In patients with rheumatoid arthritis (RA), the reported prevalence of ANCAs has ranged from 20 to 50%, and these predominantly show a pANCA pattern [9–16]. Furthermore, the clinical relevance of ANCAs in RA patients is a rather controversial topic in the literature. It has been suggested that ANCAs occur especially frequently in RA patients who have longstanding, severe disease, early markers of progressive erosion, and who are positive for rheumatoid factor (RF) and antinuclear antibody (ANA) [12, 13, 17]. An association of ANCAs with extra-articular manifestations such as vasculitis, pulmonary involvement and nephropathy has been reported [13, 18, 19]. However, no association between disease activity and ANCAs has been reported [10, 12]. On the other hand, ethnic differences in the incidence of ANCA-associated vasculitis (AAV) and/or the prevalence of PR-3 ANCA and MPO-ANCA have been reported between Japan and European countries [20, 21]. Therefore, it is important to evaluate the prevalence of ANCAs in Japanese RA patients in order to understand ethnic differences in AAV.

In this work, we investigated ANCAs for 5 antigens in sera from Japanese RA patients in order to study the prevalence of these ANCAs, and to determine whether certain ANCA antigens are closely related to clinical manifestations of RA, especially vasculitis and/or extra-articular manifestations.

Patients and methods

All patients were diagnosed and treated at the Department of Rheumatology, Juntendo Hospital, from 1995 to 1996. Patient records and sera were investigated consecutively. One hundred twenty-five patients with RA fulfilled the 1987 American College of Rheumatology (ACR) classification criteria for RA [22]. The average age of these RA patients was 55.8 ± 13.6 years (range 18–80), and 74% of the patients were female (F:M 93:32). Patients were classified by Steinbrocker radiographic stage as follows: 8 patients with stage I, 52 patients with stage II, 22 patients with stage III and 43 with stage IV. Eight of the 125 RA patients were diagnosed with rheumatoid vasculitis (RV) and/or extra-articular involvement according to the diagnostic criteria for malignant rheumatoid arthritis (MRA) proposed by the Research Committee of Intractable Vasculitis, the Ministry of Health, Labor and Welfare of Japan [23] (see "Appendix"). Several disease-modifying anti-rheumatic drugs (DMARDs) were used by the RA patients: aurothiomalate sodium was used by 22 patients, auranofin by 9 patients, bucillamine by 68 patients, methotrexate by 4 patients, mizoribine by 10 patients, lobenzarit disodium by 4 patients, actarit by 4 patients, penicillamine by 2 patients, and steroids by 39 patients. Twenty-four patients were only treated with nonsteroidal antiinflammatory drugs (NSAIDs). Four patients were treated with steroids only. Eleven patients were treated with plasmapheresis [double-filtration plasmapheresis (DFPP) and/or granulocytapheresis]. Two patients had not been started on RA treatment. Twenty-four patients with systemic lupus erythematosus (SLE), 15 with systemic sclerosis (Ssc), 14 with Sjögren syndrome (Sjs), 15 with mixed connective tissue disease (MCTD) [24], and 15 with polymyositis/dermatomyositis (PM/DM) fulfilled the ACR classification criteria described elsewhere.

Sera were frozen at −30°C before use. Since indirect immunofluorescent (IIF) staining patterns are commonly observed to be nonspecific, especially for sera from patients with RA and other rheumatic diseases, we decided to use enzyme-linked immunosorbent assay (ELISA) to investigate ANCA antigens [18]. MPO ANCA and PR-3 ANCA were determined by ELISA using a commercial kit, the Varelisa ANCA kit (ELIAS, Freiberg, Germany). As indicated in the instruction manual, the cut-off points were set to >10 IU/ml serum for MPO ANCA and >5 IU/ml for PR-3 ANCA. Lactoferrin (LF), CG and elastase (EL) were purchased from Sigma (Sigma–Aldrich, Japan KK, Tokyo, Japan), and 10 μg/ml of antigen was coated on the ELISA well and reacted with 1:50 diluted sera. The subsequent ELISA procedure was as described previously [18]. An optical density of more than the mean + 3SD (where the mean value was derived from sera from 18 healthy volunteers) was used as the positive cutoff. All ANCAs were found to be of the IgG isotype. Joint score was determined by the method reported by Lansbury et al. [25]. To establish whether the observed differences were statistically different, the Chi-square test, Fisher's extract test, and the nonparametric Mann–Whitney *U* test were used.

Results

Prevalence of ANCAs among rheumatic diseases

ANCAs against 5 antigens (LF, CG, EL, MPO and PR-3) were tsted for in sera from RA patients and patients with other rheumatic diseases. Thirty of the 125 (24.0%) sera samples from the RA patients were found to be positive for at least one of 5 ANCA antigens (Table 1). Only 2 of the 8 MRA patients were found to be ANCA positive: the serum from one patient was positive for anti-LF and that from the other patient was positive for anti-MPO.

ANCAs against LF, CG, EL, MPO and PR-3

ANCAs against LF, CG, EL, MPO and PR-3 were determined in the sera of the RA patients and the control disease group. Twenty-one of the 125 (16.8%) sera samples from

Table 1 The prevalence of ANCAs in sera from Japanese patients with various rheumatic diseases

Disease of patient	*n*	Number of ANCA-positive patients (%)[a]
Rheumatoid arthritis (RA)[b]	125	30 (24.0)
Malignant rheumatoid arthritis (MRA)	8	2 (25)
Systemic lupus erythematosus (SLE)	24	8 (33.3)
Sjögren syndrome (Sjs)	14	3 (21.4)
Scleroderma (Scl)	15	6 (40.0)
Mixed connective tissue disease (MCTD)	15	4 (26.0)
Polymyositis/dermatomyositis (PM/DM)	15	4 (26.4)

[a] Number of patients with sera that were found to be positive for at least one of the 5 ANCA antigens by ELISA

[b] Including 8 MRA patients (as described by the Japanese criteria in "Appendix")

Table 2 ANCAs against lactoferrin, cathepsin G, elastase, MP0 and PR-3 in sera from RA patients and control patients

ANCA antigen	Rheumatoid arthritis patients $n = 125$ (%)	Control patients[a] $n = 83$ (%)	*p*
Lactoferrin (LF)	21 (16.8)[b]	8 (9.6)	0.16
Catepsin G (CG)	5 (4.0)	9 (10.8)	0.58
Elastase (EL)	8 (6.4)	11 (13.3)	0.68
Myeloperoxidase (MPO)	6 (4.8)	2 (2.4)	0.48
Proteinase 3 (PR-3)	5 (4.0)	4 (4.8)	1.0

[a] Control patients consisted of 24 patients with systemic erythematosus, 14 with Sjögren syndrome, 15 with scleroderma, 15 with mixed connective tissue disease, and 15 with polymyositis/dermatomyositis

[b] Anti-LF positivity was significantly more common than positivity for the ANCAs of the other 5 antigens in RA patients ($p < 0.05$)

RA patients contained anti-LF, whereas only a few of the sera samples showed CG, EL, MPO or PR-3 (Table 2). The percentage of anti-LF-positive patients was not significantly higher than the percentage of anti-LF-positive patients with control diseases. Among the patients with control diseases, four of the 15 sera samples (26.7%) from PM/DM patients reacted to LF and 4 of the 24 (15.7%) sera from SLE patients reacted to EL (Table 3). The average values for the optical density (OD) of anti-LF antibodies in RA patient sera and control patient sera were not significantly different (data not shown).

Clinical manifestations and laboratory parameters of ANCA-positive and ANCA-negative RA patients

Since ANCAs may be pathologically involved in vasculitis or interstitial pneumonia in RA patients, the clinical manifestations of 30 ANCA-positive patients and 95 ANCA-negative patients were investigated. No statistically significant difference between these groups in terms of the presence of interstitial pneumonitis (IP), cutaneous vasculitis (CV), rheumatoid cutaneous nodules (RCN) and mononeuropathy multiplex (MNM) was observed. However, the joint score was significantly increased in the ANCA-positive group (40.8 ± 43.3 vs. 24.3 ± 26.2, $p < 0.05$) (Table 4).

Laboratory data for 125 ANCA-positive and ANCA-negative RA patients were investigated. The erythrocyte sedimentation rate (ESR) was found to be significantly higher for the ANCA-positive group than for the ANCA-negative group ($p < 0.05$). No statistically significant differences in C-reactive protein (CRP), RF, immunoglobulin (Ig) G, CH50 and IgG-RF were noted (Table 4).

Clinical manifestations, laboratory parameters and treatment of RA patients in comparison with ANCA positivity/negativity

Clinical manifestations and laboratory parameters of 125 RA patients were studied in relation to ANCA positivity/negativity. More of the patients with IP, CV, RCN, and MNM were found to be anti-LA, anti-CG, anti-EL, anti-MPO, and anti-PR-3 negative. Joint score was found to be statistically significantly higher in the anti-LF- and PR-3-positive groups than in the corresponding negative groups ($p < 0.05$, respectively) (Table 5). No statistically significant difference was found between the positive and negative groups for ANCAs against various antigens in terms of the other parameters. CRP and ESR had a tendency to be higher in the anti-LF-positive group than in the anti-LF-negative group (Table 5). No statistically significant difference was observed between the positive and negative groups for ANCAs against various antigens in terms of DMARDS, steroids, or plasmapheresis.

Case report of an MRA patient with anti-lactoferrin antibody

A 35-year-old man was diagnosed with RA in 1989. He had been treated with steroids, aurothiomalate sodium and NSAIDs, but that therapy had not been effective. He was treated with bucillamine and mizoribine in May 1994, and predonisolone was given at doses of 5–20 mg/day. He was started on DFPP in October 1994 and granulocytapheresis in July 1995 because the disease activity had not been controlled by the above medications. After granulocytapheresis the disease activity decreased and CRP and ESR were normalized. In the summer of 1996, we diagnosed him with malignant RA, considering his mononeuritis multiplex, rheumatoid nodule and decreasing level of serum complement. Increases in CRP and ESR were noted and his clinical manifestations worsened in May 1997. His

Table 3 ANCAs against the 5 antigens in sera from patients with various rheumatic diseases

ANCA antigen	SLE $n = 24$ (%)	SjS $n = 14$ (%)	SSc $n = 15$ (%)	MCTD $n = 15$ (%)	PM/DM $n = 15$ (%)
Lactoferrin (LF)	1 (4.2)	0 (0)	1 (6.7)	2 (13.3)	4 (26.7)[a]
Catepsin G (CG)	2 (8.3)	1 (7.1)	3 (20.0)	2 (13.3)	1 (6.7)
Elastase (EL)	4 (15.7)[b]	1 (7.1)	2 (13.3)	3 (20.0)	1 (6.7)
Myeloperoxidase (MPO)	0 (0)	0 (0)	1 (6.7)	1 (6.7)	0 (0)
Proteinase 3 (PR-3)	2 (8.3)	1 (7.1)	0 (0)	1 (6.7)	0 (0)

[a] Not significant compared to the corresponding value for SLE

[b] Not significant compared to the corresponding value for PM/DM

Table 4 Clinical manifestations and laboratory parameters for the ANCA-positive and ANCA-negative patients with RA

Clinical manifestation	ANCA positive $n = 30$ (%)	ANCA negative $n = 95$ (%)
Interstitial pneumonia	2 (6.7)	8 (8.4)
Cutaneous vasculitis	2 (6.7)	2 (2.1)
Rheumatoid nodules	1 (3.3)	5 (5.3)
Mononeuropathy multiplex	1 (3.3)	5 (5.3)
Joint score[a]	40.8 ± 43.3[b]	24.3 ± 26.2
Laboratory parameter	**ANCA positive $n = 28$, mean ± SD**	**ANCA negative $n = 87$, mean ± SD**
WBC (/μL)	8303.6 ± 1878.0	7048.2 ± 2251.2
ESR (mm/h)	44.4 ± 22.4[c]	28.9 ± 23.6
CRP (mg/dl)	3.79 ± 3.60	2.78 ± 4.32
RF (IU/ml)	95.3 ± 173.5	92.7 ± 155.7
IgG (mg/dl)	1852.0 ± 246.7	1763.1 ± 338.9
CH50 (U)	48.5 ± 10.4	45.0 ± 8.2
IgG-RF (U)	1.27 ± 0.9	1.35 ± 1.18

Normal values of the laboratory parameters are: WBC, 4000–8000/μL; ESR, 1–7 mm/h (male), 3–11 mm/h (female); CRP, <0.3 mg/dl; RF, <20 IU/ml; IgG, 748–1694 mg/dl; CH50, 25–54 U/dl; IgG-RF, <2.0 U

[a] Determined by Landsbary's method [25]

[b,c] Significantly higher than that for the ANCA-negative group ($p < 0.05$)

serum level of anti-lactoferrin antibody was slightly raised at the first DFPP (0.03 OD, normal range: <0.006 OD), and his anti-LF antibody rose to 0.176 OD when his disease activity increased. After we shortened the interval between DFPP to once a week, his clinical manifestations and joint score decreased and his CRP and ESR normalized. His serum level of anti-LF antibody decreased from 0.176 to 0.064 OD. Levels of RF, RAPA and ANCAs against other four antigens did not rise, but IgG-RF increased slightly when his disease activity increased. Rises in the levels of ANCAs against the other four antigens were not observed during the course of this patient (Fig. 1).

Discussion

The prevalence of pANCAs in RA patients based on the results of IIF has varied from 16 to 50% [13–19]. The reported prevalence of MPO ANCAs among RA patients has ranged from 1 to 20%, and that for anti-LF from 3.2 to 53% [14–18, 26, 27]. IIF was not performed in this study, and only antigen-specific ANCAs were determined. We found that 24% (30/125) of our RA patients were positive for at least one of the 5 ANCA antigens considered in this study (Table 1). Six of the 125 (4.8%) RA patients had anti-MPO antibodies and 21/125 (16.8%) were positive for anti-LF (Table 2). Only 2 of the 8 serum samples from patients with MRA (RA with vasculitis and/or extra-articular involvement) were ANCA positive. According to a previous Japanese report, 9 of 52 (17.3%) RA patients were positive for pANCAs according to IIF [28]. Based on these results, the prevalences of ANCAs, MPO ANCAs and anti-LF in the RA patients in this study were not very different from those previously reported from other countries, although a variance among reports was noted.

The most important aim of this study was to investigate relationships between clinical feature, especially extra-articular manifestations and the presence of ANCAs for specific antigens. The rates of interstitial pneumonitis (IP), cutaneous vasculitis (CV), rheumatoid (cutaneous) nodules (RN), and mononeuropathy multiplex (MNM) were not different between the ANCA-positive and the ANCA-negative groups. Eight of the 9 sera for which we had previously reported elevated levels of soluble CD154 in RA with vasculitis were identical [29]. A higher JS was observed in the ANCA-positive group and in anti-LF- and anti-PR-3-positive patients (Tables 4, 5). The only statistically significant difference in laboratory findings between the ANCA-positive and -negative groups was found for the ESR. Although ESR and CRP were found to be elevated in the anti-LF-positive and anti-PR-3-positive groups compared with the anti-LF-negative and anti-PR-3-negative groups, respectively, the differences were not statistically significant (Table 5).

ANCAs have been reported to be relevant to vascular and/or extra-articular involvement in patients with RA

Table 5 Clinical manifestations and laboratory parameters for RA patients who are positive or negative for ANCAs against LF, CG, EL, MPO and PR-3

Clinical manifestation	Lactoferrin		Cathepsin G		Elastase		Myeloperoxidase		Proteinase 3	
	Positive ($n = 21$)	Negative ($n = 104$)	Positive ($n = 5$)	Negative ($n = 120$)	Positive ($n = 8$)	Negative ($n = 117$)	Positive ($n = 6$)	Negative ($n = 119$)	Positive ($n = 5$)	Negative ($n = 120$)
Interstitial pneumonia	2	8	0	10	1	9	2	8	1	9
Cutaneous vasculitis	2	3	0	5	0	5	0	5	1	4
Rheumatoid nodules	1	5	0	6	0	6	0	6	0	6
Mononeuropathy multiplex	1	5	0	6	1	5	1	5	0	6
Joint score	47.3[a]	24.8	30.0	28.2	22.1	28.8	34.0	28.0	45.6[a]	27.4

Laboratory parameter	Lactoferrin		Cathepsin G		Elastase		Myeloperoxidase		Proteinase 3	
	Positive ($n = 19$)	Negative ($n = 98$)	Positive ($n = 5$)	Negative ($n = 112$)	Positive ($n = 8$)	Negative ($n = 109$)	Positive ($n = 5$)	Negative ($n = 112$)	Positive ($n = 5$)	Negative ($n = 112$)
WBC (/μL)	8354	7159	8920	7287	7975	7312	8240	7319	7850	7341
ESR (mm/h)	48.9	39.4	37.7	32.7	34.8	32.7	30.2	32.9	43.5	32.4
CRP (mg/dl)	4.2	2.79	2.6	3.04	3.18	3.01	1.96	3.07	5.1	2.95
RF (IU/ml)	115.4	89.0	280.0	224.6	48.8	96.9	20.0	96.1	94.5	93.3
IgG (mg/dl)	1865	1768	1851	1782	1765	1786	1534	1795	1903	1780
CH50 (U)	47.5	44.5	49.0	45.7	47.7	45.7	50.9	45.6	52.1	45.6
IgG-RF (U)	1.52	1.3	1.33	1.33	1.42	1.32	0.75	1.34	1.05	1.34

[a] $p < 0.05$

[7, 13, 18, 19]. According to our results, anti-LF antibody is not associated with extra-articular manifestations such as IP, CV, RN and MNM, but it is closely related to active joint inflammation, as previously reported by several investigators [2, 12, 16, 28]. Chikazawa et al. [15] indicated that anti-LF antibody is associated with lung fibrosis. Coremans et al. [18] suggested that the presence of anti-LF antibody is associated with vasculitis.

We could not prove the association of extra-articular manifestations with the presence of ANCAs for specific antigens from the small number of patients with extra-articular manifestations included in our study. However, an association of extra-articular manifestations and inflammation with anti-LF antibody is suggested by the case of a particular MRA patient, whose serum level of anti-LF antibody increased when his disease activity increased, while the levels of the ANCAs for the other four antigens did not increase. This study was actually designed to investigate disease duration [12, 27] and/or radiological changes [17]; further investigations will elucidate the main roles of anti-LF.

The pathological implications of LF and anti-LF have been reported mainly by a Swedish group. Nonstimulated leukocytes were activated by anti-LF and bound to endothelial cells [28]. In relation to heat shock protein, a similarity in amino acid sequence between mycobacterium/human HSP-60 and MPO has been demonstrated [30], LF shows cross-reactivity with mycobacterial HSP-65, while T cells from rats with mycobacterium-induced adjuvant arthritis proliferate on exposure to LF [31].

Autoantibodies against heat-shock protein 65 have been identified in RA patients [32]. However, these reports of cross-reactivity are still controversial [33].

Recent advances in molecular science have shown that lactoferrin is a multitasking protein: it exhibits antibacterial, antiviral and antitumor activities, regulates cell growth and differentiation, exhibits antiinflammatory activity, and modulates immune response [34]. The protective effects of LF may involve inhibiting the production of several proinflammatory cytokines, including tumor necrosis factor-α, interleukin-1β, and IL-6. LF has been shown to directly bind and attenuate the immunostimulatory responses of LPS, soluble CD14, and unmethylated CpG bacterial DNA. LF translocates to the nucleus and prevents NF-κB activation. LF stimulates the production and/or activation of NK cells [35]. Treatment of chronic hepatitis C infection by oral administration of LF resulted in a decreased HCV RNA titer, but the clearance of HCV RNA is impaired in patients who have anti-LF [36]. Based on these biological roles of LF, the associations of anti-LF antibody with several extra-articular involvements in RA patients described in previous reports, and the results of this study, anti-LF antibodies bind to LF and block the anti-inflammatory activity of LF, which implies a deterioration in host defense against microorganisms and inflammation.

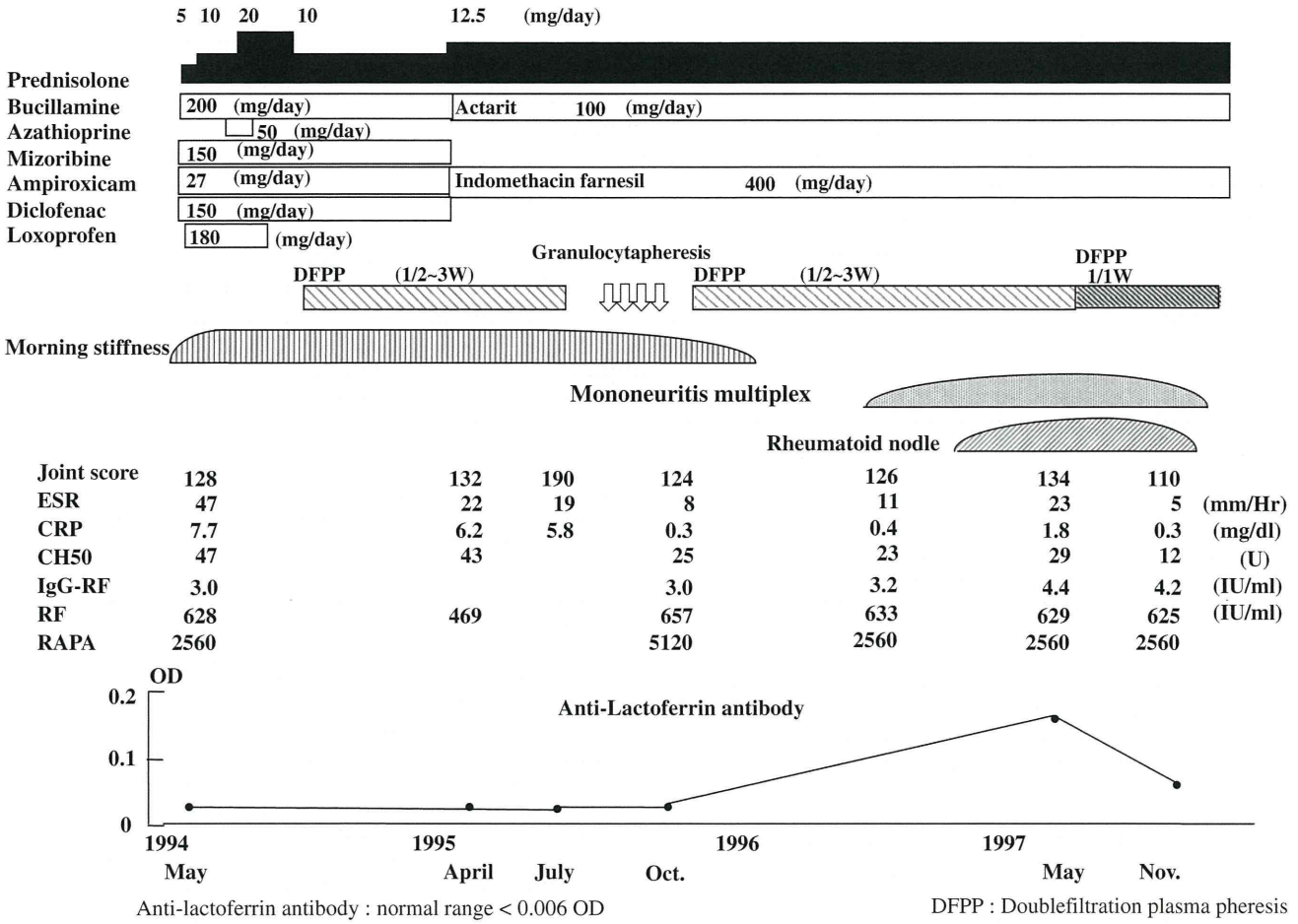

Fig. 1 Clinical course of the MRA patient with anti-lactoferrin antibody

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Conflict of interest None.

Appendix

See Table 6.

Table 6 Diagnostic criteria for malignant rheumatoid arthritis (MRA)

Fulfilment of the 1987 ACR criteria for RA and the following criteria:	
I. Clinical and laboratory judgment	
1. Neuropathy	
2. Cutaneous vasculitis	Cutaneous ulcer, gangrene or infarction of finger or toes
3. Subcutaneous rheumatoid nodules	Clinical judgment
4. Sclerotis, eposcleritis	Clinical judgment by ophthalmologist and not due to other causes
5. Pericarditis, pleuritis	Clinical judgment by physician and not due to infection or other causes
6. Myocarditis	Clinical judgment, myogenic enzyme and ECG, UCG
7. Pulmonary fibrosis	Clinical judgment by physician and chest X-ray; pulmonary function tests

Table 6 continued

8. Vasculitis involving other organs	Infarction of cardiac muscle, bowel and lung and clinical judgment by organ specialist
9. Increased level of rheumatoid factor	Found to be more than double the normal level
10. Decreased level of serum complement or positive immune complex	C3, C4, CH50 are in low level or C1q is positive more than twice
II. Biopsy	
Positive biopsy from nerve, skin, muscle, and other organs, such as necrotizing vasculitis, endarteritis obliterans and granulomatous angiitis	
Diagnosis	Fulfilled at least 3 items from I1–10 or 1 item from I and an item from II
Differential diagnosis	Infections, secondary amyloidosis and side effects of drugs (especially gold, D-pc, bucillamine, etc.), Sjögren's syndrome (about 10% of MRA cases are complicated with Sjögren's syndrome), Felty's syndrome, and overlapping syndromes with other rheumatic diseases (SLE, PSS, PM, DM)

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ORIGINAL ARTICLE

Performance of serum cystatin C versus serum creatinine as a marker of glomerular filtration rate as measured by inulin renal clearance

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Abstract
Introduction Serum cystatin C was recently proposed as an alternative marker of glomerular filtration rate (GFR), with a suggested better performance than creatinine. However, detailed studies are limited. We evaluated the performance of cystatin C as a GFR marker.
Methods GFR was measured by inulin clearance in 763 Japanese subjects. Factors other than GFR influencing serum cystatin C or serum creatinine were analyzed by multivariate analyses.
Results After adjustment for GFR, the value of serum creatinine was 25.2% lower in females than males, and decreased by 5.2% for every 20 years of age. Serum cystatin C was 8.2% lower in females, and did not change significantly with aging. Creatinine but not cystatin C was significantly affected by body weight, height and body mass index after adjustment for GFR, gender and age. The correlation coefficient between GFR and 1/cystatin C was significantly higher than that of 1/creatinine in total subjects (0.866 and 0.810, respectively, $p < 0.001$). Unlike serum creatinine, serum cystatin C did not increase in association with the reduction of GFR in subjects with very low GFR. The regression line of 1/cystatin C against GFR showed a significantly negative intercept of about -8 ml/min/1.73 m^2.
Conclusion The performance of serum cystatin C was not good in the subjects with very low GFR. Non-renal elimination of cystatin C may contribute to the result. The correlation between reciprocal cystatin C and GFR suggested its superiority in predicting GFR compared to creatinine in subjects with normal and mildly reduced GFR.

Keywords Creatinine · Cystatin C · Inulin clearance · Glomerular filtration rate

Introduction

Serum creatinine level has been used to assess renal function, but is often affected by muscle mass, which is dependent on age, weight, and gender [1]. Serum cystatin C was recently proposed as an alternative marker of glomerular filtration rate (GFR), and its higher performance compared with creatinine has been suggested from a meta-analysis [2]. However, detailed studies on the comparison between cystatin C and serum creatinine as markers of GFR are limited. In the present study, we compared the performance of serum cystatin C and serum creatinine as a GFR marker in subjects stratified by gender and age. We confirmed the better performance of cystatin C but also found an apparent non-renal elimination of cystatin C in subjects with low GFR that may affect the performance of cystatin C as a GFR marker.

On behalf of the collaborators for developing Japanese equation for estimating GFR.

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Methods

Subjects and measurements

To compare the performance of serum cystatin C with that of serum creatinine, we used same data set from which the GFR equation for Japanese was developed. Details of the subjects have been reported previously [3, 4]. A total of 763 Japanese patients in 80 medical centers were included. We stratified the subjects according to gender and into three age groups (18–39, 40–59 and 60–79 years old). GFR was measured by inulin renal clearance [3], and serum creatinine was measured by the IDMS-traceable enzymatic method [3]. In the present study, we analyzed the serum cystatin C values which were measured with serum creatinine previously in a single laboratory. Cystatin C was measured by nephrometric assay (Dade Behring).

Multivariate analyses

Factors other than GFR influencing serum cystatin C levels were analyzed by multivariate linear regression analyses. Cystatin C levels and GFR were log-transformed. Age, height, weight and body mass index (BMI) were used as the raw data. Gender was expressed as a binary factor. After adjusting for GFR, the percent change in serum level of cystatin C for a change of 20 years of age, female gender and one unit in the variables such as height, weight, and BMI was analyzed. We also examined the same analyses using serum creatinine levels.

Correlation coefficient between GFR and 1/cystatin C

The correlation coefficient between GFR and 1/cystatin C was analyzed in total subjects and in subjects stratified by gender and age groups (18–39, 40–59 and 60–79 years old). To evaluate an apparent non-renal elimination of cystatin C, the intercept of the linear regression line in the reciprocal plot was calculated. The same analyses were performed for reciprocal serum creatinine. The relationship between GFR and reciprocal creatinine curved upwards slightly, and therefore linear regression lines for 1/creatinine and 1/cystatin C were separately calculated in subjects with serum levels higher than 1.5 mg/dl and 1.5 mg/L, respectively.

Statistical analysis

Data were expressed as means ± SD. $p < 0.05$ was considered statistically significant. Statview version 4.02 (SAS Institute) and JMP 8.01 (SAS Institute) were used for statistical analyses. Smoothed lines fit to the data (Figs. 2, 3, 5) were calculated using spline model of JMP 8.01 (SAS Institute).

Results

Table 1 shows the characteristics of the study subjects. Mean measured GFR of males was significantly lower than that of females (54 ± 34 and 65 ± 36 ml/min/1.73 m^2, respectively). Mean measured GFR in the older age group was significantly lower than that in the younger age group in both males and females.

Multivariate analyses

Serum creatinine was 25.2% lower in females than in males, and decreased by 5.2% for every 20 years of age after adjustment for GFR (Table 2). Serum creatinine was significantly increased in association with increase in body weight, height, and BMI after adjustment for GFR, gender and age. Serum cystatin C was 8.2% lower in females than in males, and was not significantly changed by age after adjustment for GFR. Cystatin C was not significantly

Table 1 Characteristics of the study subjects

	N	Age	GFR (ml/min/1.73 m^2)	Cystatin-C (mg/L)	Creatinine (mg/dl)	Weight (kg)	Height (cm)
Male							
18–39 years old	119	30 ± 6	77 ± 37	1.34 ± 0.89	1.41 ± 1.32	69 ± 13	171 ± 5
40–59 years old	145	52 ± 6	54 ± 31	1.63 ± 1.02	1.81 ± 1.68	68 ± 11	169 ± 7
60–79 years old	189	68 ± 5	41 ± 26	2.01 ± 1.04	2.17 ± 1.73	63 ± 11	164 ± 7
Total	465	54 ± 17	54 ± 34	1.72 ± 1.04	1.87 ± 1.64	66 ± 12	167 ± 7
Female							
18–39 years old	91	30 ± 6	83 ± 33	1.07 ± 0.81	0.91 ± 0.96	53 ± 8	159 ± 6
40–59 years old	106	51 ± 5	64 ± 37	1.41 ± 0.95	1.22 ± 1.22	54 ± 12	155 ± 5
60–79 years old	94	68 ± 5	50 ± 28	1.66 ± 1.12	1.37 ± 1.17	51 ± 8	151 ± 6
Total	298	51 ± 17	65 ± 36	1.40 ± 0.99	1.18 ± 1.13	53 ± 10	155 ± 6

Table 2 Factors affecting serum cystatin C and creatinine after adjustment for GFR

Variable	Cystatin C % change (95% CI)	*p*	Creatinine % change (95% CI)	*p*
Adjusted for GFR				
Age (20 years)	−0.1 (−2.0 to 2.2)	0.9	−5.2 (−7.9 to −2.5)	0.0002
Female	−8.2 (−11.2 to −5.2)	<0.0001	−25.2 (−28.1 to −22.4)	<0.0001
Height (cm)	0.4 (0.2 to 0.6)	<0.0001	1.5 (1.3 to 1.7)	<0.0001
Weight (kg)	0.2 (0.0 to 0.3)	0.006	0.9 (0.7 to 1.0)	<0.0001
BMI (kg/m^2)	0.2 (−0.3 to 0.6)	0.5	1.4 (0.9 to 2.0)	<0.0001
Adjusted for GFR, age and gender				
Height (cm)	0.2 (−0.1 to 0.5)	0.1	0.6 (0.3 to 0.9)	0.0001
Weight (kg)	0.0 (−0.1 to 0.2)	0.8	0.4 (0.2 to 0.6)	<0.0001
BMI (kg/m^2)	−0.1 (−0.5 to 0.4)	0.8	0.8 (0.3 to 1.3)	0.001

% change percent change in serum level of creatinine or cystatin C for a change of 20 years of age, female gender and one unit in the variable (height, weight and BMI)

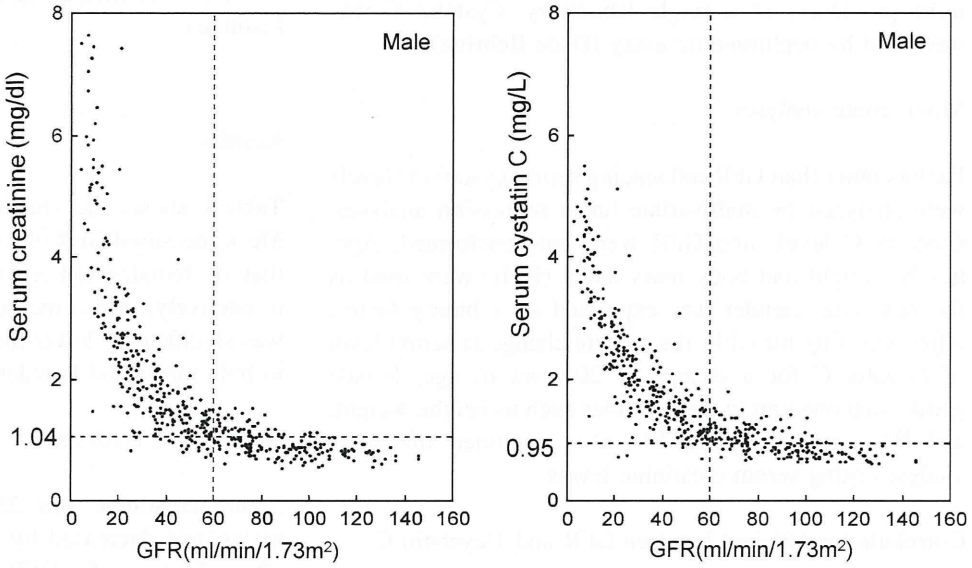

Fig. 1 Relationship between GFR and serum concentration of creatinine or cystatin C. *Left* GFR versus serum concentration of creatinine in male subjects. *Dotted lines* show upper reference limit of serum creatinine and lower reference limit of GFR (1.04 mg/dl and 60 ml/min/1.73 m^2, respectively). *Right* GFR versus serum concentration of cystatin C in male subjects. *Dotted lines* show upper limit of serum cystatin C and lower reference limit of GFR (0.95 mg/dl and 60 ml/min/1.73 m^2, respectively)

changed by body weight, height and BMI after adjustment for GFR, gender and age.

Relationship between GFR and serum concentration of cystatin C

The relationship between GFR and serum concentrations of cystatin C is shown in Fig. 1. The plot of serum cystatin C showed a curvilinear pattern that was similar to that of serum creatinine. The relationship between GFR and 1/cystatin C was almost linear, while the plots of 1/creatinine seemed to be curving upwards slightly (Figs. 2, 3). The correlation coefficient in the reciprocal plot of cystatin C was significantly higher compared with that of creatinine in total subjects ($r = 0.866$ and 0.810, respectively) (Table 3). The correlation coefficients of cystatin C were consistently higher than the values of creatinine in subjects stratified by gender and three age groups, although statistically not significant.

Relationship between serum concentration of creatinine and serum concentration of cystatin C

The relationship between serum concentration of creatinine and serum concentration of cystatin C was not linear (Fig. 4). Unlike serum creatinine, serum cystatin C did not increase in association with reduction of GFR in subjects with GFR below 15 ml/min/1.73 m^2 (Fig. 5). To evaluate the apparent non-renal elimination of cystatin C, the linear regression line of the reciprocal plots was calculated in subjects with serum cystatin C higher than 1.5 mg/L. Intercepts (95% confidence interval [CI]) of the regression lines in male and female were −8.4 (−12.1, −4.8) ml/min/1.73 m^2 and −9.1 (−15.5, −2.6) ml/min/1.73 m^2, respectively (Table 4). The values were significantly lower than zero ($p < 0.01$). The linear regression line of the reciprocal plots of creatinine that was calculated in subjects with serum creatinine higher than 1.5 mg/dl intersected near the origin. Intercepts (95% CI) of the regression lines in

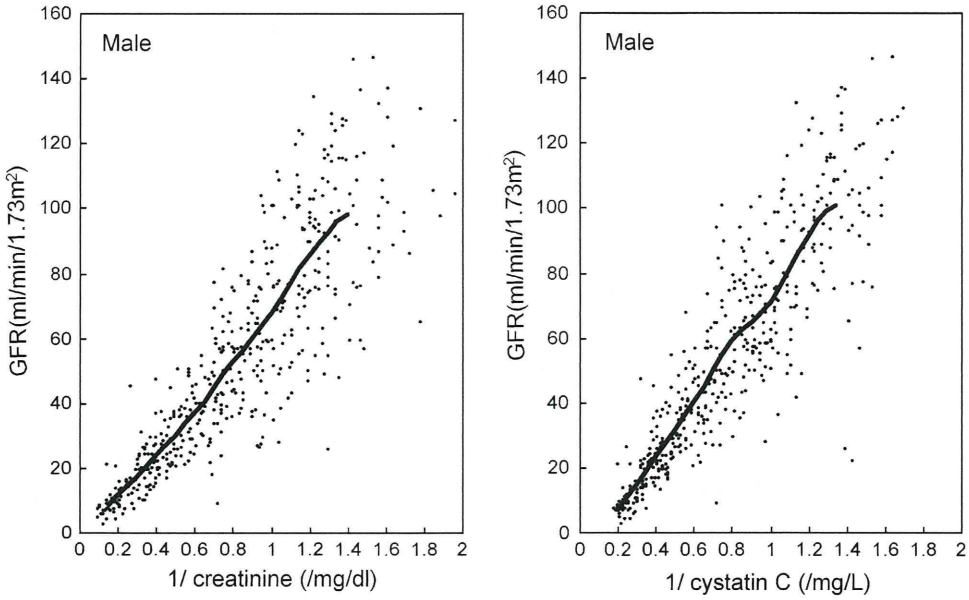

Fig. 2 Reciprocal plots of creatinine and cystatin C in male subjects. *Left* GFR versus 1/creatinine. *Right* GFR versus 1/cystatin C. *Smooth lines* show the fit of the data

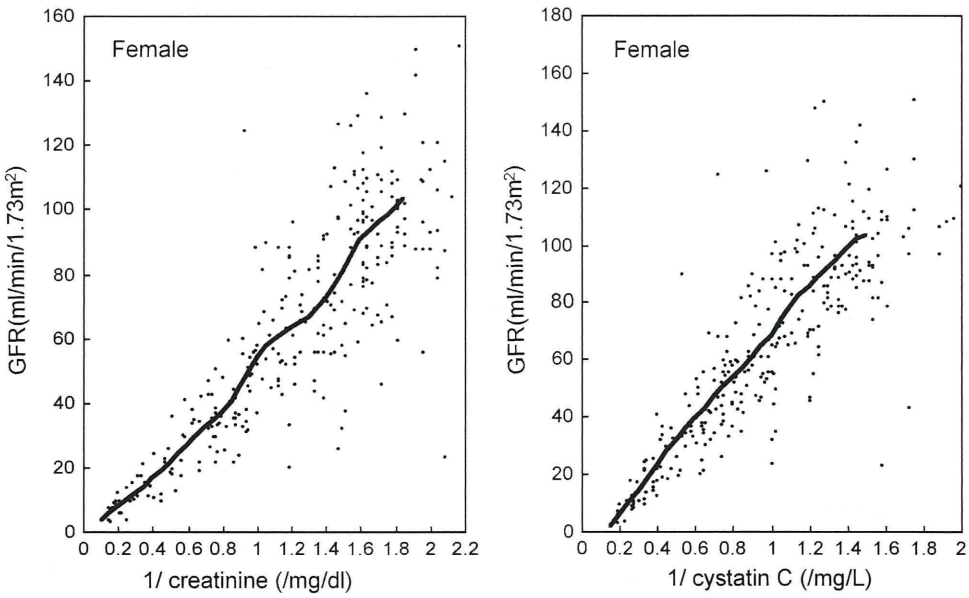

Fig. 3 Reciprocal plots of creatinine and cystatin C in female subjects. *Left* GFR versus 1/creatinine. *Right* GFR versus 1/cystatin C. *Smooth lines* show the fit of the data

males and females were −0.9 (−3.8, 2.0) ml/min/1.73 m^2 and −2.2 (−6.5, 2.1) ml/min/1.73 m^2, respectively. The values were not significantly different from zero.

Discussion

Serum creatinine and cystatin C are well-known markers of GFR. However, few studies have investigated their comparative performance relative to GFR measured by inulin renal clearance. In present study, we observed a significant difference in performance of creatinine and cystatin C as GFR markers. Gender and age effects were more prominent in creatinine. Serum creatinine was 25.2% lower in females, and declined by 5.2% for every 20 years of age, while serum cystatin C was 8.2% lower in females, and was not significantly associated with age. These results are almost consistent with the reports of Stevens et al. and Knight et al. [5, 6]. Stevens et al. reported that older age was associated with lower serum cystatin C level after adjustment of GFR measured by iothalamate clearance [5]. On the other hand, Knight et al. reported that older age was associated with higher serum cystatin C level after adjusting for creatinine clearance [6]. The backgrounds of the study population such as ethnicity, renal function and physique were different between the studies. The variable factors and methods of GFR measurement may influence the results of the studies.

Table 3 Correlation coefficients between GFR and 1/cystatin C or 1/creatinine in subjects stratified by gender and age groups

	N	GFR (ml/min/1.73 m^2)	Correlation coefficient		*p*
			1/cystatin C	1/creatinine	
Male					
18–39 years old	119	77 ± 37	0.919	0.880	0.11
40–59 years old	145	54 ± 31	0.848	0.833	0.67
60–79 years old	189	41 ± 26	0.865	0.837	0.32
Total	465	54 ± 34	0.890	0.854	0.09
Female					
18–39 years old	91	83 ± 33	0.797	0.786	0.84
40–59 years old	106	64 ± 37	0.815	0.750	0.22
60–79 years old	94	50 ± 28	0.856	0.846	0.80
Total	298	65 ± 36	0.832	0.794	0.17
Male + female					
Total	763	58 ± 35	0.866	0.810	0.0002

p values: difference between cystatin C and creatinine

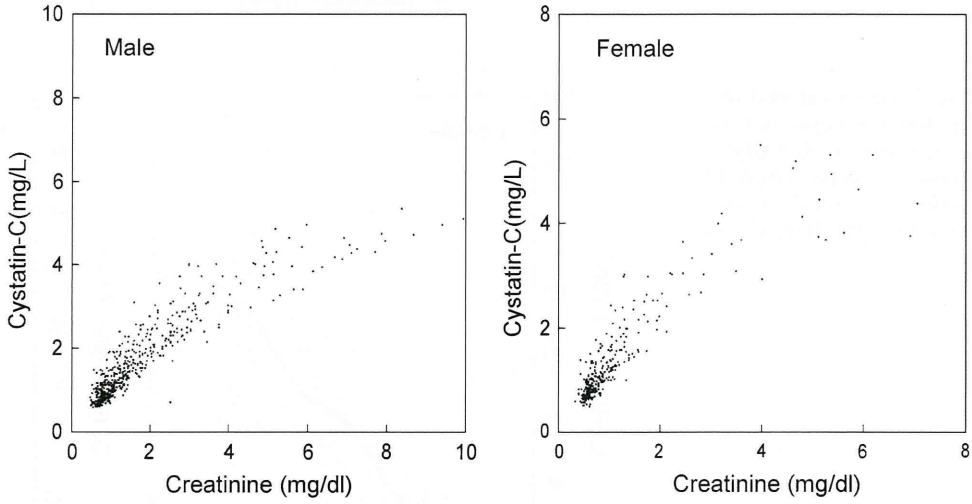

Fig. 4 Relationship between serum concentration of creatinine and serum concentration of cystatin C

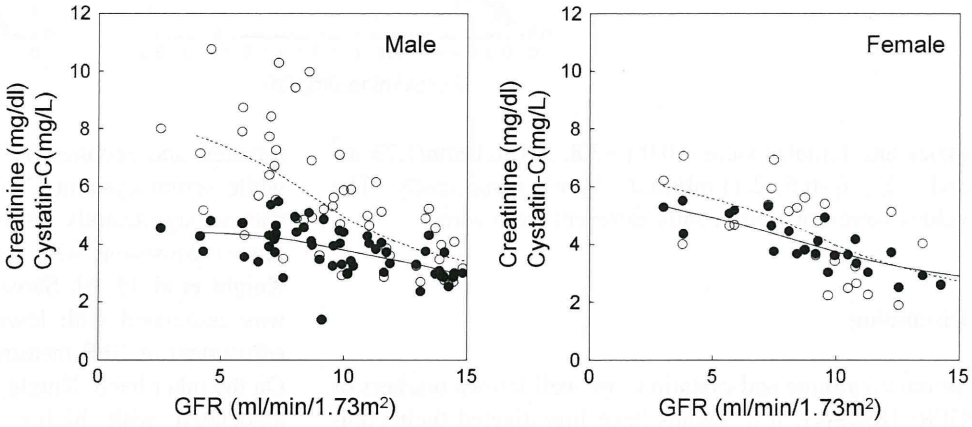

Fig. 5 Serum concentrations of creatinine and cystatin C in subjects with GFR under 15 ml/min/1.73 m^2. Unlike serum creatinine (*open circle*), serum cystatin C (*closed circle*) did not increase as much in association with reduction of GFR in subjects with GFR under 15 ml/min/1.73 m^2. *Smooth lines* show the fit of the data

Generation of creatinine is affected by muscle mass, which is dependent on physique such as weight and height. Higher serum creatinine was associated with higher body weight, height, and BMI after adjustment for GFR, gender and age (Table 2). The association of these parameters with serum cystatin C level was much smaller than with creatinine. These results are consistent with the report of Stevens et al. [5] except for BMI. They reported that the

Table 4 Intercept of the regression line between GFR and 1/cystatin C or 1/creatinine in subjects stratified by gender and age groups

	1/cystatin C			1/creatinine		
	N	Intercept (95% CI)	*p*	*N*	Intercept (95% CI)	*p*
Male						
18–39 years old	34	−11.2 (−19.7 to −2.7)	0.01	30	−0.1 (−8.9 to 8.7)	0.9
40–59 years old	50	−7.4 (−15.1 to 0.3)	0.06	57	−0.5 (−5.3 to 6.2)	0.9
60–79 years old	118	−9.5 (−14.5 to −4.6)	0.0002	100	−1.8 (−5.6 to 2.0)	0.3
Total	222	−8.4 (−12.1 to −4.8)	<0.0001	194	−0.9 (−3.8 to 2.0)	0.5
Female						
18–39 years old	9	−8.3 (−18.0 to 1.3)	0.08	6	−2.7 (−15.8 to 10.4)	0.6
40–59 years old	35	−10.6 (−20.6 to −5.2)	0.04	18	−1.3 (−7.0 to 4.5)	0.6
60–79 years old	37	−7.6 (−19.9 to 4.8)	0.2	25	−2.1 (−10.1 to 6.0)	0.6
Total	87	−9.1 (−15.5 to −2.6)	0.007	51	−2.2 (−6.5 to 2.1)	0.3

Regression lines were made using subjects with serum cystatin C over 1.5 mg/L or serum creatinine over 1.5 mg/dl

Table 5 Simulation models for reciprocal plots of creatinine and cystatin C

	Model A			Model B			Model C	
GFR	CCR/GFR	G1	P1	N-CL	G2	P2	G2	P2
100	1.20	0.96	0.80	8	0.096	0.80	0.0800	0.80
90	1.25	0.96	0.85	8	0.096	0.88	0.0793	0.88
80	1.30	0.96	0.92	8	0.096	0.98	0.0785	0.98
70	1.35	0.96	1.02	8	0.096	1.11	0.0775	1.11
60	1.40	0.96	1.14	8	0.096	1.27	0.0762	1.27
50	1.45	0.96	1.32	8	0.096	1.49	0.0745	1.49
40	1.50	0.96	1.60	8	0.096	1.80	0.0720	1.80
30	1.55	0.96	2.06	8	0.096	2.27	0.0682	2.27
20	1.60	0.96	3.00	8	0.096	3.09	0.0617	3.09
10	1.65	0.96	5.82	8	0.096	4.80	0.0480	4.80

GFR glomerular filtration rate (ml/min), *Model A* simulation model for serum creatinine, *CCR/GFR* CCR/GFR ratio, *G1* generation of creatinine (mg/min), *P1* plasma concentration of creatinine (mg/dl), *Model B* and *Model C* simulation models for serum cystatin C, *N-CL* non-renal clearance of cystatin C (ml/min), *G2* generation of cystatin C (mg/min), *P2* plasma concentration of cystatin C (mg/L)

percent change of cystatin C was higher than the value of creatinine according to the change in BMI, and speculated an association between fat mass and cystatin C levels. The mean and interquartile range of BMI in the study by Stevens et al. were 27.7 and 7.3 kg/m^2, while the values in our study were much lower (22.9 and 4.8 kg/m^2, respectively). The lower prevalence of obesity in our subjects may contribute to the inconsistent results regarding the association with BMI.

The relationship between GFR and 1/cystatin C was almost linear, evaluated by the smoothed line fit to the data (Figs. 2, 3). On the other hand, the relationship between GFR and 1/creatinine was slightly upwardly curving. This could be one of the reasons that reduces the performance of creatinine in the correlation analysis. Creatinine is secreted from tubules as well as filtered from glomeruli. Therefore, the creatinine clearance (CCR) exceeds the GFR. About 20% of creatinine is secreted from the tubule in normal subjects [1], and the tubular secretion increases up to about 50% with reduction in GFR [1]. The change in tubular secretion may contribute to the upward curve of the reciprocal plot of creatinine. We made a simulation model in which the CCR/GFR ratio was increased from 1.2 to 1.7 according to the reduction in GFR with constant generation of creatinine (Table 5; Fig. 6, model A). The simulation model confirmed the effect of tubular secretion on the upward curving of the reciprocal plot.

The reciprocal plot of serum cystatin C had a significantly negative intercept, suggesting an apparent non-renal elimination of the marker. Assuming that the generation of cystatin C is independent of GFR, non-renal elimination of cystatin C is estimated as the intercept of the reciprocal plot. We obtained an apparent non-renal clearance of cystatin C of about 8 ml/min/1.73 m^2. Sjostrom et al. [7, 8] reported similar results using the reciprocal plot of cystatin C. They measured GFR by plasma clearance of iohexol and reported the apparent non-renal elimination of cystatin C as 22.3 ml/min/1.73 m^2 in a preliminary

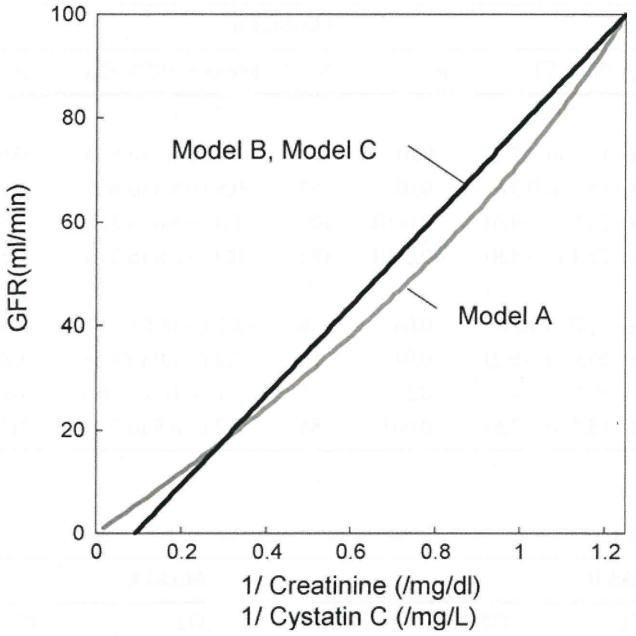

Fig. 6 Simulation models for reciprocal plots of creatinine and cystatin C. Detailed values for simulation models are described in Table 5. Model A is a simulation model for the reciprocal plot of creatinine. CCR/GFR ratio increases according to reduction of GFR. This leads to the upward curve of the reciprocal plot of creatinine. Model B is a simulation model for the reciprocal plot of cystatin C with constant non-renal clearance. This model shows the linear relationship between GFR and 1/cystatin C. Model C is a simulation model for the reciprocal plot of cystatin C without non-renal clearance. Controlled change in the generation of cystatin C is required to keep the linear relationship between GFR and 1/cystatin C

study and 14.1 ml/min/1.73 m^2 in a second study[7, 8]. We made two simulation models to explain the apparent non-renal elimination of cystatin C. The first is a simulation model with constant non-renal clearance set at 8 ml/min. The generation of cystatin C was set as the value at which serum cystatin C was 0.8 mg/L when GFR was 100 ml/min. The simulation model with constant non-renal clearance shows a linear relationship between GFR and reciprocal cystatin C with negative intercept (Table 5; Fig. 6, model B). In the second simulation model, the generation of cystatin C was decreased according to reduction in GFR. The same straight line as seen in model B could be drawn. In this case, generation of cystatin C decreases at an accelerated pace according to reduction of GFR (Table 5; Fig. 6, model C). This suggests that non-renal elimination of cystatin C is a more plausible mechanism than rapid reduction of cystatin C generation. Tenstad et al. [9] investigated renal handling of radiolabeled human cystatin C in rat. A considerable amount of extra-renal plasma clearance of labeled cystatin C was observed in nephrectomized rats, that contributed about 15% of the total plasma clearance of cystatin C. A relatively high uptake of radioactivity was recorded in the spleen. They speculated about the removal of cystatin C in the reticuloendothelial system.

Unlike serum creatinine, serum cystatin C did not increase in association with reduction of GFR in subjects with GFR below 15 ml/min/1.73 m^2. The performance of serum cystatin C was not good in these subjects, and therefore,serum cystatin C should not be used for evaluating GFR in subjects with end-stage renal disease. Apparent non-renal elimination of cystatin C contributed to the low performance of the marker. On the contrary, even if non-renal elimination of cystatin C is present at a comparable level, percentage of non-renal elimination in the total clearance would be small in subjects with normal and mildly reduced GFR, suggesting that the effect of non-renal elimination on serum levels of cystatin C is small and difficult to detect. Although the difference between the correlation coefficients of reciprocal cystatin C and reciprocal creatinine was small, cystatin C had a better correlation with GFR than creatinine in total subjects, suggesting that cystatin C concentration may be better than creatinine at predicting GFR in subjects with normal and mildly reduced GFR.

Eriksen et al. [10] showed that cystatin C is not a better estimator of GFR than creatinine, based on the testing of several GFR equations. They showed a large bias of GFR equations from cystatin C compared with GFR equations from creatinine, such as the CKD–EPI equation in the general population. The authors mentioned that the most important factor of the large bias of the cystatin C equations was probably that GFR equations from cystatin C were all developed in populations with CKD and low GFR. The influence of non-GFR factors on plasma cystatin C may differ between these patients and the general population. Also, standardization between assays and laboratories is lacking for cystatin C.

We agree with the comments of Eriksen et al. When the equation from cystatin C was developed from subjects with lower GFR, non-GFR elimination of cystatin C may influence the performance of the equation in subjects with normal or mildly reduced GFR. We have to study the model for GFR estimation including factors such as non-renal clearance. The measurement of cystatin C has not been standardized. There is up to 20% difference in the cystatin C values among various reagent companies [11, 12]. When the cystatin C value has a 20% positive bias compared with the value that was used for development of a GFR estimation equation, the measurement bias leads to about 20% underestimation of GFR and lower performance of the equation. The standardization of the measurement is a fundamental problem of the accuracy of the GFR equations derived from cystatin C. Standardization of the measurement of cystatin C is nowin progress worldwide [13], after which, development and validation of GFR equations from cystatin C will be required.

There are several limitations. The study subjects were almost all patients with native kidney disease, so it may not be possible to generalize the results to healthy subjects. It has been reported that cystatin C levels were influenced by factors other than GFR, such as thyroid function [14, 15], inflammation [5, 6], smoking [6, 8] and immunosuppressive therapy [16]. Multivariate analysis was not adjusted for the above factors in the present study.

Conclusion

The performance of serum cystatin C was not good in subjects with very low GFR; non-renal elimination of cystatin C may contribute to the result. Reciprocal cystatin C had a better correlation with GFR than creatinine in total subjects. Age, gender, body weight, height and BMI had a much smaller effect on cystatin C level than creatinine. These results suggested the superiority of cystatin C over creatinine in predicting GFR in subjects with normal and mildly reduced GFR.

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Appendix

The following investigators participated in the project for developing the Japanese equation for estimating GFR: Asahikawa Medical University; Kenjiro Kikuchi, Masakazu Haneda: Hokkaido University Hospital; Seiji Hashimoto: Sapporo Medical University; Nobuyuki Ura: Tohoku University Hospital; Sadayoshi Ito, Hiroshi Sato: Fukushima Medical University; Tsuyoshi Watanabe: Jichi Medical University; Yasuhiro Ando, Eiji Kusano: Gunma University; Yoshihisa Nojima: University of Tsukuba; Kunihiro Yamagata, Chie Saitoh: Toride Kyodo General Hospital; Tatsuo Shiigai, Yoshitaka Maeda: Saitama Medical University; Hiromichi Suzuki, Yusuke Watanabe: Saitama Medical University; Tetsuya Mitarai, Hajime Hasegawa: The University of Tokyo; Toshiro Fujita: Jikei University; Tatsuo Hosoya: Nihon University; Koichi Matsumoto, Takayuki Fujita: Tokyo Women's Medical University; Kousaku Nitta: Tokyo Women's Medical University Medical Center East; Tsutomu Sanaka: Tokyo Medical and Dental University; Eiichiro Kanda, Sei Sasaki: Juntendo University; Yasuhiko Tomino: Tokyo Medical University Hospital; Toshiyuki Nakano: Showa University; Tadao Akizawa, Hirokazu Honda: Showa University Fujigaoka Hospital; Yoshihiko Inoue, Ashio Yoshimura: Toranomon Hospital; Hiroshi Tsuji: Toho University; Yasushi Ohashi: Teikyo University; Hideaki Nakajima Shunya Uchida: Kyorin University; Akira Yamada: Yokohama City University; Satoshi Umemura, Mai Yanagi: St. Marianna University; Hiro Yamakawa, Kenjiro Kimura: Niigata University; Shinichi Nishi, Fumitake Gejyo: Hamamatsu University; Akira Hishida, Hideo Yasuda, Tomoyuki Fujikura: Nagoya University; Seiichi Matsuo: Aichi Medical University; Hirokazu Imai, Wataru Kitagawa: Fujita Health University Hospital; Satoshi Sugiyama: Mie University; Shinsuke Nomura, Eiji Ishikawa: Toyama Prefectural Central Hospital; Masahiko Kawabata: Hiroyuki Iida: Kanazawa University Hospital; Takashi Wada: Kanazawa Medical University; Hitoshi Yokoyama: University of Fukui Hospital; Haruyoshi Yoshida: Shiga University of Medical Science; Takashi Uzu, Masayoshi Sakaguchi: Kyoto University Hospital, Atsushi Fukatsu: Osaka University; Yasuyuki Nagasawa, Shiro Takahara: Osaka City University; Eiji Ishimura, Yoshiki Nishizawa: Kobe University; Masafumi Fukagawa, Michio Umezu: Hyogo College of Medicine; Nakanishi Takeshi, Izumi Masaaki: Okayama University, Hitoshi Sugiyama, Hirofumi Makino: Kawasaki Medical School; Naoki Kashihara, Tamaki Sasaki: Hiroshima University; Noriaki Yorioka: University of Tokushima; Toshio Doi: Kagawa University; Masakazu Kono: Ehime University; Takafumi Okura: Kyushu University Hospital; Kazuhiko Tsuruya, Akiko Ono: Fukuoka University; Takao Saito, Yasuhiro Abe: Nagasaki University; Akira Furusu: Kurume University; Seiya Okuda: Kumamoto University; Kimio Tomita: University of Miyazaki; Shouichi Fujimoto: University Hospital of The Ryukyus; Iseki Kunitoshi, Schuichi Takishita: Koto Hospital; Isao Ebihara, Yuko Shima: Nara Medical University; Koji Harada, Yoshihiko Saito: Dokkyo Medical University; Atsushi Numabe, Toshihiko Ishimitsu: Kitano Hospital; Eri Muso, Toshiyuki Komiya: Musashino Red Cross Hospital; Ryoichi Ando: Toyonaka Municipal Hospital; Megumu Fukunaga: Ohmihachiman Community Medical Center; Tsuguru Hatta: Sendai Shakaihoken Hospital; Osamu Hota: Tosei General Hospital; Inaguma Daijo: Matsuyama Clinic; Kazuhiro Matsuyama: Rokko Island Hospital; Naoyuki Nakano: Shuwa General Hospital; Masashi Inoshita: Kanagawa Rehabilitation Hospital; Masahisa Kusaka, Masato Mizuguchi.

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NEPHROLOGY - ORIGINAL PAPER

Histological heterogeneity of glomerular segmental lesions in focal segmental glomerulosclerosis

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Abstract Focal segmental glomerulosclerosis (FSGS) involves considerable histological heterogeneity in terms of location and quality of the glomerular segmental lesions. The present study investigated the heterogeneity of segmental lesions in each variant of FSGS, determined by the Columbia classification, and its clinical relevance. All glomerular segmental lesions of 80 cases of primary FSGS were evaluated histologically based on location [tip (TIP), perihilar (PH), or not otherwise specified (NOS)], and quality (cellular or fibrous). Among the 1,299 glomeruli of the 80 biopsy specimens, 210 glomeruli (16.2%) had segmental lesions, comprising 57 (27%) cellular TIP, 4 (2%) fibrous TIP, 42 (20%) cellular NOS, 86 (41%) fibrous NOS, and 21 (10%) fibrous PH lesions. Each case was also classified into one of the five histological variants of the Columbia classification: collapsing (COL), TIP, cellular (CEL), PH, or NOS. Overlap of segmental lesions in different location categories was seen in the COL, TIP, and PH variants, and heterogeneity of quality was apparent in the COL and CEL variants. Histological findings of the CEL variant (endocapillary hypercellularity) were observed in nine of the 13 COL variants. Both location and quality correlated with disease duration, degree of proteinuria, and histological severity of global glomerular sclerosis and tubulointerstitial lesions. These results demonstrated the histological heterogeneity of glomerular segmental lesions in all variants of the Columbia classification, except NOS. However, the fidelity of location and dominance of histological features were generally conserved in the TIP and PH variants. The COL and CEL variants warrant further investigation because of their overlapping histological findings and apparent histological heterogeneity in the glomerular segmental lesions.

Keywords Focal segmental glomerulosclerosis · Columbia classification · Segmental lesion · Perihilar · Tip · Collapsing · Not otherwise specified · Cellular · Fibrous

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Introduction

Focal segmental glomerulosclerosis (FSGS) is a morphologically defined renal disease characterized by diverse clinical manifestations. Many patients with FSGS display a progression to advanced glomerular sclerosis and subsequent end-stage renal disease (ESRD) [1]. The etiology of FSGS is typically unknown but recent investigations have revealed that many distinct factors, including a genetic abnormality, are involved in the pathogenesis of secondary FSGS, affecting the disturbance of podocytes and slit diaphragm morphology and function [2].

Recently, a working formulation for the morphological classification of primary FSGS, the "Columbia classification," was proposed by D'Agati et al. [3]. It includes five histological variants: collapsing (COL), tip (TIP), cellular (CEL), perihilar (PH), and not otherwise specified (NOS). Several studies have reported significant differences in the clinical features and outcomes among patients with different histological variants of FSGS, based on the Columbia classification [4–8]. The utility of the Columbia classification in renal transplant allografts has been studied recently [9, 10]. In one study, the majority of recurrent FSGS presented the same variant as the original FSGS in the native kidney [9], whereas another study found that the variant of FSGS in the native kidneys was not predictive of either recurrence or the type of FSGS observed in the allograft [10]. Thus, it remains unclear whether different Columbia variants represent different pathophysiological entities of FSGS or different stages of the same pathophysiological entity.

A detailed histological observation of glomerular segmental lesions in FSGS indicated that patients with severe clinical manifestation often presented similar histological features, such as proliferation of intrinsic glomerular cells comprising epithelial and endothelial cells. The accumulation of infiltrating leukocytes and foam cells within the glomerular capillary was also observed in the segmental lesions. Conversely, patients with advanced glomerular sclerosis and impaired renal function generally exhibited a consolidation of glomerular tufts with an increase of extracellular matrix, resulting in segmental glomerular sclerosis [11]. These histological qualities of the segmental lesions are reflected by a mixture of activity and chronicity of the glomerular inflammatory process. The histological activity and chronicity significantly correlate with the clinical manifestations and outcomes in various glomerular diseases. A recently revised classification for lupus nephritis [12] and similar attempts for IgA nephropathy [13–17] that consider the histological activity and chronicity of glomerular lesions appear to be beneficial for the clinical management of kidney diseases.

In the Columbia classification, the variants COL, TIP, and CEL predominantly display different aspects of histological activity, such as glomerular epithelial cell proliferation and intracapillary hypercellularity, whereas the variants PH and NOS usually show histological chronicity, such as consolidation of glomerular tufts. However, it is uncertain whether variants contain a uniform or mixed histological quality (activity/chronicity) of segmental lesions, and whether the histological quality has implications for clinical manifestation. Furthermore, it is not known whether a mutual relationship exists between the location and quality of glomerular segmental lesions.

In the present study, we evaluated histologically the glomerular segmental lesions from 80 cases of primary FSGS with respect to location and histological quality. Each case was further classified into one of the five histological variants of the Columbia FSGS classification, and the histological heterogeneity of each variant was analyzed. Our findings indicate the presence of histological heterogeneity in each variant of the Columbia classification and the importance of quality and location for assessing disease activity in patients with FSGS.

Materials and methods

Eighty biopsy-proven cases of primary FSGS seen at the Department of Medicine, Kidney Center, Tokyo Women's Medical University between 1982 and 2007 were enrolled in the study. Five glomeruli per biopsy specimen was the minimum requirement for enrollment in this study. The average number of glomeruli per one biopsy specimen was 16.2 ± 11.7. FSGS was diagnosed based on the presence of glomerular segmental lesions in at least one glomerulus in 40 consecutive sections. A segmental lesion was defined as a consolidation of the glomerular tuft due to luminal collapse and a subsequent increase of cellular

components and extracellular matrix. Hyalinosis was occasionally associated with the segmental lesions. The cellular components consisted of intrinsic glomerular cells, such as glomerular epithelial, endothelial and mesangial cells, and infiltrating leukocytes and foam cells (macrophages).

The diagnostic algorithm of FSGS is described in Fig. 1. Patients diagnosed with other primary glomerular diseases, collagen diseases, systemic vasculitis, or diabetes were excluded. No patient was diagnosed with malignant hypertension, morbid obesity, viral infection, or drug-induced nephropathy. The subjects in this study were all adults (range, 18–77 years-old, average age: 39.4 ± 1.7). There was no familial clustering of FSGS in these patients. Thus, the subjects were diagnosed mostly with primary (idiopathic) FSGS, but the possibility of secondary FSGS, mediated by adaptive glomerular changes to hypertension or non-morbid obesity, could not be completely excluded.

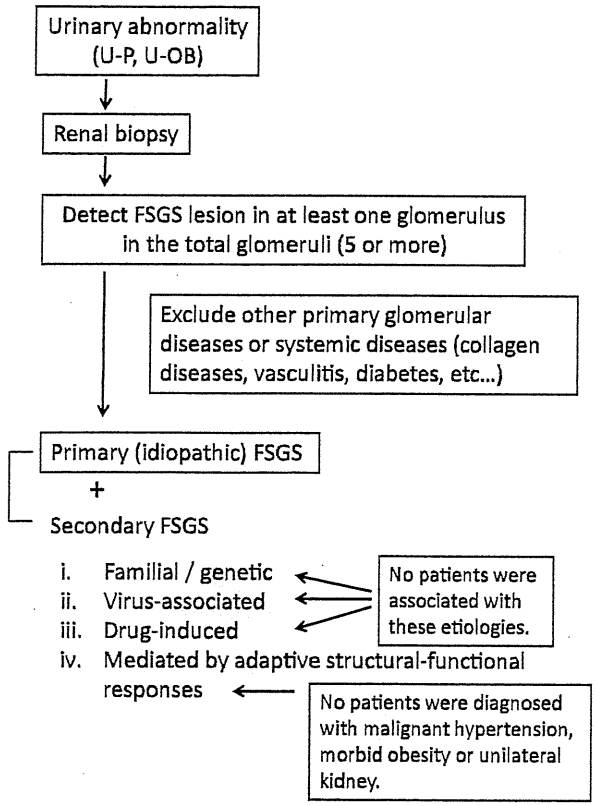

Fig. 1 Screening program for diagnosis and etiologies of FSGS. FSGS was diagnosed based on the presence of glomerular segmental lesions in at least one glomerulus. Patients diagnosed with other primary glomerular diseases or systemic disease, such as collagen diseases, vasculitis, or diabetes, were excluded. No patient was identified with a cause for secondary FSGS. Thus, the subjects of this study were diagnosed mostly with primary (idiopathic) FSGS

All subjects agreed to participate in this study and provided written informed consent.

Clinical data of the 80 patients with primary FSGS

Renal function was evaluated based on the serum creatinine levels (sCr, mg/dl) at the time of biopsy and the end of follow-up. The level of proteinuria (g/day) was also evaluated at biopsy and final observation. The interval (months) between the discovery of proteinuria and the kidney biopsy was regarded as the disease duration. Nephrotic-range proteinuria was defined as urinary protein excretion (U-p): ≥ 3.0 g/day. Hypertension was defined as systolic blood pressure of ≥ 150 mmHg and diastolic blood pressure of ≥ 90 mmHg. Malignant hypertension was defined as diastolic blood pressure of ≥ 130 mmHg accompanied by papilledema or retinal hemorrhage. Obesity was defined as a body mass index (BMI) ≥ 30 kg/m^2, and morbid obesity was defined as a BMI ≥ 40 kg/m^2. Remission of FSGS was defined as a reduction of proteinuria to less than 0.5 g/day. End-stage renal disease (ESRD) was defined as a clinical state requiring regular renal replacement therapy.

Variant classification and histological evaluation of glomerular segmental lesions in FSGS

Three pathologists (ST, KH, and MN) jointly reviewed the glomerular segmental lesions using a multi-head optical microscope to reach a consensus diagnosis for location and quality of lesions, and the variant of the Columbia classification. Glomerular segmental lesions were evaluated by their location and quality, according to the following procedures: The location of segmental lesion in each glomerulus was first categorized into three types: tip (TIP), perihilar (PH), or not otherwise specified (NOS). The TIP area was defined as the outer 25% of the glomerular tuft around the tubular pole, the PH area was defined as the proximal 25% of the glomerular tuft around the vascular pole, and any location that was not in the TIP or PH areas was considered to be NOS. Next, the quality of each segmental lesion was

subtyped as cellular or fibrous, based on its predominant histological features. The cellular subtype was characterized by a proliferation of glomerular epithelial cells and/or intracapillary occlusion with hypercellularity, including foam cells and infiltrated leukocytes. The fibrous subtype was characterized by a consolidation of the glomerular tuft with increased extracellular fibrous matrix (sclerosis). Hyalinosis was observed in both the cellular and fibrous subtypes. Finally, each case was classified as one of the five histological variants of the Columbia classification: collapsing (COL), tip (TIP), cellular (CEL), perihilar (PH), or NOS [3].

Determination of dominant location and quality of segmental lesions

The dominant location and quality of the segmental lesions in all cases was determined using the following methods. The most frequent location pattern among all segmental lesions in a given case was assigned as the dominant location category of that case. When the number of lesions in each location category were the same, the priority order was defined as TIP > PH > NOS. The quality of the segmental lesions in each case was determined to be cellular when all lesions were cellular, fibrous when all lesions were fibrous, and fibrocellular when cellular and fibrous subtypes co-existed in the same biopsy specimen. Clinical and histological features were compared according to the dominant location category or quality subtype, respectively.

Grading of other histological lesions

The number of glomeruli exhibiting global sclerosis (GS) or segmental sclerosis (SS) was calculated as a percentage of the total number of glomeruli, GS or SS, respectively, in each specimen. Interstitial fibrosis and tubular atrophy (IF/TA) were graded semi-quantitatively, based on the affected cortical area, as a percentage of the total cortical area, where 0 = absent or < 5%, 1 + = 6–25%, 2 + = 26–50%, and 3 + = >50%.

Statistical analysis

Continuous variables are presented as means ± standard error (SEM). Statistical analysis between two groups was performed using Student's *t*-test or the Mann–Whitney *U*-test, and analysis among multiple groups was performed using one-way or two-way ANOVA. Multiple comparisons (*post hoc*) using Scheffe's test were performed to compare continuous variables between two groups. The ratio of patients with ESRD was compared by Ryan's test. Analyses were performed using the SAS software (ver. 9.1.3; SAS Institute, Inc., Cary, NC, USA), and Ryan's test was performed using the 'R' statistical analysis package. Statistical significance was accepted for *P*-values < 0.05.

Results

Histological variants according to the Columbia classification

The 80 cases of FSGS were classified according to the Columbia classification as follows: COL, 13 cases (16.2%); TIP, 24 cases (30.0%); CEL, 11 cases (13.8%); PH, 13 cases (16.2%); and NOS, 19 cases (23.8%; Fig. 2). The correspondence between the variant obtained by Columbia classification and the category determined by the dominant location and quality factor is shown in Table 1. The 13 cases of the COL variant included cases categorized as TIP cellular (2 cases), NOS cellular (4), NOS fibrocellular (5), and PH fibrocellular (2). Most of the 24 TIP variant cases were categorized as TIP cellular (18 cases), with three cases of TIP fibrocellular, one of

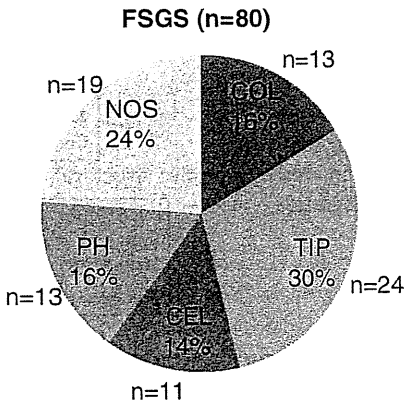

Fig. 2 Histological variants according to the Columbia classification. The 80 cases of FSGS were classified according to the Columbia classification as follows: COL, 13 cases (16.2%), TIP, 24 cases (30.0%), CEL, 11 cases (13.8%), PH, 13 cases (16.2%), and NOS, 19 cases (23.8%)