The detection and measurement of microalbuminuria: a challenge for clinical chemistry

By Douglas E. Busby, MD, MSc, and Robert C. Atkins, MD

Albumin can be detected in the urine in very small amounts in “normal,” healthy people. The term microalbuminuria (MA) refers to a range of urinary albumin excretion (UAE) that is above “normal” levels but below amounts referred to as macroalbuminuria or proteinuria, which indicate overt kidney damage. MA is found in 6% of the general population. Of the people with MA, 63% have hypertension, 38% have chronic kidney disease, and 26% are diabetic.1 MA is an important risk factor for kidney and for cardiovascular disease (CVD) in persons with and without diabetes. Therefore, MA should be detected and measured at high levels of sensitivity and specificity2 so that diabetic nephropathy and CVD can be treated as soon as this risk factor appears. Preventing, delaying, or reducing albuminuria is a key therapeutic goal for kidney and cardiovascular protection.

There is no consensus on why albuminuria is an independent risk marker, but there are several likely factors. One is that albumin leakage indicates a general vascular dysfunction, particularly to the blood vessel walls.2 A second is that vascular inflammation may be caused by the leakage, further damaging the blood vessel walls.3 Regardless of the mechanism, there is a growing need to educate the lab community on the demonstrated value of microalbumin detection. Only 21.5% of privately insured individuals who are at risk for kidney disease get tested.4 In managed care environments, there is only about a 50% screening-compliance rate.5

This brief paper speaks to the need for the early identification of MA as a risk factor in diabetic nephropathy and CVD, then it reviews conventional and new albumin tests.

Microalbumin as a risk factor
For diabetic nephropathy
Approximately 13 million individuals in the United States have been diagnosed with diabetes mellitus,6 and this number could increase to about 14.5 million by 2010 and to about 17.4 million by
Type 1 and type 2 diabetes are the most prevalent forms of diabetes mellitus, with type 1 affecting 5% to 10% and type 2 affecting 90% to 95% of Americans who are diagnosed with diabetes.

Nephropathy is a major complication of diabetes. The gradual and progressive kidney damage that occurs in diabetic nephropathy is reflected in an increasing UAE, which is detected initially as persistent MA and subsequently as persistent macroalbuminuria (also called proteinuria or clinical albuminuria). MA indicates that kidney damage is occurring in spite of UAE being above normal and below pathological levels of kidney functioning. Consequently, MA is said to indicate “incipient” nephropathy. On the other hand, macroalbuminuria indicates “overt” nephropathy, or kidney failure, which can eventually culminate in end-stage renal disease (ESRD).

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The overall prevalence of MA in persons with a diagnosis of diabetes is 30% to 40%, MA is uncommon in type 1 diabetes during the first five to 10 years after its diagnosis and is common in type 2 diabetes when it is diagnosed because this form of diabetes often exists undiagnosed for a number of years. Once persistent MA is detected in type 1 or type 2 diabetes, its excretion increases at 4% to 9% per year, with overt nephropathy generally occurring in six to 12 years. Overt nephropathy develops in 25% to 50% of individuals with diabetes, especially if hyperglycemia and elevated blood pressure are not controlled, and is aggravated by continued hyperglycemia and development of nephropathy-related hypertension. Not only is diabetic nephropathy the leading cause of ESRD, but ESRD has been increasing in prevalence in the past 10 years due to a rise in the incidence of type 2 diabetes.

For CVD in hypertensive patients

In people older than 45 years with stage 2 or higher hypertension, MA seems to be strongly associated with several traditional and nontraditional cardiovascular risk factors and with target-organ damage. Several studies have suggested that MA occurs in about 30% of patients with mild or moderate hypertension, ranging from 7% to 40% depending on age and ethnic group.

Several retrospective and cross-sectional studies have reported that the prevalence of CVD is significantly higher among hypertensive patients with MA than hypertensive patients without MA. In a large cross-sectional study of 11,343 nondiabetic hypertensive patients, those with MA had a significantly higher prevalence of:
- coronary artery disease (31% vs. 22%);
- left ventricular hypertrophy (24% vs. 14%);
- previous stroke (6% vs. 4%); and
- peripheral vascular disease (7% vs. 5%).

In the patients with MA and CVD, the amount of albumin in the urine was also significantly higher than in those who did not present with CVD.

For CVD in the general population

In several studies involving large populations, MA has also been established as a strong, independent risk factor for CVD both in persons who have and do not have a diagnosis of diabetes. For example:
- A study involving a Danish population that did not have a diagnosis of diabetes found that the presence of MA, defined as a urinary albumin:creatinine ratio (ACR) of ≥2 mg/mmol, was associated with a relative risk (RR) of 1.75 for cardiovascular events (myocardial infarction, stroke, cardiovascular death), 1.92 for all-cause mortality, and 2.42 for hospitalization for congestive heart failure, after adjusting for other risk factors. Moreover, RRs were similar in groups with and without diabetes. For every 0.4 mg/mmol increase in the ACR level, the adjusted hazard for a cardiovascular event increased by 5.9%. Notably, this increasing risk started at urine albumin concentrations as low as 0.5 mg/mmol, well below the currently accepted detection thresholds for a diagnosis of MA.
- A study involving a Danish population that did not have a diagnosis of diabetes found that the presence of MA, defined as an ACR in the upper 10% range, or >0.65 mg/mmol, was associated with an RR of 2.3 for ischemic heart disease, independent of other risk factors for atherosclerosis. Also, MA presence more than doubled the predictive effect of these other risk factors.
- A study of a general population in the Netherlands found that the greater the UAC (urinary albumin concentration), the greater the risk of both cardiovascular and noncardiovascular mortality after adjustment for other well-recognized risk factors. A two-fold increase in UAC (e.g., from 5 to 10 mg/L, or from 20 to 40 mg/L) was associated with an RR of 1.29 for cardiovascular mortality and an RR of 1.12 for noncardiovascular mortality. Again, the relationship between UAC and cardiovascular and noncardiovascular mortality was already apparent at UAC levels that are currently considered normal.
- A study that appeared in the American Journal of Kidney Diseases generated national estimates of the prevalence of MA in the U.S. population. The prevalence of MA (urinary ACR, 30 to 299 mg/g) was 6.1% in males and 9.7% in females. MA was common even among persons without diabetes or hypertension: 5.1% of those without diabetes, hypertension, CVD, or elevated serum creatinine levels had MA. The numbers increased starting at 40 years of age, and were greater in non-Hispanic blacks and Mexican Americans aged 40 to 79 years compared to similar-aged non-Hispanic whites. MA appears to reflect microvascular disease and macrovascular disease that not only affect the kidneys, but also the cardiovascular system. Consequently, MA is a risk factor for CVD even at levels that are below the normally accepted range for incipient nephropathy. MA has joined other major, independent risk factors for CVD, including hyperglycemia, hypertension, hypercholesterolemia, and smoking, that may or may not be linked with causal processes.
Test and treatment guidelines

The American Diabetes Association has recommended a range for MA for various modes of urine collection (see Table 1). A first void or early-morning spot collection is generally preferred because of its convenience to the patient, a diurnal variation in UAE, and a higher urine concentration that enables easier detection of MA. The ACR corrects for variations in volume of urine output, and usually predicts 24-h UAE accurately.

The American Diabetes Association also has recommendations for when screening for MA should be performed on persons with the diagnosis of type 1 or type 2 diabetes. Because MA rarely occurs with type 1 diabetes of short duration, individuals with type 1 diabetes should be tested annually, beginning five years after the time of diagnosis. Because precise dating of onset of type 2 diabetes is usually not possible, persons with type 2 diabetes should be tested annually, beginning at the time of diagnosis.

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Several studies have shown that if MA is detected in the initial stages, the onset of kidney disease and CVD can be slowed, halted, and — in some cases — reversed with common blood-pressure drug therapies. Effective treatment involves control of blood pressure as well as hyperglycemia; elevated blood pressure can be a contributor to and a consequence of diabetic nephropathy, as well as a risk factor for CVD in persons with and without diabetes. MA usually precedes an elevation of blood pressure in type 1 diabetes and is commonly associated with a moderate blood-pressure elevation even when type 2 diabetes is diagnosed.

Reviews of the numerous published studies of the effectiveness of drugs in the treatment of diabetic nephropathy have concluded that ACE (angiotensin-converting enzyme) inhibitors have a renoprotective effect in type 1 and type 2 diabetes, that ARBs (angiotensin receptor blockers) have a renoprotective effect in type 2 diabetes, that ARBs may be proven also to have a renoprotective effect in type 1 diabetes, and that the renoprotective effect of ACE inhibitors and ARBs is independent of their blood-pressure-lowering effect. A study conducted by Dr. de Zeeuw and colleagues reports that cardiovascular protection in diabetics can be explained by the therapy’s antiproteinuric effect as well, irrespective of changes in all other risk factors. Monitoring lab-test readings after the initial MA diagnosis is, thus, an effective way to evaluate drug efficacy.

The American Diabetes Association has recommended use only of ACE inhibitors or ARBs for MA or macroalbuminuria in type 1 and type 2 diabetes, while recognizing that use of ARBs in type 1 diabetes lacks investigational support. It has also recommended that if one of these classes of drugs is not tolerated, the other class should be substituted.

Tests for MA

Data on drug efficacy for MA patients has motivated the research community to develop detection methods with improved accuracy. Traditionally, a variety of semiquantitative urine dipstick tests have been performed in the doctor’s office. They consist of a special chemically treated test strip that is dipped into the urine sample. Following removal, the strip’s coloring provides an indication of albumin-concentration ranges. Dipstick tests are quick and can be completed in several minutes.

One widely used dipstick is the Clinitek Microalbumin Reagent Strip (Bayer Corp., Elkhart, IN). In this test, albumin binds to a sulfonephthalein dye, and creatinine forms a complex that catalyzes a reaction between diisopropylbenzene dihydroperoxide and 3,3',5',5'-tetramethylbenzidine. Both processes produce colors that are read in a Clinitek 50 portable urine chemistry analyzer (Bayer Corp., Tarrytown, NY) and used to determine albumin- and creatinine-concentration levels. Researchers have found that using the sulfonephthalein dye results in a 10-fold increase in the albumin-binding coefficient compared to several other widely used dyes.

When a patient tests positive for MA, sophisticated laboratory methods called immunonephelometry, immunoturbidimetry, and radioimmunoassay (RIA) have traditionally been used. Their performance characteristics are compared in Table 2. Busby and Bakris have recently described these methods in the Journal of Clinical Hypertension.

- **Immunepehometry**: Albumin in the urine sample comes into contact with antibody to human albumin to produce an antigen-antibody reaction. An increase in light scatter from this reaction is analyzed optometrically to provide MA concentration.
- **Immunoturbidimetry**: Albumin in the urine sample and human albumin bound to latex particles compete for a monoclonal antibody that aggregates the latex particles. Consequently, the amount of aggregation that results is in inverse proportion to the amount of albumin in the sample. The aggregation amount is measured optometrically and converted to provide MA concentration.
- **RIA**: Albumin in the urine sample displaces isotopically labeled human albumin that has been bound to an antibody to it. Consequently, the amount of labeled albumin

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**Table 1.** Range for MA recommended by the American Diabetes Association

<table>
<thead>
<tr>
<th>Category</th>
<th>Spot collection (µg/mg creatinine)</th>
<th>24-h collection (mg/24 h)</th>
<th>Timed collection (µg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoalbuminuria</td>
<td>&lt;30</td>
<td>&lt;30</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>30-299</td>
<td>30-299</td>
<td>20-199</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td>≥300</td>
<td>≥300</td>
<td>≥200</td>
</tr>
</tbody>
</table>

*At least two of three urine specimens collected within a period of three to six months should be abnormal before a patient is considered to have MA.*
In a study of 511 type 1 diabetic and 634 type 2 diabetic individuals with diabetes, the days of the following studies: cardiovascular disease populations, as indicated by the reference in the early detection of MA in diabetic and possibly in immuno-unreactive albumin. Moreover, dipstick-test gates, while HPLC detects immuno-reactive and min fragments >12 kilodaltons and polymer albumin aggregates, while HPLC detects immuno-reactive and immuno-unreactive albumin. Moreover, dipstick-test formulations typically include an inhibitor that prevents protein fragments from binding. For these reasons, HPLC should be of considerable benefit in the early detection of MA in diabetic and possibly in cardiovascular disease populations, as indicated by the results of the following studies:

- In a study of 511 type 1 diabetic and 634 type 2 diabetic stored urine samples, HPLC and RIA methods were both used to detect MA. For type 1 patients, the mean lead-time for the HPLC assay compared to RIA was 3.9 years with a confidence interval of 2.1 to 5.6 years; for type 2 progressors, the mean lead-time was 2.4 years with a confidence interval of 1.2 to 3.5 years. Moreover, false negative results for MA. As discussed earlier, high levels of albumin indicate cardiovascular disease risk in general and at-risk populations. Whether or not both immuno-reactive albumin and immuno-unreactive albumin are excreted in individuals at increased risk for CVD, however, the case can still be made for use of HPLC in the detection and measurement of MA as a risk factor for CVD. First, HPLC performs well, having an interassay coefficient of 2.4% at a urinary albumin level of 95.8 mg/L and an albumin detection limit of 2 mg/L. Second, HPLC measurement of microalbuminuria has been shown to be invariably higher than that measured by RIA in patients with mild diabetes and also higher when compared with immunonephelometry measurement in a community survey.

Third, the low albumin detection limit of HPLC appears crucial to detecting and measuring MA as a risk factor for cardiovascular disease below the lower end of the MA range for the diagnosis of incipient nephropathy.

Cost-Benefit Analysis

New advances such as HPLC may help physicians prescribe necessary drug therapies in early-stage renal disease before dialysis is required. Early medical intervention can...
result in substantial cost savings in addition to reduced incidence of ESRD. Consideration of the cost benefits must center on the ability to detect and measure MA at low levels, especially when it first appears as a risk factor for diabetic nephropathy.

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Notes
- The sensitivity (true-positive rate) of a test for MA is its ability to detect those who are known to have MA (expressed as true positives/true positives plus false negatives). The specificity (true-negative rate) of this test is its ability to detect those who are known not to have MA (expressed as true negatives/true negatives plus false positives). The false-negative rate is equal to one minus the sensitivity, and the false-positive rate is one minus the specificity.
- Type 1 diabetes, formerly called insulin-dependent diabetes or juvenile diabetes, is due to an absolute insulin deficiency. It most commonly occurs in childhood or adolescence. Type 2 diabetes, formerly called non-insulin-dependent diabetes or adult-onset diabetes, is due to insulin resistance and, usually, a relative insulin deficiency. It is associated with older age, obesity, inactivity, and race/ethnicity (e.g., Native American, African American, Hispanic/Latino). Because it develops gradually, it often goes undiagnosed for many years.
- By definition, ESRD patients require renal dialysis or transplant.

References