INTEREST OF CLINITEK® MICROALBUMIN IN SCREENING FOR MICROALBUMINURIA: RESULTS OF A MULTICENTRE STUDY IN 302 DIABETIC PATIENTS

J.P. LE FLOCH (1), M. MARRE (2), M. RODIER (3), PH. PASSA (4)

SUMMARY - A prospective survey was performed in 302 consecutive diabetic outpatients from 3 French diabetic centres to study the sensitivity and specificity of screening for microalbuminuria using Clinitek® Microalbumin. Urinary samples with positive (at least one +) proteinuria, hematuria, leucocyturia, or nitrates using the Multistix® strip were excluded from the study. Results obtained with Clinitek® Microalbumin were compared to those observed with the reference method of the biological laboratory of the centre on the same urinary sample. A positive result was defined as an albumin-to-creatinine ratio $\geq 30$ mg/g. Results were described in terms of sensitivity, specificity, positive and negative predictive values and likelihood ratio. Agreement rates were compared with the Kappa test.

In the study population, 48 patients (17%) had a positive microalbuminuria with reference assay. However, different rates were found in each site (25%, 11%, and 15%, respectively, p < 0.001). Using the Clinitek® Microalbumin, a positive result was found among 86 patients (29%), (39%, 26%, and 23%, respectively). A good agreement was observed in the population as a whole (81%, K = 0.47 ± 0.06) and in each site (77%, 81%, and 84%, respectively). Sensitivity was 79% (82%, 80%, 75%), specificity 81% (76%, 81%, 85%), positive predictive value 46% (53%, 35%, 46%), negative predictive value 95% (93%, 97%, 95%), and positive likelihood ratio 4.2 (3.4, 4.3, 5.0, respectively). Due to the excellent negative predictive value, these results suggest that Clinitek® Microalbumin is a good screening test for microalbuminuria. Positive results should be confirmed using a reference assay.

Key-words: diabetes, microalbuminuria, nephropathy, hypertension, screening test.

RÉSUMÉ - Intérêt du Clinitek® Microalbumin pour le dépistage de la microalbuminurie : résultats d’une étude multicentrique chez 302 patients diabétiques.

Afin d’évaluer la sensibilité et la spécificité du dépistage de la microalbuminurie au moyen du Clinitek® Microalbumin, une étude a été conduite prospectivement en ambulatoire chez 302 diabétiques consultant consécutivement dans 3 centres français. Les échantillons urinaires contenant une protéinurie positive, du sang, une leucocyturie ou des nitrates à la bandelette Multistix® étaient exclus. Les résultats ont été comparés à ceux obtenus sur les mêmes échantillons urinaires par la méthode de référence du laboratoire de chaque centre. Un résultat positif a été défini par un rapport albumine/créatinine urinaire $\geq 30$ mg/g. Les résultats sont exprimés en sensibilité, spécificité, valeur prédictive positive et négative, et rapport de vraisemblance. Les taux de concordance ont été comparés par test Kappa.

Dans la population d’étude, 48 patients (17%) présentaient un résultat positif pour la méthode de référence, avec une hétérogénéité des sites (25 %, 11 %, et 15 % de positifs, respectivement, p < 0.001). Un résultat positif était trouvé chez 86 patients (29 %) avec le test Clinitek® Microalbumin (39 % : 26 % ; et 23 %, respectivement). Une bonne concordance était observée globalement (81 %, K = 0.47 ± 0.06) et dans chaque site (77 %, 81 % : 84 %, respectivement). La sensibilité était de 79 % (82 %, 80 %, 75 %), la spécificité de 81 % (76 %, 81 %, 85 %), la valeur prédictive positive de 46 % (53 %, 35 %, 46 %), la valeur prédictive négative de 95 % (93 %, 97 %, 95 %), avec un rapport de vraisemblance positif de 4,2 (3,4, 4,3, 5,0). Compte tenu de l’excellente valeur prédictive négative de la méthode, le Clinitek® Microalbumin est un bon test de dépistage de la microalbuminurie. Les résultats positifs doivent être confirmés par un dosage utilisant une méthode de référence.

Mots-clés : diabète, microalbuminurie, néphropathie, hypertension, test de dépistage.
Microalbuminuria is considered a predictor of diabetic angiopathy and, more precisely, of nephropathy and cardiovascular mortality in diabetic patients [1-4]. Screening for microalbuminuria is required at least once a year [5, 6]. Evidence of microalbuminuria leads to improve the treatment of diabetes and of possibly associated hypertension [7, 8].

Screening for microalbuminuria is classically done using 24 hours urinary excretion. Because of the difficulty to obtain a complete collection, collecting over shorter period of time has been suggested, often associated with a creatinine assay, which is considered a good indicator of urinary excretion. Finally, albumin to creatinine ratio (ACR) was found the best index for studying microalbuminuria, easy to perform for patients (partial urinary collection is allowed), reliable for physicians (albuminuria being standardised when rated to creatininuria) [9, 10].

In France, screening for microalbuminuria is not commonly performed. Biological assays are performed using expensive reference methods, only available in specialised laboratories. When done in other biological laboratories, the reliability of these assays can be questioned, due to the little number of tests performed. In addition, intra-individual variability of albumin urinary excretion [10, 11] often requests repeated measurements leading to important cumulated cost. Screening methods, based on semi-quantitative assays with strips, have been developed. They are cheaper, but positive results should be confirmed by laboratory assay [12-14].

Clinitek® Microalbumin (BAYER) is a screening test for microalbuminuria performed using a strip read with a device, and leading to a semi-quantitative result of ACR. Result can come out < 30 mg/g (considered as normal), between 30 and 300 mg/g (microalbuminuria), or > 300 mg/g (macroalbuminuria) [14, 15]. It could be interesting when screening for microalbuminuria. The aim of this study was to analyse the ability of Clinitek® Microalbumin to screen for microalbuminuria in diabetic outpatients.

**MATERIAL AND METHODS**

The study was conducted in 3 University hospitals: Angers, Nîmes, and Paris. Urinary samples were collected in 389 consecutive diabetic outpatients. A first test was done by a trained nurse, using Multistix® strip. Samples with results of “+” or more hematuria, proteinuria, leucocyturia or nitrates were excluded. Samples with “+/–” results were maintained in the study. Finally, 302 samples were used, 88, 91 et 122, respectively in either centre.

Samples under study were tested for microalbuminuria and creatininuria using both Clinitek® Microalbumin and the reference assay commonly used in the biological laboratory of the centre. Measurement with Clinitek® Microalbumin was done by the same nurse and on the same urinary sample, following manufacturer recommendations. Results were read immediately using the Clinitek® 50 or Clinitek® 100 device. Semi-quantitative results for albuminuria were got in 4 classes: 10, 30, 80 or 150 mg/l, given result being that of the closer class. Similarly, creatinine was given in 5 categories: 10, 50, 100, 200 or 300 mg/dl (0.1; 0.5; 1; 2 or 3 g/l). Finally, ACR was given in 3 categories: < 30, [30-300], > 300 mg/g, the ratio being computed from real values.

Biological laboratories assayed urinary albumin and creatinine excretion and computed their ratio using their common methods: immunonephelemetry (Behring kit), in 2 centres, and radio-immuno-assay (Pharmacia Upjohn kit) in Nîmes.

In both cases, a positive result (microalbuminuria) was defined as ACR ≥ 30mg/g. Results obtained with Clinitek® Microalbumin were compared to those from reference assays, in terms of sensitivity, specificity, positive and negative predictive values, and positive likelihood ratio (Table I). Agreement rates were analysed with the Kappa test [16, 17]. All analyses were conducted with SAS 6.12 statistical software (SAS Institute, Cary, USA) using a bilateral significance level of 5%.

**RESULTS**

In the study population, urinary albumin excretion estimated with reference assay was (mean ± SD) 15.7 ± 19.0 mg/l (18.5 ± 22.4; 13.5 ± 15.1; 15.3 ± 19.0 mg/l in either centre, respectively). On average, creatinine excretion was 0.95 ± 0.73 g/l (0.82 ± 0.49; 0.95 ± 0.49; 1.06 ± 0.99 g/l, respectively), and ACR 23.1 ± 40.2 mg/g (32.6 ± 54.3; 14.8 ± 15.8; 22.4 ± 39.7 mg/g, respectively). With this reference assay, 17% of the patients had a positive result of microalbuminuria.
Screening for microalbuminuria was performed on urinary samples from unselected diabetic patients from three university hospitals. Reliability of the results obtained with the Clinitek® Microalbumin test was assessed by reference to laboratory assays of the centres. A good sensitivity (79%) and specificity (81%), were found. Negative predictive value was excellent (95%). This result suggests that the Clinitek® Microalbumin test is a reliable test for negative results. Conversely, positive predictive value was poor (46%), suggesting that an important rate of positive results were false positive results. Thus, Clinitek® Microalbumin is a good screening test for microalbuminuria, leading to a low rate of false negative results, but positive results must be proved using a reference assay. In addition, this assay will result in quantified level of microalbuminuria.

Variations between centres were observed, but should be attributed to fluctuations in the prevalence of microalbuminuria observed in patients from different sites.

In this study, an important number of patients was included, probably representative of the diabetic population from university hospital. However, this population could differ from that of general diabetic patients. A high rate of patients with type 1 diabetes, a longer duration of diabetes, more frequent degenerative complications are expected. Thus, the prevalence of microalbuminuria could be higher in this population. In the general diabetic population, a different prevalence should alter the predictive values of the test. Sensitivity and specificity, which reflect intrinsic characteristics of the test, should not change significantly.

The same urinary sample was used to screen for microalbuminuria with both the Clinitek® Microalbumin test and the reference method. Urinary sampling and testing were performed by trained nurses strictly following manufacturer recommendations. In clinical practice, urinary collection and testing could be less accurate, whatever the method used. On the other hand, mass screening supports the interest of semi-quantitative methods. In addition, screening for microalbuminuria is usually done after strip-screening for macroalbuminuria. It is only when this first screening is negative that screening for microalbuminuria is justified.

Urinary sample collection was used in our study. Urinary collection over 24 hours, which defined microalbuminuria as an urinary excretion > 30 mg/d, is considered poorly reliable in clinical practice, because of partial collection. Overnight collection has been proposed, but should be interpreted according to variations in urinary excretion [19] and uncertain duration of collection. Sample collection, commonly performed on first morning miction, with estimation of ACR finally appeared as the best solution, especially for outpatients. ACR appears to be a good indic-

**DISCUSSION**

Diabetes, the prevalence of which increases with increased life expectancy, is yet one of the main aetiology of chronic renal failure. Its severity and its cost make it a main concern in public health. Microalbuminuria is the first sign of nephropathy. Thus screening for microalbuminuria is a medical requirement, especially in type 2 diabetic patients, because it is an indice of morbi-mortality [1, 2, 9].

Prevalence of microalbuminuria depends on the type and duration of diabetes and on the possible association with hypertension [18]. Recent studies showed that renal failure could be delayed, especially when early therapeutic intervention was performed, from the moment microalbuminuria was diagnosed [3, 7, 8]. This way, semi-quantitative screening tests, such as Clinitek® Microalbumin should be interesting.

Microalbumin is a good screening test for microalbuminuria, leading to a low rate of false negative results, but positive results must be proved using a reference assay. In addition, this assay will result in quantified level of microalbuminuria.
cator of the function of the nephron. Threshold for positive result is then 30mg of albumin per g of creatinine.

Detecting microalbuminuria in diabetic patients leads to important therapeutic goals: optimal metabolic control, even using intensive treatment, improved life behaviors, physical exercise, blood pressure normalisation, including with angiotensin converting enzyme inhibitors, treatment of associated risk factors [20-22]. Screening for microalbuminuria, however, is so far performed in 10% of French diabetic patients only [23]. Improving this rate is a main goal, which can be achieved using reliable, cheap and easy to perform semi-quantitative methods [24].

REFERENCES
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