Albuminuria vs Urinary Total Protein for Detecting Chronic Renal Disorders

Z. K. Shihabi,^1 J. C. Konen,^2 and M. L. O’Connor^1

A literature review and our own data are presented to demonstrate that urinary albumin (UA) excretion increases in many renal disorders and that it offers a far more sensitive indicator than the commonly used urinary total protein (UTP) for the early detection of renal involvement in many chronic diseases such as diabetes mellitus, hypertension, and systemic lupus erythematosus. In many individuals with these disorders, UA increases severalfold, even while UTP remains within the reference interval. UA is also more suited than UTP for following therapeutic responses in these slowly progressive renal disorders. Increases in UA are associated with increased mortality. UTP measurements are plagued with many analytical problems, whereas UA is much easier to standardize. We recommend that both UA and UTP be measured when quantitative urine protein assays are ordered, especially when the UTP is <300 mg/g of creatinine.

Additional Keyphrases: diabetes mellitus · hypertension · systemic lupus erythematosus · pre-eclampsia

Urinary total proteins (UTP) are commonly assayed for diagnosing and monitoring various primary and secondary nephropathies. In many systemic and metabolic disorders, e.g., hypertension, diabetes mellitus, and systemic lupus erythematosus, the kidney may undergo slow but progressive deterioration, leading eventually to renal failure. Renal involvement in these disorders is often first manifested by a gradual increase in proteinuria, for which sensitive assays are therefore desirable. Highly sensitive assays specific for urinary albumin (UA) have also been used, but most attention has focused on the importance of UA in diabetes. We will illustrate, using published data as well as our own experience, that the UA assay is more specific and sensitive than the commonly used UTP assay for early detection and subsequent monitoring of chronic renal disorders in general. In this context, we propose the routine addition of UA assays to UTP assays even when UTP is within its reference interval.

Clinical Significance

Urinary proteins consist of a variable mixture of an ultrafiltrate of serum plus some proteins produced by the urogenital tract. The UTP reference interval in our laboratory is 0–200 mg/day, or 0–200 mg/g of creatinine. The major single protein detectable by electrophoresis of urine of normal individuals is albumin. The central 95% reference interval for UA in our laboratory is 0.4–2.8 g/mol of creatinine (5–32 mg/g of creatinine). This reference interval for UA is based on assays of UA in random (untimed) daytime urine specimens from 80 healthy adults (1) and is in close agreement with the reference interval of 0.2–2.8 g/mol of creatinine reported by Silver et al. (2). UA excretion has been also reported as mg/24 h or µg/min. Many workers use 30 mg/24 h as the upper limit of the UA reference interval (3–6), although different reference intervals or cutoff values may be set for special studies. Even with a fivefold increase in UA, the UTP values may remain <200 mg/g of creatinine (i.e., within the UTP reference interval) and be interpreted, falsely, as clinically normal. The most common proteinuria screen is the "dipstick," with a sensitivity for urinary protein (mainly albumin) of 150–300 mg/L, which is suitable only for detecting relatively gross albuminuria.

In 1982 Viberti et al. (7), using a radioimmunoassay, found that an increased rate of albumin excretion could predict the onset of nephropathy in insulin-dependent diabetics, even when the UTP test was within the reference interval. This observation has been confirmed by several other groups (4, 8–10). Viberti et al. (7) coined the term "microalbuminuria" to refer to the measurement of an increased UA with UTP within the reference interval or with a negative dipstick result. Microalbuminuria has been defined also as albumin excretion of 30–300 mg/24 h (4, 5). This term has become very common in the literature and, unfortunately, is likely to remain with us even though, in traditional protein nomenclature, the prefix "micro" is used to indicate a small-molecular-mass protein such as β2-microglobulin.

In diabetes, increased UA excretion is associated with renal glomerular hyperfiltration; it occurs early in the course of the disease (11), before renal histological or structural changes are detectable (12, 13). Among the Pima Indians, an inbred tribe with a very high prevalence of diabetes, increased UA excretion can be detected in some individuals before the onset of hyperglycemia (5). This finding is important because, in the early stages of diabetes, increased UA and renal hyperfiltration may be reversed by rigorous treatment (6, 14). Early detection and intensified treatment may thus be able to delay, if not completely prevent, the progression of proteinuria and renal failure (6).
Other studies have shown that increased UA excretion, while UTP remains within its reference interval, occurs in many other slowly progressive disorders affecting the kidney. About one-fourth of hypertensive patients have increased concentrations of UA (15, 16) (Tables I and 2) that decrease in response to medications (17). Many patients with systemic lupus erythematosus show increased albumin excretion before developing structural renal changes (18); again, the albumin excretion decreases in response to cortisol treatment (18). Increased UA excretion occurs in pre-eclampsia (19). We have found increased UA in two patients with rheumatoid arthritis.

About one-third of type II diabetics exhibit increased UA, which correlates with glyceria, blood pressure, and duration of illness (20). However, UA in type II diabetes correlates best with cardiovascular disease (21), a major cause of death in this group of patients (22). Even in nondiabetic patients, albuminuria may signify an increased risk of cardiovascular disease (23). We have been studying a group of patients older than 65 years as part of a special cardiovascular risk study; many of these individuals showed increased UA excretion (Tables 1 and 2). Although UA increases have been detected in some febrile patients with immune complexes (9, 24), the significance of UA in these disorders has not been adequately investigated.

Increased UA occurs in response to several physiological factors: catecholamines and growth hormone (11, 25), increased systemic and transglomerular pressure (25), changes in blood rheology (26), and changes in charge and pore size of glomerular membranes (11, 27). According to the Steno hypothesis (27), UA excretion in diabetes reflects widespread vascular damage, leading to increased transcapillary escape of proteins.

Although albuminuria is generally considered a glomerular proteinuria, this is not always the case. About 90% of the filtered albumin is reabsorbed by the tubular cells (28). Thus, in tubular proteinuria, a modest UTP as great as 500 mg/g 24 h can represent a 15-fold increase in UA (29). Tubular proteinuria is also often combined with glomerular proteinuria. The effect of tubular proteinuria on UA has not been studied adequately with the non new sensitive UA immunosay. We have seen a nearly eightfold increase in UA above the reference interval in six hospital patients taking gentamicin or tobramycin, drugs known to cause tubular proteinuria. Tubular proteinuria is, however, detected more specifically by increases in urine excretion of low-molecular-mass proteins and some enzymes, e.g., β2-microglobulin, retinol-binding protein, N-acetyl-β-glucoseaminidase, and lysozyme (30).

A modest increase in albumin excretion also occurs in overflow proteinuria, e.g., Bence Jones proteinuria, myoglobinuria, and hemoglobinuria (Table 2). For proper diagnosis of these disorders, specific protein assays (immunoassays and electrophoresis) are more suitable. A greater increase in UTP than in UA suggests an overflow proteinuria. Benign proteinuria (mainly albuminuria) will be detected by either UA or UTP assays. Acute renal failure is usually associated with a rapid onset of clinical symptoms and a greater increase of UTP, with albumin the major protein. In these cases, UA assays yield the same information as UTP assays.

As renal disease progresses in diabetes and hypertension, the UA/UTP fraction is quite variable but generally increases. For example, when UTP is less than 100 mg/g of creatinine, UA/UTP is about 20%. As UTP approaches 200 mg/g of creatinine, the UA/UTP increases to about 50%. With some exceptions, when the UTP exceeds 1000 mg/g of creatinine, UA/UTP approaches 80% (1). Thus, a UTP of 1000 mg/L may represent only a fivefold increase over the reference range for UTP but a 25-fold increase for UA. For this reason, UA will be increased more than UTP and also in a greater percentage of the patients, especially when UTP is only marginally increased (Table 2). The UA/UTP ratio may prove useful for evaluating renal status.

In diabetics, the filtration of some proteins such as transferrin (31) and free κ light chains (32, 33) increases more than that of albumin because of their higher isoelectric point (31). Measurement of urinary excretion of these proteins has, as yet, no demonstrated advantage over UA determination. Compared with UA, these proteins are more difficult and thus less convenient to assay. Furthermore, our preliminary data show that, whereas albumin excretion is increased in essential hypertension in humans, transferrin excretion is not.

### Table 1. Percentage of Patients* with Increased Albuminuria (>32 mg/g of Creatinine) but Normal UTP

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>% above normal</th>
<th>x-fold increase *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I diabetics</td>
<td>81</td>
<td>11.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Type II diabetics</td>
<td>271</td>
<td>19.3</td>
<td>7.2</td>
</tr>
<tr>
<td>Hypertensives</td>
<td>131</td>
<td>18.9</td>
<td>2.2</td>
</tr>
<tr>
<td>Geriatrics</td>
<td>294</td>
<td>8.6</td>
<td>4.4</td>
</tr>
</tbody>
</table>

* Signifies at our family practice clinic. * Mean of the above-normal values/upper reference range.

### Table 2. Percentage of Total Patients with Above-Normal Values* for Albumin and UTP

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Albumin/UTP</th>
<th>Dipstick</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I diabetics</td>
<td>81</td>
<td>39.5</td>
<td>34.6</td>
</tr>
<tr>
<td>Type II diabetics</td>
<td>271</td>
<td>33.2</td>
<td>22.5</td>
</tr>
<tr>
<td>Hypertensives</td>
<td>131</td>
<td>25.2</td>
<td>6.9</td>
</tr>
<tr>
<td>Geriatrics</td>
<td>294</td>
<td>13.9</td>
<td>14.6</td>
</tr>
<tr>
<td>Bence Jones</td>
<td>11</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Myoglobinuria</td>
<td>12</td>
<td>92.6</td>
<td>92.6</td>
</tr>
</tbody>
</table>

* UTP >200 mg/g of creatinine; albumin >32 mg/g of creatinine, or dipstick positive. * Mean of the above-normal values/upper reference range.

### Analytical Aspects

The assay of UTP is plagued by several major problems: many interfering substances, poor reproducibility at low ranges, and different sensitivities to different proteins by various assay techniques. Many colorimetric
methods are used to assay UTP in addition to several common semiquantitative screening methods, e.g., dipsticks, precipitation, and heat and acid tests. The lack of sensitivity of the dipsticks for UTP is evident from the data in Table 2. A recent survey of the College of American Pathologists showed a CV of 21.5% among the means of various methods used to assay a sample with an above-normal concentration of protein (mean 1434 mg/L). The CV among laboratories for one method for that sample was as high as 33.8%. Most imprecision has to do with standardization of the test. A similar survey for urine protein in the United Kingdom also showed poor performance among the participant laboratories (34). The UTP assay is considered one of the worst analyses performed by the laboratories (34).

These problems diminish the clinical significance of UTP values obtained for samples with borderline increases and have led to proposals to replace UTP by UA (34, 35).

Analytically, it is easier to measure one well-defined protein than a complex mixture of several proteins. For UA determinations, the concentration of albumin standards can be assigned by mass or by molar absorbitivity at 280 nm (5). For many years, UA has been estimated by electrophoretic analysis after concentration of the sample. However, this technique is not sensitive enough to detect the low concentrations present in normal subjects and in the early stages of diabetes. Radioimmunoassays have been used to measure low concentrations of UA (2, 8). As the importance of measuring these low concentrations has become established, simpler UA immunoassays have been introduced. Nephelometric (36), turbidimetric (1, 37, 38), and latex particle (39) immunoassays have been described for UA determination, involving the use of polyethylene and latex particles to enhance the sensitivity and the speed of the reaction. Radial immunodiffusion (1), fluoroimmunoassay (2, 40), and enzyme immunoassay (41) methods have also been described for determining UA.

Advantages and disadvantages of these various methods have been discussed (5, 42).

UA assays are adaptable to the automated instruments used in the routine laboratory with a reagent cost per test for UA similar to that for UTP. Some UA immunoassays are, however, subject to problems from the prozone effect in the presence of antigen excess. Because the UA concentration can range over several orders of magnitude, special care should be taken to select methods that can detect antigen excess. Specific colorimetric and fluorometric assays would be desirable. In the near future, automated capillary electrophoresis may prove suitable for UA measurement; it has the sensitivity to measure low values and can incorporate internal standards.

UA excretion is quite variable in the individual, with reported CVs ranging from 45% (3, 9) to 100% (5). Random (untimed) urine samples have more variability than do 24-h collections, but indexing the UA of untimed samples to creatinine excretion reduces some of the variability. Because of this increased variability, the reference interval for untimed samples is slightly wider than that based on 24-h collections (5). From a practical point of view, untimed samples are more convenient for the patient and thus are recommended by some workers (5); some workers prefer a morning sample (43). Exercise (4, 9, 31), posture (44), and diuresis (45) affect UA. The effects of diet (e.g., salt or high protein) on UA have not been studied adequately. Because UA changes over a very wide range (up to 100-fold), a slight increase in an untimed sample might not be significant. Given the variability in UA excretion, some workers use two to three samples (3, 9) to verify above-normal values. For slowly progressive disorders, the change in UA over time may be more important than an isolated single value (9).

Conclusion

UA is a more specific and sensitive indicator of a wide variety of renal disorders than is UTP. In the Framingham study (46), individuals with persistent proteinuria (mainly UA) and increased risk for cardiovascular disease had a higher mortality rate. A recent editorial in Diabetic Medicine (47) stated that measurements of UA and glycohemoglobin were the two major advances of the 1980s in care for diabetes patients. Because of the lack of studies of UA as a marker of renal disorders other than in diabetes, UA has not been widely used in general clinical medicine. Yet, in our experience, about 20% of the hospital patients’ samples requested for UTP that yield values between 100 and 300 mg/g of creatinine will have about a threefold increase above the upper limit of the reference interval for UA.

Answers to several practical and basic questions also must precede the widespread use of this test. What is the best sample to collect? How long can samples be stored? What medication and dietary and physiological factors affect the excretion of albumin? What is the basic mechanism for increased excretion in different disorders? Additionally, it is important to investigate the various renal disorders, including tubular and acute renal disorders, that might cause increases in UA and to assess the clinical value of this information for patient treatment. These questions should not be discouraging, but rather should be seen as an exciting new opportunity for the clinician, pathologist, and clinical chemist to pursue knowledge to improve patient care.

Thus, we advocate the addition of a UA assay to the measurement of UTP for patients presenting with normal UTP or a borderline increase in UTP (100–300 mg/g of creatinine) and for patients who might be at risk of developing chronic renal disease. This practice will detect these disorders with better specificity and greater sensitivity. Because UA increases in a wide variety of renal disorders, UA concentrations can be used to follow the course of disease and signal the necessity for early treatment when the disease might be reversible. Results must be interpreted in light of the patient’s history. However, UA is most useful in following the course of slowly progressive disorders. The ratio of UA/UTP adds
extra information for patient diagnosis. The addition of UA testing to samples with normal UTP values would augment the clinical significance of UTP and enhance the detection of chronic renal disorders.

We thank Dra. R. W. Prichard, S. Iskander, R. Appel, and R. Bloomfield for reviewing this manuscript before submission. This study was supported in part by a grant from the Centers for Disease Control (U32CCU-403318).

References