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Serum Cystatin C May Predict the Prognostic Stages of Patients With IgA Nephropathy Prior to Renal Biopsy

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The relationship between the levels of serum cystatin C and the prognostic stages of IgA nephropathy was determined in a multicenter trial in Japan. The levels of serum cystatin C in patients with IgA nephropathy were measured using the Dade Behring N Latex Cystain C assay. In 1995, the Joint Committee of the Special Study Group on Progressive Glomerular Diseases, Ministry of Health and Welfare of Japan, and the Japanese Society of Nephropathy reported four prognostic stages. These are: good prognosis group (Group I), relatively good prognosis group (Group II), relatively poor prognosis group (Group III), and poor prognosis group (Group IV), for this disease. Three-hundred and six patients with IgA nephropathy and other glomerular diseases were examined. There were no significant changes in the levels of serum creatinine (Cr) or creatinine clearance (CCr) between Group I and Group II. The mean levels of serum cystatin C in Group II were significantly higher than those in Group I ($P < 0.05$). The mean levels of serum cystatin C in Group III or IV were significantly higher than those in Group I ($P < 0.001$, $P < 0.005$, respectively). These suggest that the measurement of serum cystatin C may predict the prognostic stages of patients with IgA nephropathy prior to renal biopsy. J. Clin. Lab. Anal. 15:25–29, 2001.

Key words: serum cystatin C; prognostic stage; IgA nephropathy

INTRODUCTION

In 1995, the Joint Committee of the Special Study Group on Progressive Glomerular Diseases, Ministry of Health and Welfare of Japan, and the Japanese Society of Nephropathy reported four prognostic stages: the good prognosis group, the relatively good prognosis group, the relatively poor prognosis group, and the poor prognosis group, for this disease (1). Recently, we reported that the serum IgA/C3 ratio was

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also a more useful marker for distinguishing IgA nephropathy from non-IgA nephropathy together with serum IgA levels of more than 315 mg/dl using a new, widely accepted reference material (IFCC/CRM470) (2). However, the levels of serum IgA and/or the serum IgA/C3 ratio did not predict the prognostic stages of IgA nephropathy in our study.

Cystatin C is a small nonglycosylated 13-kD basic protein of the cystatin superfamily of cysteine proteinase inhibitors, which are produced by various nucleated cells (3–5). The stable production rate of cystatin C strongly indicated that the glomerular filtrastion rate (GFR) is the major determinant of cystatin C levels in sera (6,7). The objective of the present study was to determine the relationship between the levels of serum cystatin C in patients with IgA nephropathy and the prognostic stages of this disease in a multicenter trial in Japan.

MATERIALS AND METHODS

Serum Samples

Serum samples from 195 patients with IgA nephropathy and 111 patients with other glomerular diseases (non-IgA nephropathy) were obtained from our hospitals. All patients were non-nephrotic. In patients with IgA nephropathy, biopsy specimens stained predominantly for IgA in the glomerular mesangial areas. Patients with SLE, Henoch-Schönlein purpura (HSP) nephritis, liver cirrhosis, or other systemic diseases were excluded. HSP nephritis patients who had purpura, arthralgia, and/or abdominal symptoms (pain, melena, or diarrhea) were excluded from the present study. Among IgA nephropathy patients, 100 were males and 95 were females. The ages of the patients ranged from 22 to 71 years old (mean: 37.9 years). Among non-IgA nephropathy patients, 51 were males and 60 were females. The ages of these patients ranged from 12 to 79 years old (mean: 42.3 years). None of the patients was being treated with antiplatelet drugs, anti-inflammatory drugs, corticosteroids, and/or immunosuppressants at the time of renal biopsy.

Serum samples were obtained from the patients before renal biopsy, and were stored at –200°C or –700°C prior to use.

Prognostic Criteria for Patients With IgA Nephropathy

IgA nephropathy patients were divided into four groups at the time of renal biopsy (1). The good prognosis group (Group I) had almost no possibility of dialysis. Slight mesangial proliferation and increased matrix were observed. Glomerulosclerosis, crescent formation, or adhesion to Bowman's capsule was not observed. Prominent changes were not seen in the interstitium, renal tubuli, or blood vessels. The relatively good prognosis group (Group II) had a possibility of dialysis that was relatively low. Slight mesangial proliferation and increased matrix were observed. Glomerulosclerosis, crescent formation, or adhesion to Bowman's capsule were observed in less than 10% of all biopsied glomeruli. Interstitial and vascular findings were the same as in Group I. In the relatively poor prognosis group (Group III), dialysis was likely to be required within 5 to 20 years. Moderate, diffuse mesangial cell proliferation and increased matrix were observed. Glomerulosclerosis, crescent formation, or adhesion to Bowman's capsule were seen in 10 to 30% of all biopsied glomeruli. Cellular infiltration was slight in the interstitium except around some sclerosed glomeruli. Tubular atrophy was slight, and mild vascular sclerosis was observed. In the poor prognosis group (Group IV), the possibility of dialysis within 5 years was high. Severe, diffuse mesangial cell proliferation and increased matrix were observed. Glomerulosclerosis, crescent formation, or adhesion to Bowman's capsule were seen in more than 30% of all biopsied glomeruli. When sites of sclerosis are totaled and converted to global sclerosis, the sclerosis rate was more than 50% of all glomeruli. Some glomeruli also showed compensatory hypertrophy. The sclerosis rate was the most important index in the evaluation of prognosis. Interstitial cellular infiltration and tubular atrophy, as well as fibrosis were seen. Hyperplasia or degeneration may be present in some intrarenal arteriolar walls. Glomeruli often show a mild-to-moderate increase in mesangial cells and matrices. Electron-dense deposits were mainly observed in the glomerular mesangial areas (Fig. 1a; Group I). Finely granular mesangial deposits are typically situated between the mesangial cells and the GBM. The GBM were usually normal in thickness (Fig. 1b; Group II). Subendothelial, intramembranous, and subepithelial deposits have been observed in rare cases. Mesangial expansion and cell infiltration were observed in a glomerulus (Fig. 1c; Group III). Mesangial interposition to inside of the GBM was marked (Fig. 1d; Group IV). Crescentic formation was associated with focal compression of the glomerular tufts. There was often mild foot process fusion and villous transformation of the visceral epithelial cell cytoplasm.

When such parameters as blood pressure, serum creatinine (s-Cr), creatinine clearance (CCr) and urinary protein tend to be aggravated, they become important auxiliary parameters in the evaluation of prognosis in addition to renal biopsy.

Detection of Serum Cystatin C

The levels of serum cystatin C were measured using the Dade Behring Cystatin C assay with the automated Dade Behring Nephelometer II (BNII) (software version 2.0) (8). The N Latex Cystatin C kit (Lot No. 29577, Dade Behring Diagnostics, Marburg, Germany) was used in this study. The assay is a fully automated particle-enhanced nephelometric immunoassay for measuring serum cystatin C. The values of cystain C obtained from 276 healthy controls ranged from 0.5 to 0.9 mg/dl (mean ± SE; 0.66 ± 0.01 mg/dl) (9).

Clinical Activity

Levels of serum creatinine (s-Cr) and creatinine clearance (CCr) were measured by routine methods in each hospital.

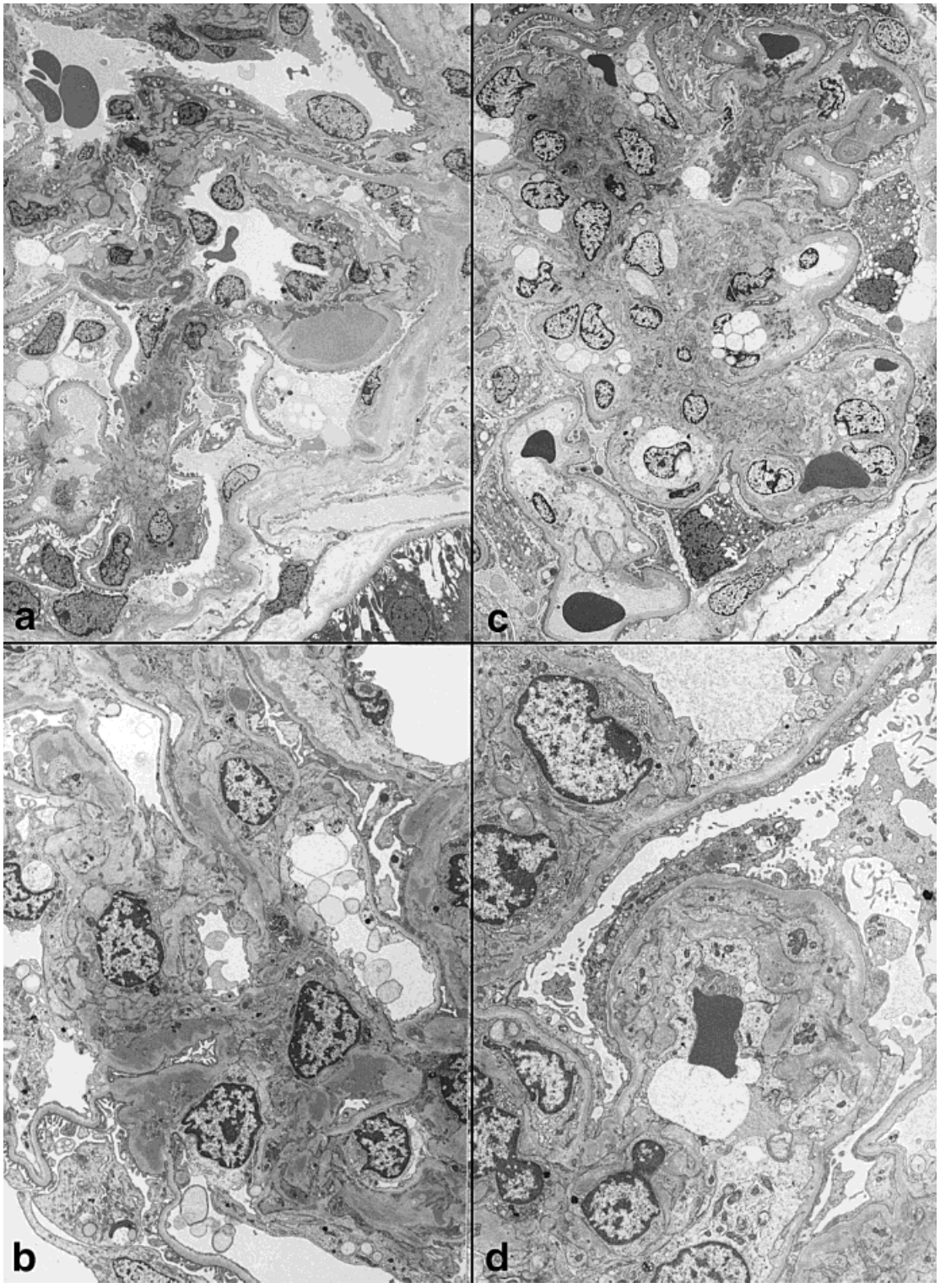

Fig. 1. Electron microscopic findings in patients with IgA nephropathy. Electron-dense deposits were mainly observed in the glomerular mesangial areas. Fig. **1a**; good prognosis group (Group I). Finely granular mesangial deposits are typically situated between the mesangial cells and the glomerular basement membranes (GBM). The GBM were normal in thickness. Fig. **1b**; relatively good prognosis group (Group II). Mesangial expansion and cell infiltration were observed in a glomerulus. Fig. **1c**; relatively poor prognosis group (Group III). Mesangial interposition to inside of the GBM was marked. Fig. **1d**; poor prognosis group (Group IV).

TABLE 1. Mean levels of serum cystatin C in patients with IgA nephropathy and non-IgA nephropathy, and healthy controls

Disease	Mean ± SE (mg/dl)
IgA nephropathy (n = 195)	0.90 ± 0.03
Group I (n = 30)	0.68 ± 0.03
Group II (n = 63)	0.82 ± 0.04
Group III (n = 80)	0.99 ± 0.05
Group IV (n = 19)	1.06 ± 0.14
Non-IgA nephropathy (n = 111)	0.97 ± 0.05
Healthy controls[a] (n = 276)	0.66 ± 0.01

[a]Cited from ref. 9.

The levels of serum IgA and C3 were measured by the automated Dade Behring Nephelometer II (BNII) using a nephelometric immunoassay (10).

Statistical Analysis

Statistical analysis was performed using STAT FLEX (version 5.0) (11). The Student's *t*-test was also used in statistical comparisons between individual study groups. $P < 0.05$ was regarded as significant.

RESULTS

Levels of Serum Cystatin C in Patients With IgA Nephropathy and Non-IgA Nephropathy

The mean values of serum cystatin C in patients with IgA nephropathy and non-IgA nephropathy are shown in Table 1. There were no significant differences in the levels of serum cystatin C between patients with IgA nephropathy and those with non-IgA nephropathy. The mean levels of serum cystatin C in Group II IgA nephropathy patients were sig-

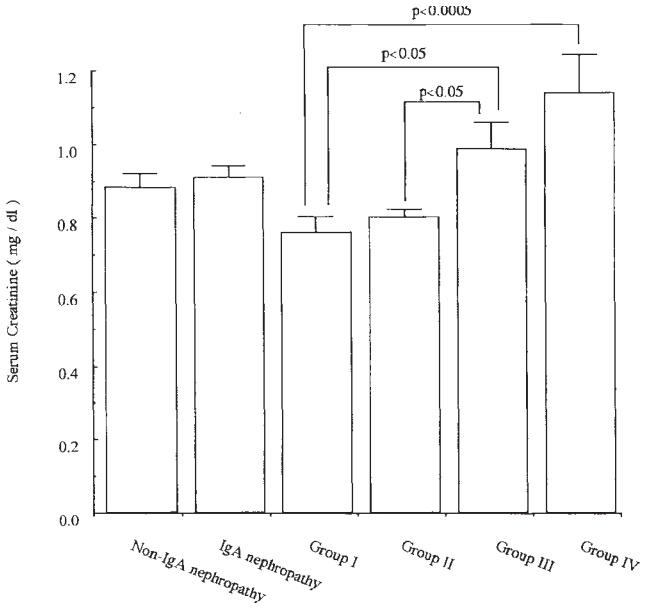

Fig. 3. Mean levels of serum creatinine (Cr) in patients in the good prognosis group (Group I), relatively good prognosis group (Group II), relatively poor prognosis group (Group III), and poor prognosis group (Group IV) of IgA nephropathy and non-IgA nephropathy.

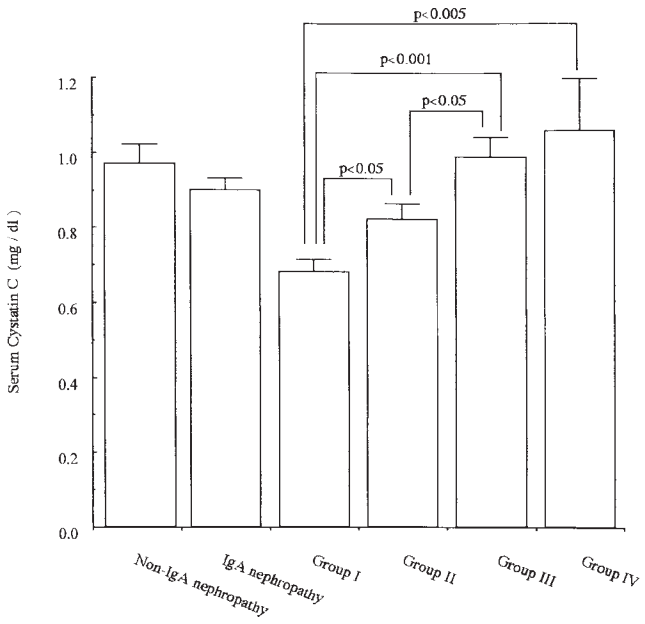

Fig. 2. Mean levels of serum cystatin C in patients in the good prognosis group (Group I), relatively good prognosis group (Group II), relatively poor prognosis group (Group III), and poor prognosis group (Group IV) of IgA nephropathy and non-IgA nephropathy.

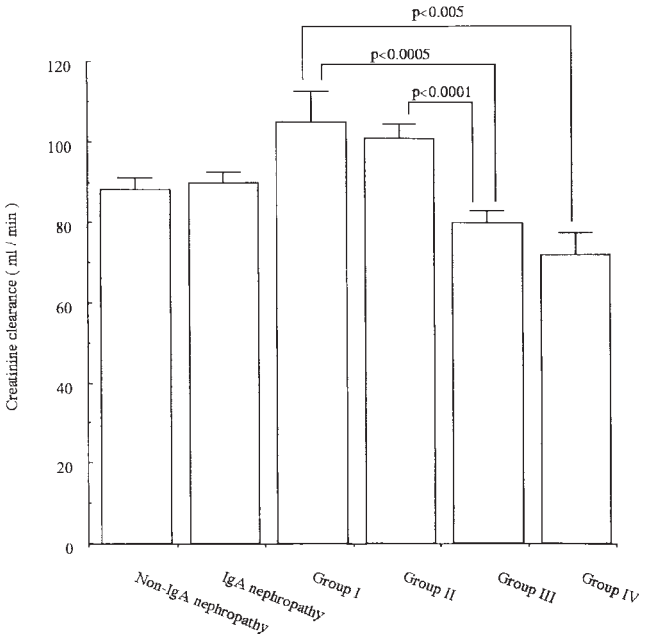

Fig. 4. Mean levels of creatinine clearance (CCr) in patients in the good prognosis group (Group I), relatively good prognosis group (Group II), relatively poor prognosis group (Group III), and poor prognosis group (Group IV) of IgA nephropathy and non-IgA nephropathy.

TABLE 2. Levels of serum IgA and serum IgA/C3 ratio in patients[a]

	Serum IgA (mg/dl, mean ± SD)	Serum IgA/C3 ratio (mean ± SD)
Group I (n = 30)	332.8 ± 131.1	2.82 ± 1.05
Group II (n = 63)	347.4 ± 148.3	2.97 ± 1.15
Group III (n = 80)	341.5 ± 115.1	3.19 ± 1.21
Group IV (n = 19)	293.7 ± 112.2	2.77 ± 1.13

[a]Group I, good prognosis; Group II, relatively good prognosis; Group III, relatively poor prognosis; Group IV, poor prognosis.

nificantly higher than those in Group I ($P < 0.05$). The mean levels of serum cystatin C in Group III IgA nephropathy were significantly higher than those in Group II ($P < 0.05$). The mean levels of serum cystatin C in Group III or IV IgA nephropathy were significantly higher than those in Group I ($P < 0.001$, $P < 0.005$, respectively). There were significant changes in the levels of cystatin C between Groups I and II , or Groups II and III ($P < 0.05$). However, there were no significant changes in the levels of cystatin C between Groups III and IV (Fig. 2).

Clinical Activity in Patients With IgA Nephropathy

The levels of serum creatinine (Cr) in Group III or IV IgA nephropathy were significantly higher than that in Group I or II (Fig. 3). The levels of creatinine clearance (CCr) in Group III or IV IgA nephropathy were significantly lower than that in Group I or II. There were no significant changes in the levels of CCr between Group I and II IgA nephropathy (Fig. 4). There were no significant changes in the levels of serum IgA or serum IgA/C3 ratio among the four groups (Table 2).

DISCUSSION

As discussed, four prognostic stages, Groups I–IV, were reported for this disease in 1995. (1). Recently, we reported that the serum IgA/C3 ratio was also a more useful marker for distinguishing IgA nephropathy from non-IgA nephropathy together with serum IgA levels of more than 315 mg/dl using a new, widely accepted reference material (IFCC/CRM470) (2). However, the levels of serum IgA and/or the serum IgA/C3 ratio did not predict the prognostic stages of IgA nephropathy in this study. Groups I–IV show increasing levels (with Group I having the lowest level) of serum creatinine (Cr) in patients with IgA nephropathy. On the other hand, the levels of creatinine clearance (CCr) decreased in the four groups, with Group 1 having the highest level. These results confirmed that this classification reflected appropriately the prognostic stages of this disease. However, the levels of serum Cr and CCr were not clear in the early stages of IgA nephropathy, i.e., Groups I and II. There have been several reports in recent years suggesting that cystatin C measurement in sera correlates with the glomerular filtration rate (GFR) (6,7). Newman et al. (12) reported that serum cystatin C has been shown to be in all likelihood a more sensitive marker of early deterioration of GFR than serum creatinine (Cr). The mean levels of serum cystatin C in Group II IgA nephropathy were significantly higher than those in Group I in this study. The mean levels of serum cystatin C in Group III or IV IgA nephropathy were also significantly higher than those in Group I. Thus, the levels of serum cystatin C were statistically correlated with the prognostic stage of IgA nephropathy in contrast with serum creatinine and creatinine clearance. Especially, the potential utilization of serum cystatin C was observed in the early stage of IgA nephropathy. It appears that the levels of serum cystatin C may predict the early prognostic stages of patients with IgA nephropathy prior to renal biopsy.

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REFERENCES

1. Sakai H, Abe K, Kobayashi Y, et al. Clinical guidelines of IgA nephropathy. Jpn J Nephrol 1995;37:417–421.
2. Tomino Y, Suzuki S, Imai H, et al. Measurement of serum IgA and C3 may predict the diagnosis of patients with IgA nephropathy prior to renal biopsy. J Clin Lab Anal 2000;14:220–223.
3. Barrett AJ, Davies ME, Grubb A. The place of human γ-trace (cystein C) amongst the cystein proteinase inhibitors. Biochem Biophys Res Commun 1984;120:631–636.
4. Grubb A, Lofberg H: Human γ-trace, a basic microprotein: amino acid sequence and presence in the adenohypophysis. Proc Natl Acad Aci U S A 1982;79:3024–3027.
5. Barret AJ, Fritz H, Grubb A, et al. Nomenclature and classification of the proteins homologous with chicken cystatin. Biochem J 1986(abstr); 236:312.
6. Simonsen O, Grubb A, Thysell. The blood serum concentration of cystatin C (γ-trace) as a measure of the glomerular filtration rate. Scand J Clin Lab Invest 1985;45:97–101.
7. Grubb A, Simonsen O, Sturtfelt G, Truedsson L, Thysell H. Serum concentration of cystatin C, factor D and β2-microglobumin as a measure of glomerular filtration rate. Acta Med Scand 1985;218:499–503.
8. Erlandsen EJ, Randers E, Kristensen JH. Evaluation of the Dade Behring Latex Cystatin C assay on the Dade Behring Nephelometer System. Scand J Clin Lab Invst 1999;59:1–8.
9. Ohara T, Nakai K, Orisaka M, Itoh T, Saito K, Itoh K. A trial for establishing reference intervals of serum cystatin C in normal individuals. Medicine and Pharmacology 2000;43:835–844 (in Japanese).
10. Fink PC, Romer M, Haeckel R, et al. Measurement of proteins with the Behring nephelometer. aA multicenter evaluation. J Clin Chem Clin Biochem 1989;27:261–276.
11. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993;39:561–577.
12. Newman DJ, Thakkar H, Edwards RG, et al. Serum cystatin C measured by automated immunoassay: a more sensitive marker of changes in GFR than serum creatinine. Kidney Int 1995;47:312–318.