Comparison of Serum Concentrations of Cystatin C versus Creatinine in Patients with IgA Nephropathy

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Serum cystatin C (Scyst) has been proposed as a novel indicator of glomerular filtration rate (GFR). The present study was performed to evaluate practical use of a commercially available kit to measure Scyst as a new marker of GFR in patients with IgA nephropathy. A total of 179 patients aged 34.2 ± 11.5 years (male/female: 67/112) were enrolled in this study. We simultaneously measured the Scyst levels and other GFR markers such as inulin clearance (Cin) and serum levels of β_2- and α_1-microglobulin. A highly significant correlation was found between Scyst and serum creatinine (Screat) ($r = 0.892$, $p < 0.0001$), and Scyst and Cin ($r = 0.738$, $p < 0.0001$). No significant difference was detected in the correlation coefficient in glomerulosclerosis rate and Scyst or Screat. There were no significant changes in Scyst and Screat when the extent of interstitial fibrosis increased. The area under the receiver operating characteristics (ROC) curve was significantly greater for Scyst than for Screat ($p = 0.0002$). This study suggests that Scyst is more sensitive than Screat for detecting a mild renal impairment and Scyst could be proposed as a confirmatory test for IgA nephropathy patients with elevated Screat

Introduction

Measurement of glomerular filtration rate (GFR) as an index of renal function is a useful tool in clinical nephrology. Urinary clearance of inulin remains the gold standard for accurate determination of GFR. Clearance of inulin (Cin) has in common to be expensive and have to require accurate timing of urine collection. Assessment of renal function is commonly secured in clinical practice by estimating GFR from serum creatinine (Screat) measurement or from formulas as that proposed by Cockcroft and Gault[1].

More recently, serum cystatin C (Scyst) has been proposed as an indicator of GFR. Cystatin C is a 13.3 kDa unglycosylated polypeptide member of the cysteine proteinase inhibitor super-family[2]~[5]. Cystatin C is a 120 amino-acid basic micro-protein (isoelectric point of 9.3). The physical properties (low molecular weight and positive charge at physiological pH) sug-gest that this substance is easily filtered through the glomerulus[5]. Cystatin C gene belongs to the housekeeping gene type, thus cystatin C is produced by all investigated nucleated cells, and its production rate is stable in each individual throughout time[6], not influenced by sex, muscular mass, age or inflammation. Several reports have shown that Scyst is related to GFR as measured by various isotopic methods and may improve diagnosis of renal failure[7]~[13]. However, most of Scyst measurements have been performed by particle-enhanced immunonephelometry on the nephelometer system.

The aim of this study was to evaluate the specificity and sensitivity of Scyst using a commercially available kit as compared with Screat and GFR (Cin) in patients with IgA nephropathy.

Patients and Methods

Patients

One hundred-seventy nine patients underwent

renal biopsy in 1999 to March 2002 were enrolled in this study. All patients were diagnosed clinically and histologically as IgA nephropathy. Mean age was 34.2 ± 11.5 years ranging from 15 to 64 years. Sex ratio was (67 male/112 female). Patients were divided into 4 groups according to Cin, an indicator of GFR. Group I consisted of patients with a remarkable reduction of GFR (Cin $<$ 30 ml/min/1.48 m^2), group II consisted of patients with a moderate reduction of GFR (30 $<$ Cin $<$ 60 ml/min/1.48 m^2), group III consisted of a normal GFR (60 $<$ Cin $<$ 90 ml/min/1.48 m^2) and group IV consisted of patients with a high GFR (90 $<$ Cin ml/min/1.48 m^2). Scyst levels of 310 healthy volunteers (202 males and 108 females) were also assessed.

Analytical methods

Scyst was measured by latex immunonephelometry (Mitsubishi Kagaku Iatron, Inc., Tokyo). Intra-assay coefficient of variation was less than 10%. Screat concentrations were assayed by an enzymatic method (Iatro LQ CRE, Mitsubishi Kagaku Iatron). Intra-assay coefficient of variation was less than 5%. Serum levels of β_2-microglobulin (β_2-m) and α_1-microglobulin (α_1-m) were measured by using SPERIOL BMG II and ALPHA I (Diatron Co., Tokyo).

Assessment of Cin measurement

All GFR measurements were performed in fasting patients. A standard clearance technique was used for measurement of Cin. After a prime dose of inulin (Inutest; 25%, Laevosan Gesellschaft, Vienna, Austria) 64 mg/kg body weight, a continuous intravenous infusion of 1-2 mg/kg per min inulin was given. Urine samples were collected by spontaneous micturition at 60-min intervals, and blood samples were drawn midway between each urine collection period. The baseline GFR corresponds to the mean values of the two periods. Urine and plasma concentrations of inulin were measured by using a fully enzymatic method. The clearance was calculated as Cin $= U \times V/S \times dt$ (U : urinary concentration, dt : duration of the period, V : urine volume, S : arithmetic mean of serum concentrations). Cin were indexed to 1.48 m^2 body surface was calculated.

Histological examination of renal biopsy specimens

All specimens were obtained by the percutaneous needle biopsy method. The specimens were fixed with 10% phosphate-buffered formalin (pH 7.2), embedded in paraffin, and cut into 4 μm sections. Hematoxylin and eosin, periodic acid Schiff (PAS), silver methenamine, and Masson trichrome stainings were performed for light microscopy. Each specimen was evaluated on glomerular and interstitial changes. The changes were scored semiquantitatively by two independent observers without any knowledge of the clinical data. The percentages of glomeruli exhibiting glomerulosclerosis were estimated. The extent of interstitial fibrosis was semiquantitatively graded into four categories according to the proportion of fibrotic lesion to total cortical area: grade 0, less than 5% of total cortical area; grade 1, 5-20%; grade 2, 20-40%; and grade 3, more than 40%. The definition of glomerulosclerosis includes focal and/or segmental sclerosis of glomeruli.

Statistical analysis

All data are expressed as mean $\pm$ SD. The significance of differences between groups was determined by using Mann-Whitney U test and p value $<$ 0.05 was considered significant. Pearson's correlation analysis was used to observe the relationship between Scyst and other clinical parameters. Sensitivity and specificity of Scyst and Screat were assessed by receiver-operating characteristic (ROC) curves according to the procedure of Hanley and McNeil[14]. ROC curves demonstrate the trade-off between sensitivity and specificity at different values for each variable. An ideal diagnostic test should have a cut-off point near the upper left-hand corner of the graph, at a point where both sensitivity and specificity are maximized.

Results

Characteristics of Scyst in the normal population

Normal population was the healthy volunteers who had not any laboratory abnormalities without medication. The upper cut-off values of Scyst and Screat in our laboratory were 1.0mg/l and 1.3mg/

Table Markers of GFR in IgA patients

Markers	Group I (n = 13)	Group II (n = 62)	Group III (n = 79)	Group IV (n = 25)
Creatinine (mg/dl)	1.67 ± 0.43	1.07 ± 0.30	0.81 ± 0.18	0.87 ± 0.27
Cystatin C (mg/l)	1.63 ± 0.24	1.06 ± 0.21	0.80 ± 0.13	0.78 ± 0.12
β_2-microglobulin (mg/dl)	3.83 ± 1.38	2.14 ± 0.85	1.47 ± 0.42	1.45 ± 0.51
α_1-microglobulin (mg/dl)	41.52 ± 9.89	26.04 ± 6.91	19.20 ± 5.10	19.76 ± 6.78
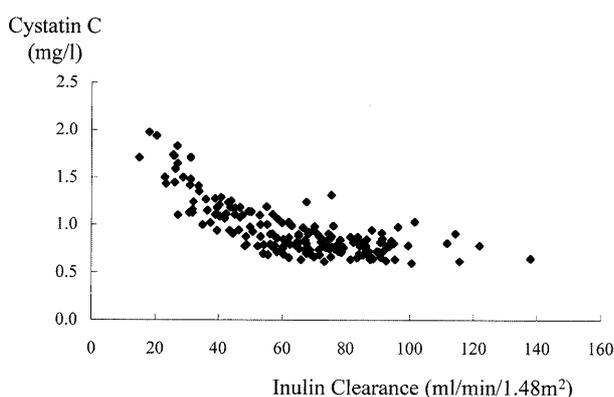
Fig. 1 Relationship between serum cystatin C and inulin clearance in patients with IgA nephropathy (n = 179)

dl, respectively. The Scyst level was stable for at least 2 days when stored at room temperature, for up to 1 weeks at 4 °C, at least 1 week at −20 °C.

Correlation between Scyst and other GFR markers in patients with IgA nephropathy

The Scyst levels in the patients ranged from 0.46 to 1.97 mg/l, and Screat levels ranged from 0.48 to 2.26 mg/dl. Mean values of Scyst and other biochemical markers for different ranges of Cin are shown in Table. Scyst increased in accordance with the reduction of renal function.

The relationship between Scyst and Cin is shown in Fig. 1, indicating the classical curvilinear relationship demonstrated by Scyst and Screat. As shown in Fig. 2, correlation between the reciprocal Scyst and Cin (r = 0.738, 1/Scyst = 0.0087 × Cin + 0.573) was higher than that between the reciprocal Screat and Cin (r = 0.582), the reciprocal β_2-m and Cin (r = 0.651) and the reciprocal α_1-m and Cin (r = 0.558).

There was a positive correlation between Scyst and Screat (r = 0.892, p < 0.0001), Scyst and β_2-m (r = 0.832, p < 0.0001), Scyst and α_1-m (r = 0.846, p < 0.0001). Scyst levels were significantly correlated

with Screat and serum levels of β_2-m and α_1-m (Fig. 3).

Correlation between Scyst and pathological markers in patients with IgA nephropathy

Figure 4 compares the correlation between glomerulosclerosis rate and Scyst or Screat. The correlation coefficients in two parameters were similar.

We also compared Scyst and Screat according to the grade of interstitial fibrosis score. Scyst and Screat tended to be increased when the extent of interstitial fibrosis increased (Fig. 5).

ROC analysis for Scyst and Screat in patients with IgA nephropathy

We chose a cut-off value of 60 ml/min/1.48 m² for the definition of renal impairment. To assess the diagnostic accuracy of the Scyst and Screat in predicting reduced GFR, we conducted the ROC plots. We estimated that significant, with a type 1 error rate, could be taken to be 0.05 (two-tailed) and a power of 80%. As shown in Fig. 6, the area (0.8698 ± 0.0286) under the Scyst curve was significantly larger than those (0.7955 ± 0.0348) under the Screat curve (p = 0.0002), demonstrating that the diagnostic accuracy of the Scyst is superior to that of Screat in identifying individuals with reduced GFR.

Discussion

Screat and creatinine clearance are usually used in the assessment of GFR. Previous reports have emphasized the effects of differences among individuals in the rates of tubular secretion and total renal excretion of creatinine[15)16)]. This hampers the reliability of Screat as a marker for GFR. Since the tubular secretion of creatinine increases as the GFR decreases, Screat systematically underestimates the severity of the renal disease[17)]. Moreover, a variety of non-renal events may influence the Screat
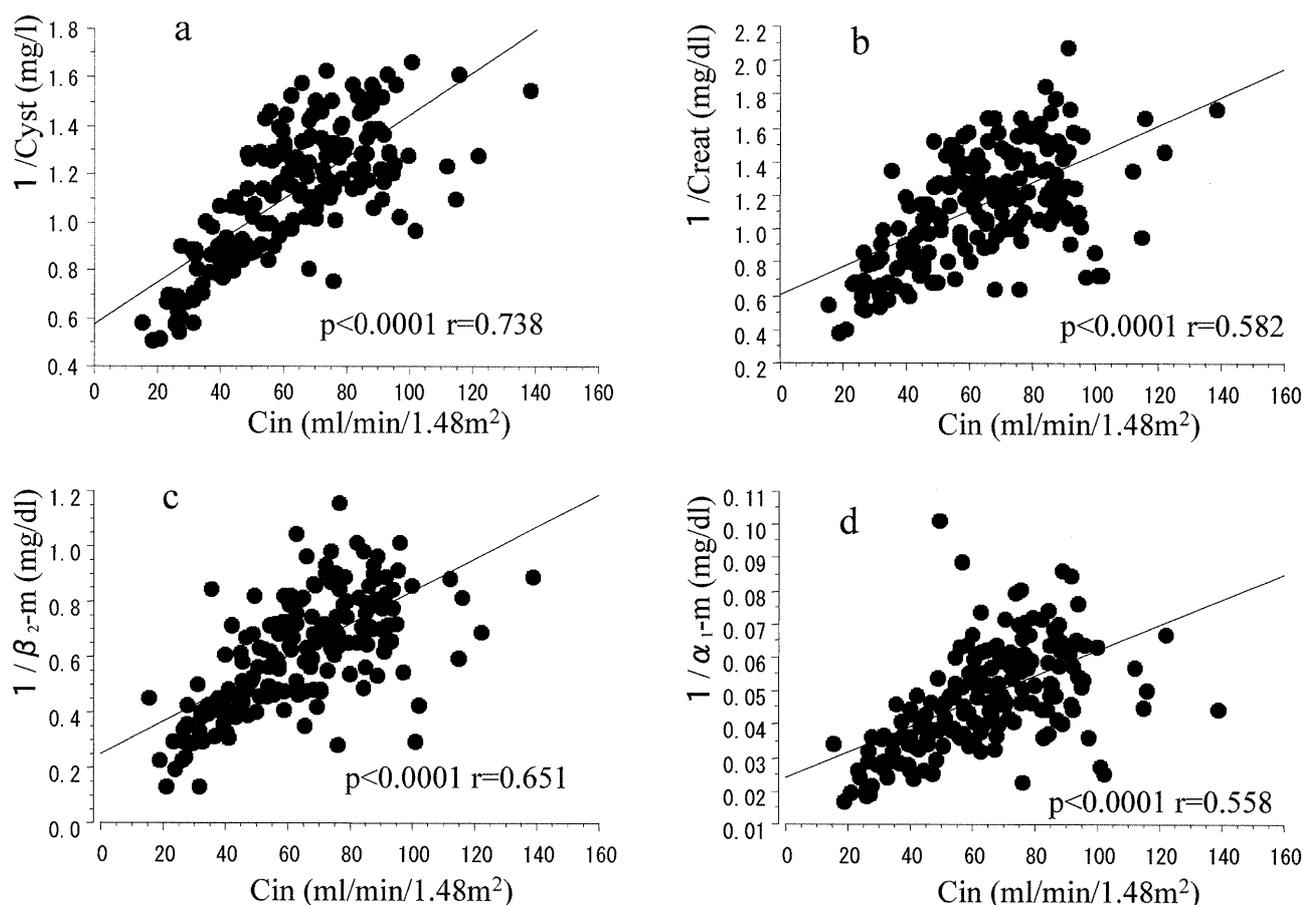
Fig. 2 Correlation between inulin clearance (Cin) and reciprocal concentrations of cysta-
tin C (a), creatinine (b), β_2-microglobulin (c) and α_1-microglobulin (d) in patients with
IgA nephropathy (n = 179)
Cyst; cystatin C, Creat; creatinine, β_2-m; β_2-microglobulin, α_1-m; α_1-microglobulin.

levels determined. Drugs, like trimethoprimsulfa
and cimetidine, compare with creatinine related to
tubular secretion[18] and cause interference in some
procedures for creatinine determination resulting in
spurious high or low creatinine values. Therefore, it
is necessary to determine a serum marker able to
detect renal function impairment, especially at in-
itial phase.

Since cystatin C, a low molecular weight protein,
is produced by all investigated nucleated cells and
its production rate is unaltered in inflammatory
conditions, the measurement of cystatin C for esti-
mating GFR have been studied by several investi-
gators[7]~[13]. The wide variation of reference inter-
vals reflects the difficulties of standardizing an im-
munological method. This was thought to be due to
the differences in assignment of values to the cali-
brators, and a reduced bias has been reported re-

cently[19]~[23]. However, most of Scyst measurements
have been performed by particle-enhanced immu-
nonephelometry on the nephelometer system. The-
refore, a commercially available kit is required to
detect Scyst by autoanalyzer used for routine labo-
ratory testing.

The present study has demonstrated that meas-
urement of Scyst could be useful to evaluate GFR in
patients with IgA nephropathy. There was a signifi-
cant positive correlation between Scyst and other
GFR markers. However, no significant difference
was detected in the correlation coefficient between
Scyst and glomerulosclerosis rate or Screat and
glomerulosclerosis rate. To evaluate the diagnostic
accuracy of the Scyst and Screat, we conducted
ROC plots and found that the diagnostic accuracy of
the Scyst was superior to that of Screat in the IgA
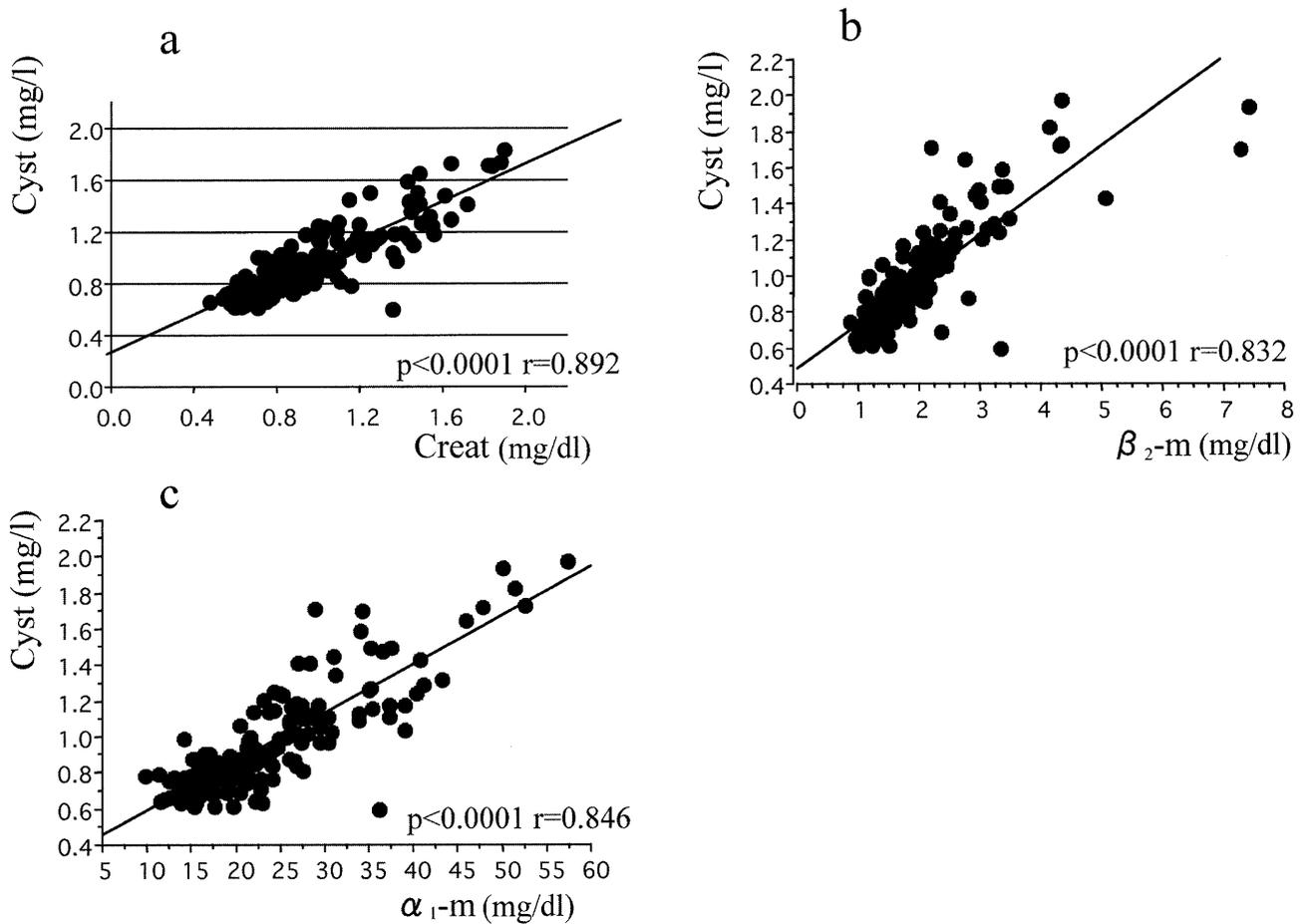nephropathy patients studied.

a

b

c

Fig. 3 Correlation between Scyst and serum levels of creatinine (a), β₂-microglobulin (b)
and α₁-microglobulin (c) in patients with IgA nephropathy (n = 179)
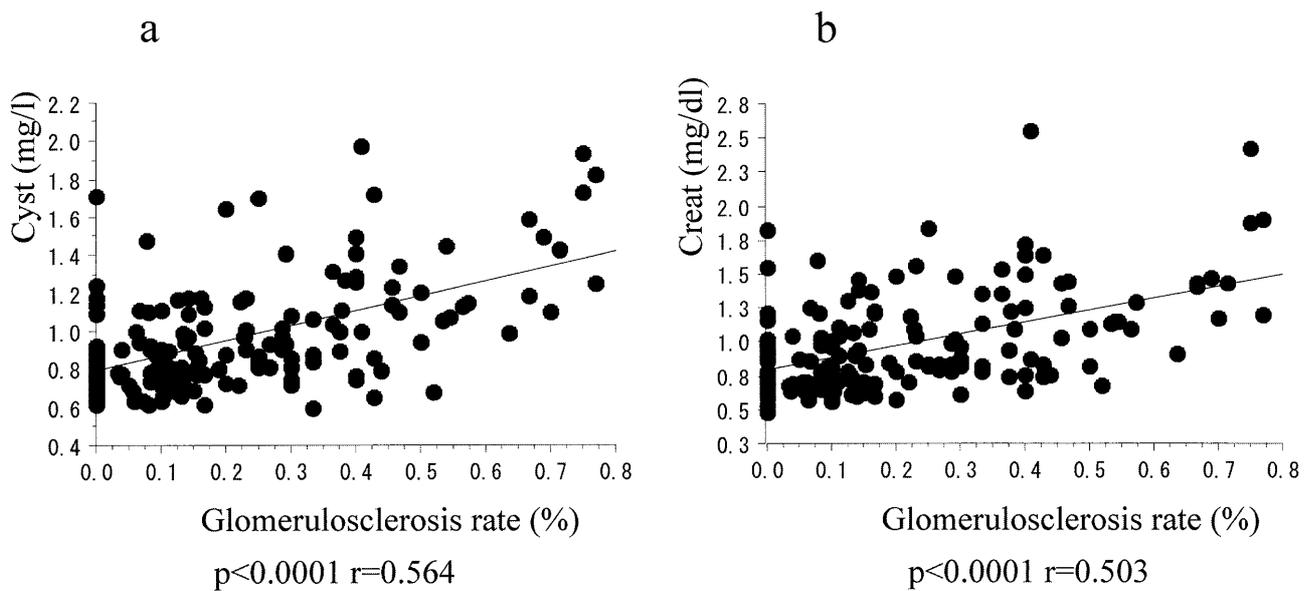Cyst; cystatin C, Creat; creatinine, β₂-m; β₂-microglobulin, α₁-m; α₁-microglobulin.

a

Glomerulosclerosis rate (%)

$p < 0.0001$ r=0.564

b

Glomerulosclerosis rate (%)

$p < 0.0001$ r=0.503

Fig. 4 Correlation between glomerulosclerosis rate and serum levels of cystatin C (a) and
creatinine (b) in patients with IgA nephropathy (n = 179)
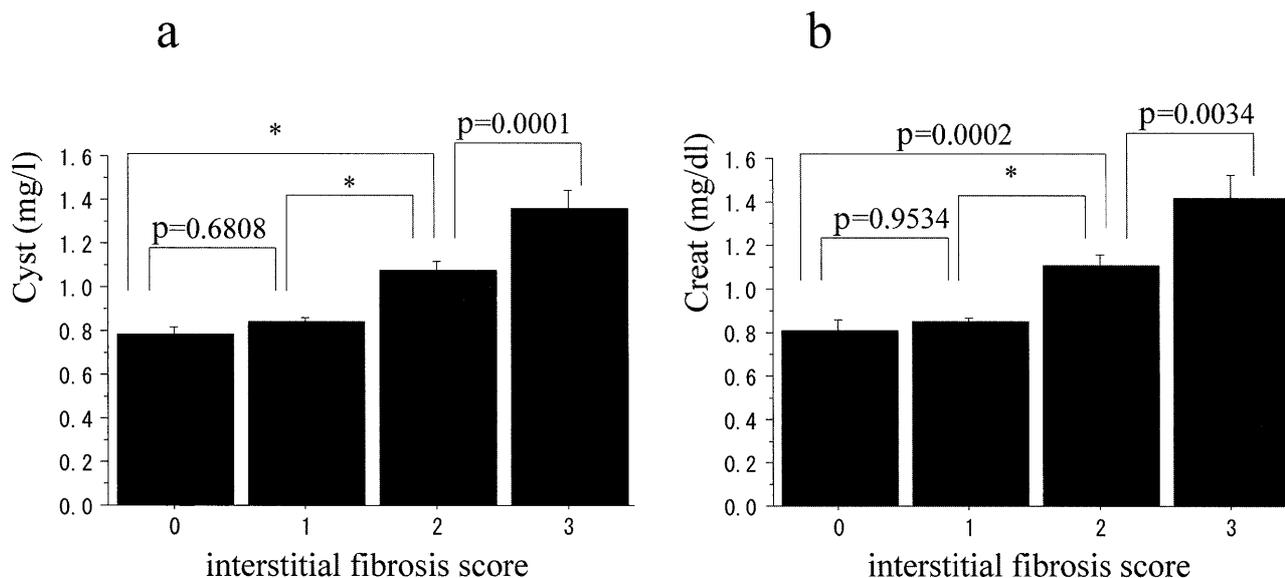Cyst; cystatin C, Creat: creatinine

a

b

Fig. 5 Relationship between interstitial fibrosis score and serum levels of cystatin C
(a) and creatinine (b) in patients with IgA nephropathy (n = 179)
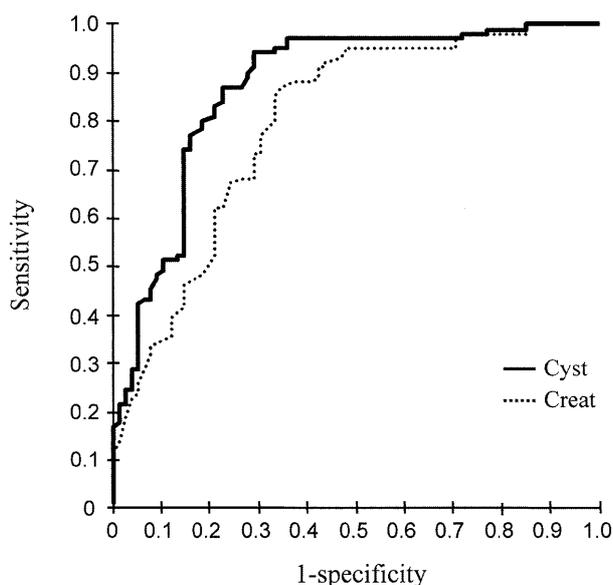Cyst; cystatin C, Creat; creatinine, *p<0.0001

Fig. 6 Receiver-operating characteristic (ROC) plots
showing the area under the serum cystatin C curve
and serum creatinine curve in patients with IgA
nephropathy (n = 179)

If Scyst is to replace Screat or creatinine clear-
ance as the routine method of choice, it is important
to determine the correlation of results with those
using a reference method. Newman et al[10] in study-
ing 206 patients with various types of renal disease
demonstrated that the increase in Scyst occurred

earlier than with Screat as the GFR value fell, using
^{51}Cr-EDTA single injection technique as the refer-
ence method. Tian et al[24] similarly found that a mild
reduction in GFR was more readily detected by a
change in Scyst than in Screat. In the current study,
we have shown that measurement of Scyst is useful
to detect a mild reduction of GFR in patients with
renal diseases[25]. However, renal diseases consisted
of glomerular disorders, which showed heterogene-
ous glomerular damages. Therefore, we selected
the patients with IgA nephropathy. This is the first
observation that demonstrates the usefulness of
Scyst measurement using a commercially available
kit in patients with IgA nephropathy. A recent re-
port has shown that the high sensitivity of Scyst
and its diagnostic accuracy at low Screat levels
makes it a good predictor of later renal impairment
in diabetic patients[26].

Most of Scyst measurements have been per-
formed by particle-enhanced immunonephelometry
on the nephelometer system. It has limited applica-
tion and is not readily available due to relatively
high initial and maintenance costs in many hospi-
tals. Therefore, this commercially available kit
should be easily used as a routine measurement in
most of hospitals in Japan. Further studies are re-

quired to test the hypothesis that Scyst measured by a commercially available kit is superior to useful to detect a mild reduction of GFR in patients with homogeneous renal diseases.

In summary, measurement of Scyst may confer any real advantages over determination of either Screat of Cin for detection of mild to moderate renal failure. However, false negatives of either Screat or Scyst are observed in different subjects. Thus, in patients with mild to moderate increase in Screat, Scyst could be used as a confirmation test for diagnosis of renal impairment in patients with IgA nephropathy. Studies in specific populations where Scyst is known to be a poor marker of GFR, i.e., edematous states, cirrhosis, acute renal failure, or acute hypercatabolic states must be done to determine the actual place of this biological marker in clinical practice.

Acknowledgements

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IgA腎症患者におけるシスタチンCとクレアチニンの血清濃度の比較

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IgA腎症患者の糸球体濾過値（GFR）のマーカーとして，シスタチンCとクレアチニンの血清濃度（Scyst vs. Screat）を比較した．対象は，臨床的および病理学的にIgA腎症と診断された179例である．平均年齢は34.2±11.5歳で，男女比は男性：女性＝67：112であった．標準GFR測定法として，イヌリン・クリアランス（Cin）を施行し，Cin値により対象を4群(I群：$Cin<30ml/min/1.48m^2$，II群：$30<Cin<60ml/min/1.48m^2$，III群：$60<Cin<90ml/min/1.48m^2$，IV群：$90ml/min/1.48m^2<Cin$）に分けた．Scystはlatex immunonephelometry法で，Screatは酵素法で測定した．他のGFR関連マーカーとして，β_2-ミクログロブリン（β_2-m）とα_1-ミクログロブリン（α_1-m）も同時に測定した．病理学的指標として，糸球体硬化率と間質線維化度を用いた．ScystおよびScreatのカットオフ値は，それぞれ$1.0mg/l$と$1.3mg/dl$であった．Cinとの相関係数は，1/Scyst（r＝0.738），1/Screat（r＝0.582），$1/\beta_2$-m（r＝0.651）および$1/\alpha_1$-m（r＝0.558）で，Scystで最も相関性が高かった．Scystとの相関係数は，Screat（r＝0.892），β_2-m（r＝0.832）およびα_1-m（r＝0.846）とほぼ同等であった．Cin値で4群に分けて，ScystとScreat濃度の変化と糸球体硬化度および間質線維化度との関係を比較したが，有意な関連性は見出せなかった．$Cin＝60ml/min$を基準としたROC曲線による解析では，Screat曲線下面積（0.7955±0.0348）に比し，Scyst曲線下面積（0.8698±0.0286）は有意に広く（p＝0.0002），診断の特異性および感受性が優れていることが判明した．これらの結果より，IgA腎症においては，ScreatよりScystがより正確にGFRを反映していると考えられた．