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Urinary β-trace protein

A unique biomarker to screen early glomerular filtration rate impairment

Carlo Donadio, MD*, Laura Bozzoli, MD

Abstract

The screening for chronic kidney disease (CKD) patients needs the measurement of serum markers like creatinine. Our previous results indicated that urinary excretion of β-trace protein (BTP), a low-molecular-weight protein (23–29 kDa), is increased in CKD patients from stage 2. The aim of this study was to assess the major determinants of urinary excretion of BTP and to evaluate its feasibility as noninvasive marker of glomerular filtration rate (GFR) impairment.

We studied 355 CKD patients (198 males), aged 15 to 83 years, in stable clinical conditions, classified in the different stages of CKD on the basis of GFR (renal clearance of ^{99m}Tc-diethylenetriamine penta-acetic acid). At the same time, we measured serum and urinary creatinine and BTP, and urinary albumin. Urinary excretion of BTP and albumin was expressed as mg/g urinary creatinine. Fractional clearance of BTP was calculated as the ratio of BTP clearance to creatinine clearance (%).

Urinary excretion of BTP is mainly determined by its serum concentration and by the level of GFR, and to a lower extent by urinary albumin excretion. In fact, urinary BTP (U-BTP) and fractional clearance of BTP progressively and significantly increased along with the reduction of GFR and the concurrent rise in serum BTP (S-BTP). The relationship of U-BTP with GFR was very similar to that of S-BTP with GFR: U-BTP mirrors S-BTP. The accuracy of U-BTP to screen patients with GFR <90 mL/min/1.73 m^2 was good (area under the curve 0.833), its sensitivity was 76.9%, specificity 80%, and positive predictive value 84.9%. Sensitivity of U-BTP was quite similar to that of S-BTP and serum creatinine.

The major determinants of urinary excretion of BTP are S-BTP and GFR. U-BTP may be a suitable noninvasive marker to screen the general population for detection of GFR <90 mL/min/1.73 m^2.

Abbreviations: AKI = acute kidney injury, AUC = area under the curve, B2M = β2-microglobulin, BTP = β-trace protein, CKD = chronic kidney disease, DTPA = diethylenetriamine penta-acetic acid, GFR = glomerular filtration rate, LMWP = low-molecular-weight protein, MW = molecular weight, NPV = negative predictive value, PPV = positive predictive value, ROC = receiver-operating characteristic, S-BTP = serum β-trace protein, S-Cr = serum creatinine, U-Alb = urinary albumin, U-BTP = urinary β-trace protein.

Keywords: β-trace protein, chronic kidney disease, glomerular filtration rate, low-molecular-weight proteins, noninvasive screening, sensitivity, specificity

1. Introduction

The identification of chronic kidney disease (CKD) patients at early stages of impairment of renal function may slow the progression of CKD and possibly reduce the number of incident patients who need the replacement of renal function. Furthermore, a beneficial effect on cardiovascular system is expected, since the cardiovascular risk increases with the impairment of glomerular filtration rate (GFR). Finally, the measurement, or the estimate, of GFR is essential to establish the stage of CKD and to reduce the burden of drug nephrotoxicity, even in patients without evidence of CKD. Urinalysis and urinary albumin (U-Alb) excretion, which are useful markers of kidney disease, cannot quantify the impairment of GFR. Therefore, the screening for subjects with impaired GFR needs the measurement of serum markers like serum creatinine (S-Cr) and/or cystatin C, or the measurement of creatinine clearance. Low-molecular-weight proteins (LMWPs) are cleared from the blood mainly through glomerular filtration, followed by an almost complete reabsorption by proximal tubular cells.[1,2] After degradation to smaller peptides and amino acids, their fragments are reabsorbed into peritubular circulation, whereas urinary excretion of LMWPs is null. Due to this peculiar renal handling, the serum concentration of cystatin C, β2-microglobulin (B2M), and other LMWPs has been proposed as a serum marker of GFR impairment, whereas the increase in their urinary excretion is considered a marker of tubular damage. Indeed, an increased urinary excretion of LMWPs can be observed also in patients with very low GFR, at end-stage renal disease.[3,4] β-trace protein (BTP), which is also known as lipocalin-type prostaglandin D synthase, is a small

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protein (23–29 kDa, depending on the different glycosylation of the molecule) isolated primarily from cerebrospinal fluid.[5–7] Like other LMWPs, BTP is taken up by tubular cells and actively degraded within their lysosomes to produce the N-terminal-truncated form.[8] Previous studies demonstrated that serum BTP (S-BTP) is an adequate marker of GFR impairment with a diagnostic accuracy similar to those of S-Cr, cystatin C, and B2M.[9–12] Our previous data indicate that urinary excretion of cystatin C, B2M, and retinol-binding protein is increased when GFR is below 30 mL/min/1.73 m^2, whereas an increase in urinary BTP (U-BTP) is observed already in patients at CKD stage 2.[13] These results suggest the feasibility of LMWPs and namely BTP as a urinary marker to screen for patients with GFR impairment.

The aim of this study, performed in a group of CKD patients with different levels of GFR, was to assess the major determinants of urinary excretion of BTP and to evaluate its feasibility as a noninvasive marker of GFR impairment.

2. Patients and methods

2.1. Patients

Setting was the laboratory for the evaluation of GFR of the division of nephrology at the Department of Clinical and Experimental Medicine of the University of Pisa.

Inclusion criteria were as follows: measurement of GFR for the assessment of renal function in CKD patients clinically stable; in renal transplant recipients; in potential living kidney donors, and in patients with cancer scheduled for chemotherapy. Exclusion criteria were as follows: recent administration (within 2 weeks) of potentially nephrotoxic drugs or contrast media; acute kidney injury (AKI).

Four hundred eighty-five patients were examined (Fig. 1). The great majority of examined patients were on ambulatory follow-up of already diagnosed kidney diseases. Ninety patients did not fulfill the inclusion criteria. The measurement of the index test (U-BTP, mg/g creatinine) was obtained in 386 patients. The measurement of GFR (reference test) was not adequate in 34 patients, mainly due to technical reasons (inability of some patients to urinate on command, or inadequate volume of blood sampling). The remaining 355 CKD patients affected by different kidney diseases, in stable clinical conditions, with various degree of impairment of renal function, are analyzed in the present study (Table 1, Suppl File Urinary BTP Medicine.xls, http://links.lww.com/MD/B441). The ethnicity was Caucasian for all patients.

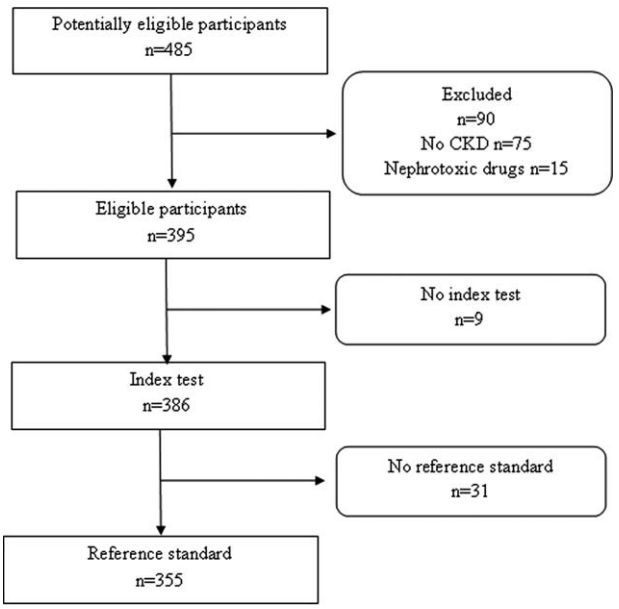

Figure 1. Flow of participants during the study.

Table 1

Demographic and clinical characteristics of the 355 examined patients (males 198, females 157), and underlying kidney disease.

	Range	Median	IQR 25–75
Age, y	15–83	53	41.3–64.0
Weight, kg	36.6–131	71	60.7–82.5
Height, cm	140–195	164	157.0–171.8
Body surface area, m^2	1.25–2.53	1.76	1.62–1.93
Body mass index, kg/m^2	16.3–40.0	26.1	23.2–29.4
Serum creatinine, mg/dL	0.4–12.1	1.35	0.9–2.6
Kidney disease			
Glomerulonephritis	91 (25.6)		
Chronic renal failure	65 (18.3)		
Ischemic nephropathy	50 (14.1)		
Interstitial nephropathy	42 (11.8)		
Diabetic nephropathy	30 (8.5)		
Polycystic and other cystic kidney disease	25 (7.0)		
Renal transplant recipients	19 (5.4)		
Kidney donors	9 (2.5)		
Others	24 (6.8)		

Data are expressed as number (percent), range, median, and interquartile range (IQR) 25–75.

The study was approved by the Institutional Ethical Committee of Azienda Ospedaliero-Universitaria Pisana and was conducted in accordance with guidelines of Helsinki declarations. All patients gave their informed consent.

2.2. Methods

Glomerular filtration rate was measured, in the morning before breakfast, with a radio-isotopic method, as the renal clearance of ^{99m}Tc-diethylenetriamine penta-acetic acid (DTPA).[14,15]. The measurement of ^{99m}Tc-DTPA clearance is a reference method for the measurement of GFR. In fact, its renal clearance is only slightly lower than inulin clearance, due to a modest link to plasma albumin. The coefficient of variation of ^{99m}Tc-DTPA GFR, tested on duplicate measurement, was approximately 8% (our laboratory data). The results were adjusted, as usual, to the standard body surface of 1.73 m^2. Patients were classified in the different stages of CKD on the basis of the value of the GFR measurement, using the modified classification of CKD, which divides the stage 3 into 3a (GFR 45–60 mL/min/1.73 m^2) and 3b (GFR 30–45 mL/min/1.73 m^2).[16]

Blood and urine samples were drawn at the time of GFR measurement. Serum and urine samples were divided into Eppendorf tubes, which were hermetically closed and stored at −20°C, up to the time of biochemical determinations.

Serum and urinary concentrations of creatinine were measured with a rate-blanked creatinine/Jaffé method (CREA Roche/Hitachi automated analysis for Hitachi 917, Roche Diagnostics, Mannheim, Germany; reference intervals for serum concentration are 0.50–0.90 mg/dL in women and 0.70–1.20 mg/dL in men).

Serum and urinary concentrations of BTP were measured with a particle-enhanced immune-nephelometric method (N Latex BTP assay, Siemens Healthcare GmbH, Erlanger, Germany). Upper reference range (95 percentile), men and women: serum = 0.70 mg/L, urine = 3.75 mg/L; coefficient of variation within laboratory <6.6%.[17]

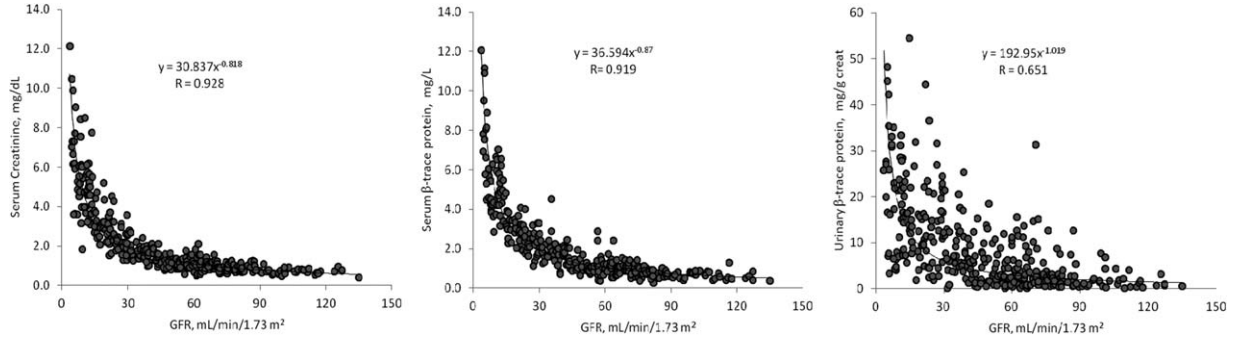

Figure 2. Correlation with glomerular filtration rate (GFR) of serum creatinine, serum, and urinary β-trace protein (BTP). The data of individual patients and the regression lines are plotted. The regression equations and the coefficients of correlation with GFR are reported.

Different reference intervals are reported from literature for S-BTP (2.5%–97.5%) are 0.37 to 0.77 mg/L in men and 0.40 to 0.70 mg/L in women[12]; upper reference limits (97.5 percentile) for U-BTP were 7.79 mg/L for men and 3.13 mg/L for women[18]; upper reference limits (90 percentile) for U-BTP were 3.5 mg/g creatinine for men and 2.5 mg/g creatinine for women.[19]

Urinary albumin was measured with an immune-nephelometric method (N antiserum to human albumin, Siemens).

Fractional clearance of BTP was calculated as: 100 × (U-BTP [mg/L] × S-Cr [mg/dL])/(S-BTP × urinary creatinine).

The renal threshold for tubular reabsorption of BTP was estimated from the plot of the logarithm of U-BTP (mg/L) versus S-BTP (mg/L).[20]

2.3. Statistical analysis

The correlation coefficients between GFR and the serum and urinary markers were measured. The significance of the differences among correlation coefficients was tested.[21] The significance of the differences between 2 independent samples and between 2 paired samples was tested using the nonparametric Mann–Whitney and Wilcoxon tests, respectively. The diagnostic accuracy of markers was assessed using receiver-operating characteristic (ROC) analysis. On the basis of the value of the area under the curve (AUC), the accuracy was considered excellent (AUC = 0.90–0.99), good (AUC = 0.80–0.89), fair (AUC = 0.70–0.79), and poor (AUC = 0.60–0.69). Stepwise multiple regression analysis was used to establish the determinants of U-BTP excretion.[22]

Statistical analysis was performed using MedCalc (version 16.2.0; MedCalc Software, Mariakerke, Belgium). $P < 0.05$ was considered significant.

3. Results

Urinary excretion of BTP (U-BTP), which was modest in patients with GFR >90 mL/min/1.73 m^2, progressively increased in patients with lower GFR. The statistically significant negative correlation found between U-BTP and the value of GFR ($r = 0.650$, $P < 0.000001$) is shown in Fig. 2, where are represented, for comparison, also the correlations of S-Cr and S-BTP with GFR in the same patients. S-Cr, serum concentrations, and urinary excretion of BTP were significantly higher in men than in women ($P < 0.0001$), and the values of GFR were significantly lower in men ($P < 0.05$) (Table 2). No significant difference was found for age and U-Alb excretion according to the sex of patients. The values of U-BTP in patients at CKD stage 2 (GFR 60–90 mL/min/1.73 m^2) were already significantly higher ($P < 0.05$) than in patients at CKD stage 1 (GFR >90 mL/min/1.73 m^2) (Table 3). The difference versus patients at CKD stage 1 progressively increased and became highly significant in patients with lower GFR ($P < 0.001$ at stage 3a, $P < 0.0001$ at stages 3b, 4, and 5). Fractional clearance of BTP also progressively and significantly increased in patients with reduced GFR, indicating that tubular reabsorption of BTP decreases according to the stage of CKD (Table 3). U-Alb excretion resulted significantly higher ($P < 0.05$) only in patients with GFR <45 mL/min/1.73 m^2 versus those with GFR >90 (Table 3). The negative correlation between U-BTP and GFR became even more significant when patients were clustered in groups according to their CKD stage. The exponential terms of the increase of U-BTP and of fractional clearance of BTP with the reduction of GFR were similar to that of S-BTP with GFR (Fig. 3). The renal threshold for tubular reabsorption of BTP is reached for a value of approximately 1 mg/L of S-BTP (Fig. 4).

Multiple regression analysis (stepwise) indicated that the determinants of U-BTP are mainly S-BTP and GFR values, and to a lower extent U-Alb excretion. Stepwise analysis excluded the other tested parameters (age, body weight, and height) from the model (Table 4). The regression equation was: U-BTP (mg/g creatinine) = 6.4 + (2.46 × S-BTP [mg/L]) − (0.0698 × GFR [mL/min/1.73 m^2]) + (0.0082 × U-Alb [mg/g] creatinine); R^2 adjusted = 0.52.

The accuracy of U-BTP to screen patients with GFR lower than 90 mL/min/1.73 m^2 was quite satisfactory. In fact, using as cut-off value of U-BTP >2.24 mg/g creatinine, the accuracy was good (AUC 0.833, 95% confidence interval [CI] 0.790–0.870, $P < 0.00001$), the sensitivity was 76.9%, and the specificity 80.0% (Table 5).[22]

The accuracy of S-Cr (AUC = 0.928, 95% CI 0.896–0.953, $P < 0.00001$) and S-BTP (AUC = 0.909, 95% CI 0.873–0.937, $P < 0.00001$) resulted excellent, and significantly better than that of U-BTP ($P < 0.01$ vs S-Cr, $P < 0.05$ vs S-BTP). Anyway, the sensitivity of U-BTP was identical to those of S-Cr and S-BTP. Assuming the prevalence of 58.3% of subjects with GFR <90 mL/min/1.73 m^2, found in epidemiological analysis of general population,[23] the positive predictive value (PPV) becomes 84.9%, indicating this probability that a patient with a positive test (U-BTP >2.24) has a GFR <90 mL/min/1.73 m^2. As expected, a screening based on blood test has better PPV (97.1% for both S-Cr and S-BTP), whereas the negative predictive values (NPVs) were quite similar to that of U-BTP.

Table 2

Serum creatinine concentrations, serum concentrations, and urinary excretion of β-trace protein, urinary albumin excretion, and values of glomerular filtration rate.

	n	Range	Median	IQR 25–75
All patients				
Age, y	355	15–83	53	41.3–64.0
Serum creatinine, mg/dL	355	0.4–12.1	1.35	0.9–2.6
Serum β-trace protein, mg/L	347	0.3–12.1	1.35	0.9–2.8
Urinary β-trace protein, mg/g creatinine	355	0.07–54.5	5.69	1.9–12.7
Urinary albumin, mg/g creatinine	353	0–1950	12.4	3.4–93.3
Glomerular filtration rate, mL/min/1.73 m^2	355	3.6–134.9	46.85	23.3–70.6
Men				
Age, y	198	15–79	51.74	42–64
Serum creatinine, mg/dL	198	0.60–12.13	1.73**	1.10–3.22
Serum β-trace protein, mg/L	194	0.36–12.06	1.68**	1.03–3.11
Urinary β-trace protein, mg/g creatinine	198	0.07–54.53	7.46**	2.82–15.52
Urinary albumin, mg/g creatinine	197	0–1950.0	11.8	2.7–91.7
Glomerular filtration rate, mL/min/1.73 m^2	198	3.6–127.4	41.5 °	21.1–65.8
Women				
Age, y	157	17–83	54.0	40.8–65
Serum creatinine, mg/dL	157	0.40–7.02	0.97	0.75–1.67
Serum β-trace protein, mg/L	153	0.27–11.15	1.01	0.74–2.30
Urinary β-trace protein, mg/g creatinine	157	0.12–48.21	3.64	1.44–8.36
Urinary albumin, mg/g creatinine	156	0–1769.2	12.8	5.0–96.9
Glomerular filtration rate, mL/min/1.73 m^2	157	4.6–134.9	53.2	29.6–75.2

The results obtained in all patients and separately in men and women are reported. Data are expressed as number, range, median, and interquartile range (IQR) 25 to 75. The significance of the differences between men and women are reported ($^{**}P<0.0001$; $^{***}P<0.05$).

Table 3

Serum creatinine concentrations, serum concentrations, urinary excretion, and fractional clearance of β-trace protein, urinary excretion of albumin, and values of glomerular filtration rate.

	GFR >90 mL/min/1.73 m^2	GFR >60 to <90 mL/min/1.73 m^2	GFR >45 to <60 mL/min/1.73 m^2	GFR >30 to <45 mL/min/1.73 m^2	GFR >15 to <30 mL/min/1.73 m^2	GFR <15 mL/min/1.73 m^2
Number of patients	30	103	49	55	60	58
Age, y	35.4 ± 12.4	48.7 ± 15.1**	56.9 ± 13.2**	54.6 ± 15.2**	56.9 ± 12.7**	56.9 ± 13.5**
Serum creatinine, mg/dL	0.70 ± 0.15	0.96 ± 0.27**	1.10 ± 0.25**	1.67 ± 0.41**	2.67 ± 0.89**	5.48 ± 2.00**
Serum β-trace protein, mg/L	0.66 ± 0.20	0.91 ± 0.30**	1.10 ± 0.45**	1.82 ± 0.60**	2.66 ± 0.69**	5.61 ± 2.01**
Urinary β-trace protein, mg/g creatinine	1.86 ± 1.89	4.14 ± 4.73***	4.94 ± 4.33*	7.60 ± 5.52**	14.35 ± 8.89**	20.24 ± 11.79**
Fractional clearance of β-trace protein,%	2.03 ± 1.99	4.52 ± 5.67***	5.24 ± 4.90*	7.65 ± 6.55**	14.73 ± 9.73**	19.82 ± 10.56**
Urinary albumin, mg/g creatinine	40.2 ± 61.9	70.4 ± 226.2	71.0 ± 136.5	82.5 ± 183.6***	112.4 ± 160.0***	164.4 ± 287.6***
GFR, mL/min/1.73 m^2	107.7 ± 11.9	72.6 ± 8.2**	53.2 ± 4.3**	37.8 ± 4.4**	23.0 ± 4.6**	9.5 ± 3.1**

Patients are clustered according to the stages of chronic kidney disease. Data are expressed as number, mean ± standard deviations. The significance of the differences versus the patients with GFR >90 mL/min/1.73 m^2 are indicated: $^{*}P<0.001$; $^{**}P<0.0001$; $^{***}P<0.05$.

The values of AUC, sensitivity, and specificity of the 3 index tests as indicators of GFR <60 mL/min/1.73 m^2 were similar to those found as indicators of GFR <90 mL/min/1.73 m^2, whereas the cut-off values were higher. Due to the lower prevalence (8.1%) of patients with GFR <60 mL/min/1.73 m^2,[23] the PPVs were quite low, whereas the NPVs were definitely high. Similar results of sensitivity, specificity, PPV, and NPV of U-BTP were found versus GFR predicted using modification of diet in renal

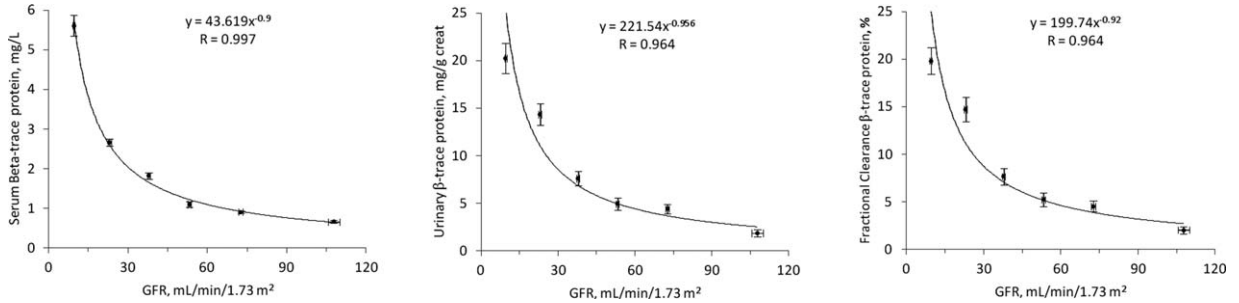

Figure 3. Correlation with glomerular filtration rate (GFR) of serum concentration, urinary excretion, and fractional clearance of β-trace protein (BTP). Patients are clustered according to chronic kidney disease (CKD) stage. Mean values and standard errors of the mean are represented for BTP and for GFR.

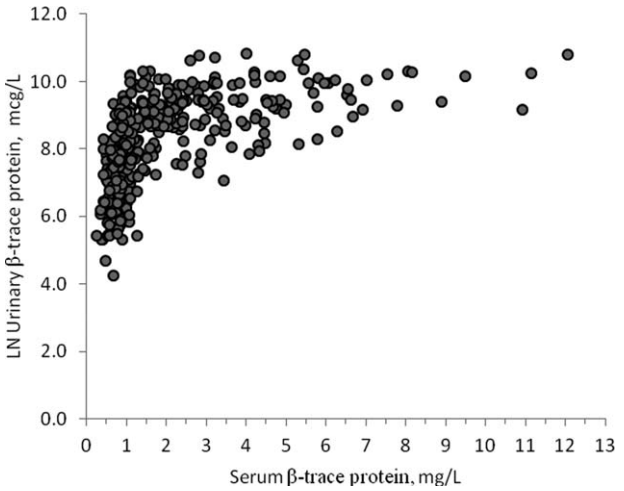

Figure 4. β-trace protein (BTP): urinary versus serum concentrations (semilogarithmic scale).

disease (4 variables, isotope dilution mass spectrometry), CKD-EPI Cr, and CKD-EPI Cr-Cys formulae (Suppl Table, http://links.lww.com/MD/B442).

The sensitivity of the examined test, to screen the patients with GFR <90 mL/min/1.73 m^2, increases to ~90% using lower cut-off values: U-BTP 1.08 mg/g creatinine, S-BTP = 0.71 mg/L; S-Cr = 0.8 mg/dL. In the mean time, the specificity and PPVs of the tests decrease, whereas NPVs increases.

The accuracy of U-BTP, to screen for a GFR <90 mL/min/1.73 m^2, was higher in men than in women (AUC 0.866 and 0.783, respectively) (Fig. 5). Also, the cut-off value was different in men (U-BTP >4.32 mg/g creatinine) than in women (2. 24 mg/g creatinine).

4. Discussion

It is generally acknowledged that a more significant reduction in the progression of CKD patients to advanced renal failure is linked to an earlier detection of CKD, possibly when the impairment in GFR is still moderate. Furthermore, the identification of patients with impaired renal function is relevant to reduce the risk of cardiovascular disease, to decrease the burden of nephrotoxicity due to the administration of inadequate doses of drugs or xenobiotics, and to reduce the risk of postsurgical AKI. Note that a reduction in GFR is frequent in aged patients, independently from the presence of CKD, and S-Cr underestimates the impairment of GFR in aged patients with reduced muscle mass.

A screening for early impairment of GFR responds to both classic and revisited Wilson and Jungner screening criteria.[24,25] Many factors affect the possibility of an efficient screening for functional renal impairment. First, the performance of the diagnostic test, that is its sensitivity and specificity, which, together with the prevalence of the disease in the examined population, determine the PPV and NPV of the test. In the screening procedure, it is important to avoid either high percentages of false-positive results, which may lead to negative psychological consequences for patients, or of false-negative results. Also relevant, for the practical execution, is the simplicity of the administered test. Thus, a screening based on a urinary marker has an evident advantage, being noninvasive, in comparison with a screening based on a blood marker. In fact, using a free self-test for screening albuminuria in the general population resulted in a large response and a number of newly detected diseases.[26]

Table 4

Multiple linear regression modeling (stepwise) for urinary β-trace protein excretion (mg/g creatinine) based on serum β-trace protein concentration (mg/L), sex, GFR (mL/min/1.73 m^2), urinary albumin excretion (mg/g creatinine), age, body weight, height, and body mass index.

Independent variables	Coefficient	Standard error	*t*	Probability
Constant	6.33			
Serum β-trace protein, mg/L	2.39	0.273	8.773	<0.0001
Sex, male	2.06	0.717	2.878	<0.005
Glomerular filtration rate, mL/min/1.73 m^2	−0.0682	0.0174	−3.915	0.0001
Urinary albumin, mg/g creatinine	0.0085	0.00175	4.859	<0.0001

Variables not included in the model: age, body weight, height, and body mass index.
GFR = glomerular filtration rate.

Table 5

Receiver-operating curve (ROC) analysis of serum creatinine and serum and urinary β-trace protein as indicators of a glomerular filtration rate (GFR) lower than 90 or 60 mL/min/1.73 m^2.

	Cut-off	AUC	AUC, 95% confidence interval	Sensitivity, %	Specificity, %	PPV, %	NPV, %
GFR <90 mL/min/1.73 m^2, prevalence 59.3%							
Serum creatinine, mg/dL	>0.94	0.928*	0.896–0.953	76.9	96.7	97.1	74.2
Serum β-trace protein, mg/L	>0.89	0.909*	0.873–0.937	77.0	96.7	97.1	74.2
Urinary β-trace protein, mg/g creatinine	>2.24	0.833*	0.790–0.870	76.9	80.0	84.9	70.4
GFR <60 mL/min/1.73 m^2, prevalence 8.1%							
Serum creatinine, mg/dL	>1.25	0.928*	0.896–0.952	79.73	93.23	50.9	98.1
Serum β-trace protein, mg/L	>1.42	0.918*	0.885–0.945	75.12	96.92	68.3	97.8
Urinary β-trace protein, mg/g creatinine	>4.38	0.816*	0.771–0.865	77.03	77.44	23.1	97.5

The cut-off values, the area under the curve (AUC), and the values of sensitivity and specificity of the tests are reported. On the basis of the prevalence in the general population of the GFR values, we calculated the positive predictive values (PPVs), the negative predictive values (NPVs), and the likelihood ratios of the different tests. The statistical significance of the AUC values is indicated: *$P < 0.00001$.

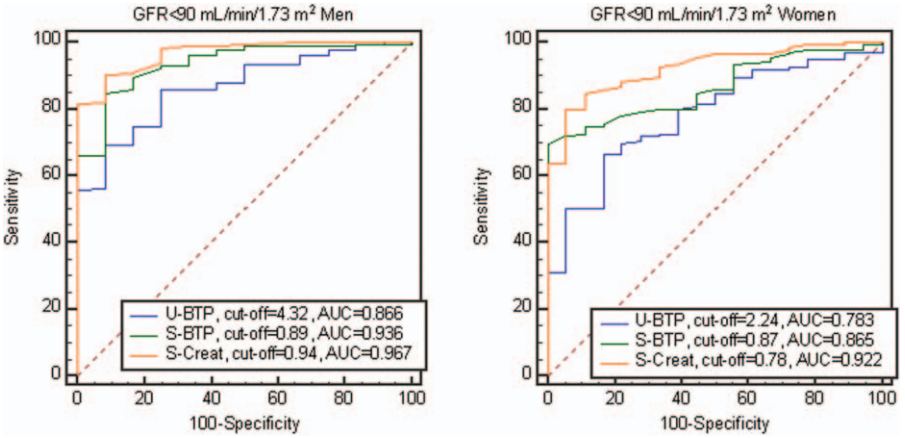

Figure 5. Receiver-operating curve (ROC) analysis in men (A) and in women (B) of serum creatinine and serum and urinary β-trace protein (BTP) as indicators of a glomerular filtration rate (GFR) lower than 90 mL/min/1.73 m^2. The values of cut-off and the areas under the curve (AUCs) are reported.

Up to now, urinary biomarkers have been proposed for the early diagnosis and prognosis of AKI,[27] to predict cardiovascular risk or progression of CKD,[28] or to evaluate tubular damage and nephrotoxicity.[29,30] No urinary biomarker has been validated up to now for the early diagnosis of GFR impairment in CKD patients. Our previous data indicated that U-BTP increased in CKD patients with GFR <90 mL/min/1.73 m^2.[13]

The present study, for the first time, aimed to evaluate the determinants of U-BTP, the relationship between U-BTP and measured GFR, and the feasibility of BTP as a urinary biomarker to screen early GFR impairment. The study was performed in a group of clinically stable patients with different degrees of impairment in GFR (from normality to advanced renal failure). We excluded patients with inherited tubular disease or acquired tubular malfunction, since high values of U-BTP may be found in these patients. The weakness of this study is the lack of an external control group. One limitation with measuring BTP, as unlike other novel biomarkers like cystatin C, is the lack of reference standard. Hence, there may be significant inter and intralaboratory variation. The strength of the study is the number of examined patients and the direct measurement of GFR with a "gold standard" method.

Our results indicate that, differently from other LMWPs, BTP is present in the urine of patients with normal GFR, and its urinary excretion progressively increases along with the reduction of GFR and the concurrent rise in S-BTP. In the mean time, the fractional clearance of BTP progressively increases with the reduction of GFR, suggesting a decrease in its tubular reabsorption. In fact, urinary excretion of BTP, as demonstrated in multiple regression analysis, is determined by its serum concentration, by albumin excretion, and, inversely, by GFR. A positive correlation between U-BTP and U-Alb had been already found in diabetic patients[19] and also in renal patients.[31] The interpretation of this finding may be different: from increase in glomerular permeability to competition at tubular level for the same transport system.[32,33] BTP and albumin possibly compete for the same receptor complex megalin/cubilin/amnionless.[34] A competition with albumin has already been demonstrated for other LMWPs.[33] After glomerular filtration, BTP also undergoes proximal tubular reabsorption.[8] The system appears already saturated for the transport of BTP at normal GFR, as indicated by the measurable excretion of BTP in these patients. On the contrary, the filtration coefficient of BTP (molecular weight [MW] 23–29 kDa) is probably lower than those of smaller molecules like creatinine and B2M. This hypothesis is supported by the fact that fractional clearance of BTP does not exceed 40%, even in patients at CKD stage 5. In these patients, if glomerular filtration of BTP was free, tubular reabsorption of BTP should be null and its fractional clearance should approximate 100%. In fact, fractional clearance of B2M (MW 11.8 kDa, filtration coefficient ~1) reaches the value of 90% in patients with very low GFR.[35]

The results of the present study are in large agreement with other studies that evaluated the excretion of BTP in renal disease. In fact, literature data indicate a higher urinary excretion of BTP in hypertensive patients, increasingly along with advance in renal dysfunction, in diabetic patients with subclinical renal injury or with cardiovascular complications, in lupus nephritis patients according to the activity of disease and efficacy of treatment, and also in Anderson–Fabry disease.[36–43]

Other data strongly suggest that BTP may be useful as a diagnostic marker for early detection of renal tubular damage.[18,44] Urinary excretion of BTP was also correlated with urinary excretion of other LMWPs, commonly used as markers of tubular damage, but was also significantly correlated with the impairment of estimated GFR.[31]

Different literature data indicate a difference in U-BTP, linked to sex. In fact, U-BTP was higher in men than in women, either in normal subjects or in CKD patients.[18,19] Similarly, the best cut-off values of U-BTP to predict renal disease or diabetic microalbuminuria resulted higher in men (3.2–4.2 mg U-BTP/g creatinine) than in women (2.9–2.8 mg/g).[19] These cut-off values are very similar to those that we found as the best values to screen for GFR <90 mL/min/1.73 m^2 in men (4.32 mg/g creatinine) and in women (2.24 mg/g creatinine).

The novelty of the present paper is the finding that the relationship between U-BTP and GFR is very similar to that between S-BTP and GFR: U-BTP mirrors S-BTP. The possible explanation of this behavior is that any increase in the glomerular and then tubular charge of BTP escapes tubular reabsorption, which is already saturated at normal GFR and is excreted into the urine due to the low renal threshold for tubular reabsorption of BTP.

Our results also indicate that U-BTP may be an adequate indicator of GFR <90 mL/min/1.73 m^2, with a sensitivity similar to those of S-BTP and S-Cr. The PPV of U-BTP was also similar to

those of S-BTP and S-Cr, assuming that a GFR <90 mL/min/1.73 m^2 has a prevalence of 58.3%, as found in the general population.[23] Note that the prevalence of subjects with eGFR <90 mL/min/1.73 m^2 was even higher (75.6%) in a community-based screening for CKD among populations older than 40 years.[45] The higher incidence of the disease would increase the PPV of the test, whereas a lower cut-off value would increase sensitivity and decrease specificity and PPV. To the contrary, a higher cut-off value (U-BTP >4.32 mg/g creatinine) strongly suggests a GFR <60 mL/min/1.73 m^2. The development of a simple "point-of-care" urine dip test based on urine BTP could simplify the screening for renal diseases. In fact, the concurrent determinations of U-BTP and U-Alb should meliorate the accuracy of the screening and might single out the presence of CKD and/or of GFR impairment. On the basis of our data, the positivity of both U-Alb and U-BTP suggests the presence of kidney disease, with an impairment in GFR. The negativity of both tests indicates absence of kidney disease and no GFR impairment. The positivity of U-Alb, whereas U-BTP is negative, indicates the presence of kidney disease without impairment in GFR. The negativity U-Alb associated with a positive U-BTP should indicate an impairment in GFR without kidney disease. These possibility needs to be confirmed by further studies.

5. Conclusions

Urinary BTP seems to be an adequate biomarker to screen the general population for a slight impairment in GFR (<90 mL/min/1.73 m^2). Further studies in general population and in high-risk populations are warranted to prove this hypothesis.

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