Urinary free light chain is a potential biomarker for ISN/RPS class III/IV lupus nephritis

Original article

Urinary Free Light Chain is a Potential Biomarker for ISN/RPS Class III/IV Lupus Nephritis

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List of Abbreviations

dsDNA = double-stranded DNA; eGFR = estimated glomerular filtration rate; ESRF = end-stage renal failure; FLCs = free light chains ; IAs = immunosuppressive agents; ISN/RPS = the International Society of Nephrology and the Renal Pathology Society; LN = Lupus nephritis; SLE = Systemic lupus erythematosus;

Abstract

Objectives. To evaluate the use of urinary free light chains (FLCs) as a biomarker for proliferative lupus nephritis (LN) and the potential association between the intensity of the plasma cell infiltration of the kidney and the urinary FLC levels in LN.

Methods. Forty-three SLE patients were consecutively enrolled in the study. These patients were divided into an ISN/RPS class III/IV LN subset (n = 18) and an ISN/RPS class I/II/V (class non-III/IV) LN subset (n = 25). The expression of κ-LCs, λ-LCs, CD19 and CD138 in kidney specimens was also evaluated with immunohistochemical staining. To measure the FLC levels before and after treatment, an additional 6 patients with the class III/IV LN were consecutively enrolled.

Results. The urinary FLCs were significantly higher in the class III/IV LN subset than in the class non-III/IV LN subset. The urinary λ-FLC levels were significantly correlated with the urinary protein-to-creatinine ratio in the class III/IV LN subset (r_s = 0.67, P <0.01). Moreover, the LC-secreting $CD19^-/CD138^+$ cell counts in the kidney specimens were higher in the class III/IV LN subset than in the class non-III/IV LN subset. The total urinary FLC levels were correlated with the numbers of CD138+ cells in the kidney (r = 0.71, P = 0.03). Following treatment, urinary λ-FLCs could not be detected in any of the patients.

Conclusions. The intensity of the plasma cell infiltration of the kidney is associated with the urinary FLC levels. Urinary FLCs are potentially useful biomarkers in ISN/RPS class III/IV LN or proliferative LN.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease with multiple organ manifestations, including skin lesions, arthritis, serositis, nephritis, and neuropsychiatric and hematological disorders. Lupus nephritis (LN) is a common complication of SLE, and the frequency of LN is approximately 31-65% among SLE patients in the United States and Europe and 45-86% in Japan [1]. The long-term prognosis for LN has improved [2]. However, WHO class IV LN is one of the most common contributors to end-stage renal failure (ESRF). The frequency of ESRF is 40.9% in patients with WHO class IV LN, compared with 2.6% in patients with non-class IV LN [3]. In general, combination therapy with corticosteroids and immunosuppressive agents (IAs), such as cyclophosphamide and mycophenolate mofetil, is recommended for class III/IV LN, as defined by the International Society of Nephrology and the Renal Pathology Society (ISN/RPS). The early diagnosis and appropriate management of ISN/RPS class III/IV LN is critical for improving the renal and overall survival of SLE patients.

Conventional clinical parameters of SLE, such as the levels of serum complement, anti-double-stranded DNA (dsDNA) antibodies, creatinine, and proteinuria, are assessed to evaluate the disease activity and predict the complications of ISN/RPS class III/IV LN in SLE patients [4]. However, these markers are not always sufficiently sensitive or specific to detect ongoing disease activity and early relapses of LN. Numerous novel biomarkers, such as serum and urinary cytokines, chemokines, adhesion molecules and growth factors, have been evaluated for monitoring treatment response and detecting early renal flares in LN [5]. In particular, urinary biomarkers are more promising than serum biomarkers, possibly

because the former result directly from kidney inflammation or injury [5].

It was recently reported that large numbers of anti-dsDNA antibody-secreting plasma cells were present in the kidneys of NZB/W mice and that differentiated (long-lived) plasma cell infiltration of the kidney medulla was associated with more severe LN in SLE patients [6]. A normal immunoglobulin molecule is composed of two light chains (LCs) and two heavy chains. During normal immunoglobulin synthesis by B cells/plasma cells, most LCs bind to heavy chains. Unbound LCs are released from B cells/plasma cells as free light chains (FLCs) [7]. Serum FLC levels are strongly correlated with global disease activity in SLE and may have applications as biomarkers [8]. Furthermore, urinary FLC levels may be useful as quantitative markers of *in vivo* polyclonal B cell activity [9]. Moreover, more severe renal inflammation and a higher risk of disease relapse are associated with increases in urinary FLCs in SLE patients [10, 11]. The findings of the above studies indicate that FLC synthesis by B cells/plasma cells are activated both systematically and locally (in the kidneys) in LN.

However, relationships between serum and urinary FLC levels; associations between serum or urinary FLC levels and pathohistological findings, such as ISN/RPS class III/IV LN and class non-III/IV LN; and correlations between FLC levels and the infiltration of B cells/plasma cells in the kidney have not been so far evaluated by the previous studies in LN. In the present study, we measured both serum and urinary FLC levels and investigated the differences between ISN/RPS class III/IV LN and ISN/RPS class non-III/IV LN at the FLC level.

Materials and methods

Patients

A total of 43 patients with SLE were enrolled in the study. These patients were admitted to our hospital between 2004 and 2006 and consecutively underwent renal biopsies after written informed consent was obtained. All of the patients were diagnosed with SLE based on the criteria of the American College of Rheumatology (ACR) [12] and were admitted to our institution because of active SLE-associated symptoms. Of these 43 patients, 41 were Japanese, and 2 were non-Japanese Asians.

To evaluate the serum and urinary FLC levels before and after treatment, another 6 Japanese patients with ISN/RPS class III/IV LN were consecutively enrolled from 2009 to 2010. This study was approved by the ethics committee of our institution, in accordance with the Declaration of Helsinki.

Data collection

Information that included clinical manifestations and laboratory data was obtained from the patients' medical records. Urinary measurements, including proteinuria and hematuria (by dipstick), urine sediment, protein-creatinine ratio, serum albumin, creatinine, complement components (C3 and C4), immunoglobulin G (IgG) and anti-dsDNA antibodies, were evaluated upon admission and prior to the renal biopsy. C3 and C4 were measured using the standard method. Anti-dsDNA antibodies were detected with a radioimmunoassay, with less than 6 IU/ml considered normal. Antibodies against SS-A, U1-snRNP and Sm were measured with double immunodiffusion. Antibodies against cardiolipin and β2-

glycoprotein I were measured with an enzyme-linked immunosorbent assay. The estimated glomerular filtration rate (eGFR) was calculated according to a previously described method, using parameters that included serum creatinine levels, age and sex [13]. The SLE disease activity of each patient at admission was assessed using the SLEDAI-2K [14]. The SLEDAI-2K renal scores included urinary casts (heme-granular or red blood cell casts), hematuria (>5 red blood cells/high-power field), proteinuria (urinary protein-creatinine ratio >0.5) and pyuria (>5 white blood cells/high-power field).

Measurement of serum and urinary free light chains

Sera and spot urine samples were obtained shortly before the renal biopsies were performed and were stored at -80 degrees. Both serum and urinary FLCs were measured with a nephrometric assay (the Freelite®). The normal values for serum κ-FLCs and λ-FLCs were 3.3-19.4 mg/L and 5.7-26.3 mg/L, respectively [15]. The normal values for urinary κ-FLCs and λ-FLCs had not been previously determined, although FLCs are present in the urine of healthy individuals only at extremely low concentrations [7]. The FLC values from the spot urine samples correlated well with those from the 24 h urine samples ($r = 0.71$, $P < 0.01$, data not shown). Therefore, the FLC values from the spot urine samples were not corrected for urinary creatinine, as with the urinary protein-creatinine ratio.

Evaluation of renal pathohistology

The renal pathohistological findings were categorized according to the 2003 ISN/RPS classification system [16, 17]. In addition, we evaluated the infiltration of immunoglobulin

LC-secreting B cells/plasma cells into the kidney cortex and medulla in ISN/RPS class III/IV LN and compared the measurements with those from the ISN/RPS class V samples. We selected biopsy samples that included sufficient portions of the medulla (with a cortex/medulla proportion < 1). Ultimately, 5 ISN/RPS class III/IV samples and 4 ISN/RPS class V samples fulfilled the criteria described above.

Immunohistochemical staining was performed for κ-LCs (DAKO, Tokyo, Japan, A0191), λ-LCs (DAKO, A0193), CD19 (DAKO, M7296) and CD138 (DAKO, M7228) using the standard method. To evaluate the intensity of the cellular infiltration, positively stained cells were counted in all the fields of the samples. The total cell count of each sample was divided by the entire area of the sample (cell count per mm^2).

Statistical analyses

Statistical analyses were performed using a chi-squared test to compare frequencies, a *t*-test to compare mean values and the Mann-Whitney U test to compare median values. Correlation coefficients were calculated as a Pearson's correlation coefficient or Spearman's rank correlation if applicable. The urinary protein-creatinine ratio, the anti-dsDNA antibody levels and the serum and urinary FLC levels before and after treatment were compared using the Wilcoxon signed-rank test. The data were analyzed with JMP® software (SAS Institute, Cary, NC, USA). P values <0.05 indicated statistical significance.

Results

Comparison of clinical manifestations between the ISN/RPS class III/IV and class non-III/IV LN subsets

The 43 enrolled patients were divided into the following two subsets: an ISN/RPS class III/IV LN subset and an ISN/RPS class I, II or V (class non-III/IV) LN subset. The combined classes III and V and classes IV and V were referred to as class III and class IV, respectively. The frequencies of LN classified as ISN/RPS classes I, II, III, IV and V were 9 (21%), 7 (16%), 8 (19%), 10 (23%) and 9 (21%), respectively. As shown in Table 1, the median age, sex, frequency of prednisolone (PSL) or IA administration and PSL dosage did not differ between the two subsets. The urinary protein-creatinine ratio was higher ($P = 0.01$) and the serum albumin level was lower ($P = 0.03$) in the ISN/RPS class III/IV LN subset than in the ISN/RPS class non-III/IV LN subset. The complement level was lower, and the anti-dsDNA antibody titer and the SLEDAI-2K total score were higher in the ISN/RPS class III/IV LN subset.

Comparison of serum and urinary FLC levels between the ISN/RPS class III/IV and class non-III/IV LN subsets

As shown in Table 2, the serum κ-FLC levels were significantly higher in the ISN/RPS class III/IV LN subset than in the ISN/RPS class non-III/IV LN subset, although the median values and interquartile ranges were within the normal limits in each subset. There were no significant differences in serum λ-FLCs between the two subsets.

In contrast, both urinary κ-FLCs and λ-FLCs were significantly higher in the ISN/RPS class III/IV LN subset than in the ISN/RPS class non-III/IV LN subset ($P = 0.02$

for both). In the ISN/RPS class non-III/IV LN subset, no urinary λ-FLCs were detected in 13 (52%) of 25 patients.

Relationship between serum and urinary FLCs

No significant correlations were found between the serum and urinary κ-FLCs in both the ISN/RPS class non-III/IV LN subset and the ISN/RPS class III/IV LN subset (Figure 1A). In addition, there were no significant correlations between the serum and urinary λ-FLCs in either subset (Figure 1B).

Associations between SLEDAI-2K scores and serum/urinary FLCs

We analyzed the correlations between the SLEDAI-2K total or renal score and serum or urinary FLCs, in all of the 43 enrolled patients. The serum FLC levels were not significantly correlated with the SLEDAI-2K total score (κ-FLC: $r_s = 0.13$, P = 0.40, and λ-FLC: $r_s = 0.20$, P = 0.20). There was a correlation between the SLEDAI-2K total score and the urinary λ-FLC levels ($r_s = 0.40$, P = 0.02), although no significant correlation existed between the SLEDAI-2K total score and the urinary κ-FLC levels (κ-FLC: $r_s = 0.23$, P = 0.15) and between the SLEDAI-2K renal score and urinary κ or λ-FLC levels. Moreover, there was no significant difference of the serum or urinary FLC levels between the active disease (SLEDAI-2K total score >4) subset and the inactive disease subset in both the non-ISN/RPS class III/IV LN subset and the class III/IV LN subset.

Association between urinary FLCs and conventional biomarkers

Significant correlation was found between urinary λ-FLC and anti-dsDNA Ab (r_s = 0.42, P = 0.01), although there was no significant correlation between urinary λ-FLC and C3 (r_s = -0.14, P = 0.44) in the ISN/RPS class III/IV subset. There were no statistically significant differences in the urinary FLC levels between the present of active urinary sediments (RBC > 5/HPF, WBC >5/HPF or heme-granular or RBC casts) subset and the absent subset.

Correlation between urinary FLCs and urinary protein-creatinine ratio

The serum κ-FLCs and λ-FLCs were not correlated with the urinary protein-creatinine ratio in either the ISN/RPS class non-III/IV LN subset or the ISN/RPS class III/IV LN subset. As shown in Figure 2A, there was also no significant association between the urinary κ-FLCs and the urinary protein-creatinine ratio in the ISN/RPS class III/IV LN subset (r_s = 0.46, P = 0.06), although the urinary κ-FLCs correlated with the urinary protein-creatinine ratio in the non-SN/RPS class III/IV LN subset (r_s = 0.42, P = 0.03). The urinary λ-FLCs were significantly positively correlated with the urinary protein-creatinine ratio in both the ISN/RPS class non-III/IV LN subset (r_s = 0.61, P < 0.01) and the ISN/RPS class III/IV LN subset (r_s = 0.67, P < 0.01) (Figure 2B). On the other hand, the C3 and anti-dsDNA antibody levels were not associated with the urinary protein-creatinine ratio.

To investigate an association between the levels of urinary FLC and proteinuria in detail, we compared the ISN/RPS class III/IV subset with the class V subset. The median value of the urinary protein-creatinine ratio was 0.8 and 0.9 in the ISN/RPS class III/IV subset and the class V subset, respectively. There was no statistically significant difference in the urinary protein-creatinine ratio between the two subsets. In contrary, the ratio of the

urinary κ or λ-FLC levels to the urinary proteinuria concentration was higher in the class III/IV subset than in the class V subset ($p = 0.05$ and 0.01 in urinary κ-FLC and urinary λ-FLC, respectively).

Immunohistochemical staining of kidney specimens with immunoglobulin light chains, CD19 and CD138 in ISN/RPS classes III/IV and V LN

To compare the ISN/RPS class III/IV LN patients (n = 5) with the ISN/RPS class V LN patients (n = 4) in terms of the infiltration of B cells/plasma cells and the status of the LCs synthesized by those cells in the kidney, immunohistochemical staining was performed for κ-LCs, λ-LCs, CD19 and CD138 in both patient subsets. As shown in a representative ISN/RPS class III/IV LN case (Figure 3A), κ-LCs and λ-LCs were synthesized mainly by $CD138^+$ cells. The $\kappa\text{-LC}^+$ cell counts were significantly higher ($P = 0.03$) in the ISN/RPS class III/IV LN subset than in the ISN/RPS class V LN subset (Figure 3B). In addition, the $\lambda\text{-LC}^+$ cell counts and the $CD\ 138^+$ cell counts were higher in the class III/IV LN subset than in the class V LN subset, although this difference was not significant (Figure 3C and 3D). The CD19+ cell counts did not differ between the two subsets (Figure 3E). Among all of the infiltrating CD138+ cells, there were no significant differences between the κ-LC+ cell counts and the λ- LC+ cell counts. Moreover, the CD138+ cells expressed virtually no CD19 in the kidney specimens from the ISN/RPS class III/IV LN subset. Almost all of the CD138+ cells were located around the glomeruli or the tubulointerstitium of the margin between the renal cortex and medulla.

Correlation between the urinary FLC levels and the numbers of CD138+ cells infiltrating the kidney

As shown in Figure 3F, the total (κ+λ) urinary FLC levels were significantly correlated ($r = 0.80$, $P < 0.01$) with the numbers of CD138+ cells infiltrating the kidney of 9 patients described above (5 patients with ISN/RPS class III/IV LN and 4 with ISN/RPS class V LN), although there were no such associations with the total serum FLC levels.

Comparison of clinical parameters before and after immunosuppressive treatment

As shown in Figure 4A and 4B, the urinary protein-creatinine ratio and the anti-dsDNA antibody titer were lower after treatment than before treatment ($P = 0.03$ and 0.06, respectively) in 6 patients with ISN/RPS class III/IV LN. Both the serum κ-FLCs and the serum λ-FLCs decreased, although their values were almost within normal limits prior to treatment (Figure 4C and 4D). Both the urine κ-FLCs and the urine λ-FLCs also decreased after treatment, compared with before treatment (Figure 4E and 4F). Notably, no urine λ-FLCs could be detected in any patient after treatment.

Discussion

The present study demonstrated that the urinary FLC levels were elevated and were associated with the intensity of the plasma cell infiltration of the kidney in ISN/RPS class III/IV LN patients. During normal immunoglobulin synthesis, B cells/plasma cells release FLCs, which pass through the glomerular filtration barrier rapidly (with a serum half-life of 2-6 hours) [7]. The urinary FLC levels are low in healthy individuals [18,

19]. Serum FLC levels are elevated in many autoimmune/inflammatory diseases, and FLC levels are strongly correlated with other markers of B cell/plasma cell activation [8, 20, 21]. Urinary FLC levels might represent quantitative markers of real-time, *in vivo* polyclonal B cell/plasma cell activity; SLE relapse has been associated with increases in urinary FLCs [9, 10]. According to the previous report described by Tsai et al, urinary FLCs were not detected in patients with inactive lupus nephritis (24h urinary excretion: range 3-20g/day) [11]. In the present study, the ratio of the urinary FLC levels to the proteinuria levels was higher in the ISN/RPS class III/IV LN subset than the ISN/RPS class V LN subset, although there was no significant difference in the levels of proteinuria between the two subsets. These results indicate that urinary FLC levels were lead to not only the synthesis of FLCs or the urinary protein excretion concentrations but also the disease activity of LN. Urinary FLC levels may reflect the disease activity of LN more real-time than conventional markers.

A recent study revealed that plasma cell infiltration into the renal medulla is correlated with the amount of inflammation and the disease severity of patients with LN. A study of NZB/W mice revealed that anti-dsDNA antibodies were produced by long-lived plasma cells located in the kidney [6]. $CD138^+$ cells that express low levels of CD19 are compatible with a long-lived plasma cell phenotype [22]. Long-lived plasma cells are capable of continuously secreting antibodies. These cells live primarily in the bone marrow and secondarily in niches in the spleen and chronically inflamed tissues, such as the kidneys in SLE or the synovia in rheumatoid arthritis [23]. The present study demonstrated that LCs were synthesized mainly by $CD19^-/CD138^+$ cells and that the total urinary FLC

levels were significantly correlated with the numbers of kidney-infiltrating $CD138^+$ cells in ISN/RPS class III/IV LN. In contrast, the ISN/RPS non-class III/IV (e.g., class V) LN patients exhibited low or undetectable urinary FLC levels, and the $CD138^+$ cells infiltrating their kidneys were scarcely detectable. These findings revealed that the infiltration of long-lived plasma cells into the kidney may play a role in local inflammation and that the urinary FLC levels reflect the intensity of the plasma cell infiltration of the kidney in proliferative types of LN, such as ISN/RPS class III/IV LN.

In the present study, urinary λ-FLCs were not detected after immunosuppressive therapy in any of the ISN/RPS class III/IV LN patients. In contrast, the urinary κ-FLC levels were significantly decreased (but detectable) after treatment, although there was no significant difference between the κ-LC^+/$CD138^+$ cell counts and the λ-LC^+/$CD138^+$ cell counts in the ISN/RPS class III/IV LN kidney specimens. κ-LCs and λ-LCs are encoded by different chromosomes. λ-FLCs have a dimeric structure, whereas κ-FLCs have a monomeric structure [24]. In AL amyloidosis, λ-LCs are involved in amyloid deposition more often than κ-LCs [25]. Although the precise molecular functions of each type of FLC remain unknown, the molecular differences between κ-LCs and λ-LCs might cause the discrepancies in the serum and urinary levels between κ-FLC and λ-FLC.

A previous study reported that serum FLC levels were correlated with global disease activity in SLE [8]. However, the present study found no correlation between the SLEDAI-2K scores and the serum FLC levels. A bias in the selection of enrolled patients may have contributed to the differences between the results of the previous study and ours. In this study, urinary FLC levels were correlated with urinary protein/creatinine ratio,

although there were no significant correlations between urinary protein/creatinine ratio and serum biomarkers such as FLC, anti-dsDNA Ab and C3. Urinary FLC could reflect the activity of LN. However, urinary FLC is not a specific marker to LN. Urinary FLC may be increasing in the other glomerulonephritis involved with plasma cells infiltration. Moreover, serum and urinary FLC levels can be influenced by several factors, including renal dysfunction and hypergammopathies, such as infections and multiple myeloma [7, 10],

There are some limitations in our study. First, the serum and urine samples were stored for long-term. This might affect the result of the serum and urinary FLC levels. Second, sample size was small in this study. We didn't longitudinally evaluate the urinary FLC level after treatment. So it has been unsure whether urinary FLC is a useful biomarker as a predictor for renal relapse.

In conclusion, urinary FLC is a noninvasive biomarker for ISN/RPS class III/IV LN or proliferative LN. The intensity of the plasma cell infiltration of the kidney is also associated with urinary FLC levels in LN.

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TABLES

Table 1. Comparison of the clinical manifestations of the ISN/RPS class III/IV LN subset and the class non-III/IV LN subset

	Class III/IV (n = 18)	Class non-III/IV (n = 25)	P value
Age at renal biopsy, years	32 (23-48)	31 (25-42)	0.66
Female, no. (%)	18 (100)	24 (96)	1.00
Patients who received PSL, no. (%)	5 (28)	11 (44)	0.35
Dosage of PSL, mg/day	0 (0-15)	5 (0-17.5)	0.43
Patients who received IA, no. (%)	1 (6)	2 (8)	1.00
Urinary protein-creatinine ratio	0.85 (0.10-1.54)	0.07 (0.03-0.24)	0.01
Serum albumin, g/dl	3.3 (2.9-3.5)	3.8 (3.3-4.1)	0.03
Serum creatinine, mg/dl	0.54 (0.45-0.59)	0.51 (0.46-0.58)	0.68
eGFR, mL/min/1.73 m^2	115 (89-124)	114 (91-135)	0.79
C3, mg/dl	44.0 (32.5-70.3)	65.0 (44.8-86.5)	0.06
C4, mg/dl	4.0 (2.3-7.5)	9.0 (3.8-15)	0.03
IgG, mg/dl	1956 (1635-2706)	1908 (1467-2564)	0.64
Anti-dsDNA Ab, IU/ml	298.5 (150.3-623.8)	20.5 (7.5-29.3)	<0.01
Anti-SS-A Ab positivity, no. (%)	11 (61)	10 (40)	0.22
Anti-U1 snRNP Ab positivity, no. (%)	6 (33)	15 (60)	0.12
Anti-Sm Ab positivity, no. (%)	1 (6)	10 (40)	0.01
Anti-CLβ2GP I Ab positivity, no. (%)	2 (11)	2 (8)	1.00
SLEDAI-2K total score	15 (10-21)	9 (4-12)	0.01

Except for the percentages, all of the numbers listed above represent median values and IQRs.

The P values were established using a chi-squared test or the Mann-Whitney U test.

ISN/RPS = International Society of Nephrology/Renal Pathology Society; PSL= prednisolone; IA = immunosuppressive agents; eGFR = estimated glomerular filtration rate; Ab = antibody; CLβ2GP I = cardiolipin and β2-glycoprotein I; SLEDAI-2K = Systemic lupus erythematosus disease activity index 2000; SLEDAI-2K renal score = SLEDAI score with renal involvement, including urinary casts, hematuria, proteinuria and pyuria.

Table 2. Comparison of the serum and urinary FLC levels of the ISN/RPS class III/IV subset and the ISN/RPS class non-III/IV subset

	Class III/IV (n = 18)	Class non-III/IV (n = 25)	P value
Serum κ-FLC (mg/L)	11.3 (8.8-17.1)	9.2 (5.9-11.7)	0.04
Serum λ-FLC (mg/L)	27.4 (22.8-32.9)	21.3 (17.0-36.4)	0.13
Urinary κ-FLC (mg/L)	4.05 (2.16-8.01)	1.0 (0.47-1.99)	0.02
Urinary λ-FLC (mg/L)	2.69 (1.04-6.25)	0 (0-1.11)	0.02

Each number listed above represents a median value or IQR.

The P values were established using the Mann-Whitney U test.

FLC = free light chain; ISN/RPS = International Society of Nephrology/Renal Pathology Society

FIGURE LEGENDS

Figure 1. Associations between serum and urinary FLCs in the ISN/RPS class non-III/IV LN subset and in the ISN/RPS class III/IV LN subset. An association between serum κ-FLC and urinary κ-FLC (A), and an association between serum λ-FLC and urinary λ-FLC (B) in each subset.

Figure 2. Correlations between urinary protein-creatinine ratios and urinary FLCs in the ISN/RPS class non-III/IV LN subset and in the ISN/RPS class III/IV LN subset. A correlation between urinary protein-creatinine ratios and urinary κ-FLC (A), and a correlation between urinary protein-creatinine ratios and urinary λ-FLC (B) in each subset.

Figure 3. Immunohistochemical staining for immunoglobulin light chains (LCs), CD19 and CD138 in ISN/RPS class III/IV and class V LN kidney specimens. In a representative ISN/RPS class III/IV LN case, κ-LC and λ-LC were synthesized mainly by $CD138^+$ cells (**A**). Immunohistochemical staining for κ-LC (**B**), λ-LC (**C**), CD138 (**D**) and CD19 (**E**) in the class III/IV LN subset (n = 5) and in the class V LN subset (n = 4). There was a positive correlation between the total urinary FLC levels and the numbers of $CD138^+$ cells infiltrating the kidney (**F**).

Figure 4. Clinical parameters before and after treatment. The urinary protein-creatinine ratios (**A**), anti-dsDNA antibody titers (**B**), serum κ-FLC levels (**C**), serum λ-FLC levels (**D**), urinary FLC levels (**E**) and urinary λ-FLC levels (**F**).

Key Messages.

- Urinary FLCs are useful as a noninvasive biomarker for the response to treatment of ISN/RPS class III/IV LN.
- The intensity of the plasma cell infiltration of the kidney is associated with urinary FLC levels in LN.