Brief Communication

Conventional dipsticks in the screening of microalbuminuria and urinary tract infections. *Killing 2 birds with one stone?*

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The amount of proteins excreted in the urine by normal adults (<150 mg/24 hours) is the result of collection of proteins from serum or renal origin and their degradation products. Under normal physiological conditions the most prevalent of the urine proteins excreted (up to 70 mg per day) is produced in the kidney, urine proteins from serum origin only accounting for up to 22 mg per day. Glomerular filtration barriers indeed markedly limit the filtration of normal to high-molecular weight serum proteins, and the proximal tubule efficiently reabsorbs low-molecular weight serum proteins (<40 kDa) filtered by the glomeruli. Therefore, an albumin excretion above 20 mg/L (microalbuminuria), increases the albumin to total protein ratio,[1] and is considered a diagnostic marker for chronic kidney disease (CKD) even in the presence of normal glomerular filtration rate.[2] Microalbuminuria is now also part of the strategy for cardiovascular risk assessment and immunometric systems specific for albuminuria are gradually replacing multiparametric conventional dipstick (MCD) in epidemiological studies.[3] However, the increased albumin excretion may also let the total urine protein concentration reach the first turning point of the MCD.[4] The semi-quantitative assessment with MCD indeed marks trace results in response to a protein concentration of as little as 150 mg/L and a distinct color change of the 1+ level at around 300 mg/L. The possibility to rule out urinary tract infections (UTI) with MCD was also reported.[5] The present study was thus performed to investigate the sensibility and specificity of MCD to estimate microalbuminuria and UTI in epidemiological studies.

Urine specimens arriving at the Central Laboratory of Careggi Hospital, Florence, Italy from February through May 2009 for total protein assay (n=280; 59% males; mean age 57 years, range 16-78), urinary albumin evaluations (n=454; 57% males; mean age 53, range 13-79) or suspected UTI (n=179; 43% males; mean age 46, range 14-68) were used. In patients with suspected UTI, urine was collected by the midstream clean-catch technique after preliminary exclusion of the subjects who had either taken antibiotics in the past 72 hours or symptomatic vaginal discharge (standardized instructions). All samples were processed within 2-4 hours after arrival. Test strip urinalysis was carried out using Aution sticks 10EA (Menarini Diagnostic, Florence, Italy) according to the manufacturer's instructions. Data were expressed as ordinal scale ("normal," "negative," "positive"; nominal concentrations). Total protein was measured with Pyrogallol red complex procedure (Advia 2400 analyser; Siemens Healthcare Diagnostic, Tarrytown, NJ, USA). Albumin in urine was measured with the immuno-nephelometric method (Immage 800, Beckman-Coulter, Brea, CA, USA). Urine culture was performed with an automated system (Robobact System, DIESSE Diagnostica Senese S.p.A., Siena, Italy). Independent predictors of UTI were investigated using 10^4 and 10^5 colony forming units (CFU)/mL as criteria for positivity of culture.[5] Statistical analysis was performed using the Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA). A p-value <0.05 was considered significant. Kappa for nominal data was used to assess concordance between raters. Different assay methods were compared with Chi^2 test for discrete readings, or linear regression analysis, and Pearson's correlation coefficients for continuous variables. Diagnostic accuracy was assessed by Receiver Operating Characteristic (ROC) curves. Predictors of UTI were investigated with stepwise logistic regression using MCD parameters (relative density, pH, nitrite, leukocyte esterase, hemoglobin, or protein) as independent variables.

The relationship between total protein and albumin urinary concentrations was preliminary assayed in 80 urine samples with normal protein electrophoresis values. Notwithstanding the close correlation between total protein and albumin urinary concentration (y = 0.643 x -37.11; r = 0.9572; p=0.009), a non-uniform relationship was confirmed with slope change at around 150 mg/L total protein and 20 mg/L albumin concentrations (**Figure 1**). The impact of rating in ranking of proteinuria readings was then assessed in the first 84 samples. Fifty out of the 84 dipsticks (59.5%) were allocated in the same group by the 3 operators, 32 (38.1%) received a different allocation by one of the operators. Only 2 strips (2.4%) received a different allocation by the 3 readers. Kappa for nominal data revealed a significant concordance of raters (p=0.009).

Accuracy of the stick to discriminate positive and negative responses for proteinuria and the uniformity of correct/incorrect results compared with reference methods along the measurement range (0-12,000 mg/L) are reported in **Table 1**. A Chi^2 test for correct/incorrect readings for total protein showed good discriminating capacity (p=0.001). Furthermore, Chi^2 tests on the over/correct/under readings for the 6 protein ranges showed homogeneity of response (p=0.001 for all). In particular, 157 out of 280 specimens (56%) had urinary

Disclosure. This study was supported by a research grant from the "Ministero dell'Università e della Ricerca, Direzione Generale per le strategie e lo sviluppo dell'inte rnazionalizzazione della ricerca scientifica e tecnologica" and "Ministero degli Affari Esteri" within the frame of the Executive Programme of Scientific and Technological Cooperation between Italy and Yemen for the years 2006-2009 (Grant #269/P/0116202).

total protein levels over 150 mg/L. The diagnostic accuracy of MCD for total proteins (>150 mg/L) revealed 100% and 91% sensitivity with 58% and 69% specificity at the score levels of trace and 1+ (area under the curve [AUC] 91%) (**Table 1**). The drawback of conventional dipstick test is that urine concentration/dilution modifies the results. However, the correction of protein values for 24 hour urinary excretion only lightly shifted results (sensitivity 91%, specificity 69% for the trace level). When urinary samples were tested for albumin with the reference method, 138 out of the 454 specimens (30%) showed albumin concentrations over 20 mg/L. Sensitivity and specificity of MCD for microalbuminuria (urinary albumin concentration >20 mg/L) were 88% and 81% at the score levels of trace (AUC 88%) (**Table 1**). Ninety-two out of the 179 specimens fulfilled the 10^4, and 61 fulfilled the 10^5 CFU/mL criteria for positivity of urine culture. Nitrites and leukocyte esterase strip tests were significantly associated with UTI at Chi^2 test (p=0.001 for both), both tests being selected as independent predictors of UTI at binary logistic regression analysis. The combined positivity to nitrites and leucocyte esterase had a sensitivity of 72%, and specificity of 82% for the 10^4 CFU/mL diagnostic criteria, and 72% and 85% for the >10^5/mL criteria.

In conclusion, the main result of the present study is that the first turning point for protein (trace), corresponding to the threshold level of 150 mg/L, also detects subjects with over 20 mg/L of urinary albumin with 88% sensitivity and 80% specificity, thus suggesting the potential value of MCD in the screening of microalbuminuria. Although optical MCD may be less sensitive and specific than instrumental assessment, the potential source of variability connected to operator reading did not introduce any further bias so that optical readings have a good discriminating capacity to allocate results in correct range categories of protein excretion and can be used in epidemiological studies. In addition, due to the low cost of MCD when compared to microalbuminuria dipstick (0.30€ and 1.30€) the potential value as a mass screening tool should be reconsidered. Current guidelines recommend excluding the presence of UTI before assessing protein urinary excretion. The presence of UTI can be hardly preliminarily excluded in epidemiological door-to-door studies, and MCD might be of help to select patients with UTI. However, the sensitivity of the combination of nitrites and leukocyte-esterase to screen UTI is low (72%) as reported in other different patient groups (68-88%).[5] However, in door-to-door studies, the distance of the laboratory and time required to despatch samples might induce more analytical errors than those associated with the use of a simple point of care method.

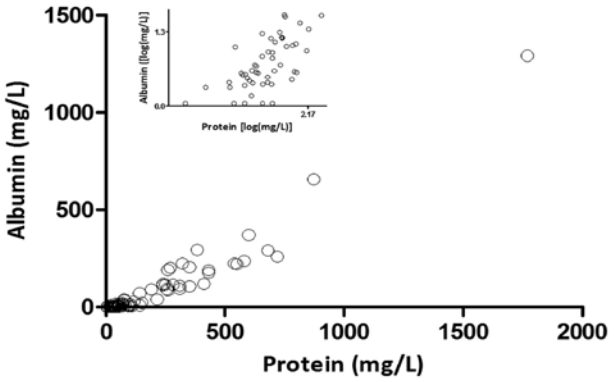

Figure 1 - Relationship between total protein and albumin urinary concentration assessed with reference methods subjects with normal electrophoresis of urinary proteins (n=80) (y = 0.643 x -37.11; r = 0.9572; p=0.009). Inset reports in logarithmic scale data present in the region of the cut-off limits of total protein (150 mg/L = 2.17) and albumin (20 mg/L = 1.3).

Table 1 - Classification of auction-stick optical readings and diagnostic performance by total protein (n=280) and albumin (n=454).

Variable	Auction-stick optical readings						Total
	0	trace	1	2	3	4	
Protein (mg/L)							
<150	71	45	7	0	0	0	**123**
150-299	0	47	15	0	0	0	**62**
300-499	0	5	32	11	1	0	**49**
500-999	0	1	0	14	3	1	**19**
1000-2999	0	0	0	0	5	2	7
>3000	0	0	0	0	3	17	**20**
Total	**71**	**98**	**54**	**25**	**12**	**20**	**280**
Albumin (mg/L)							
<20	257	56	2	1	0	0	**316**
>20	16	75	19	12	8	8	**138**
Total	**273**	**131**	**21**	**13**	**8**	**8**	454

Received 24th January 2010. Accepted 22nd March 2010.

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