Screening for Microalbuminuria in Type 2 Diabetic Patients: the Evaluation of a Dipstick Test in General Practice

W.J.C. de Grauw, E.H. van de Lisdonk, H.J.M. van den Hoogen, W.H.E.M. van Gerwen, W.J.H.M. van den Bosch, J.L. Willems, C. van Weel

*Department of General Practice and Social Medicine, University of Nijmegen, and †Department of Clinical Chemistry, University Hospital, University of Nijmegen, the Netherlands

To evaluate the Micral test, a semiquantitative dipstick test, in a general practice setting, 317 Type 2 diabetic patients completed a screening for microalbuminuria by means of the Micral test as well as immuno-nephelometry with the Disc 120 immuno-nephelometer (Hyland, Nivelles, Belgium). Data were collected in 10 general practices performing the Nijmegen Monitoring Project. At a regular check-up each Type 2 diabetic patient was asked to collect first morning urine samples on three consecutive days. The sensitivity of the Micral test was 67%, the specificity 93%. Between the practices the sensitivity ranged from 58% to 81%, the specificity from 87% to 95%. Microalbuminuria, defined as a mean urine albumin concentration > 20 mg/l on three consecutive days, was found in 66 patients (21%). The first Micral test correctly picked out these patients with microalbuminuria in 70% of the cases and in 90% those patients without microalbuminuria. The diagnostic performance of the Micral test was further proved by a Receiver Operating Characteristic (ROC) curve. The Area Under the Curve (AUC) of the Micral test was 0.84 (95% CI 0.78-0.90). Micral test results of 0 and 10 should be regarded as negative.

Key words: Microalbuminuria, Type 2 diabetes, General Practice, Micral test

Introduction

Microalbuminuria is defined as an albumin excretion rate (AER) of 20–200 µg min⁻¹ in an overnight collection, or 30–300 mg 24 h⁻¹. It is thought to be related to a number of risk factors for cardiovascular disease and predicts the development of clinical proteinuria, early cardiovascular mortality, proliferative retinopathy, and nephropathy. Although only 3–8% of the Type 2 patients with nephropathy develop end-stage renal disease, the prevalence of this type of diabetes turns renal failure into a quantitative relevant problem. Approximately 50% of all diabetes patients requiring dialysis or transplantation therapy are Type 2 diabetes patients. It is obvious that renal replacement therapy negatively influences patients health, quality of life, and economic aspects. Antihypertensive treatment will at least slow down the progression from microalbuminuria to persistent proteinuria. The rate at which albumin excretion increases is related to long-term glycaemic control. However, it remains to be established whether antihypertensive treatment or strict blood glucose control will reduce the development of renal failure and cardiovascular morbidity and mortality. Moreover, the potential benefits of any screening and intervention have to be weighted against the costs of screening and treatment. Although early disease detection and treatment seems simple, in practice many prerequisites have to be fulfilled. Gilbert recently reviewed the prognostic and therapeutic implications of microalbuminuria in diabetes mellitus. In agreement with former recommendations he concluded that screening for microalbuminuria seems justified. Moreover, he addressed the fact that screening helps to identify patients at high risk for retinopathy and cardiovascular diseases. Such patients in particular may benefit from more frequent and tight control.

As most Type 2 diabetic patients are treated in general practice, an instrument to screen for microalbuminuria should be feasible in general practice. For this reason the collection of 24-h or timed samples is inconvenient, especially when repeated measures are proposed. Moreover, an albumin concentration in a first morning urine sample of 20 µg/l is a reasonable initial screening cut-off point for identifying patients with microalbuminuria. Recently a cheap and simple to handle dipstick test for microalbuminuria (Micral test) was evaluated in a clinical setting. In the hands of trained laboratory technicians the following were found: a sensitivity of 75–100%, a specificity of 80–97% compared to radioimmunoassay, a predictive value of a positive test result of 55–72%, and of a negative test result of 97%. The critical time of contact between stick and urine and reading may be a major source of error in the use of the strip under general practice conditions.
The aim of this study was to assess the feasibility of screening a large diabetic population for microalbuminuria by means of the Micral test in a general practice setting. This study compares measuring albumin concentration in first morning urine on three consecutive days by means of the Micral test and by immuno-nephelometry.

Patients and Methods

Patients and Study Design

First morning urine samples on three consecutive days of 401 Type 2 diabetic patients were tested for microalbuminuria by means of the Micral test and by immuno-nephelometry. The immuno-nephelometric results served as reference values.

Patients were selected from the Nijmegen Monitoring Project (NMP).36 This is a longitudinal registration aimed at studying the course of Type 2 diabetes mellitus and other chronic diseases in general practice. Since 1985 all Type 2 diabetic patients in 10 general practices, including those who are under specialist medical care, have been included in this registration if the diagnostic evidence is in agreement with the WHO criteria.37 Patients who were treated with insulin within 1 year of diagnosis and who remained on it were regarded as Type 1 diabetic patients. All other patients were regarded as Type 2, irrespective of their current treatment. Monitoring consists of the follow-up of the level of metabolic control, diabetes-related complications, cardiovascular morbidity, cardiovascular risk factors, and mortality.

For this study all Type 2 diabetic patients in the Nijmegen Monitoring Project under control of their general practitioner were invited to participate in the screening for microalbuminuria. The screening took place at their routine diabetes check-ups over a period of 1 year. All patients were asked to:

1. collect first morning urine samples on three consecutive days;
2. store these samples at 4 °C (as this preserves the albumin concentration for up to 2 weeks38);
3. bring them to the practice on the morning of the third day.

All patients received instructions from the practice assistants and written explanation about the standardized procedures how to collect and store the urine samples. On the morning the patients brought their urine samples to the practice, these samples were first tested for nitrite and proteinuria by a dipstick test (N-combur test, Boehringer Mannheim, Germany). Samples with positive nitrite or albumin tests were excluded. All other samples were tested by the practice assistant by means of the Micral test. Aliquots of these samples were stored at 4 °C and analysed by immuno-nephelometry within 1 week of collection.

Measurements of Microalbuminuria

The Micral test is an immunochemical strip test specific for albumin. (Boehringer Mannheim, Germany) The reagent part of the test strip should be dipped into the urine for 5 s, then laid down horizontally and read after 5 min. The intensity of the colour produced is proportional to the albumin concentration in the urine. The colour formed is compared with the reference chart on the vial. There are five colour blocks, reflecting categories of albumin concentrations of 0, 10, 20, 50, 100 mg l⁻¹. Careful initial training was given to the general practitioners (GPs) and practice assistants on the standardized procedures and performance of the Micral test. As reference values, albumin concentrations were also measured with the Disc 120 immuno-nephelometer (Hyland, Nivelles, Belgium) with an anti-serum raised in New Zealand white rabbits. Details of the immunization procedure and the immunological nephelometric method were published earlier.38 The coefficient of variation between batches was 3 % and 10 % in high concentration (mean 70.1 SD 2.1) and low concentration (mean 5.3, SD 0.6), respectively. The coefficient of variation within batches was 1.8 %. Detection limit was 1 mg l⁻¹.

Analysis

The day-to-day variation of the albumin concentrations of each patient was calculated by assessing the agreement between the albumin concentration on first, second, and third mornings measured by nephelometry (Bland-Altman plot).39 A cut-off point of ≥ 20 mg l⁻¹ was used to differentiate subjects with microalbuminuria from subjects without microalbuminuria. The sensitivity, specificity, predictive values, and likelihood ratios of the Micral test were based on the Micral test results of the first morning urine sample and the albumin concentrations measured in the same sample by immuno-nephelometry. The likelihood assesses the diagnostic power of a test. It is the quotient of the chance to find a positive test result in a sick individual (nominator) and the chance of a positive test result in a healthy subject (denominator). For a test which does not discriminate between sick and healthy individuals the nominator and denominator are equal, resulting in a likelihood ratio of 1.0. Test discrimination is better when the likelihood ratio for a positive test result is greater than 1.0 and for a negative test result when approximating zero. We calculated the reliability of the Micral test to single out those patients without microalbuminuria, defined by the mean nephelometry result for the 3 days.

A Receiver Operating Characteristic (ROC) curve was constructed by graphing the sensitivity on the y-axis against the false positive fraction (1-specificity) on the x-axis. The area under this curve gives a measure for the diagnostic power of a test. For a test which does not discriminate between sick and healthy people the resulting ROC curve coincides with the diagonal and...
**Table 1. The day-to-day variation of the urine albumin concentration as the mean differences from the mean on three consecutive days in the same patient**

<table>
<thead>
<tr>
<th>Level of albumin concentration</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4.9 (n = 174)</td>
<td>0.02 (1.3)</td>
<td>0.00 (1.2)</td>
<td>-0.03 (1.4)</td>
</tr>
<tr>
<td>10-19.9 (n = 77)</td>
<td>2.1 (6.2)</td>
<td>-1.5 (4.2)</td>
<td>-0.5 (5.1)</td>
</tr>
<tr>
<td>20-49.9 (n = 48)</td>
<td>0.4 (9.6)</td>
<td>-0.6 (10.2)</td>
<td>0.3 (10.8)</td>
</tr>
<tr>
<td>50-99.9 (n = 5)</td>
<td>-4.9 (8.9)</td>
<td>-9.5 (5.9)</td>
<td>11.5 (8.3)</td>
</tr>
<tr>
<td>100-300 (n = 13)</td>
<td>23.5 (71.2)</td>
<td>-15.2 (62.8)</td>
<td>-8.3 (44.9)</td>
</tr>
</tbody>
</table>

Results as mg l⁻¹ with standard deviation in parentheses.

*Mean urine albumin concentration on 3 consecutive days measured by nephelometry.

**Table 2. Urine albumin concentrations as a result of first Micral test and immuno-nephelometry (Hyland-Disc 120)**

<table>
<thead>
<tr>
<th>Micral category</th>
<th>Urine concentration by nephelometry (mg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-10</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
</tr>
</tbody>
</table>

Results as absolute numbers.

*All samples from 1 of the 10 participating practices.

**Results**

A complete set of three consecutive first morning urine samples was collected from 401 Type 2 diabetic patients. Using the N-Combur-test, nitrite was found in one or more of the samples in 22 patients and albumin in 64 patients. Of the remaining 317 patients three urine samples were tested by the Micral test and immuno-nephelometry. The day-to-day variation of albumin concentrations in the same patient was assessed by the mean difference between the urine albumin concentration measured by nephelometry on first, second, and third morning and their mean (Table 1). The day-to-day variation seems to increase with the level of microalbuminuria.

Sensitivity, specificity, predictive values, and likelihood ratios are based on the first Micral test (Tables 2 and 3). It was reasonable to present only the result of the first morning sample of each patient as the other samples give the same information. A threshold of 20 mg l⁻¹ gives optimal balance between sensitivity and specificity. A threshold of 10 mg l⁻¹ gives a poor specificity (44%), a threshold of 50 mg l⁻¹ a poor sensitivity (31%). If the categories 0 and 10 are regarded as negative test results, the Micral test is a good instrument to identify subjects without microalbuminuria (specificity 93%). The test was less suitable for identifying patients with an albumin excretion ≥ 20 mg l⁻¹ (sensitivity 67%).

We analysed the data under three conditions for microalbuminuria:

1. Mean value of the three measurements for nephelometry ≥ 20 mg l⁻¹ (Table 4).
2. Median value of the three measurements for nephelometry ≥ 20 mg l⁻¹.
3. Two out of three nephelometry results ≥ 20 mg l⁻¹.

As results were virtually the same, we presented the mean values.

Microalbuminuria, defined as a mean albumin concentration ≥ 20 mg l⁻¹ by nephelometry for three consecutive first morning urine samples, was present in 66 patients (21%). Of these 66 patients, 12 had a Micral test result ≥ 20 mg l⁻¹ in none of the samples; 54 had one positive result, 47 had two and 39 three. The first Micral test correctly picked out these patients with microalbuminuria in 70% of the cases and in 90% those patients without microalbuminuria (Table 4).

The diagnostic performance of the Micral test was further proved by a ROC curve (Figure 1). The AUC of the Micral test was 0.84 (95% CI 0.78-0.90).

**Discussion**

We found that in a general practice setting the Micral test has good specificity and moderate sensitivity at the cut-off point of a urine albumin concentration of 20 mg l⁻¹. The frequency of false positive results (1-specificity) was 7%, occurring in 8% of the study population. Since the Micral test is a screening test and not a diagnostic test, this number of false positive results is acceptable. The sensitivity of the Micral test was lower in our study than in the clinical setting. The frequency of false negative results (1-sensitivity) was 33%, occurring in 5% of the study population. This means that the Micral test has limited value for identifying patients with microalbuminuria. In screening, a fairly high false negative rate is only acceptable if at the next screening the missed cases are detected still at a pre-clinical stage. Cooper found that the mean duration of the
Table 3. Evaluation of the Micral test compared to immuno-nephelometry (Hyland-Disc 120)

<table>
<thead>
<tr>
<th>Screening cut-off point (mg l⁻¹)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PV⁺</th>
<th>PV⁻</th>
<th>LR⁺</th>
<th>LR⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 20</td>
<td>67</td>
<td>93</td>
<td>74</td>
<td>90</td>
<td>9.6</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Results of sensitivity, specificity, and predictive values as percentages.

*Predictive value of a positive test result.
*Predictive value of a negative test result.
*Likelihood ratio of a positive test result.
*Likelihood ratio of a negative test result.

Table 4. Reliability of the Micral test to identify patients with microalbuminuria defined as a mean urine albumin concentration ≥ 20 mg l⁻¹ in three consecutive first morning urine samples measured by nephelometry

<table>
<thead>
<tr>
<th>Micral testᵃ</th>
<th>Microalbuminuria (mean ≥ 20 mg l⁻¹) based on nephelometryᵇ</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positiveᶜ</td>
<td>45</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>20</td>
<td>228</td>
<td></td>
</tr>
</tbody>
</table>

ᵃMicral test result of the first urine sample.
ᵇMean based on three nephelometry results.
ᶜPositive is defined as a result in the category 20, 50 or 100.

Figure 1. Receiver Operating Characteristic (ROC) curve of the Micral test in general practice (n = 317). The AUC of the Micral test was 0.84 (95% CI 0.78–0.90)

The specificity of the Micral test was high (85–97 %) in all settings. However he found a decrease in sensitivity from almost 90% in laboratory technicians to 66 % in the general practice setting.35 Jury showed that the depth of dipping the strip into the urine sample and the time of reading the colour were critical.31 Marshall studied the time dependency of the test by comparing the results obtained for strips that were in contact with urine for 2 and 5 s and by reading at 4, 5 and 6 min. Contact with urine for 2 s rather than the recommended 5 s resulted in an underestimation of the urine albumin concentration, as did taking readings earlier than the recommended 5 min.²⁹ This time dependency is a serious problem in general practice as the practice assistant has to perform diagnostic tests during other activities, such as telephone calls and patient contacts. Tiu demonstrated a considerable inter-observer variation in the matching of colours for the Micral test.²⁸

These, as well as our results, clearly demonstrate the necessity of careful initial training, follow-up training, and a quality-control system to ensure continuing satisfactory performance of the Micral test. A limitation of the Micral test, as of all methods that measure urine albumin concentration, is the influence of diuresis. The wide day-to-day variations of diuresis and urine albumin excretion make it necessary to confirm an abnormal albumin
albunin concentration in first morning urine with a second test. In order to confirm diagnosis repeated abnormal results require ideally a timed urine collection. However, the correct collection of timed samples, even if the patient knows that he or she is at risk for nephropathy, may be very difficult. The measurement of albumin/creatinine ratios may be more reliable.

Based on the screening strategy proposed by the St Vincent declaration and our findings, for general practice we suggest the screening strategy outlined in Figure 2. Patients with a Micral test result of 0 are highly unlikely to have a urinary albumin concentration $\geq 20 \text{ mg} l^{-1}$, so these patients only need to be re-screened after 1 year. Approximately 40% of the Type 2 diabetic patients in our study had a Micral test result of $10 \text{ mg} l^{-1}$. Nearly 20% of them had an albumin concentration $\geq 20 \text{ mg} l^{-1}$. It seems sensible to perform another Micral test in those patients within 4 weeks. If the second Micral test result falls into the category $\geq 20 \text{ mg} l^{-1}$ further diagnosis is required. In all other cases re-screening after 1 year seems justified.

Micral test results of 20, 50 or 100 mg l$^{-1}$ are clearly found almost exclusively in those patients with an albumin concentration $\geq 20 \text{ mg} l^{-1}$. In those cases we propose a second Micral test within 4 weeks. If the second Micral test result falls into the category $\geq 20 \text{ mg} l^{-1}$ further diagnosis is required. In all other cases re-screening after 1 year seems justified.

We compared two screening strategies in terms of cost effectiveness. Both are based on albumin concentrations in first morning urine samples and using an albumin concentration threshold of $20 \text{ mg} l^{-1}$: Strategy 1: screening using nephelometry and Strategy 2: screening using Micral test. The cost of the nephelometry assay was £10.40 and that of the dipstick £1.28, based on the Dutch real costs (assay-dipstick cost ratio 8.1). The cost-effectiveness of this screening strategy was calculated in a fictitious cohort of 100 diabetic patients.

**Strategy 1:** all patients screened with nephelometry. The costs include a first test for all patients and a second test for patients with results on first screening $\geq 20 \text{ mg} l^{-1}$ (about 20%): $(100 + 20) \times 10.40 = £1248$ (exclusive of costs due to the storage and the transport at 4°C of the urine samples from the practice to the laboratory).

**Strategy 2:** all patients screened with Micral test. The costs include a first dipstick for all patients and a second dipstick for patients with results on first screening $> 0$ (about 65%): $(100 + 65) \times 1.28 = £211.20$.

Floch studied the cost-effectiveness of screening for microalbuminuria using the Micral test or laboratory assay in a fictitious cohort of 10 000 diabetic patients (Types 1 and 2). He stated that the cost-effectiveness of the Micral test was strongly related to the frequency of false negative results. In that study the frequency of false negative results was 9.2% (the Micral test was performed in a clinical setting); if increased to 15–20% the effectiveness decreased dramatically. The frequency of false negative results in our study was even higher. Although the practice assistants and GPs received initial training, follow-up training and quality control might have contributed to a higher sensitivity.

**Figure 2.** Screening strategy Micral test in general practice. Patients with a Micral test result of 0 are highly unlikely to have a urinary albumin concentration $\geq 20 \text{ mg} l^{-1}$, so these patients only need to be re-screened after 1 year. In patients with a Micral test result of 10 it is sensible to perform another Micral test within 4 weeks. If the second Micral test result falls into the category $\geq 20$ a timed quantitative measurement is recommended. In all other cases re-screening after 1 year seems justified. In patients with Micral test results of 20, 50 or 100 we propose a second Micral test within 4 weeks. If the second Micral test result falls again into the category $\geq 20$ a timed quantitative measurement is recommended. In all other cases re-screening after 1 year seems justified.
Apart from the cost reduction in strategy 2 of almost 85%, the Micral test has the practical advantage that the result is immediately available during the patient's visit. Moreover, the urine samples do not have to be stored at 4 °C and sent to a laboratory.

In conclusion, screening for microalbuminuria in Type 2 diabetic patients is still a controversial topic because it has to be proven whether interventions can delay the development of renal failure, cardiovascular morbidity, and mortality. However, there is growing evidence in favour of screening for microalbuminuria. As most Type 2 diabetic patients are treated in general practice such a screening should be easily applicable in that setting. Our results suggest that the use of the Micral test can only be supported if strict standardized procedures are followed and repeated measurements are performed.

References


