Review Paper

Comparison of Commonly Used Assays for the Detection of Microalbuminuria

Douglas E. Busby, MD, MSc;[1] George L. Bakris, MD[2]

There are a variety of methods for assessing urinary albumin excretion, extending from the very low-range microalbuminuria to higher ranges extending into macroalbuminuria or proteinuria. The recommendation for the initial screening of a new patient is to use a urine dipstick to assess for microalbuminuria. If positive, a spot urine for albumin:creatinine should be measured and reassessed annually. All patients with kidney disease, diabetes, or hypertension and metabolic syndrome should be screened for albuminuria. New methodologies using high-performance liquid chromatography are much more sensitive and specific when compared with older methods of detection and may prove very useful for earlier identification of high-risk patients. This is important since studies have shown that albuminuria levels below the microalbuminuria range, determined by conventional methodologies in uncomplicated essential hypertensive men, are associated with an adverse cardiovascular and metabolic risk profile. High performance liquid chromatography methodology, in contrast to older studies, detects all intact albumin and enables clinicians to assess disease severity and monitor therapeutic effectiveness with confidence in the accuracy of the microalbuminuria data reported to them. (J Clin Hypertens. 2004;6(11 suppl 3):8–12)

From LaPorte Regional Hospital and Health System, La Porte, IN;[1] the Department of Preventive Medicine, Rush University Medical Center, Hypertension/Clinical Research Center, Chicago, IL[2]
Address for correspondence:
George L. Bakris, MD, Rush University Hypertension Center, Rush University Medical Center, 1700 West Van Buren Street, Suite 470, Chicago, IL 60612
E-mail: george_bakris@rush.edu

www.lejacq.com ID: 4237

Microalbuminuria (MA) represents a range of urinary albumin excretion that is an important marker of cardiovascular (CV) risk in persons with and without diabetes. Moreover, progression to macroalbuminuria or proteinuria indicates an increased CV risk and the presence of kidney disease. Therefore, MA should be looked for and measured with a procedure that offers a high level of sensitivity and specificity, so that diabetic nephropathy and CV disease can be treated as soon as this risk factor appears.

To fully understand the differences between the tests used to determine MA, it is important to remind oneself of the definitions of sensitivity and specificity. The sensitivity (true positive rate) of a test is its ability to detect individuals who are known to have a disease or finding (expressed as true positives/true positives plus false negatives). The specificity (true negative rate) of a test is its ability to detect persons who are known not to have a disease or finding (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. This paper presents an overview of common tests used to screen for MA. It focuses on newer tests that have a higher sensitivity and specificity to detect lower levels of MA.

CONVENTIONAL TESTS FOR MA

Dipstick Measurement

MA is usually defined as 30–300 mg/d, whereas the albuminuria detected with the usual dipstick test represents a finding of >300 mg/d. The traditional test in the office setting to screen for MA has utilized a variety of semiquantitative dipsticks. These tests involve wetting a chemically impregnated test strip with a sample of urine. If a test is

Table. Performance Characteristics of Immunonephelometry, Immunoturbidimetry, and Radioimmunoassay Methods Used for the Detection and Measurement of Microalbuminuria in Persons With Diabetes[6]

Method	Interassay Coefficients of Variation	Detection Limit for Albumin	False Negatives vs. HPLC	False Positives vs. HPLC
HPLC	2.4% at 95.8 mg/L	2 mg/L		
Immunonephelometry (Beckman Array Analyzer [Global Medical Instrumentation, Inc., Ramsey, MN])	4.2% at 12.1 mg/L 5.3% at 45 mg/L	2 mg/L	ND	ND
Immunoturbidimetry (Dade-Behring Turbimeter [Dade Behring, Inc., Deerfield, IL])	4.1% at 10.6 mg/L 2.2% at 77.9 mg/L	6 mg/L	36%	0%
Radioimmunoassay	9.2% at 12.2 mg/dL 4.8% at 33 mg/L	16 µg/L	23%	0%

HPLC=high performance liquid chromatography; ND=not determined

positive for MA, it can be confirmed and the MA accurately quantified by various laboratory methods. These laboratory methods have also been used to evaluate the accuracy of the dipstick tests.

One example of a dipstick test for MA is the Clinitek Microalbumin Reagent Strip (Bayer Corporation, Tarrytown, NY). In this test, albumin binds to a sulfonephthalein dye, and creatinine forms a copper-creatinine complex with peroxidase-activity that catalyzes the reaction of diisopropylbenzene dihydroperoxide and 3,3′,5,5′-tetramethylbenzidine.[1,2] Both of these reactions produce colors that are read in a Clinitek 50 portable urine chemistry analyzer (Bayer Corporation, Tarrytown, NY) and reported as albumin concentrations of 10, 30, 80, or 150 mg/L; creatinine concentrations of 0.9, 4.4, 8.8, 17.7, or 26.5 mmol/L (10, 50, 100, or 200, mg/dL); and as an albumin:creatinine (A:CR) ratio <30, 30–300, or >300 mg/g. Evaluations comparing this test to reference laboratory testing have shown that:

- In a total of 144 urine samples from individuals with diabetes and/or renal disease, and with an upper limit of normal of <20 mg/L for albumin concentration, this test gave a sensitivity of 95.4% and a specificity of 78.9%.[1]
- In a total of 302 urine samples from consecutive patients with diabetes, and with an upper limit of normal of <30 mg/g for A:CR, this test gave a sensitivity of 79% and a specificity of 81%.[3]
- In a total of 200 urine samples from children, adolescents, and young adults with type 1 diabetes, and with an upper limit of normal of <30 mg/L for albumin concentration, this test gave a sensitivity of 89% and a specificity of 73%.[2]

Another example of a dipstick test for MA is the Micral-Test II test strip (Boehringer Mannheim, Indianapolis, IN). In this test, albumin passes via a wick fleece into a conjugate fleece, where it binds to specific, gold-labeled antibodies and then flows to a detection pad.[1,4] A chemical reaction in the detection pad produces a color that is compared visually to color blocks, with colors representing albumin concentrations of 0, 20, 50, and 100 mg/L. Evaluations comparing this test to reference laboratory testing have shown that:

- In a total of 2228 urine samples from diabetic patients, and with an upper limit of normal of <20 mg/L for albumin concentration, this test gave a sensitivity of 96.7% and a specificity of 71%.[5]
- In a total of 411 urine samples from consecutive patients with diabetes, and with an upper limit of normal of <20 mg/L for albumin concentration, this test had a sensitivity of 93% and a specificity of 93%.[4]
- In a total of 96 urine samples from individuals with diabetes and/or renal disease, and with an upper limit of normal of <20 mg/L for albumin concentration, this test gave a sensitivity of 97.1% and a specificity of 33.3%.[1]

Immunologically-Based Assays

Traditionally, three laboratory methods—immunonephelometry, immunoturbidimetry, and radioimmunoassay—have been used for the confirmation and measurement of MA. The performance characteristics of these methods are listed in the Table.

- Immunonephelometry: Albumin in the urine sample comes into contact with an 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 urine sample. The aggregation amount is measured optometrically and converted mathematically to an MA concentration.

- Radioimmunoassay: Albumin in the urine sample displaces isotopically-labeled human albumin that has an antibody bound to it. Consequently, the amount of labeled albumin that remains bound to the antibody is in inverse proportion to the amount of albumin in the sample. The "free" and "bound" labeled albumin can be separated in several ways for radioactivity measurement. Radioactive counts are compared with a calibration or standard curve to provide MA concentration.

Notably, comparisons of these laboratory methods for the detection and measurement of albumin in the urine of persons with diabetes have demonstrated that the results from these methods can vary considerably from one another. In one study, immunonephelometry gave values that were approximately three-fold lower than immunoturbidimetry, meaning that an albumin concentration of about 30 mg/mL (MA range) with immunoturbidimetry would only register as about 10 mg/mL (normoalbuminuric range) with immunonephelometry.[6] In other studies, radioimmunoassay gave values that were 1.4-fold lower than immunonephelometry and over six-fold lower than immunoturbidimetry,[7] and immunonephelometry gave values that were 1.6-fold lower than immunoturbidimetry.[8]

The American Diabetes Association[9] has recommended that if a laboratory is not readily available to screen for MA, dipstick testing may be used since it shows "acceptable sensitivity (95%) and specificity (95%) when carried out by trained personnel." The National Academy of Clinical Biochemistry,[10] however, has recommended that the sensitivity of qualitative or semiquantitative dipstick testing exceed 95% in order minimize the false negative rate, and, consequently, that negative, as well as positive, results be confirmed by a laboratory method. Moreover, the Academy has suggested that this testing be based on a urinary albumin:creatine (UA:C) of 20 mg/L, as an upper limit of normal, to ensure detection of MA as measured by laboratory methods.

Data from evaluations of the two dipstick tests described above indicate that such tests may *not* fulfill efficacy requirements for detecting the early appearance of MA as a risk factor for diabetic nephropathy when MA is near the lower end of its recommended range for diagnosis. Moreover, these tests do not appear to fulfill these requirements for detecting MA as a risk factor for CV disease when MA is below the lower end of this range. Conventionally, then, precise detection and measurement of these critical risk factors should be assured with laboratory testing.

High-Performance Liquid Chromatography-Based Measurement

Recent studies have found that dye- and immunologically-based dipstick and immunologically-based laboratory methods have not been analyzing all intact albumin in the urine, which raises the potential for false negatives in detecting and measuring MA. Four important discoveries have prompted a reassessment of how MA should be detected. First, albumin is excreted in the urine as a complex mixture of components, including immunoreactive intact albumin, albumin fragments and polymer albumin aggregates, and immuno-unreactive intact albumin.[11–14] Second, immuno-unreactive albumin increases in incipient diabetic nephropathy.[15,16] Third, the high performance liquid chromatography (HPLC)-based laboratory test detects both immunoreactive and immuno-unreactive intact albumin, whereas dye and immunologically based dipstick tests and immunologically based laboratory methods detect only immunoreactive intact albumin fragments >12 kDa, and polymer albumin aggregates.[6,15,16] Lastly, dye- and immunologically-based dipstick tests and immunologically-based laboratory methods underestimate urinary albumin concentrations in persons with diabetes, resulting in significant lag times for the diagnosis and treatment of incipient diabetic nephropathy.[6,17,18] The HPLC methodology is used in the Accumin test (AusAm Biotechnologies, Inc., New York, NY) for MA detection and enhances the potential for detecting and measuring *all* intact urinary albumin, particularly for individuals with diabetes mellitus.

The advantage of using Accumin rather than a conventional dipstick test or laboratory method for detecting MA in individuals with diabetes was demonstrated by two recently reported studies.

- False negative rates for the detection of MA (A:CR ≥30 mg/g) by the Clinitek Microalbumin Reagent Strip vs. Accumin were determined for urine samples from a group of 115 patients with diabetes and a group of 106 volunteers without diabetes.[17] The false negative rate for the samples from the group with diabetes was 42.9%.
- False negative rates for the detection of MA (A:CR ≥30 mg/g) by immunoturbidimetry, as compared with Accumin, were also determined for the urine

samples from the group of 115 patients with diabetes and the group of 106 volunteers without diabetes.[17] The false negative rate for the samples from the group with diabetes was 36.3%. Since the urine samples from the volunteer group would not be expected to contain immuno-unreactive albumin to be detected by Accumin, the false negative rate of this group was 0%.

- The differential lead times for detecting MA at A:CR ≥30 mg/g for Accumin vs. radioimmunoassay were determined in groups of patients with type 1 and type 2 diabetes.[18] An analysis was performed on 511 urine samples collected over a 13-year period from a total of 42 patients with type 1 diabetes, 17 of whom progressed from normoalbuminuria to MA, and 25 of whom continued to have normoalbuminuria. The mean lead time for Accumin vs. radioimmunoassay for these patients was 3.9 years (95% confidence interval [CI] of 2.1–5.6 years). An analysis was also performed on 634 urine samples collected over the same period from a total of 49 patients with type 2 diabetes, 24 of whom progressed from normoalbuminuria to MA, and 25 of whom continued to have normoalbuminuria. The mean lead time for Accumin vs. radioimmunoassay for these patients was 2.4 years (95% CI, 1.2–3.5 years).
- UA/C measured with immunonephelometry and HPLC were compared using 24-hour urine samples collected from 1484 subjects in the Prevention of Renal and Vascular End-stage Disease (PREVEND) study.[19] A UA/C <20 mg/L was considered normal. Whereas 13 (2.0%) of the 666 subjects who were classified as normoalbuminuric by HPLC were classified as microalbuminuric by immunonephelometry, 337 (34.2%) of the 986 subjects who were classified as normoalbuminuric by immunonephelometry were classified as microalbuminuric by HPLC. Mean UA/Cs for the 998 subjects who would have been considered normoalbuminuric by immunonephelometry were 6.78 mg/L for immunonephelometry and 17.6 for HPLC, a 159% difference.

The comparative effectiveness of Accumin to other conventional tests in the detection and measurement of MA as a risk factor for CV disease remains to be determined. MA in individuals with and without diabetes appears to reflect a widespread vasculopathy that manifests as an increased renal epithelial/endothelial permeability for albumin.[20–23] Therefore, unless biochemical processing of filtered albumin is different in persons with and without diabetes, one would expect that both immunoreactive albumin and immuno-unreactive albumin would be excreted as risk markers for CV disease as well as diabetic nephropathy.

As with any dipstick test or laboratory method for the detection and measurement of MA, consideration of the cost benefits of Accumin must center on its ability to detect and measure MA at low levels, especially when it first appears as a risk factor for diabetic nephropathy. Conventional dipstick testing with laboratory confirmation has been viewed as a cost-effective means of screening for MA in persons with diabetes[24–27]; however, little attention has been given to the negative impact of the dipstick test failing to detect MA in a substantial number of individuals with diabetes (false negatives).[6,10] These "missed" individuals are placed at increased risk of end-stage renal disease, which costs them greatly in quality and quantity of life and costs the health care system at least $37,000 per individual per year in the United States, based on year 2000 data.[28] The health care cost savings of early medical intervention in diabetic nephropathy to slow or stop its progression to end-stage renal disease is apparent in light of the fact that, in the year 2000, >41,000 Americans with diabetes initiated treatment for end-stage renal disease, and >129,000 Americans underwent renal dialysis or transplantation.[29] Clearly, the cost of accurately screening for MA, even of the entire at-risk and known diabetic population, would be greatly offset by these savings.

SUMMARY

There are a variety of methods for assessing MA. New HPLC methodologies are much more sensitive and specific when compared with older methods of detection and may prove useful for earlier identification of high-risk patients. As has been noted in a recent study, high-normal albuminuria levels determined by conventional methodologies in uncomplicated essential hypertensive men were associated with an adverse CV and metabolic risk profile.[30] HPLC methodology, in contrast to older test procedures, detects all intact albumin and enables clinicians to assess disease severity and monitor therapeutic effectiveness with confidence in the accuracy of the MA data.

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