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Cystatin C, β2-microglobulin, and retinol-binding protein as indicators of glomerular filtration rate: comparison with plasma creatinine

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Abstract

Background: The aim of this study was to assess the diagnostic accuracy of plasma levels of three low-molecular weight proteins cystatin C, β2-microglobulin, and retinol-binding protein, as indicators of impairment of glomerular filtration rate in comparison with plasma creatinine. *Methods:* Glomerular filtration rate (GFR) was measured in 110 patients (51 M and 59 F, aged 18–79 years); creatinine (Creat), cystatin C (Cys), β2-microglobulin (β2M), and retinol-binding protein (RBP) were determined on the same day. The correlation coefficients between the different markers and GFR were determined. Receiver-operating characteristics (ROC) analysis was performed to assess their diagnostic accuracy. Furthermore, the relationship between plasma levels of the examined markers of GFR and body weight, height, fat-free mass (FFM) and body cell mass (BCM) was determined. FFM and BCM were calculated by means of total body electrical impedance measurement. *Results:* Serum concentrations of Cys, β2M and RBP increase progressively with the reduction of GFR. The magnitude of the increase in blood levels of Creat and β2M was higher than the increase of Cys, and much more than that of RBP, in particular in patients with GFR < 20 ml/min/1.73 m^2. The correlation coefficients between GFR and 1/plasma concentrations were 0.647 for Creat, 0.651 for Cys, 0.731 for β2M, and 0.406 for RBP. ROC analysis indicated that the accuracy of β2M and Cys, as indicators of different degrees of GFR impairment (< 80, < 60, and < 40 ml/min per 1.73 m^2), was similar to that of Creat, while the diagnostic accuracy of RBP resulted significantly lower than that of Creat for any level of GFR. In patients without renal failure (GFR > 40 ml/min per 1.73 m^2), plasma concentrations of Creat were positively correlated with body weight ($P < 0.01$), height ($P < 0.01$), FFM ($P < 0.001$) and BCM ($P < 0.001$). Serum concentrations of RBP resulted correlated with FFM ($P < 0.05$) and BCM ($P < 0.05$), while no correlation was found between anthropometric data and Cys and β2M. *Conclusion:* Cystatin C and β2-microglobulin have a diagnostic accuracy very similar to that of creatinine, while retinol-binding protein is not an adequate marker of glomerular filtration.

Keywords: Cystatin C; β2-Microglobulin; Retinol-binding protein; Creatinine; Glomerular filtration rate; Fat-free mass; Body cell mass

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1. Introduction

The ideal marker of glomerular filtration rate (GFR) should be an endogenous molecule which, being produced at a constant rate, is cleared solely by the kidneys via free glomerular filtration, without being neither secreted by tubular cells, nor reabsorbed into peritubular circulation.

Plasma concentration of creatinine is the most commonly used test to evaluate an impairment of GFR. However, creatinine does not completely fulfil the characteristics of an ideal marker of GFR. In fact, besides glomerular filtration, creatinine is also secreted by renal tubules. Furthermore, at least in patients with advanced renal failure, creatinine is also eliminated by extra-renal clearance [1].

Many low molecular weight (MW) proteins are cleared from the plasma mainly by the kidneys via glomerular filtration, followed by complete tubular reabsorption and complete catabolization inside tubular cells [2–4]. Due to such renal handling, the measurement of plasma concentration of various low MW proteins has been proposed as a useful tool to evaluate an impairment of GFR [5–10]. Furthermore, published data suggest that some of these proteins, namely cystatin C and β2-microglobulin, could be better markers of GFR than creatinine [7,11–18].

The aim of this study was to assess the diagnostic accuracy of serum levels of three low MW proteins cystatin C (MW 13.3 kDa), β2-microglobulin (MW 11.8 kDa), and retinol-binding protein (MW 21.2 kDa), as indicators of impairment of GFR, in comparison with plasma creatinine.

2. Patients and methods

2.1. Patients

One hundred and ten adult renal patients (51 M and 59 F; aged 18–79 years, mean 51.6; body weight 38–114 kg, mean 69.5) with various kidney diseases with stable renal function and different degree of functional impairment (plasma creatinine 0.45–14.4 mg/dl, mean 1.41) participated in this study. There was a clinical indication for all patients to undergo the measurement of GFR for the assessment of renal function or to evaluate the progression of kidney disease.

2.2. Methods

2.2.1. Measurement of plasma concentration of creatinine, and of serum cystatin C, β2-microglobulin, and retinol-binding protein

Plasma concentrations of creatinine and serum concentrations of the three low MW proteins were measured with the following commercially available methods:

Creatinine (Creat) with an autoanalyzer method (Boehringer Mannheim automated analysis for Hitachi 717/911);

Cystatin C (Cys) by particle enhanced immune-nephelometry (N Latex Cystatin C, Dade Behring);

β2-Microglobulin (β2M) with an immune-enzymic method (AxSym β2-microglobulin, Abbott);

Retinol-binding protein (RBP) by immune-nephelometry (N Antiserum to human retinol-binding protein, Dade Behring).

2.2.2. Measurement of renal function

Glomerular filtration rate was measured, by a radio-isotopic method, as the renal clearance of ^{99m}Tc-DTPA (diethylenetriaminepentaacetic acid) [19,20]. The results were adjusted, as usual, to the standard body surface of 1.73 m^2.

2.2.3. Body composition analysis: measurement of fat-free mass and of body cell mass

Total body electrical impedance (resistance and reactance) was measured using a tetrapolar impedance plethysmograph (BIA 109, Akern, Firenze, Italy). The measurement of bioimpedance, which takes only a few minutes, was performed in supine position in fasting patients with the four electrodes placed on the right hand and foot [21]. The values of fat-free mass (FFM) and body cell mass (BCM) were obtained from bioimpedance data and measurement of height and weight of the patients, according to the manufacturer's equations.

The measurement of blood concentrations of Creat, Cys, β2M, and RBP, and the analysis of body composition were performed simultaneously to GFR measurement.

2.2.4. Statistical analysis

Linear correlation between GFR and 1/plasma concentrations of Creat, Cys, β2M, and RBP was evaluated from the values of coefficients of correlation.

The linear correlation between blood concentration of Creat and of the three low MW proteins was also evaluated.

Receiver-operating characteristics (ROC) analysis was used to assess the diagnostic accuracy of Cys, β2M, and RBP as indicators of different degrees of GFR impairment, in comparison with Creat.

Student's *t* test was employed to evaluate the significance of the difference of mean values of blood levels of Creat, Cys, β2M, and RBP in patients with different values of GFR.

3. Results

Serum concentrations of Cys, β2M and RBP increased progressively with the reduction of GFR (Fig. 1). Similarly to plasma creatinine, in patients with GFR lower than 40 ml/min per 1.73 m^2, serum concentrations of Cys and β2M were higher than the upper limits (2SD over the mean) of values found in the 39 patients with GFR > 80 ml/min per 1.73 m^2, i.e. 1.3 mg/dl for Creat, 1.4 mg/l for Cys, 2.3 mg/l for β2M, and 7.7 mg/dl for RBP. Normal reference values of our laboratory for plasma concentrations of Creat were 0.5–1.2 mg/dl (females) and 0.7–1.4 mg/dl (males); references values for the three low MW proteins were 0.6–1.45 mg/l for Cys, 0.8–2.5 mg/l for β2M, and 3–6 mg/dl for RBP, without differences between males and females. The increase in serum concentrations of RBP appeared less clearly related to the reduction of GFR. In fact, when we examined the correlation plots between 1/concentrations of Creat, Cys, β2M, RBP and the value of GFR, the

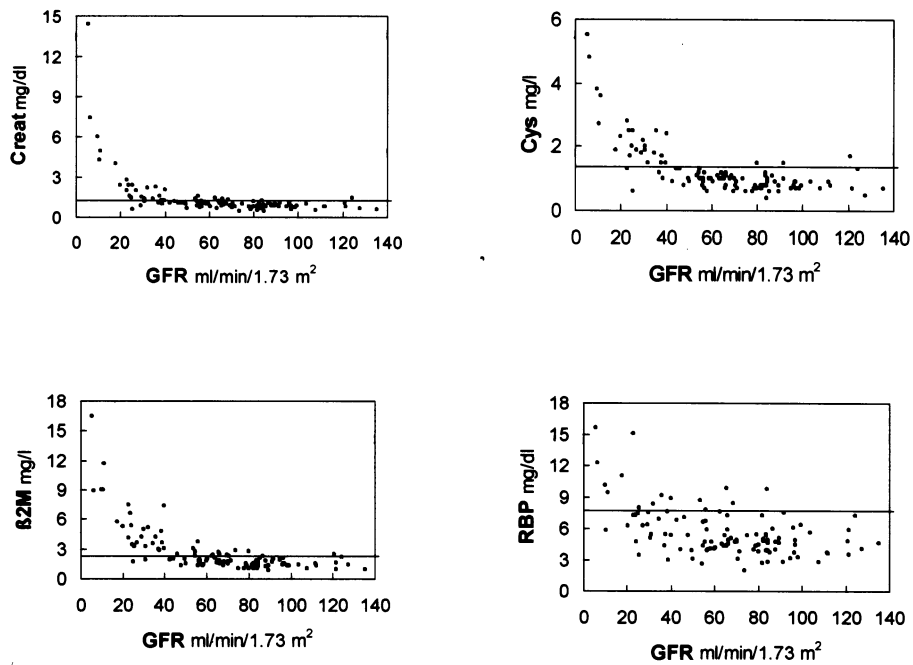

Fig. 1. Plasma concentrations of creatinine (Creat), cystatin C (Cys), β2-microglobulin (β2M), and retinol-binding protein (RBP) plotted vs glomerular filtration rate (GFR). The solid lines represent the upper limit of values found in the group of 39 patients with GFR > 80 ml/min per 1.73 m^2 (mean + 2SD): 1.3 mg/dl for Creat, 1.4 mg/l for Cys, 2.3 mg/l for β2M, and 7.7 mg/dl for RBP.

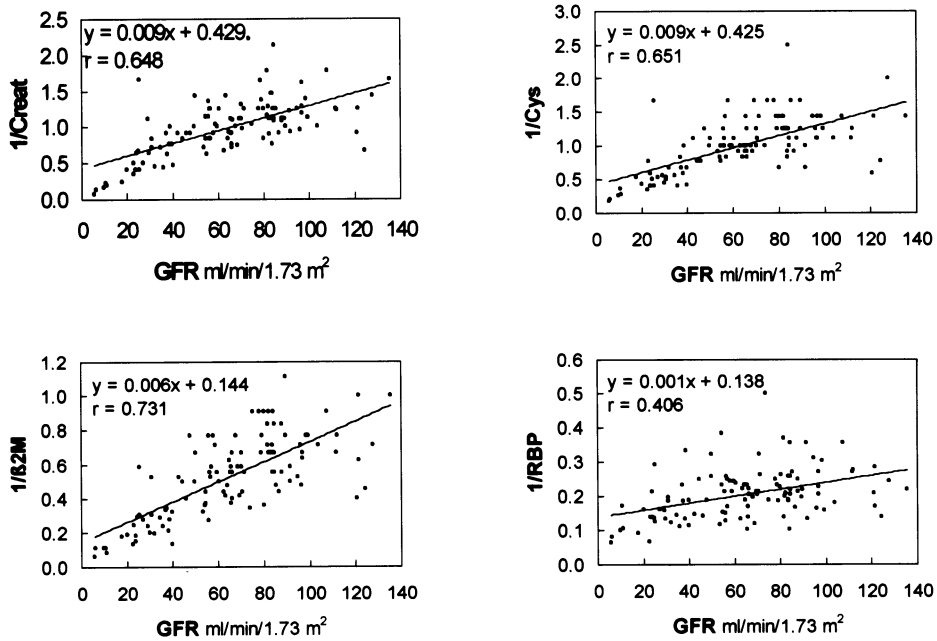

Fig. 2. Correlation plots between 1/plasma concentrations of creatinine (Creat), cystatin C (Cys), β2-microglobulin (β2M), retinol-binding protein (RBP) and glomerular filtration rate (GFR).

Table 1
Mean values (± SD) of plasma concentrations of creatinine (Creat), cystatin C (Cys), β2-microglobulin (β2M), and retinol-binding protein (RBP) [a]

GFR ml/min/1.73 m^2	N	Creat mg/dl	Cys mg/l	β2M mg/l	RBP mg/dl
<20	6	6.8 ± 3.9***	3.7 ± 1.3***	10.1 ± 3.6***	10.7 ± 3.3***
20–40	21	1.7 ± 0.6***	1.8 ± 0.5***	4.1 ± 1.4***	6.6 ± 2.5**
40–60	20	1.1 ± 0.3**	1.1 ± 0.4*	2.3 ± 1.4**	5.4 ± 1.8
60–80	24	1.0 ± 0.3*	0.9 ± 0.2	1.9 ± 0.5**	5.1 ± 1.7
>80	39	0.9 ± 0.2	0.9 ± 0.3	1.5 ± 0.4	4.7 ± 1.5

[a] Patients were divided in groups according to their glomerular filtration rate (GFR, ml/min per 1.73 m^2) (*$P<0.05$, **$P<0.001$, ***$P<0.0001$ indicate the significance of the difference vs the group of patients with GFR > 80 ml/min per 1.73 m^2, Student's t-test).

coefficients of linear correlation r were 0.648 for Creat, 0.651 for Cys, 0.731 for β2M, and 0.406 for RBP (Fig. 2).

A significant increase in plasma Creat and in serum β2M was already observed in the group of patients with GFR between 60 and 80 ml/min per 1.73 m^2 in comparison with the group of patients with GFR > 80 ml/min per 1.73 m^2 ($P < 0.05$ and $P < 0.001$, respectively) (Table 1). Mean values of serum Cys became significantly higher than normal in patients with GFR 40–60 ml/min per 1.73 m^2 ($P < 0.05$), and mean values of RBP were higher than normal values only in patients with GFR < 40 ml/min per 1.73 m^2 ($P < 0.001$).

The magnitude of the increase in blood levels of Creat and β2M was higher than the increase of Cys, and much more than that of RBP (Table 2 and Fig. 3). For comparison, in Fig. 3 is plotted the theoretical relationship with GFR of an hypothetical ideal marker of GFR, that is and endoge-

nous molecule produced at a constant rate, which is cleared solely by the kidneys via free glomerular filtration without tubular secretion or reabsorption.

Serum concentrations of Cys and β2M had a stronger correlation with plasma creatinine than RBP. Furthermore, in the 83 patients without renal failure (GFR > 40 ml/min per 1.73 m^2) the correlation between Creat and serum concentrations of the three low MW proteins resulted definitely weaker than in the 27 patients with renal failure (GFR < 40 ml/min per 1.73 m^2) (Fig. 4). These results suggest that, in patients without renal failure, the blood concentration of some of these glomerular markers is influenced by other factors than GFR. In fact, in these patients, plasma concentrations of Creat resulted positively correlated with body weight, height, FFM and BCM. In the same patients serum concentrations of RBP resulted correlated with FFM and BCM, while no correlation was found between anthropometric data and Cys and β2M concentrations (Table 3).

Diagnostic accuracy of Creat, Cys and β2M, for different impairments of GFR (GFR < 80, < 60 and < 40 ml/min per 1.73 m^2) resulted similar, as demonstrated by the values of the area under the curve of the ROC plot (Table 4). Conversely, the diagnostic accuracy of RBP was significantly lower than that of Creat for any level of renal functional impairment.

Table 2
Mean increases in plasma concentrations of creatinine (Creat), cystatin C (Cys), β2-microglobulin (β2M), and retinol-binding protein (RBP) with respect to plasma concentrations found in the patients with normal glomerular filtration rate (GFR >80 ml/min per 1.73 m^2)

GFR ml/min per 1.73 m^2	Creat	Cys	β2M	RBP
<20	7.95	4.33	6.75	2.26
20–40	1.93	2.12	2.72	1.40
40–60	1.28	1.24	1.54	1.13
60–80	1.14	1.08	1.27	1.08

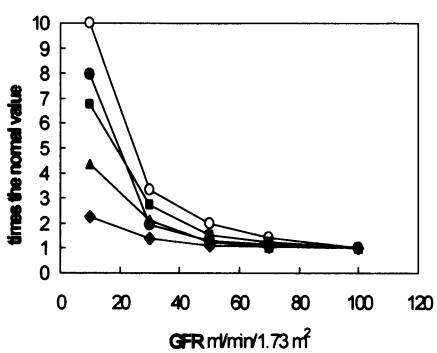

Fig. 3. Normalized values of plasma concentrations of creatinine (Creat), cystatin C (Cys), β2-microglobulin (β2M), retinol-binding protein (RBP) and glomerular filtration rate (GFR). Patients were clustered in 5 groups according to their GFR: < 20, 20–40, 40–60, 60–80 and > 80 ml/min per 1.73 m^2. The mean values of each group were normalized for the mean values found in the patients with GFR > 80 ml/min per 1.73 m^2 (○, Ideal marker of GFR; ●, Creat; ■, β2M; ▲, Cys; ◆, RBP).

4. Discussion

The results of this study in patients with different kidney diseases and a wide range of renal function, from normality to advanced renal failure, indicate that cystatin C and β2-microglobulin are indicators of GFR as reliable as creatinine, while retinol-binding protein is not an adequate marker of GFR.

Inulin is the gold standard for the measurement of GFR [22]. However, the measurement of renal clearance of inulin with the conventional method is not feasible in clinical practice since it is cumbersome and not well accepted by the patients, due to the necessity of constant IV infusion of the tracer and of bladder catheterisation to ensure an adequate urine collection. ^{99m}Tc-DTPA is a validated and widely used tracer for the assessment of GFR. The method that we used measures the renal clearance of ^{99m}Tc-DTPA, injected IV as a bolus, from the increase of bladder radioactivity, measured with external counting, thus avoiding both IV infusion and bladder catheterisation [19].

As expected, serum concentrations of cystatin C, β2-microglobulin, and retinol-binding protein were above the upper limits of normal values in patients with reduced renal function. However, their relationships with GFR resulted significantly different.

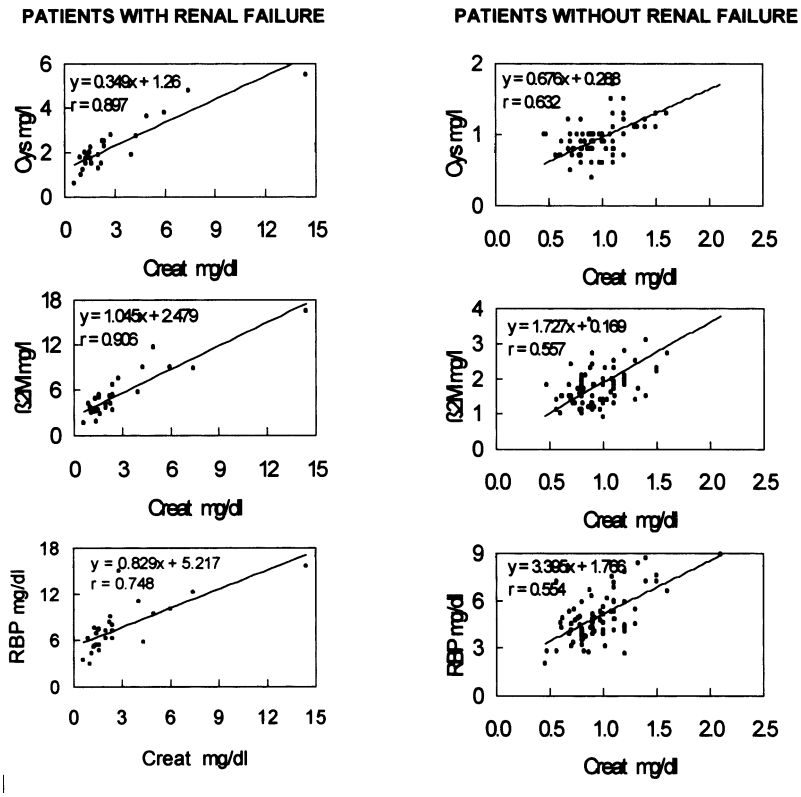

Fig. 4. Correlation plots between plasma concentrations of creatinine (Creat), and serum concentrations of cystatin C (Cys), β2-microglobulin (β2M), and retinol-binding protein (RBP) in the 27 patients with renal failure (left side) and in the 83 patients without renal failure (right side).

Neither serum cystatin C nor β2-microglobulin had a closer correlation with GFR than plasma creatinine. Conversely, the correlation of retinol-binding protein with GFR was definitely lower than those of creatinine and of the two other low MW proteins. Furthermore, the diagnostic accuracy of cystatin C and β2-microglobulin, tested with ROC analysis for different impairments of GFR (from 80 to 40 ml/min per 1.73 m^2) was very similar to that of creatinine, while the diagnostic accuracy of retinol-binding protein was always lesser than that of creatinine.

In patients without renal failure the relationships between serum concentrations of the three low MW proteins and plasma concentration of creatinine resulted weaker than in patients with renal failure (GFR < 40 ml/min per 1.73 m^2). Taking into account the positive correlation found, in the 83 patients without renal failure, between plasma concentration of creatinine and body weight, height, fat-free mass and body cell mass, it is possible to hypothesize that in the

Table 3
Correlation between anthropometric data and plasma concentrations of creatinine (Creat), cystatin C (Cys), β2-microglobulin (β2M), and retinol-binding protein (RBP) in the 83 patients without renal failure (GFR > 40 ml/min per 1.73 m^2)[a]

	Creat	Cys	β2M	RBP
Body weight	++	NS	NS	NS
Height	++	NS	NS	NS
Fat-free mass	+++	NS	NS	+
Body cell mass	+++	NS	NS	+

[a] The level of the statistical significance of a positive correlation is reported. NS, not significant; $+P<0.05$; $++P<0.01$; $+++P<0.001$.

Table 4
Diagnostic accuracy of creatinine (Creat), cystatin C (Cys), β2-microglobulin (β2M), and retinol-binding protein (RBP) for different impairments of glomerular filtration rate (GFR)[a]

GFR ml/min per 1.73 m^2	*n*	Creat	Cys	β2M	RBP
<80	71	0.783	0.775	0.839	0.670 *
<60	47	0.823	0.835	0.857	0.698 **
<40	27	0.902	0.936	0.957	0.786 *

[a] Are reported the values of the area under the curve of the R.O.C. plot; $*P<0.05$, $**P<0.01$ indicate the significance of the difference vs Creat.

patients with better preserved renal function plasma creatinine concentration, besides the level of GFR, is influenced also by these anthropometric data. This could explain the different relationship between plasma concentration of creatinine and serum concentrations of cystatin C, β2-microglobulin and retinol-binding protein in patients with or without renal failure.

Neither creatinine nor any of the examined low MW proteins behave like an ideal marker of GFR. Their blood concentrations, in particular in the group of patients with GFR < 20 ml/min per 1.73 m^2 (mean value 10.4 ± 4.7 ml/min per 1.73 m^2), were lower than that expected for an ideal marker (Fig. 3). Furthermore, relevant differences were found in the relationship between GFR and the four examined markers of GFR. Various factors may help to explain these differences. Creatinine, besides glomerular filtration, is secreted by renal tubular cells. Furthermore, at least in patients with advanced renal failure, creatinine has also an extrarenal clearance and probably a reduced production, due to the malnutrition of these patients [23]. Both these factors may determine the lower than expected increase in plasma concentration of creatinine in patients with renal failure. Data in rat indicate that cystatin C has an extrarenal clearance which approximate 15% of total plasma clearance [24]. The relationship between GFR and cystatin C resembles that predicted for a substance with a similar degree of extrarenal clearance, i.e. 17 ml/min (Fig. 5). The relationship between β2-microglobulin and GFR appears similar to that of a substance with an extrarenal clearance of 6 ml/min (Fig. 5). However, β2-microglobulin is generally believed to be eliminated solely by the kidneys [8]. On the other hand, a similar behavior could be determined by a glomerular sieving coefficient lesser than 1 for this protein [25], or by a reduced production of β2-microglobulin in renal failure patients [26]. The interpretation of the results obtained for retinol-binding protein is even more complex. Most probably, due to its MW of 21.2 kDa, retinol-binding protein is not freely filterable. Furthermore, in the plasma retinol-binding protein circulates free or bound to transthyretin (approximately 20%), and various forms of retinol-binding protein, with different renal clearance, have been demonstrated in the plasma of patients with renal failure [27–29].

In conclusion, the ideal marker of glomerular filtration rate is not yet available. Cystatin C and β2-microglobulin, whose serum concentration are not influenced by anthropometric data, have a diagnostic accuracy very similar to that of crea-

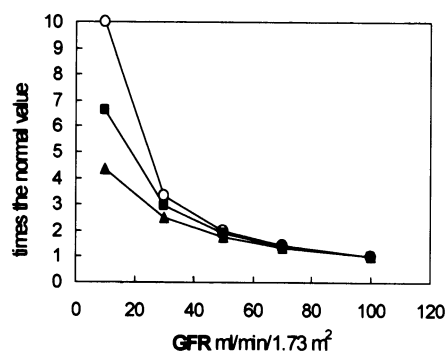

Fig. 5. Predicted plasma concentrations of hypothetical substances with different extra-renal clearance (○ Ideal marker of GFR without extra-renal clearance; ■ molecule with an extra renal clearance = 6 ml/min; ▲ molecule with an extra-renal clearance = 17 ml/min.

tinine, while retinol-binding protein is not an adequate marker of GFR. Finally, it is important to take in account that, for the moment, creatinine dosage is really less expensive than the others.

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