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Rapid Immunochromatographic Strip Test for the Detection of Albuminuria and Brief Literature Review on Albuminuria Screening

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Abstract
Albuminuria is a powerful predictor of cardiovascular events in diabetic and hypertensive patients as well as in the general population. In the present study we evaluated the diagnostic performance of the new PreventID® Albumin test, a semi-quantitative immunochromatographic dipstick device for the rapid detection of low concentrations of urinary albumin (microalbuminuria). 88 randomly selected fresh urine samples from the central clinical laboratory of the Charité, Berlin/Germany were analysed. The diagnostic accuracy of the PreventID® Albumin test for the detection of urinary albumin excretion >18 mg/l was assessed by comparing the results with urinary albumin excretion determined by immunoturbidimetry as golden standard. In comparison with immunoturbidimetry the PreventID® Albumin test had a sensitivity of 96.2% and specificity of 97.1%. False negative results were found in 2.3% and false positive results were obtained in 1.1% of specimens. These findings suggest that the PreventID® Albumin test may be a useful and valid method for the screening of albuminuria. However, it should not be regarded as a diagnostic test. Positive results should be followed by quantification of urinary albumin or albumin/creatinine ratio by a laboratory based method.

Key words: Albuminuria, microalbuminuria, predictive marker, cardiovascular diseases, renal diseases, dipstick test, rapid test, sensitivity, specificity, validity.

Abbreviations: ACR, albumin/creatinine ratio; ROC, receiver operating characteristic

Brief Literature Review on Albuminuria Screening

Albuminuria is a powerful predictor of cardiovascular events in diabetic and hypertensive patients [1-5] as well as in the general population [6-11].

Measurement of urinary albumin is simple, relatively inexpensive, painless, and, along with blood pressure, cholesterol, and glucose, provides opportunity to identify patients with small-blood-vessel disease in the kidney, which is likely reflective of both small and large-blood-vessel disease throughout the body [12-14].

Recent post-hoc analyses of data from clinical trials in both diabetic and non-diabetic patients with early kidney disease are quite consistent in demonstrating that reduction of albuminuria or proteinuria is predictive of less likelihood of progression of kidney disease as well as fewer cardiovascular events [15-18].

The current recommendations by United States guideline committees (including the American Diabetes Association and JNC 7) include screening for albuminuria in patients with diabetes or evidence of kidney disease at regular intervals. European (including German) guidelines have concurred with this approach [19].

As mentioned above, albuminuria predicts cardiovascular events even in the general population. However, instead of screening the entire population, it has been advocated to screen those subjects aged 25 or older, with any of the following risk factors: male gender, low birth weight, diabetes, hypertension, obesity, smoking, and high salt and protein intake [20]. Of interest is a study showing that the presence of albuminuria more than doubles the predictive effect of the conventional atherosclerotic risk factors, be it male gender, smoking, hypertension, or hypercholesterolaemia [10]. Therapies that have been shown to lower albumin excretion, such as ACE inhibitors, angiotensin II receptor antagonists, statins and optimal glycaemic control should be started early in such patients to prevent them from reaching end-stage renal failure.

Microalbuminuria has been defined as an elevated urinary albumin value above the reference range for healthy subjects, but clinically undetectable by usual dipstick tests. An albumin excretion rate of 20-200 μg/min or 30-300 mg/d (equivalent to about 18-180 mg/l in case of normal urine output) has been used as an operational definition [21]. However, even a urinary albumin excretion rate above 4.8 μg/min (7 mg/d) has recently been reported as a strong and independent determinant of coronary heart disease and death [22]. Since there is no precise lower cut-off below which there is no increased risk [6, 8, 22] it seems preferable not to categorize according to the magnitude of albuminuria (micro- vs. macroalbuminuria), but simply to screen for detection with subsequent quantification.

Microalbuminuria progresses to overt proteinuria in a substantial proportion of patients. Studies of primary care physicians in the United States reveal astonishingly low levels of albuminuria screening even in high risk diabetic patients [23, 24]. European physicians are not doing much better [25]. In Germany, the recent implementation of a disease management program (DMP) is targeted to improve albuminuria screening in diabetic patients. Explanations for the poor performance have focused on the lack of immediate results for medical decision making with both spot urine samples and 24h urine collections in addition to the associated cost, patient inconvenience and patient non-compliance with the latter method [23, 24]. Nephelometry and immunoturbidimetry are laboratory techniques that comply with the requirements of precision and analytic range but they are not readily available, especially in primary care. Consequently, modified urine dipsticks, such as Micral® test strips, have been designed to allow an immediate and accurate detection of microalbuminuria on a small urine sample. Unfortunately, the reported range of sensitivity and specificity of this method has raised some concerns of reliability [26, 27].

The aim of the present study was to evaluate the diagnostic performance of the PreventID® Albumin test, a new dipstick device for the rapid detection of elevated urinary albumin.

Material, Methods and Statistics

PreventID® Albumin test devices were provided by Preventis (Bensheim, Germany). The test is based on the binding of urinary albumin to blue coloured latex beads. This binding is caused by specific monoclonal antibodies against human albumin attached to the beads. The beads are carried up the test device by wicking action. The test strip has human albumin fixed in the lower of two bands on the strip. At low levels of albumin in the urine sample, the blue beads bind to this band producing a blue coloured band in the lower half of the test window. The test window has a second band that binds the coloured beads even if they have reacted with urinary albumin. As the amount of albumin in the urine increases, more beads pass through the first band and are bound by the second band. After three minutes, the darkness or intensity of the two blue bands in the test window is compared. If the band in the upper half of the test window is lighter than or equal to the lower band, the test is negative (no elevated urinary albumin excretion present). If the band in the upper half is darker than the lower band, the test is positive. The cut-off value stated by the manufacturer is 18 mg/l.

This study was designed to evaluate the sensitivity and specificity of the PreventID® Albumin test.

Aliquots were taken from 88 randomly selected fresh 24 h urine samples that were routinely analysed in the central clinical laboratory of the Charité, Berlin/Germany. The patients' age ranged from 16 to 100 years with a mean of 53.8 years. 60.2% of patients were male. Albumin content was immediately determined in parallel by two methods. The widely regarded quantitative immunoturbidimetric method (Tina-quant Albumin in urine) which is established in the Institute of Laboratory Medicine of the Charité (Modular P800, Roche/Hitachi) served as golden standard. The PreventID® Albumin test was performed by an untrained assistant according to the manufacturers instructions. Furthermore, urinary creatinine concentration was measured by rate-blanked Jaffe compensated assay (Modular P800, Roche/Hitachi) in order to calculate the albumin/creatinine ratio (ACR), which is an accepted alternative and confirmatory method for the detection of albuminuria (positive when >30 mg/g) [28].

Results obtained by the PreventID® Albumin test were interpreted regarding its ability to detect a urinary albumin concentration >18 mg/l or ACR >30 mg/g as determined by laboratory techniques. The diagnostic performance was also illustrated by means of a receiver operating characteristic (ROC) curve. SPSS version 11.5 (Chicago, IL, USA) was used for statistical analysis.

Results

By immunoturbidimetric method the median urinary albumin concentration was discovered to be 42.7 mg/l with a range of 0-5620 mg/l. 35 patients (39.8%) were normoalbuminuric (<18 mg/l), 23 patients (26.1%) were microalbuminuric (18-180 mg/l) and 30 patients (34.1%) were macroalbuminuric (>180 mg/l).

Table 1. Diagnostic accuracy of PreventID® Albumin test for the detection of albuminuria >18 mg/l by immunoturbidimetry.

	Immunoturbidimetry	
PreventID® test	>18 mg/l	<18 mg/l
positive	51	1
negative	2	34

When compared to immunoturbidimetry regarding its ability to detect a urinary albumin concentration >18 mg/l (Table 1) the PreventID® Albumin test had a sensitivity of 96.2% and specificity of 97.1%. For the randomly chosen samples of this study results a positive predictive value of 98.1% and a negative predictive value of 94.4%. False negative results were found in 2.3% and false positive results were obtained in 1.1% of specimens. No false negative results occurred in the group of macroalbuminuric patients. Table 2 shows the data for sensitivity, specificity and predictive values considering different thresholds of urinary albumin concentration.

The area below the ROC curve is 0.98 (95% confidence interval 0.94-1.02; Fig. 1).

The ACR, serving as a second parameter for test validation, had a median of 100.4 mg/g in the analysed samples (0-329583 mg/g). 33 patients (37.5%) were found to be negative (<30 mg/g) and 55 patients (62.5%) were positive (>30 mg/g). When compared to the ACR the PreventID® Albumin test had a sensitivity of 89.1% and a specificity of 90.9%. For the randomly chosen samples of this study results a positive predictive value of 94.2% and a negative predictive

Table 2. PreventID® Albumin test evaluation using different thresholds for definition of elevated urinary albumin concentration.

Threshold albuminuria (mg/l)	>8	>18	>28	>38
Samples above threshold (%)	71.6	60.2	56.8	52.3
Sensitivity (%)	81.0	96.2	98.0	100
Specificity (%)	96.0	97.1	92.1	85.7
Positive predictive value (%)	98.1	98.1	94.2	88.5
Negative predictive value (%)	66.7	94.4	97.2	100
Correct classifications (%)	85.3	96.6	95.5	93.2

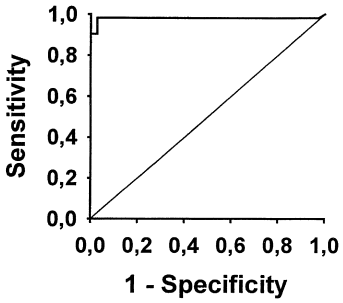

Fig. 1. Diagnostic accuracy of PreventID® Albumin test as determined by receiver operating characteristic (ROC) curve. The perfect classification rule has a ROC curve that follows the left and top borders of the graph.

value of 83.3%. False negative results were found in 6.8 and false positive results were obtained in 3.4% of specimens.

Discussion

The new PreventID® Albumin test is a rapid immunochromatographic strip test designed for the early detection of low concentrations of urinary albumin primarily in an ambulatory setting. The utility of the test depends on its ability to accurately predict which patients will be classified as either microalbuminuric or normoalbuminuric by means of a standard laboratory method.

The results of this study suggest that the PreventID® Albumin test is valid for the detection of albuminuria >18 mg/l with a high diagnostic accuracy. The high sensitivity (96.2%) assures that only few patients with elevated urinary albumin are overlooked. The number of false positive results (1.1%) is very low. This is pivotal for a screening test since costs for confirmatory testing have to be considered, especially when the indication for albuminuria screening is extended to a population less likely to have elevated urinary albumin.

A main competitor of the PreventID® Albumin test is the Micral® II test (Roche Diagnostics) which is commercially available since a few years. Several studies of different size have evaluated the Micral® II test strip in diabetic and non-diabetic patients. Study populations, study design and cut-off values vary between them. Thus, the comparison of validity parameters is not exact but gives an idea of the test performance. The sensitivity of the Micral® II test is reported to be within the range of 64-97% and reaches 100% in non-diabetic patients with kidney diseases. Specificity is reported to be in the range of 71-93% [26,27,29,30]. Validity parameters of the PreventID® Albumin test are on the upper limit or even above this range. Since its specificity appears higher, the PreventID® Albumin test might help to limit costs for the identification of false positive results. The predictive values strongly depend on the prevalence of albuminuria. This prevalence in the given clinical setting is a decisive factor in determining the utility of the PreventID® Albumin test and other test devices for the detection of elevated urinary albumin.

The PreventID® Albumin test seems applicable for the recently suggested practical screening approach to start with a dipstick test for albuminuria on a morning spot urine sample which has the advantage of being simple and relatively inexpensive [20,28]. Although the golden standard for defining whether there is an elevated urinary albumin excretion remains one (or preferably more) 24 hour urine sample(s), in daily practice a spot morning urine sample for measurement of albumin is frequently used [20]. Guidelines of the American National Kidney Foundation recommend that patients with a positive dipstick test for albuminuria should undergo confirmation by measuring the ACR on an untimed urine sample within 3 months [28]. This study demonstrates that the PreventID® Albumin test is also able to detect an elevated ACR with a sensitivity and specificity around 90%.

In summary, our findings suggest that the PreventID® Albumin test may be a useful and valid method for the screening of elevated urinary albumin excretion (microalbuminuria). However, it should not be regarded as a diagnostic test and a positive result should be followed by quantification of urinary albumin or ACR by a laboratory based method.

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