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ORIGINAL ARTICLE

Cystatin C as a predictor factor in patients with renal cell carcinoma treated by everolimus

Lubomir Bodnar[1] · Rafał Stec[1] · Małgorzata Dzierżanowska[2] · Agnieszka Synowiec[1] · Szczepan Cierniak[3] · Grzegorz Kade[4] · Cezary Szczylik[1]

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Abstract
Background We evaluated the influence of serum cystatin C (CysC) with respect to other glomerular filtration rate (GFR) markers on the treatment effect of everolimus in a phase II study in patients with metastatic renal cell carcinoma (mRCC).
Materials and methods Outcomes were from the study's primary analysis. GFR was calculated according to CKD-EPI-sCr equation, CKD-EPI-CysC equation and CKD-EPI-sCr-CysC equation, Modification of Diet in Renal Disease (MDRD) equation and Cockcroft–Gault (CG) equation, serum levels of creatinine (sCr) and CysC before the treatment.
Results We observed in 56 patients analysed patients high correlation (*R* Spearman from ±0.69 to ±1.00; $P < 0.0001$) between CysC level and GFR markers: sCr, CKD-EPI-sCr, CKD-EPI-CysC, CKD-EPI-sCr-CysC, MDRD, GFR (CG) before everolimus therapy. We observed that the adverse independent predictors for everolimus therapy were increased CysC level [HR: 2.85 (95 % CI 1.34–6.05), $P = 0.0065$], histologic grade G1/2 [HR: 3.38 (95 % CI 1.59–7.20), $P = 0.0016$] and increased LDH level [HR: 5.59 (95 % CI 2.52–12.40), $P < 0.0001$]. Worse OS was seen in multivariate analysis in patients with increased cystatin C level before treatment [HR: 2.60 (1.03–2.60), $P = 0.0428$], increased corrected calcium level [HR: 2.78 (95 % CI 1.03–7.54), $P = 0.0441$] and increased LDH level before treatment [HR: 2.34 (95 % CI 1.11–4.97), $P = 0.0262$].
Conclusion Increased serum CysC level in contrast to other studied GFR markers had predictive significance in patients with mRCC.

Keywords Renal cell carcinoma · Cystatin C · Glomerular filtration rate · Predictor factor

Introduction

Cystatin C (CysC) is a cysteine proteinase inhibitor, a 120-amino acid basic protein (molecular weight 13 kDa) that is produced by nearly all human cells and released into the bloodstream, from which it is freely filtered by the kidney glomerulus and metabolised by the proximal tubule. Cystatin C is not secreted, but reabsorbed by tubule epithelial cells and subsequently catabolised and, thus, is mainly eliminated by glomerular filtration; it has previously been supposed to represent an alternative endogenous glomerular filtration rate (GFR) marker in different clinical trials [1]. CysC is less dependent on muscle mass and diet than creatinine, and it is contemplated that cystatin C is a more accurate estimate of GFR than creatinine. Two recently published studies have shown higher values calculated with equations based on both CysC and creatinine in serum in order to classify stages of chronic kidney disease (CKD) and assess prognostic risk [2, 3].

In malignancy, an imbalance between cysteine proteases and their inhibitors, associated with a metastatic tumour

✉ Lubomir Bodnar
lubo@esculap.pl

[1] Department of Oncology, Military Institute of Medicine, 128 Szaserow St., 04-141 Warsaw, Poland

[2] Department of Laboratory Diagnostics, Military Institute of Medicine, Warsaw, Poland

[3] Department of Pathology, Military Institute of Medicine, Warsaw, Poland

[4] Department of Nephrology, Military Institute of Medicine, Warsaw, Poland

cell phenotype, is thought to facilitate tumour cell invasion and metastasis. Numerous studies have provided evidence of substantial increases in the mRNA, protein and activity of tumour cysteine proteases, accompanied by only moderately increased or unchanged concentrations of intracellular inhibitors. Enhanced extracellular secretion of cysteine proteases is another feature associated with tumour cell phenotypes [4]. A few studies have reported that cystatin C levels are enhanced in malignant tissues or in body fluids of patients with neoplastic diseases including patients with breast cancer, prostate cancer, multiple myeloma and ovarian cancer, and that this phenomenon is associated with more aggressive forms of these tumours [5–8].

To evaluate its validity in metastatic renal cell carcinoma (mRCC) patients included in everolimus therapy, serum concentrations of CysC and serum creatinine were analysed and compared with respect to estimation of the GFR and their impact on survival.

Materials and methods

Patients

The clinical trial design has previously been described [9]. In brief, a prospective phase II trial was conducted at the Department of Oncology (Military Institute of the Health Services, Warsaw, Poland).

It prospectively included 56 consecutive patients with mRCC, who were eligible for treatment with everolimus (10 mg/day). The study protocol was approved by the local ethics committee, and written informed consent was obtained from all participants.

Laboratory methods

GFR markers such as serum levels of creatinine, Cockcroft–Gault creatinine clearance (GFR-CG), Modification of Diet in Renal Disease (MDRD), CKD-EPI creatinine (CKD-EPI-sCr), CKD-EPI cystatin C (CKD-EPI-CysC) and CKD-EPI creatinine–cystatin C (CKD-EPI-sCr-CysC) formulas were recorded before starting treatment. Serum creatinine, albumin and blood urea nitrogen (BUN) concentrations were assessed by using commercial kits on a Cobas Integra 800 (Roche Diagnostics) automated analyser. CysC concentrations were determined by an immunoturbidimetric assay according to the manufacturer's recommendations (DakoCytomation). We calibrated serum cystatin C assays or measured CysC on the Cobas Integra 800 (Roche Diagnostics), traceable to the International Federation of Clinical Chemistry Working Group for Standardization of Serum Cystatin C and the Institute for Reference Materials and Measurements certified reference materials.

GFR was calculated from the Cockcroft–Gault formula:

$$\mathrm{GFR} = \frac{140 - \mathrm{age(years)} \times \mathrm{weight(kg)} \times R}{72 \times \mathrm{Cr(mg/dL)}}$$

where R is a coefficient of 0.85 for women and Cr is serum creatinine level.

GFR was calculated from the MDRD formula as:

$$\mathrm{GFR} = 170' \left[\text{serum creatinine level(mg/dL)}\right]^{-0.999} \left[\mathrm{age(years)}\right]^{-0.176}$$
$$[0.762 \text{ for women}]\,[1.18 \text{ for black women}]$$
$$[\mathrm{BUN}]^{-0.170}\,[\mathrm{ALB}]^{0.318}$$

GFR was calculated according to the CKD-EPI-sCr, CKD-EPI-CysC and CKD-EPI-sCr-CysC equations as described in detail by Inker et al. [2].

Statistical analysis

The cut-off date for our analysis was established as 31 January 2013. Demographic data are presented as median or mean with standard deviation (SD) and 95 % confidence interval (CI). Variables before treatment were compared with Student's t test or the Mann–Whitney test, where appropriate. Correlations were assessed by a nonparametric Spearman correlation test.

Overall survival (OS) was defined as the time elapsed between the date of randomisation and date of death or the date of last follow-up. Progression-free survival (PFS) was calculated from the date of the start of everolimus therapy to the first documented evidence of treatment failure; patients who were still alive without progressive disease at the time of analysis were counted on the date of their last follow-up.

Median and life tables were computed using the product-limit estimate by the Kaplan–Meier method, and the log-rank test was employed to assess statistical significance; p values less than 0.05 were considered to indicate statistical significance. Univariate analyses of variables influencing PFS or OS were carried out by log-rank test, which identified a preliminary list of significant factors. All variables that were found to be significant and factors that showed a trend towards significance ($P < 0.1$) in the univariate analysis were planned for inclusion in a multivariate analysis. Multivariate analyses of PFS and OS were carried out by Cox proportional hazard regression using the forward stepwise method; all variables found to be significant in the univariate analysis were included in the multivariate analysis.

Statistical calculations were carried out using STATISTICA for Windows version 12.0 software.

Results

Patient characteristics

The study included 58 patients of Caucasian ethnicity (32.1 % women and 67.9 % men). Due to damage of the materials of two patients under development and during storage, analysis was carried out on 56 of the 58 patients included in the study. The main histologic subtype was clear cell RCC (92.9 %). All patients had previously undergone nephrectomy. Table 1 summarises the clinical characteristics of the 56 eligible patients whose data form the basis of this report.

Table 1 Patient characteristics ($N = 56$)

Age, median, range (in years)	60 (41–78)
Gender	
Male	32.1 % (18/56)
Female	67.9 % (38/56)
Karnofsky performance status	
100	28.6 % (16/56)
90	53.6 % (30/56)
80	14.3 % (8/56)
70	3.6 % (2/56)
MSKCC risk factors for second-line therapy	
Favourable	50.0 % (28/56)
Intermediate	39.3 % (22/56)
Poor	10.7 % (6/56)
Previous treatment	
Sunitinib	55.4 % (31/56)
Sorafenib	39.3 % (22/56)
Both sunitinib and sorafenib	8.9 % (5/56)
Pazopanib	1.8 % (1/56)
Bevacizumab	12.5 % (7/56)
INF α	44.6 % (25/56)
Histologic type	
Clear cell	92.9 % (52/56)
Papillary	7.1 % (4/56)
Histologic differentiation	
G1	12.5 % (7/56)
G2	66.1 % (37/56)
G3	17.9 % (10/56)
G4	3.6 % (2/56)
Previous nephrectomy	100 % (56/56)
BMI, median, range (kg/m^2)	26.0 (18.6–39.0)
BSA, median, 95 % CI (m^2)	1.79 (1.75–1.86)

MSKCC Memorial Sloan Kettering Cancer Center, *BSA* body surface area (according to Du Bois equation), *CI* confidential interval, *BMI* body mass index, *G* grade, *INF* interferon

Renal function and other biochemical tests

At baseline, the mean (SD) of sCr and CysC was 1.32 (±0.45) and 1.74 (±0.71) mg/L, respectively. Mean GFR (SD) calculated from CKD-EPI-sCr, CKD-EPI-CysC, CKD-EPI-sCr-CysC, GFR-CG and MDRD formulas was: 54.09 (±18.93), 44.14 (±20.65), 47.46 (±18.59), 48.00 (±17.95) and 54.27 (±18.38) mL/min/1.73 m^2, respectively. Table 2 shows the values for renal function and other biochemical tests.

Correlation among measures of renal function

Figure 1 shows that there was high correlation (R Spearman from ±0.69 to 1.00; $P < 0.0001$) between CysC concentration and other GFR markers: sCr, CKD-EPI-sCr, CKD-EPI-CysC, CKD-EPI-sCr-CysC, MDRD and GFR-CG formulas before everolimus therapy.

Figure 2 shows a high degree of individual differences between assessment methods of GFR based on the CKD-EPI-sCr and CKD-EPI-CysC formulas, which indicated that the mean GFR from the first is higher than from CKD-EPI-CysC (bias −9.946; 95 % CI from −5.941 to −13.95).

CysC as a predictive factor

In univariate analysis, we found that the following clinical parameters correlated with PFS: higher than the upper limit of the norm for serum LDH and CysC levels (Fig. 3a) before treatment ($P = 0.0489$ and $P = 0.0434$, respectively). Other GFR markers indicated in CKD such as serum levels of creatinine, CKD-EPI-sCr, CKD-EPI-CysC, CKD-EPI-sCr-CysC, MDRD and Cockcroft–Gault formulas before everolimus therapy were not correlated with PFS.

It was found that the adverse independent predictors for everolimus therapy were increased CysC level before treatment [HR: 2.85 (1.34–6.05), $P = 0.0065$], histologic grade G1/2 [HR: 3.38 (95 % CI 1.59–7.20), $P = 0.0016$] and increased LDH level before treatment [HR: 5.59 (95 % CI 2.52–12.40), $P < 0.0001$]. The results are presented in Table 3.

Association between CysC and overall survival

Statistical significance was achieved for the following factors: increased CysC (Fig. 3b), corrected calcium level, LDH levels before treatment and the prognosis by MSKCC scale. Other GFR markers indicated in CKD such as serum levels of creatinine, CKD-EPI-sCr, CKD-EPI-CysC, CKD-EPI-sCr-CysC, MDRD and Cockcroft–Gault formulas before everolimus therapy were not correlated with OS.

Using Cox regression analyses, we observed that increased cystatin C level before treatment [HR: 2.60

Table 2 Renal function and other biochemical tests ($N = 56$)

Parameter	Mean	SD	−95 % CI	+95 % CI	Median	Minimum	Maximum
Cystatin C (mg/L)	1.74	0.71	1.55	1.92	1.54	0.90	3.640
Creatinine (mg/dL)	1.32	0.45	1.20	1.44	1.26	0.66	3.200
CKD-EPI-sCr (ml/min/1.73 m^2)	54.09	18.93	49.02	59.16	51.50	15.00	99.000
CKD-EPI-CysC (ml/min/1.73 m^2)	44.14	20.65	38.61	49.67	42.00	13.00	88.000
CKD-EPI-sCr-CysC (ml/min/1.73 m^2)	47.46	18.59	42.48	52.44	48.00	13.00	91.000
MDRD (ml/min/1.73 m^2)	54.27	18.38	49.35	59.20	52.69	16.01	102.890
GFR (CG) (ml/min/1.73 m^2)	48.00	17.95	43.19	52.81	44.00	16.00	95.000
BUN (mg/dL)	22.44	8.40	20.19	24.69	20.60	7.50	44.400
ALB (g/dL)	4.16	0.54	4.01	4.30	4.22	2.46	4.950
Ca^{2+} (mg/dL)	9.71	1.06	9.42	9.99	9.80	3.20	11.400
LDH (U/L)	264.16	355.29	169.01	359.31	181.00	120.00	2575.000
HGB (g/dL)	12.07	1.62	11.64	12.50	12.01	9.00	16.200

GFR(CG) Cockcroft–Gault creatinine clearance, *MDRD* Modification of Diet in Renal Disease, *CKD-EPI* Chronic Kidney Disease Epidemiology Collaboration, *CysC* cystatin C, *sCr* serum creatinine, *ALB* albumin, *BUN* blood urea nitrogen, Ca^{2+} calcium ion (2+), *LDH* lactate dehydrogenase, *HGB* haemoglobin, *GFR* glomerular filtration rate, *SD* standard deviation, *CI* confidence interval

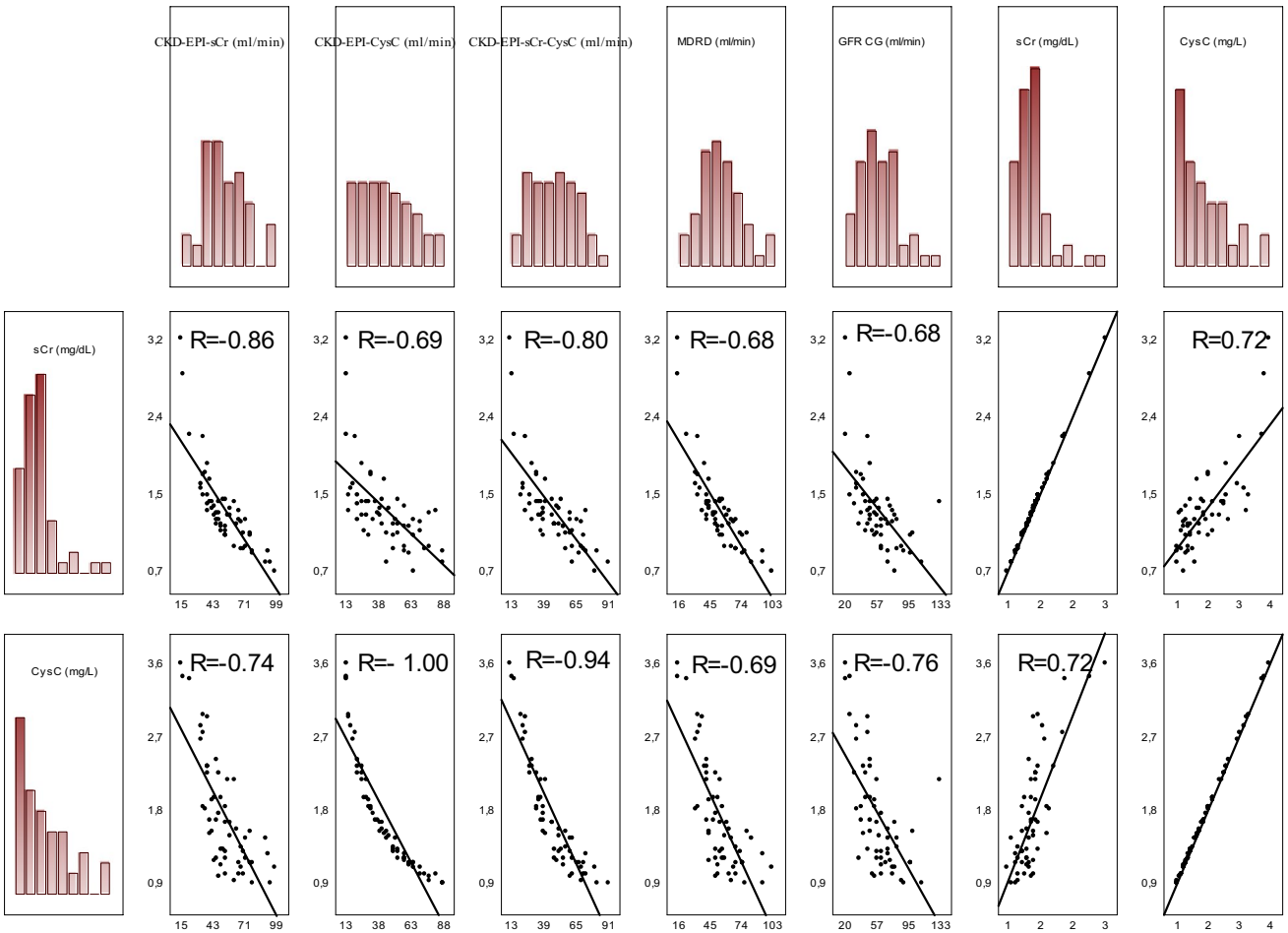

Fig. 1 Correlation between levels of creatinine, cystatin C and GFR calculated based on formulas of CKD-EPI-sCr, CKD-EPI-CysC, CKD-EPI-sCr-CysC, MDRD, Cockcroft–Gault equation (GFR-CG) before everolimus therapy (all results of R Spearman are presented with significance P value <0.0001). *GFR (CG)* Cockcroft–Gault creatinine clearance, *MDRD* Modification of Diet in Renal Disease, *CKD-EPI* Chronic Kidney Disease Epidemiology Collaboration, *CysC* cystatin C, *sCr* serum creatinine, *GFR* glomerular filtration rate, *R* correlation coefficient

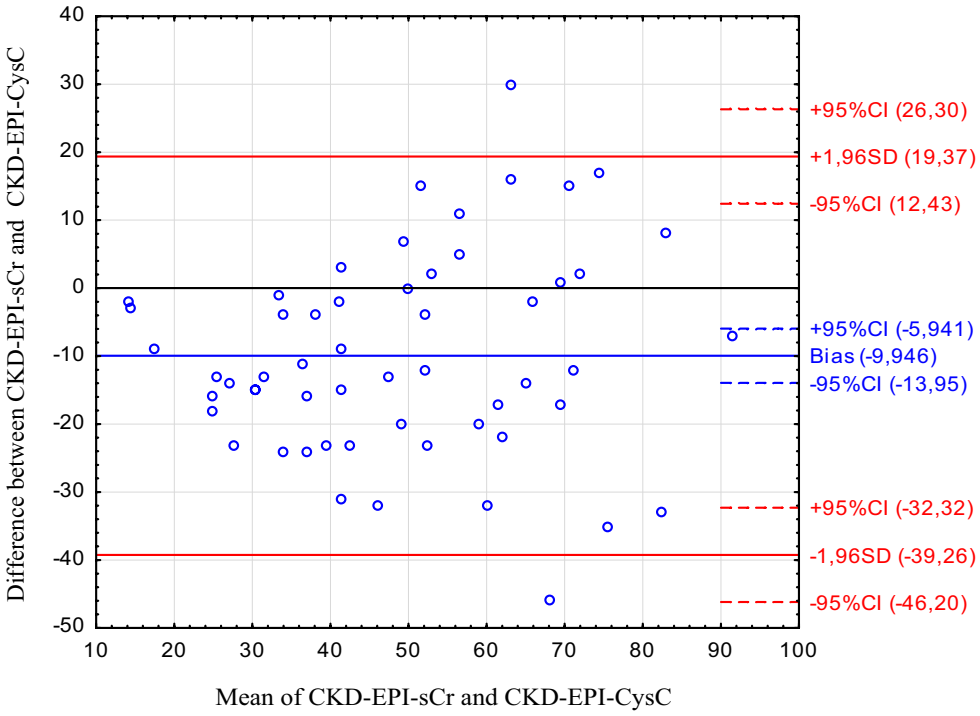

Fig. 2 Bland–Altman difference plot between CKD-EPI-sCr and CKD-EPI CysC. *CKD-EPI* Chronic Kidney Disease Epidemiology Collaboration, *CysC* cystatin C, *sCr* serum creatinine, *SD* standard deviation, *CI* confidence interval

(1.03–2.60), $P = 0.0428$], increased corrected calcium level [HR: 2.78 (95 % CI 1.03–7.54), $P = 0.0441$] and increased LDH level before treatment [HR: 2.34 (95 % CI 1.11–4.97), $P = 0.0262$] were independently correlated with worse OS (Table 4).

Discussion

We found that only cystatin C level in contrast to other studied GFR markers had predictive and independent correlated with worse OS in our population of patients with mRCC. We observed that increased cystatin C level before treatment was independent adverse predictor factor. We did not find this significance in PFS or OS of other GFR markers: sCr, CKD-EPI-sCr, CKD-EPI-CysC, CKD-EPI-sCr-CysC, MDRD or GFR-CG formulas.

Cystatin C is coded by gene CST3, a 'housekeeping' gene located on chromosome 20 (20p11.2). It is transcribed at a relatively constant level, is expressed in all nucleated cells and has multiple biological functions including control of extracellular proteolysis, modulation of the immune system and exertion of antibacterial and antiviral activities [10]. It is a low molecular weight secretory protein that inactivates members of the cathepsin family of cysteine proteases. Cystatin C has multiple biological functions in normal tissues, including regulation of protein catabolism, antigen presentation, bone resorption and hormone processing, and is coupled to cleavage of membrane and extracellular matrix proteins during tissue remodelling. In diseased tissues, particularly malignant and arthritic conditions, cathepsins stimulate cell migration, invasion and metastasis [11].

Having regard to the role of cystatin C in numerous cellular processes, particularly in malignant cells, the use of CysC for GFR estimation in oncological practice has been controversially discussed. In fact, it has been postulated that increased CysC may inhibit the proteolytic activity of extracellular cysteine proteases, a feature often associated with malignant cell phenotypes. Some reports indicate that CysC levels may be increased in oncological patients, leading to biased results in GFR estimation. Some researchers observed a prognostic role of elevated levels of CysC in lung cancer [12], myeloma multiplex [10] and colorectal cancer [13].

On the other hand, Štabuc et al. [14] showed that measurement of CysC is superior to serum creatinine determination for detection of decreased GFR in cancer patients who received cisplatin, and that this effect was independent of the presence of metastases and the type of cancer. Similarly, Bárdi et al. [15] demonstrated that CysC determination may represent a suitable marker for the estimation of the GFR in children with cancer treated with various polychemotherapeutic regimens.

Recently, a group of CKD-EPI researchers revealed that the assessment of GFR based on the equation of a combination of CysC and creatinine levels more accurately estimates GFR than on the basis of equations based on each parameter separately [2]. The KDIGO guidelines which assess estimated GFR (eGFR) based on eGFRcreatinine

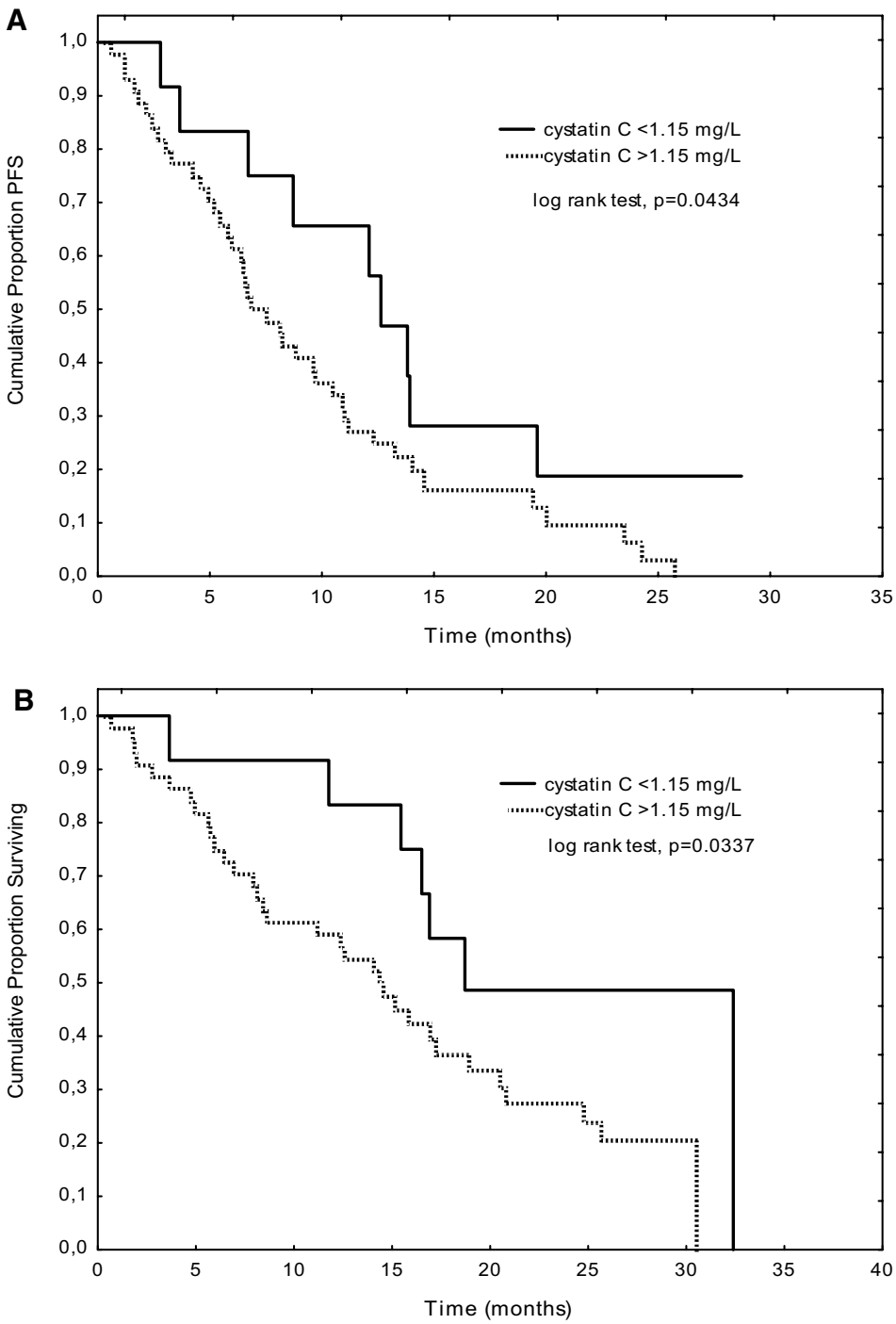

Fig. 3 Progression-free survival (**a**) and overall survival (**b**) by cystatin C serum level

and eGFRcystatin C are recommended only for clinically stable patients. The guidelines recommend using a reference method in a clinical situation where eGFRcreatinine or eGFRcystatin C is inaccurate or biased [16].

Assessment of renal function is necessary in all patients before oncology treatment. However, new targeted agents, including everolimus, used in patients with mRCC and with accompanying renal impairment do not require special dose adjustment because they are mostly excreted in the faeces [17].

Additionally, assessment of renal function in patients with cancer is necessary to carry out computed tomography (CT) tests with iodinated contrast required to evaluate the response of the tumour to treatment. Contrast-induced acute kidney injury (CI-AKI) after contrast material administration greatly depends on the specific definition

Table 3 Univariate and multivariate progression-free survival (PFS) analysis of predictor factors with assessment of renal function parameters for patients with renal cell carcinoma

Factors	Univariate analysis			Multivariate analysis	
	No. of patients	Median (months)	*P* value	Hazard ratio (95 % CI)	*P* value
Age			0.2194		
<70 years	43	8.6			
≥70 years	13	7.3			
Gender			0.7630		
Female	38	8.6			
Male	18	6.5			
Karnofsky performance status			0.2425		
≥80	54	8.0			
<80	2	4.7			
Histology			0.6257		
Clear cell	52	6.3			
Papillary	4	4.6			
Histologic differentiation			0.0644	3.38 (1.59–7.20)	0.0016*
G1-2	44	6.6			
G3-4	12	13.2			
LDH level before treatment			0.0489*	5.59 (2.52–12.40)	<0.0001*
≤ULN	43	9.4			
>ULN	13	4.3			
HGB level before treatment			0.0950		
≤ULN	30	9.8			
>ULN	26	6.2			
Corrected calcium level			0.0748		
≤10 mg/dL	50	9.0			
>10 mg/dL	6	5.4			
MSKCC prognostic index			0.0708		
Favourable	28	10.2			
Intermediate	22	6.0			
Poor	6	5.4			
Creatinine			0.6372		
≤ULN (1.2 mg/L)	23				
>ULN (1.2 mg/L)	35				
Cystatin C			0.0434*	2.85 (1.34–6.05)	0.0065*
≤ULN (1.15 mg/L)	12				
>ULN (1.15 mg/L)	44				
CKD-EPI-sCr			0.9761		
≥60 mL/min/1.73 m^2	37				
<60 mL/min/1.73 m^2	19				
CKD-EPI-CysC			0.0861		
≥60 mL/min/1.73 m^2	11				
<60 mL/min/1.73 m^2	45				
CKD-EPI-sCr-CysC			0.5169		
≥60 mL/min/1.73 m^2	17				
<60 mL/min/1.73 m^2	39				
MDRD			0.4477		
≥60 mL/min/1.73 m^2	19				
<60 mL/min/1.73 m^2	37				

Table 3 continued

Factors	Univariate analysis			Multivariate analysis	
	No. of patients	Median (months)	*P* value	Hazard ratio (95 % CI)	*P* value
GFR (CG)			0.6167		
≥60 mL/min/1.73 m^2	14				
<60 mL/min/1.73 m^2	42				

GFR (CG) Cockcroft–Gault creatinine clearance, *MDRD* Modification of Diet in Renal Disease, *CKD-EPI* Chronic Kidney Disease Epidemiology Collaboration, *CysC* cystatin C, *sCr* serum creatinine, *GFR* glomerular filtration rate, *ULN* upper limit norm, *MSKCC* Memorial Sloan Kettering Cancer Center, *LDH* lactate dehydrogenase, *HGB* haemoglobin, *G* grade

* Significance *P* value <0.05

Table 4 Univariate and multivariate analysis of overall survival (OS) with assessment of renal function parameters for patients with renal cell carcinoma

Factors	Univariate analysis			Multivariate analysis	
	No. of patients	Median (months)	*P* value	Hazard ratio (95 % CI)	*P* value
Age			0.4345		
<70 years	43	15.3			
≥70 years	13	14.3			
Gender			0.8455		
Female	38	15.3			
Male	18	14.3			
Karnofsky performance status			0.1377		
≥80	54	15.2			
<80	2	5.3			
Histology			0.5211		
Clear cell	52	15.1			
Papillary	4	9.5			
Histologic differentiation			0.3296		
G1–2	44	14.3			
G3–4	12	16.1			
LDH level before treatment			0.0458*	2.34 (1.11–4.97)	0.0262*
≤ULN	43	16.9			
>ULN	13	9.9			
HGB level before treatment			0.0970		
≤ULN	30	16.9			
>ULN	26	13.9			
Corrected calcium level			0.0235*	2.78 (1.03–7.54)	0.0441*
≤10 mg/dL	50	16.7			
>10 mg/dL	6	5.3			
MSKCC prognostic index			0.0116*		
Favourable	28	17.2			
Intermediate	22	14.3			
Poor	6	6.2			
Creatinine			0.2042		
≤ULN (1.2 mg/L)	23				
>ULN (1.2 mg/L)	35				
Cystatin C			0.0337*	2.60 (1.03–2.60)	0.0428*
≤ULN (1.15 mg/L)	12				
>ULN (1.15 mg/L)	44				

Table 4 continued

Factors	Univariate analysis			Multivariate analysis	
	No. of patients	Median (months)	*P* value	Hazard ratio (95 % CI)	*P* value
CKD-EPI-sCr.			0.8765		
≥60 mL/min/1.73 m^2	37				
<60 mL/min/1.73 m^2	19				
CKD-EPI-CysC			0.1729		
≥60 mL/min/1.73 m^2	11				
<60 mL/min/1.73 m^2	45				
CKD-EPI-sCr-CysC			0.5757		
≥60 mL/min/1.73 m^2	17				
<60 mL/min/1.73 m^2	39				
MDRD			0.1990		
≥60 mL/min/1.73 m^2	19				
<60 mL/min/1.73 m^2	37				
GFR (CG)			0.1087		
≥60 mL/min/1.73 m^2	14				
<60 mL/min/1.73 m^2	42				

GFR (CG) Cockcroft–Gault creatinine clearance, *MDRD* Modification of Diet in Renal Disease, *CKD-EPI* Chronic Kidney Disease Epidemiology Collaboration, *CysC* cystatin C, *sCr* serum creatinine, *GFR* glomerular filtration rate, *ULN* upper limit norm, *MSKCC* Memorial Sloan Kettering Cancer Center, *LDH* lactate dehydrogenase, *HGB* haemoglobin, *G* grade

* Significance *P* value <0.05

and cut-off values used. More recent evidence from controlled studies suggests that the risk is likely non-existent in patients with normal renal function, but there may be a risk in patients with renal insufficiency [18]. Therefore, defining the current renal function in patients who have very often had nephrectomy due to RCC and have renal impairment is particularly important. Oncology patients in the advanced stage of cancer tend to have reduced muscle mass. Falsely reduced serum creatinine causes falsely increased estimation of GFR [19].

In conclusion, the results obtained in our study indicate that CysC, despite the high correlation with other parameters of renal function used in daily practice, does not fully reflect only their function. The observed effect for CysC as an independent prognostic factor in patients with mRCC treated with everolimus may be in relation to its nature as a cysteine protease inhibitor and could be the result of the role of cystatin C in cancer disease.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflicts of interest.

Ethical approval This study was approved by the Institutional Review Board of the Military Institute of Medicine in Warsaw (16 December 2009; No 54/WIM/2009).

Informed consent Written informed consent was obtained from all patients.

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