THE MICRAL TEST II: A SIMPLE TEST FOR MICROALBUMINURIA
AS A PREDICTOR FOR CORONARY ARTERY DISEASE

By

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The members of the Committee appointed to examine the project of KENNETH EUGENE FRANCK find it satisfactory and recommend that it be accepted.

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Microalbuminuria has been found to be a good predictor of coronary artery disease in both the diabetic and non-diabetic population. Testing for microalbuminuria can be expensive, complex and time-consuming. Using a dipstick method can be an inexpensive, simple, and quick alternative. Research has been done to compare the various available testing methods. The Micral-Test II has been shown to be reliable and efficient. Sensitivity has been found to be as high as 100% with a specificity of 91%. Positive tests can lead to more extensive testing and preventative treatment of target populations.
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**Introduction**

Microalbuminuria is becoming widely accepted as a marker and predictor of coronary artery disease and its associated high morbidity and mortality (Niskanen, Penttila, Parviainen and Uusitupa, 1996; Mattock, Barnes, Viberti and Keen, 1998; Savage, Estacio, Jeffers and Schrier, 1996). Microalbuminuria is defined as a urinary albumin concentration of between 30-200 mg/l (Evans and Greaves, 1999).

Urine dipstick testing allows for a rapid and reliable screening for microalbuminuria. Early detection of microalbuminuria, intervention by healthcare providers, patient education and lifestyle changes may affect the quality, as well as quantity, of life in certain patient populations. Populations affected by early detection include the diabetic and non-diabetic patients with renal insufficiency. Screening for microalbuminuria may save unnecessary suffering and early death related to undiagnosed heart disease. High cost for health care will also be positively affected, as the test will predict the possibility of coronary artery disease and could conceivably reduce its associated high morbidity and mortality.

**Epidemiology/Risk Factors**

Coronary heart disease is rapidly replacing pneumonia as the number one cause of disability in the world. Estimates from 1996 show 12,000,000 Americans have some form of heart disease. Coronary heart disease will claim 787,200 lives in the year 2000 and is the single leading cause of death in America today. In America, cardiovascular disease is the leading cause of death among young women and, in 1994, accounted for 45% of all deaths in women. (http://www.americanheart.org).
Direct costs for care of people afflicted with cardiovascular diseases and stroke will be approximately $178 billion in 1999. Figures for lost productivity related to morbidity and mortality will bring that figure closer to $286 billion (http://www.americanheart.org).

The major cause of increased mortality in non-insulin-dependent diabetes mellitus (NIDDM) patients is cardiovascular disease (Gall, Borch-Johansen, Hougaard, Nielsen and Parving, 1995). Over 10,000,000 Americans have physician-diagnosed diabetes and two-thirds of people with diabetes mellitus die of some form of cardiac or peripheral vascular disease.

The Islington Diabetes survey of nondiabetic subjects found an odds ratio for ischemic heart disease of 5.7 in microalbuminuria subjects and a 24-fold increased mortality over a period of 3.5 years (Yudkin, Andres, Mohamed-Ali and Gould, 1997). Microalbuminuria has also been associated with dynamic vascular dysfunction and with significant morphological and functional cardiac alteration in insulin-dependent diabetes mellitus (IDDM) patients (Gugliemi et al., 1995). Among nondiabetic individuals, those with microalbuminuria tend to have an increased cardiovascular morbidity (Deckert et al., 1992).

Coronary heart disease represents the most prominent cause of death in Type 1 and 2 diabetes. Age-adjusted cause-specific mortality rate for coronary heart disease proved to be 2-4 times higher in diabetic patients than in comparable non-diabetic control subjects (Panzram, 1987). Cardiovascular disease is the principal cause of the excess and premature mortality seen among diabetic patients when compared to the general population (Morrish et al., 1990). The presence of microalbuminuria in NIDDM can be
regarded as an index of increased cardiovascular vulnerability and a signal for vigorous efforts at correction of known risk factors (Mattock et al., 1992). Microalbuminuria predicts not only the progression of nephropathy, but also cardiovascular morbidity and mortality (Lim, Caballero, Smakowski and LoGerfoet, 1999). The predictive value of microalbuminuria is independent of age, sex, diabetes duration or blood pressure which makes it a good screen for asymptomatic patients (Mattock, 1992). Microalbuminuria has also been found to be associated with parasympathetic neuropathy. This correlation may be related to vascular endothelial dysfunction caused by the diabetes (Szelag, et al., 1999).

Albumin excretion rates can be integrative markers of several clinical conditions related to increased risk of mortality in acute myocardial infarction (AMI). Measurement of albumin excretion rates are an inexpensive and readily obtainable prognostic index in patients with AMI, independent of other factors (Berton, Citro, Palmieri and (Petucco, 1997). Testing for microalbuminuria can be done easily and at a relatively low cost (Agrawal, Berger, Wolf and Luft, 1996, Mogensen et al., 1997). Patient’s quality of life, as well as society’s pocketbook, are greatly affected by whether or not one decides to screen for microalbuminuria in select patient groups (Statland, 1996). Treatment of microalbuminuria and proteinuria would add little to the cost of overall treating of stage I cardiovascular disease (Brown, Pedula and Bakst, 1999). Given the ease, low cost, and accurate predictability factor, primary care providers can use it reliably in the clinical setting.

Agrawal et al. (1996) believes that demonstrating the clinical utility of
microalbuminuria in a practice setting has important implications. At present, too little too late is being done against the premature mortality in Type 2 diabetics and the potential improvement calls for early and comprehensive measures (Panzram, 1987).

Qualitative microalbuminuria determinations in a practice setting would be of value in identifying high-risk patients and may play a role in their subsequent management (Agrawal et al., 1996). The Diabetes Care Journal's Standards of Medical Care for Patients with Diabetes Mellitus include testing for microalbuminuria in pubertal and post-pubertal type 1 patients who have had diabetes for at least 5 years and in all who have type 2 diabetes (Anonymous, 1999). The potential for testing in various clinical settings include identification of microalbuminuria by urine test strips in diabetes (Delaney et al., 1999).

**Physiology**

The normal physiology of protein retention in the kidneys is due to the large molecular diameter of the major plasma protein, albumin. Albumin is 7.1 nanometers which is larger than the opening of 6-7 nanometers in the basement membranes and the slit membranes of the glomerular capillaries. When the kidney is affected by diabetes, the endothelial-capsular membranes become so permeable, that plasma proteins enter the filtrate (Constanzo, 1998). Microalbuminuria is believed to reflect the glomerular component of a systemic capillary leak. In the healthy kidney, over 99% of filtered albumin is reabsorbed. A small increase in glomerular vascular permeability results in an increase in the filtered albumin presented to the renal tubules. This augmentation results in a rapid rise in albumin excretion and decrease in plasma albumin (Evans and
Ensuing hypoalbuminemia results in ischemia and causes heart failure as a consequence of cardiomyopathy. Systolic dysfunction ensues from myocyte death further complicating the already compromised heart (London and Parfrey, 1997).

**Clinical Features**

Testing for microalbuminuria should be done for non-diabetic hypertensive and nephrology patients who have been found to have renal insufficiency (Mogensen, 1997). At the time of diagnosis, all Type 2 diabetics should be tested. Microalbuminuria seldomly occurs with short term Type 1 diabetes or before puberty. For this reason testing should begin at puberty and at 5 years intervals. Variability in day-to-day albumin excretion can occur, therefore testing should consist of at least 2 out of 3 samples in a 3 to 6 month period before confirming a diagnosis of microalbuminuria (Uphold & Graham, 1998). Factors that can skew the results of testing for microalbuminuria include exercise, urinary tract infections, acute illness, cardiac failure and blood glucose. Decompensation can be detected at routine clinical and laboratory examinations (Mogensen, 1997). See Table 1 for screening and confirmatory procedures suggested by various sources.

**How the Test Works**

Microalbuminuria presents when an increase in the permeability of endothelial-capsular membranes occurs due to injury or disease, increased blood pressure or irritation of kidney cells by substances such as bacterial toxins, ether, or heavy metals (Tortora & Grabowski, 1996). Diabetes mellitus causes an increase in kidney size due to cellular hypertrophy and cellular proliferation with an increase of 20-50% in the glomerular
filtration rate (Tierney, 1998). The Laboratory Test Handbook gives critical values of 30-300 mg/24 hours (Jacobs, 1996). The Diabetes Control and Complications Trial Research Group defines microalbuminuria as >40 mg/24 hours (DCCT, 1993). The Micral-Test II defines microalbuminuria as 20-200 mg/l (Agrawal et al., 1996; Poulsen and Mogensen, 1995; Mattock et al., 1998).

Methods of determining levels include radioimmunoassay (RIA), radial immunodiffusion (RID), enzyme-linked immunosorbent assay (ELISA), immunoturbidimetric and the Micral Test II (Jacobs et al., 1996). RIA, RID, ELISA and immunoturbidimetric are all more expensive than the Micral-Test II and have to be done in a laboratory setting. See Table 2 for cost and assay time comparisons among the various tests.

The Micral-Test II test strip is a reliable semiquantitative determination of low albumin concentrations in urine samples (Mogensen et al., 1997). In a comparison with the four quantitative methods in which a double-antibody radioimmunoassay was used, the Micral-Test displayed a sensitivity of >90% and a specificity of 87% (Tiu, Lee and Cheng, 1993). Marshall, Shearing and Alberti (1992) found the Spearman rank correlation coefficient of the Micral-Test and radioimmunoassay to be 0.92 (P < 0.001). All 34 samples with albumin concentrations ≥20 mg/L and 71 of the 78 samples having albumin concentrations <20 mg/L were correctly identified by the Micral-Test, giving a sensitivity of 100%, a false-negative rate of 0%. Specificity of the Microbumintest, another available test, is lower (82.5%) than the Micral-Test but is more sensitive (100%). However, user reliability was less with the Microbumintest as it was more
difficult to match the colors. Sensitivity of the Albustix test was even less than both Micral-Test and Microbumintest (Tiu et al., 1993).

Poulsen (1995) found Micral-Test II to have a sensitivity of 95% and a specificity of 93%. Based on an abnormal albumin concentration of ≥15 mg/L, the predictive value of the positive test was 97% and predictive value of the negative test was 88%. Data thus indicates that the Micral-Test II provides an accurate test for the detection of microalbuminuria. The efficacy of the test has improved in recent years to make it ever more reliable and accurate. Figure 1 shows how the test actually performs (Mogensen et al., 1997).

Reproducibility of the Micral-Test seems good and would be a reliable initial screening test for patients with microalbuminuria (Tiu et al., 1993). The reaction of the Micral-Test II test strip takes only one minute to reach a stable reaction color. This is an advantage for busy practitioners in a clinic or practice to evaluate different urine samples simultaneously for urinary albumin in a series. Interpersonal variability of the color interpretation of the test strip showed concordant results 93% of the time (Mogensen et al., 1997).

Conclusion

Better standardization of microalbuminuria ranges could lead to a more thorough understanding of the importance of this simple test. Agreement on frequency of testing is lacking and needs further research. Agreement on predictability is widespread although Tiu et al. (1993) emphasized that these qualitative tests are useful only for preliminary screening and positive tests should be verified with quantitative assays before making a diagnosis of microalbuminuria. There is also unanimous agreement on the associated
cardiovascular morbidity and mortality that happens with microalbuminuria. All authors recommend the need for more preventative maintenance to reduce the pathology in the kidneys that lead to microalbuminuria in the diabetic populations.

In conclusion, testing for microalbuminuria is a useful, inexpensive, and simple way to predict the development of coronary artery disease and its associated morbidity and mortality in selected populations. Educating practitioners in the use of the test and the significance it can have in reducing morbidity and mortality in the patient populations needs to be initiated. More research is also needed in larger populations to help establish the validity of testing for microalbuminuria.
REFERENCES


Lim, S. U., Caballero, A. E., Smakowski, P. LoGerfoet, F. W., et. al. (1999). Soluble intercellular adhesion molecule, vascular cell adhesion molecule, and impaired microvascular reactivity are early markers of vasculopathy in type 2 diabetic individuals.... *Diabetes Care, 22*(11), 1865-1872.


<table>
<thead>
<tr>
<th>Document</th>
<th>Proposed yearly screening and confirmatory procedure</th>
<th>BP screening; demarcation for interview</th>
<th>Confounding factors for microalbuminuria</th>
<th>Comments on associated abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Vincent</td>
<td>First morning urine A/C ratio or AC, confirmed by 3 overnight UAE (or by continued monitoring of A/C ratio)</td>
<td>Yearly measurements; &gt;140/90 below 60 yr, &gt;160/90 above 60 yr</td>
<td>Heavy exercise, urinary tract infection, acute illness, cardiac failure</td>
<td>Cardiovascular disease, dyslipidemia</td>
</tr>
<tr>
<td>WHO</td>
<td>UAE (not specified) at least once a year for all with tIDDM .5 yrs and aged &gt;12, and all with NIDDM until age 70. Confirm by repeat testing and measure 6-monthly</td>
<td>BP at least annually; &gt;140/90 below 60 yr or increases in BP by 5 mm Hg</td>
<td>Heavy exercise, urinary tract infection, complications</td>
<td>Other long-term acute illness, high protein intake, metabolic decompensation, cardiac failure</td>
</tr>
<tr>
<td>Australia</td>
<td>Spot urine A/C ratio or AC for screening. Timed urine more accurate. Confirm microalbuminuria by UAE (2 out of 3). Micral test or other bedside test useful</td>
<td>140/90 in young individuals. Measured in supine position after 5 min at rest at every visit</td>
<td>--</td>
<td>Retinopathy, dyslipidemia, foot complications, discourage smoking</td>
</tr>
<tr>
<td>ADA consensus</td>
<td>UAE in a timed urine or A/C ratio in random urine. Sensitive strips may be used. Quantitative sensitive albumin determination for confirmation</td>
<td>Frequent BP monitoring, --</td>
<td>Retinopathy often present</td>
<td></td>
</tr>
<tr>
<td>National Kidney Foundation</td>
<td>At least once a year of A/C ratio in first morning urine (or random urine). Should be confirmed. Different dipstick available for measurements of low albumin concentrations</td>
<td>Monitoring advisable; 140/90</td>
<td>Heavy exercise, urinary tract infection, acute febrile illness, heart failure, non-steroidal anti-inflammatory agents</td>
<td>Dyslipidemia, discourage smoking</td>
</tr>
<tr>
<td>National Institutes of Health</td>
<td>Yearly screening, not specified</td>
<td>&gt;130/85</td>
<td>--</td>
<td>Microalbuminuria designates patients at risk for other complications</td>
</tr>
</tbody>
</table>

A/C ratio = albumin/creatinine ratio  
AC = albumin concentration  
UAE = urinary albumin excretion rate
URINE PASSES VIA A WICK FLEECE INTO THE CONJUGATE FLEECE.

ALBUMIN PRESENT BINDS SPECIFICALLY TO GOLD-LABELED ANTIBODIES.

EXCESS ANTIBODIES ARE BOUND BY IMMOBILIZED ALBUMIN IN THE CAPTURE MATRIX.

ONLY ANTIBODIES BOUND TO ALBUMIN PASS THROUGH.

GOLD-LABELED ANTIBODIES FLOW TO THE DETECTION PAD AND TURN IT RED.

REACTION TIME IS ONE MINUTE.

THE COLOR IS VISUALLY COMPARED TO COLORS ON A CHART ATTACHED TO THE VIAL.

COLORS REPRESENT 0 MG/L, 20 MG/L, 50 MG/L, AND 100 MG/L.

FIGURE 1. HOW THE MICRAL-TEST II WORKS.

Table 2 - Characteristics of RIA, RID, IT, Micral-test, and Microbumintest

<table>
<thead>
<tr>
<th>TEST</th>
<th>WORKING RANGE (MG/L)</th>
<th>~PRICE PER TEST (US $)</th>
<th>ASSAY TIME</th>
<th>EQUIPMENT REQUIRED</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIA</td>
<td>0.8 - 80</td>
<td>2.2</td>
<td>2 HOURS</td>
<td>Gamma-counter</td>
</tr>
<tr>
<td>RID</td>
<td>6 - 89</td>
<td>3</td>
<td>2 DAYS</td>
<td>RID plate-counter</td>
</tr>
<tr>
<td>IT</td>
<td>10 - 160</td>
<td>2</td>
<td>1 HOUR</td>
<td>Spectrophotometer</td>
</tr>
<tr>
<td>MICRAL-TEST</td>
<td>0 - &gt;100</td>
<td>1.0</td>
<td>5 MINUTES</td>
<td>--</td>
</tr>
<tr>
<td>MICROBUMINTEST</td>
<td>0 - &gt;80</td>
<td>1.0</td>
<td>0.5 MINUTES</td>
<td>--</td>
</tr>
</tbody>
</table>

RIA=radioimmunoassay  
RID=radialimmunodiffusion  
IT=immunoturbidimetric

Source: Adapted from Tiu, 1993.
REVIEW OF RECENT LITERATURE ON MICROALBUMINURIA

Kenneth Franck
Review of Recent Literature

Standardizing the terminology for the pathology could help researchers compare and contrast the information derived from completed and ongoing studies. Various terms are used in either titles, text or abstracts to describe the presence of albumin in the urine. These include, but are not limited to, albuminuria, proteinuria, microalbuminuria, urinary albumin excretion and albumin excretion rate. It is not apparent if all researchers are describing the same pathology, however they all do refer to the increased morbidity and mortality of these factors. Use of the terms coronary heart disease, cardiovascular disease, cardiac heart disease, cardiovascular events and coronary artery disease, also made it difficult to fully verify and correlate the findings of each author to the dependent variable, microalbuminuria.

Studies reviewed were mainly focused on sample groups within the diabetic populations including Type I and Type II. Several studies were done with both diabetic and non-diabetic groups and extrapolation of findings to the non-diabetic groups was attempted (Niskanen et al., 1996; Yudkin et al., 1997; Agrawal et al., 1996). Although most research involved male subjects, a few also included females as subjects (Panzram, 1987; Mattock et al., 1992; Morrish et al., 1990; Niskanen et al., 1996).

One study included White, AfroCaribbean, and South Asian, but reported only white NIDDM patient outcomes (Mattock et al., 1998). Differences in the definition or criteria for diagnosis of microalbuminuria causes a difficulty in interpreting and comparing the results of research done on microalbuminuria. Seven researchers used 20-200 mg/l as their determination of microalbuminuria (Agrawal et al., 1996; Deckert et al., 1992; Mattock et al., 1992; Mattock et al., 1998; Niskanen et al., 1996; Poulsen et al., 1995; Savage et al., 1996; Yudkin et al., 1997). Five researchers used 30-300 mg/24h as their determination of microalbuminuria (Berton et al., 1997; Gall et al., 1996; Gugliemi et al., 1996;
1996; Jacobs et al., 1996; Statland, 1996). One researcher chose to use a definition of proteinuria as the presence of any degree of visible precipitation on the salicylsulphonic acid test (Morrish et al., 1990). The findings of the studies coalesced together to determine that measuring microalbuminuria is an important factor in starting early preventive treatment to slow down the progression of cardiovascular disease. See Table 3 for a comparison of the criteria used by these researchers.
Comparison of criteria included in research.

<table>
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<tr>
<th>AUTHOR</th>
<th>CRITICAL VALUE DEFINITION</th>
<th>SAMPLE GROUP</th>
<th>FEMALES INCLUDED</th>
<th>ETHNIC GROUPS INCLUDED</th>
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<tr>
<td>Niskanen</td>
<td>20 - 200 mg/l</td>
<td>D, N</td>
<td>Yes</td>
<td>N/A</td>
</tr>
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<td>Yudkin</td>
<td>20 -200 mg/l</td>
<td>D, N</td>
<td>N/A</td>
<td>C</td>
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<tr>
<td>Agrawal</td>
<td>20 - 200 mg/l</td>
<td>D, N</td>
<td>Yes</td>
<td>N/A</td>
</tr>
<tr>
<td>Panzram</td>
<td>N/A</td>
<td>D</td>
<td>Yes</td>
<td>N/A</td>
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<tr>
<td>Mattock</td>
<td>20 - 200 mg/l</td>
<td>D</td>
<td>Yes</td>
<td>C, AC, SA</td>
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<tr>
<td>Morrish</td>
<td>Any visible precipitation on salicylsulphonic acid test</td>
<td>D, N</td>
<td>Yes</td>
<td>N/A</td>
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<tr>
<td>Deckert</td>
<td>20 - 200 mg/l</td>
<td>N/A</td>
<td>N/A</td>
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<td>Poulson</td>
<td>20 - 200 mg/l</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<td>Savage</td>
<td>20 -200 mg/l</td>
<td>D</td>
<td>Yes</td>
<td>C, AA, H, O</td>
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<tr>
<td>Berton</td>
<td>30 - 300 mg/l</td>
<td>D, N</td>
<td>Yes</td>
<td>N/A</td>
</tr>
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<td>Gulieimi</td>
<td>30 - 300 mg/l</td>
<td>D</td>
<td>Yes</td>
<td>N/A</td>
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<tr>
<td>Jacobs</td>
<td>30 - 300 mg/l</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<td>Statland</td>
<td>30 - 300 mg/l</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Gall</td>
<td>30 - 300 mg/l</td>
<td>D</td>
<td>Yes</td>
<td>C</td>
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</table>

AA = African-American   AC = Afro-Caribbean   C = Caucasian    H = Hispanic  
N/A = Not Available     O = Other            SA = Southeast Asian  
D = Diabetic             N = Non-diabetic