to therapy. The urine protein excretion value has consistently been shown to predict subsequent disease progression in different clinical settings; for example, protein excretion correlated with progression in patients presenting with the nephrotic syndrome^[478] and in patients with mild renal insufficiency of various causes.^[479] Similar findings have been reported in patients with IgA nephropathy,^{[480] [481] [482] [483]} membranous nephropathy,^{[480] [484] [485]} and type I membranoproliferative glomerulonephritis.^[480] The clinical course and effect of immunosuppressive therapy can also be monitored with sequential quantitation of urine protein excretion.^[486]