Plasma CD147 reflects histological features in patients with lupus nephritis

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Abstract

Objective. A glycosylated transmembrane protein, CD147, has been implicated in regulating lymphocyte responsiveness and leukocyte recruitment. As lupus nephritis (LN) often follows a relapsing-remitting disease course, accurate understanding of the disease activity would be extremely helpful in improving prognosis. Unfortunately, neither clinical nor serological data can accurately reflect the histological features of LN. The present study investigated whether CD147 can accurately predict pathological features of LN.

Methods. Plasma and spot urine samples were collected from 64 patients who underwent renal biopsy between 2008 and 2011. Disease activity for LN tissues was evaluated using biopsy activity index, and compared to levels of biomarkers including CD147.

Results. In LN tissues, CD147 induction was striking in injured glomeruli and infiltrating inflammatory cells, but not in damaged tubules representing atrophy. Plasma CD147 levels accurately reflected the histological disease activity. However, prediction using a single molecule would be quite difficult because of complex pathogenesis of LN. The diagnostic accuracy of multiplex parameters indicated that the combination including plasma CD147 might yield excellent diagnostic abilities for guiding ideal LN therapy.

Conclusion. Plasma CD147 levels might offer useful insights into disease activity as a crucial biomarker in patients with LN.

Keywords. CD147; lupus nephritis; neutrophil gelatinase-associated lipocalin; monocyte chemoattractant protein-1

Introduction

Lupus nephritis (LN) is one of the key determinants of higher mortality and poor quality of life. Diverse mechanisms of LN involving genetic, epigenetic, environmental, hormonal and immune-regulatory factors lead to a loss of self-tolerance and organ failure [1]. Current outcome for treating severe LN thus remains insufficient, despite aggressive management by immunosuppressive agents [2]. The ideal approach to seek appropriate therapeutics includes elucidation of the various underlying mechanisms involving the immune system and an understanding of disease activity. In particular, early identification of at-risk patients would be extremely helpful for improving complete remission rates, and avoiding higher toxicity and poor toleration. This will require diagnostic tools that accurately reflect the disease activity of LN, as kidney biopsy cannot be repeated at every flare. Unfortunately, neither clinical nor serological data can accurately reflect the histological features of LN. A need remains for noninvasive biomarkers that are able to predict renal flare [3].

Recently, candidate biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL) [4-7] and monocyte chemoattractant protein (MCP)-1 [8, 9] have been examined to determine whether they reflect histological features of LN, and thus might allow prediction of impending flares and selection of future therapies. Among these, urinary NGAL has been suggested as a candidate biomarker for LN in both children and adults [4-6]. However, the biological functions in LN remain unelucidated, with NGAL found in various types of renal injury [3]. Other investigators have documented that urinary MCP-1 is specific for renal flare in LN [8], and associated with fibrogenic response in kidney diseases [10]. However, the diagnostic accuracy for each biomarker remains unsatisfactory.

CD147 is a glycosylated transmembrane protein that belongs to the immunoglobulin superfamily and is distributed in various types of cells, including hematopoietic, epithelial, and endothelial cells [11, 12]. It is well documented for its ability to function as an extracellular matrix metalloproteinase (MMP) inducer [13] and has also been implicated in the regulation of lymphocyte responsiveness, monocarboxylate transporter (MCT) induction, carcinoma metastasis and spermatogenesis [14]. Relationships with a wide range of binding partners, including caveolin, cyclophilin, MCT, and CD147 itself, have already been found. Through interactions with cyclophilin, CD147 plays an important role in leukocyte recruitment and thereby chemotactic activity. T lymphocytes in patients with LN show inappropriate tissue homing and promote inflammation by secreting cytokines [15] and activating dendritic cells [16]. In patients with active systemic lupus erythematosus (SLE), CD147 is overexpressed on CD3+ T lymphocytes [17], and activated regulatory T cells (Treg) within the CD4+ FoxP3+ subset [18]. Indeed, the transcription factor FoxP3 is likely to be low in Treg from patients with active SLE [16]. The aberration of molecular mechanism involving CD147-mediated Treg function may be a crucial determinant in active LN.

We have previously demonstrated the potential roles of CD147 in ischemic acute kidney injury (AKI) through E-selectin-dependent neutrophil migration [19] and the development of renal fibrosis via MMPs activation [20], using CD147 gene-deficient mice. Given our evidences, CD147 may be involved in the pathogenesis of LN through the polarization of T lymphocytes. The present study examined whether CD147 can accurately predict the pathological features of LN as a noninvasive diagnostic tool in patients with LN compared to previously reported parameters.

Patients and Methods

Patients and Procedures

This study proceeded according to the principles of the Declaration of Helsinki, the Japanese National Ethical Guidelines, and the institutional review boards of Nagoya University Hospital and affiliated hospitals. All patients provided written informed consent to participate.

Among adult patients clinically diagnosed with SLE, 64 patients who underwent renal biopsy as part of standard-of-care therapy in Nagoya University and affiliated hospitals between 2008 and 2011 were registered. According to the American College of Rheumatology (ACR) criteria [21], diagnosis was clinically and pathologically performed using light, immunofluorescence and electron microscopy. In the same period, 14 patients only with microhematuria or a subtle amount of proteinuria were regarded as pathological controls. These have not been identified as the kidney diseases in both clinical and pathological aspects. To further prove the proper of CD147 values as a control, 16 healthy individuals were enrolled. Plasma and spot urine samples were collected on the day prior to renal biopsy. Within 7 days prior to renal biopsy, information about patient demographic characteristics, medications, and disease activity was recorded, and key parameters such as levels of complement C3, anti-DNA antibody and proteinuria were measured. The clinical disease activity of LN was evaluated using the renal domain score of SLE disease activity index (SLEDAI; range 0 as inactive LN to 16 as severe LN) [22].

Renal Histology

Kidney tissues were fixed in 10% formalin, embedded in paraffin and then cut into

4-μm sections for immunohistochemistry. Sections were stained with mouse monoclonal anti-human CD147 antibody (Abcam, Cambridge, MA), mouse monoclonal anti-human CD31 antibody (Dako, Carpinteria, CA) to endothelial cell marker, mouse monoclonal anti-human angiotensin converting enzyme (ACE) antibody (Abcam) to proximal tubule marker, sheep polyclonal anti-human Tamm Horsfall glycoprotein (THP) antibody (Serotec, Oxford, UK) to distal tubule marker, mouse monoclonal anti-human CD68 antibody (Dako), mouse monoclonal anti-human CD5 antibody (Novocastra, Newcastle upon Tyne, UK) to lymphocyte marker, mouse monoclonal anti-human neutrophil elastase antibody (Dako) and rabbit polyclonal anti-human S100A4 antibody (Thermo scientific, Yokohama, Japan), followed by a second antibody of Histofine Simple Stain Max PO (Nichirei, Tokyo, Japan). The staining was visualized with 3,3'-diaminobenzidine (Nichirei), a brown color being produced.

Morphological Assessment

LN tissues in renal biopsy specimens were classified according to the international Society of Nephrology (ISN)/Renal Pathology Society (RPS) criteria [23], and quantified the degree of LN activity and chronicity based on previously reported pathological findings [7, 24]. In brief, we verified histological features reflective of active inflammations such as endocapillary hypercellularity, leukocyte infiltration, subendothelial hyaline deposit, fibrinoid necrosis/karyorrhexis, cellular crescent and interstitial inflammation, and of chronic and degenerative injuries, including glomerular sclerosis, fibrous crescent, tubular atrophy and interstitial fibrosis. The observations of LN activity and chronicity listed above were categorized as: 0, no injured lesions; 1, injured regions comprising < 25% of glomeruli or interstitium; 2, injured regions

comprising 26-50%; and 3, injured regions comprising > 51%. Evaluations were then made using biopsy activity index (BAI; range, 0-24) and biopsy chronicity index (BCI; range, 0-12). Risk factors for poor LN prognosis were regarded as BAI scores of ≥7 and BCI scores of ≥ 4 [25-27]. All and quantifications were performed in a blinded manner by three independent expert nephropathologists.

Biomarkers Measurement

Plasma and urinary samples were centrifuged at 2000×g for 5 min to remove cellular components and debris, then equal volumes of supernatants were stored at -80°C. All samples were tested using a dipstick before centrifugation and examined by microscopy. Plasma and urinary CD147, and urinary NGAL and MCP-1 values were measured using commercial enzyme-linked immunosorbent assay kits according to the manufactures' respective instructions (Bioport Diagnostic, Gentofte, Denmark; and R&D systems, Minneapolis, MN). Measured levels in urine were then normalized to urinary creatinine (Cr) levels.

Statistical Analysis

Continuous variables are presented as means ± standard deviation (SD) and categorical variables as numbers and ratios (%). Continuous variables were compared using Student's *t*-test, the non-parametric Mann-Whitney *U*-test, one-way ANOVA test or Kruskal-Wallis test followed by Steel-Dwass post hoc multiple comparisons as appropriate. We used Spearman correlation coefficients to examine the strength of association between two variables. To assess the independent predictive ability, univariate and multivariate logistic regression analysis was examined. The investigation

of combination biomarkers was also performed in the multivariate logistic regression models. Receiver-operating characteristics (ROC) curves were constructed to assess the diagnostic accuracy of each biomarker and diagnostic performance was quantified as area under the curves (AUCs). Two-tailed *P*-values < 0.05 were considered statistically significant. Statistical analysis was performed using SPSS and STATA version 9, commercial software.

Results

Patient characteristics with LN

Table 1 summarizes the characteristics of the 64 patients with LN included in the present study. All participants were Japanese, with a mean age of 44 years (range, 15-80), and the majority (72 %) was female. The pathological control group had a mean age of 32.2±14.4 years (*P* = 0.018 versus LN patients) and was 57% female (*P* = 0.285 versus LN patients). The healthy control group showed a mean age of 38.6±8.3 years (*P* = 0.069 versus LN patients) and was 44% female (*P* = 0.034 versus LN patients). Patients with LN showed more renal dysfunction in parameters such as serum Cr level than pathological control patients (0.78±0.27 mg/dl, *P* = 0.027) and healthy individuals (0.74±0.15 mg/dl, *P* = 0.011). As expected, component C3 and anti-DNA antibody values were 54.4 mg/dl and 155.4 IU/ml, respectively. The averages of the systemic lupus activity measure (SLAM) and SLEDAI scores were 7.6 (range, 1-18) and 16.7 (range, 2-30), respectively. At the time of renal biopsy, some LN patients were treated with glucocorticoids, whereas a few received immunosuppressive therapies. Almost all histologic features in biopsy-proven LN indicated proliferative LN (ISN/ RPS class III or IV) or epimembranous deposits (ISN/RPS class V, III+V or IV+V) with

endocapillary hypercellularity (Table 2).

CD147 expression in the kidney

Pathological control patients showed strong expressions of CD147 in both proximal and distal tubules in the cortical kidneys, but to a less extent in medulla kidneys (Figure 1(a) and (d)). In particular, this expression was distributed to the basolateral side and intercellular junction of tubular epithelial cells (TECs). In glomeruli, CD147 expression was very weak (Figure 1(b)), and wasn't also found in the endothelial cells of arterioles and collecting tubules (Figure 1(c) and (d)).

In renal tissues of patients with LN, CD147 expression was markedly reduced in lesions representing tubulointerstitial injury with tubular atrophy (Figure 1(e)). Particularly, tubules with degeneration of TECs exhibited striking declines in CD147 expression, suggesting that CD147 may serve a key molecule in cell-to-cell adhesion. In contrast, this induction was strong in damaged glomeruli, including in endocapillary proliferations, adhesion to Bowman capsule and cellular crescent (Figure 1(f)). More interestingly, inflammatory cells and fibroblasts with CD147 induction showed marked infiltration into these areas (Figure 1(e) and (g)). Most CD147-positive cells infiltrating around damaged glomeruli and tubules were detected with CD68 antibody to macrophages, using serial sections (Figure 1(g)). Parts of them were lymphocytes, neutrophils and fibroblasts. Damaged arterioles expressed CD147, but interlobular arteries did not (Figure 1(h)).

Plasma and urinary CD147 levels

There were no significant differences in plasma and urinary CD147 values between

pathological control patients and healthy individuals (Figure 2(a) and (b)). In healthy individuals, plasma CD147 levels ranged from 2103.8 to 3210.7 pg/ml, and urinary CD147 levels were narrowly distributed between 6.89 and 26.66 after normalization to urinary Cr (Figure 2(a) and (b)). In contrast, both plasma and urinary CD147 levels in LN patients (mean±SD (range): plasma levels, 4983.9±3461.9 pg/ml (1215.7-18458.2); urinary levels, 41.04±31.01 (8.01-220.18)) were double those in groups of pathological controls and of healthy individuals. Of note, patients with proliferative LN (ISN/RPS class III or IV) showed more plasma and urinary CD147 induction. A significant association was evident between plasma and urinary CD147 levels (Figure 2(c)). Plasma CD147 levels correlated significantly with markers of renal function such as levels of serum Cr (Figure 2(d)), blood urea nitrogen (BUN) and renal SLEDAI scores, but not with proteinuria. Interestingly, no significant correlations existed between plasma CD147 expression and traditional parameters for systemic activity of SLE, including anti-DNA antibody, component C3 (Figure 2(e) and (f)) and SLEDAI scores (data not shown). Likewise, none of these markers correlated with urinary CD147 expression. There were no significant differences in plasma and urinary CD147 levels between LN patients treated with and without glucocorticoid therapy at the time point of renal biopsy (data not shown). We further examined plasma CD147 levels in 10 patients without LN. Those values in SLE patients without LN (3137±740 pg/ml) were significantly higher than those in healthy individuals (Supplemental figure 1). LN patients tended to exhibit the higher levels of plasma CD147 than SLE patients without LN, but not significantly. To next investigate the change of plasma CD147 from active phase to inactive status, 11 patients who are undergoing treatment for LN were assessed. Interestingly, patients with inactive LN (2650±372 pg/ml) showed the reduction of

plasma CD147 values in the therapeutic duration, compared to LN patients (3357±825 pg/ml) (Supplemental figure 2).

Comparison of LN biomarkers with BAI and BCI

To investigate the reliability and benefit of CD147 as a hallmark for LN, we next compared levels with urinary concentrations of NGAL and MCP-1, as novel biomarkers, with regard to the BAI and BCI scores in LN patients. Plasma CD147 levels showed good correlation with activity of LN, similar to that of urinary MCP-1 values (Figure 3). These data suggest that plasma CD147 might offer a reliable hallmark of LN.

As kidney biopsy cannot be repeated at every flare of LN, we further clarified the diagnostic accuracy of plasma CD147 as a non-invasive predictor reflecting renal function and histological features. BAI score is already known to be reliable for determining renal dysfunction in disease survey [28], with higher scores (BAI $\geq$ 7) indicating the status of severe inflammation [25-27]. Eleven patients (17.2%) with LN showed higher BAI scores. Plasma CD147 was likely to be one of crucial predictors of active LN (Figure 4). In addition, plasma CD147 significantly predicted the histological features in patients with active LN after adjusted for ages (years), sex (male or female), serum Cr levels (mg/dl) and glucocorticoid therapy (yes or no) in multivariate logistic regression analysis (data not shown). We further evaluated encouraging combinations of multiplex markers in patients with active LN, since a single molecule was not sufficient for achieving higher diagnostic accuracy. In the present study, higher AUC scores were shown in the combinations of marker such as plasma CD147+component C3 (AUC, 0.92) in BAI (Figure 5). The combinations of three variables weren't superior to those of two ones. Table 3 summarized the diagnostic accuracy of combination biomarkers

assessed by ROC curve analysis. Interestingly, several combinations of biomarkers involving urinary NGAL and/or MCP-1 didn't reach appropriate AUC (AUC > 0.9) for decision.

Discussion

The present study examined whether CD147 can accurately predict the pathological features of LN as a noninvasive diagnostic tool. In biopsy tissues of LN with a variety of histological features, CD147 induction was strikingly present in injured glomeruli and infiltrating inflammatory cells, but not in injured tubules representing atrophy. Most interestingly, active lesions representing cell proliferation and cellular crescent strongly expressed CD147. Despite reductions in tubules, both plasma and urinary CD147 levels in patients with LN were double those of pathological control patients and healthy individuals. A significant correlation existed between plasma and urinary CD147 levels, whereas urinary levels of CD147 were markedly higher than plasma levels. These data indicate that the part of urinary CD147 levels may be filtrated from circulating blood in glomeruli of patients with LN, but most of them might be caused by the detachment of injured TECs in both acute and chronic phase of LN. That may be the reason why urinary CD147 wasn't appropriate as a precious predictor for active LN. Plasma CD147 values correlated strongly with serum Cr levels, which represent a key independent risk factor for the development of chronic kidney disease or death [29]. The reduction of glomerular filtration caused by kidney damage may show decreased renal clearance of CD147. In this study, however, we demonstrated that plasma CD147 was the independent predictor of active LN on adjusting for serum Cr levels. Based on our basic researches, circulating soluble CD147 on inflammatory cells shed by MT1-MMP might

describe the severity of AKI. Taken together, plasma CD147 values might reflect the disease activity of LN, but not urinary CD147.

Consistent with our results, the traditional specific parameters for SLE, including anti-DNA antibody and proteinuria, did not predict disease activity in patients with LN [3]. To further evaluate the reliability of plasma CD147, we compared with sensitive biomarkers such as urinary NGAL and MCP-1, which have been already reported to be candidates for LN in child and/or adult patients [4, 5, 8, 9, 30]. Biopsy activity indices provide reliable and useful information for therapy [31]. As shown in Figure 4, plasma CD147 appears to show higher values in LN with severe inflammation, similar to urinary NGAL and MCP-1, and correlated significantly with renal disease activity. Indeed, lower levels of plasma CD147 were observed in inactive LN patients with immunosuppressive treatment and SLE patients without renal involvement, compared to active LN patients. Further verification would be needed to elucidate these findings in the near future. These data suggest that plasma CD147 levels might be a prime candidate in the clinical setting of LN and be valuable for avoiding repeated kidney biopsy.

To the best of our knowledge from basic research, however, CD147 may not be specific for LN, similar to the others. Despite numerous efforts and clinical research, no specific biomarker for the disease activity of LN has yet been identified. NGAL is also recognized as an inflammation-related biomarker in various systemic diseases, including vasculitis [32], Kawasaki disease [33], cancers [34] and ischemic-induced AKI [35]. Indeed, plasma CD147 levels significantly increased in biopsy-proven other kidney diseases. In LN representing various histological features caused by multiple complex pathogenesis, prediction using a single molecule might be quite difficult. Multiplex

markers would be therefore needed to achieve high diagnostic accuracy (AUC > 0.9) for the guidance of LN therapy [7]. The candidate biomarkers were assessed by the multivariate logistic regression analysis. In this study, the combinations of plasma CD147+component C3 (AUC, 0.92) reached at a reliable AUC level for estimating pathological LN activity. The diagnostic accuracy of multiplex biomarkers also indicates that the combination including plasma CD147 might yield excellent diagnostic abilities for guiding ideal LN therapy (Table 3). The diverse mechanisms involving CD147 for the kidney diseases need to be elucidated in the near future.

The majority of clinical studies to date have emphasized the necessity for identifying biomarkers reflecting the disease activity of LN [3, 7, 36]. Many studies exploring and validating noninvasive tool are thus underway. In the near future, a large prospective longitudinal cohort of patients with LN will be assembled to confirm standardized platforms for reliable measurement of promising biomarkers. We propose that plasma CD147 might provide key insights to understand the disease activity of LN and allow the start of preemptive medication as a crucial biomarker in patients with LN. This process should eventually result in improved complete remission rates and reduced toxicity from therapy.

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Conflict interest statement

The authors declare no conflicts of interests.

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Table 1. Patient characteristics

	Lupus n=64	Pathological control n=14	Healthy control n=16
Sex (%) female	72	57	44
Age (years)	44.1 ±17.0 (15-80)	32.2 ±14.4 (17-70)	38.56 ± 8.29 (29-63)
Height (cm)	158.7 ± 8.7 (141.0-180.0)	163.4 ± 11.1 (145.8-183.1)	164.4 ± 8.0 (153.0-177.0)
Weight (kg)	54.0 ± 11.2 (36.0-100.0)	62.2 ± 23.9 (32.8-120.0)	59.9 ± 12.1 (41.0-82.4)
Mean BP (mmHg)	97.3 ± 17.3 (66.7-146.7)	86.8 ± 14.91 (66.7-120.0)	85.4 ± 9.12 (70.7-103.3)
Serum Cr (mg/dl)	1.09 ± 1.05 (0.35-6.00)	0.78 ± 0.27 (0.44-1.29)	0.74 ± 0.15 (0.47-0.97)
eGFR (mL/min/1.73m^2)	74.76 ± 37.20 (7.84-171.58)	91.27 ± 26.58 (52.50-156.09)	
Proteinuria (g/day)	2.51 ± 2.09 (0.12-10.50)	0.36 ± 0.21 (0.10-0.71)	
Hb (g/dl)	11.2 ± 2.1 (6.8-15.6)	14.1 ± 1.7 (10.9-17.3)	14.3 ± 1.0 (13.4-16.0)
HbA1c (%)	5.2 ± 0,5 (4.2-6.6)	5.4 ± 0.5 (4.6-6.0)	
CRP (mg/dl)	0.7 ± 1.1 (0.0-4.5)	1.0 ± 2.5 (0.0-7.7)	0.08 ± 0.06 (0.03-0.24)
C3 (mg/dl)	54.4 ± 25.9 (12.0-123.2)	101.8 ± 28.3 (58.0-153.0)	112.3 ± 24.7 (84.0-181.0)
SLAM (score)	7.6 ± 3.8 (1-18)		
SLEDAI (score)	16.8 ± 6.1 (2-30)		
Anti-DNAantibody (IU/ml)	155.4 ± 227.3 (2-903)		

Data are expressed as mean±SD (range).

Cr, creatinine.

eGFR, estimated glomerular filtration rate.

Hb, hemoglobin.

CRP, C-reactive protein.

C3, components C3.

SLAM, the systemic lupus activity measure.

SLEDAI, the systemic lupus erythematosus disease activity index.

Table 2. Histological features of patients with LN

		n of N (%)
ISN/RPS class	I	1 (1.6%)
	II	1 (1.6%)
	III	6 (9.4%)
	IV	21 (32.8%)
	V	10 (15.6%)
	III + V	12 (18.8%)
	IV + V	13 (20.3%)
Histological features on biopsy	Endocapillary hypercellularity	59 (92.2%)
	Leukocyte infiltration	10 (15.6%)
	Subendothelial hyaline deposits	24 (37.5%)
	Fibrinoid necrosis/karyorrhexis	11 (17.2%)
	Cellular crescents	17 (26.6%)
	Interstitial inflammation	29 (45.3%)
	Glomerular sclerosis	28 (43.8%)
	Fibrous crescents	21 (32.8%)
	Tubular atrophy	26 (40.6%)
	Interstitial fibrosis	39 (60.9%)

Table 3. Comparison of multiplex markers predicting active features in LN

model outcome variable	predictor variables	AUC (95% CI)
	CD147, C3	0.92 (0.84-0.99)
	CD147, anti-DNA Ab	0.88 (0.78-0.97)
	MCP-1, C3	0.87 (0.75-0.99)
	CD147, MCP-1	0.86 (0.75-0.98)
	CD147, NGAL	0.86 (0.76-0.97)
	CD147, UP	0.84 (0.70-0.97)
	MCP-1, anti-DNA Ab	0.83 (0.68-0.98)
BAI score ≥ 7	C3, anti-DNA Ab	0.78 (0.65-0.92)
	NGAL, C3	0.78 (0.65-0.91)
	MCP-1, NGAL	0.76 (0.58-0.94)
	NGAL, anti-DNA Ab	0.76 (0.64-0.89)
	C3, UP	0.75 (0.56-0.94)
	MCP-1, UP	0.73 (0.49-0.98)
	NGAL, UP	0.56 (0.33-0.79)
	anti-DNA Ab, UP	0.45 (0.23-0.66)

CD147, plasma CD147; MCP-1, urinary MCP-1; NGAL, urinary NGAL;

C3, components C3; anti-DNA Ab, anti-DNA antibody; UP, urinary protein;

AUC, the area under the receiver operating characteristic (ROC) curve; 95%CI, 95% confidence interval

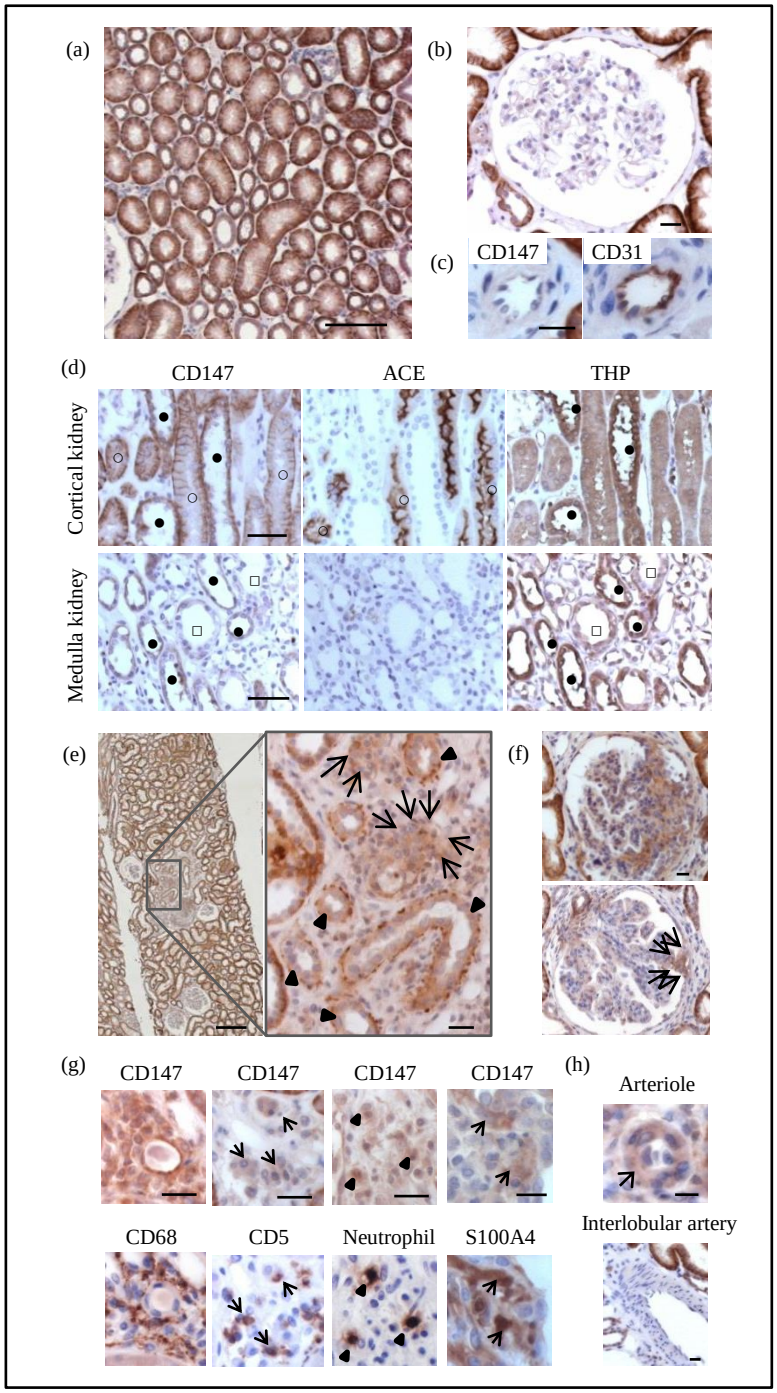

Figure 1. CD147 expression in patients with lupus nephritis

(a) Immunohistochemical staining of CD147 expression in the tubulointerstitium of control patients. Bar, 100 μm. **(b)** CD147 expression in glomeruli. Bar, 20 μm. **(c)** CD147 expression and CD31 expression. Serial sections were used. Bar, 20 μm. **(d)** open circles, proximal tubule; closed circles, distal tubule; open boxes, collecting tubule. Bar, 50 μm. **(a)-(d)** control patients. **(e)** Immunostaining of CD147 expression in patients with lupus nephritis. This expression is decreased in damaged regions. Bar, 200 μm (low magnification). Injured tubules show marked reductions in CD147 expression (arrowhead). Migration of various cells is seen around injured tubules. (arrow). Bar, 20 μm (high magnification). **(f)** CD147 expression in injured glomeruli. Upper panel: cellular proliferation. Lower panel: adhesion (arrow). Bar, 20 μm. **(g)** Upper panels: CD147 expression. Lower panels: CD68 expression, CD5 expression, neutrophil elastase expression and S100A4 expression. Serial sections were analyzed using immunohistochemical staining. Bar, 20 μm. **(h)** CD147 expressions in arteriole and interlobular artery. Bar, 20 μm. **(e)-(h)** LN patients.

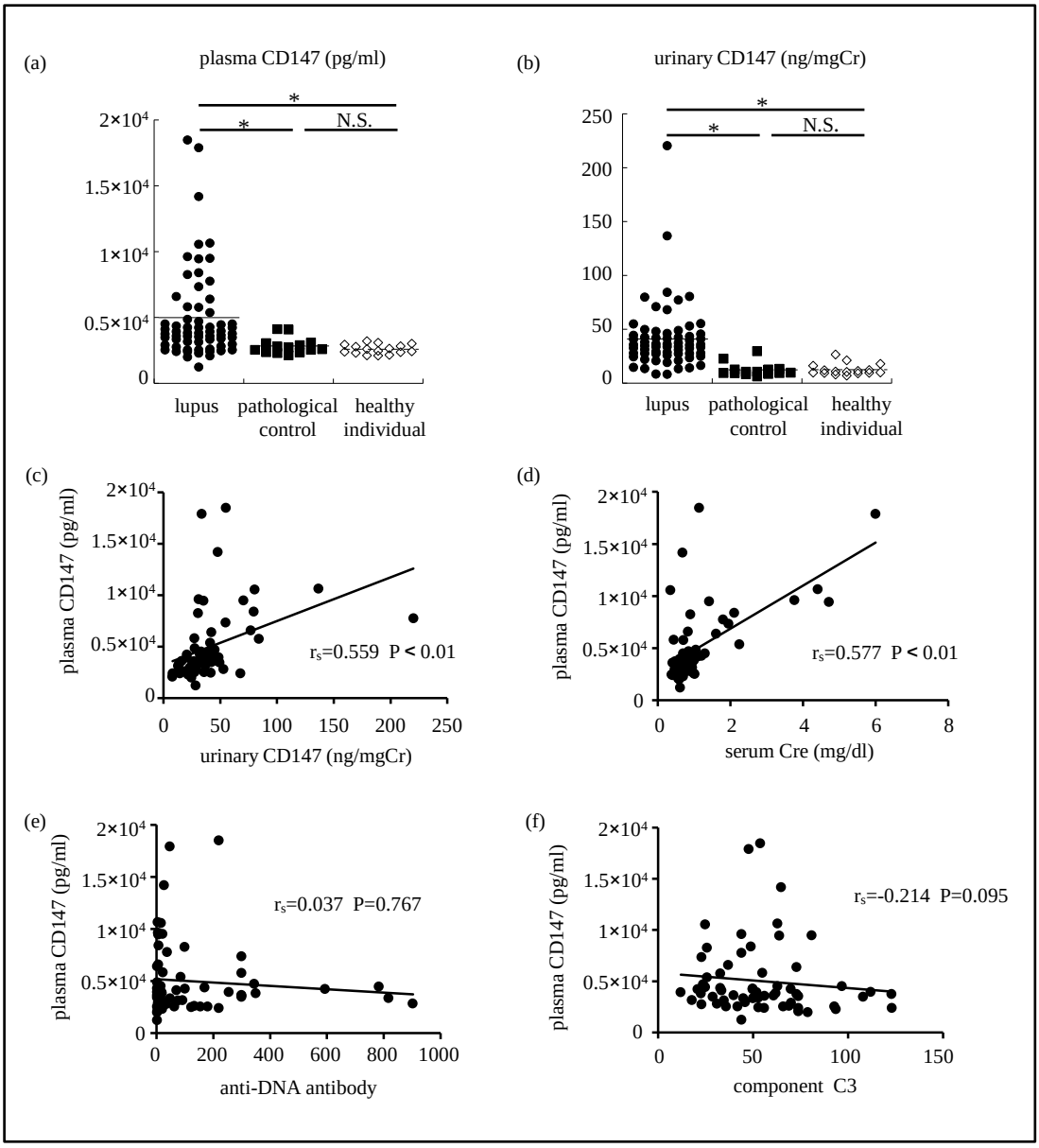

Figure 2. Plasma and urinary CD147 values

(a) Plasma CD147 levels (pg/ml). **(b)** Urinary CD147 values (pg/mgCr). *, $p < 0.01$. **(c)** Correlation between plasma and urinary CD147 levels. **(d)-(f)** Correlation between plasma CD147 levels, renal function and traditional parameters. Plasma CD147 is strongly associated with renal function **(d),** but not traditional parameters for LN, such as anti-DNA antibody **(e)** and component C3 **(f)**.

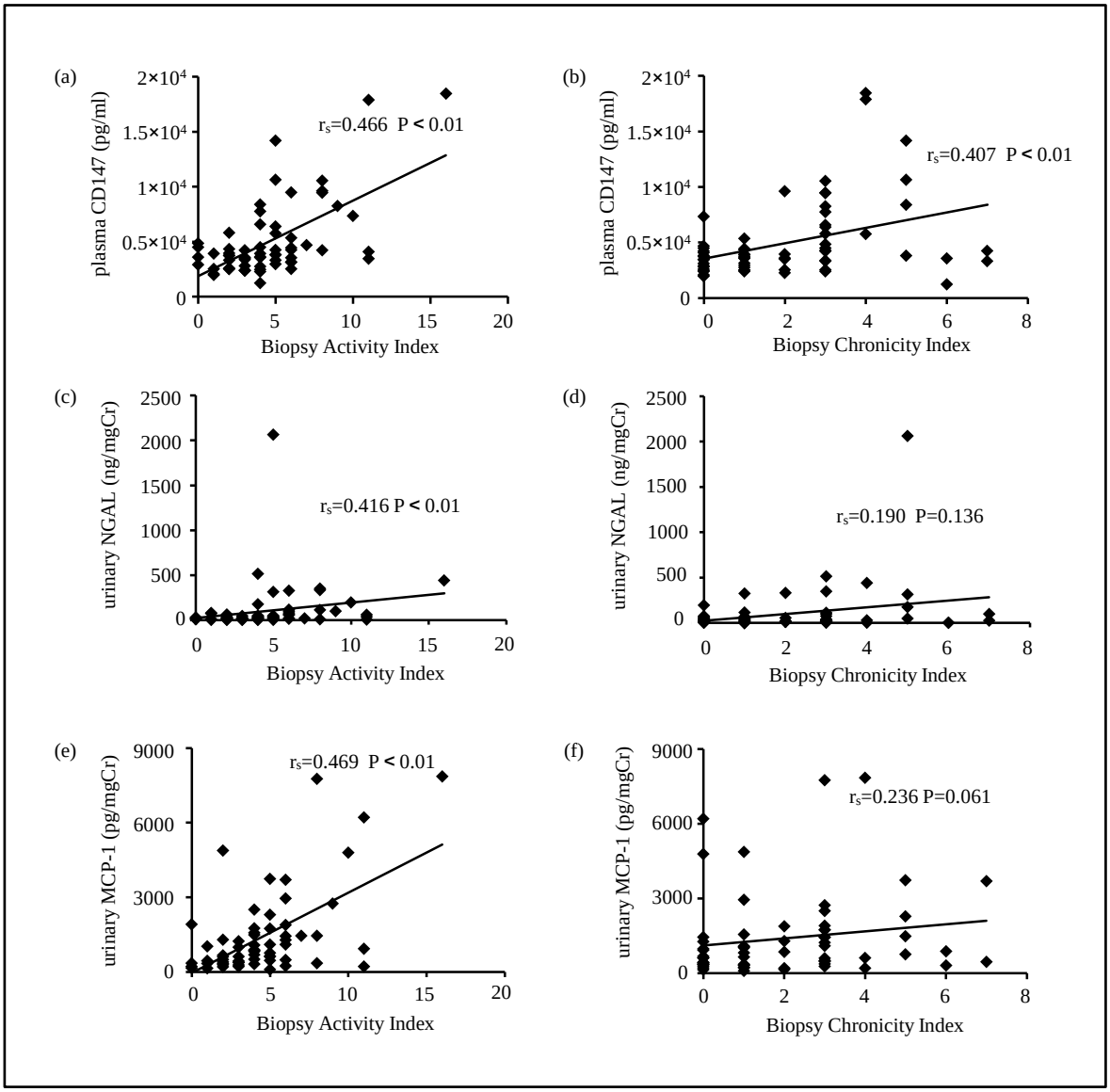

Figure 3. Comparison of LN biomarkers in BAI and BCI

Left panels show the combination between BAI scores and plasma CD147 (a), urinary NGAL (c) and MCP-1 (e). These biomarkers exhibited good correlation with pathological activity of LN. Right panels show the combination between BCI scores and plasma CD147 (b), urinary NGAL (d) and MCP-1 (f).

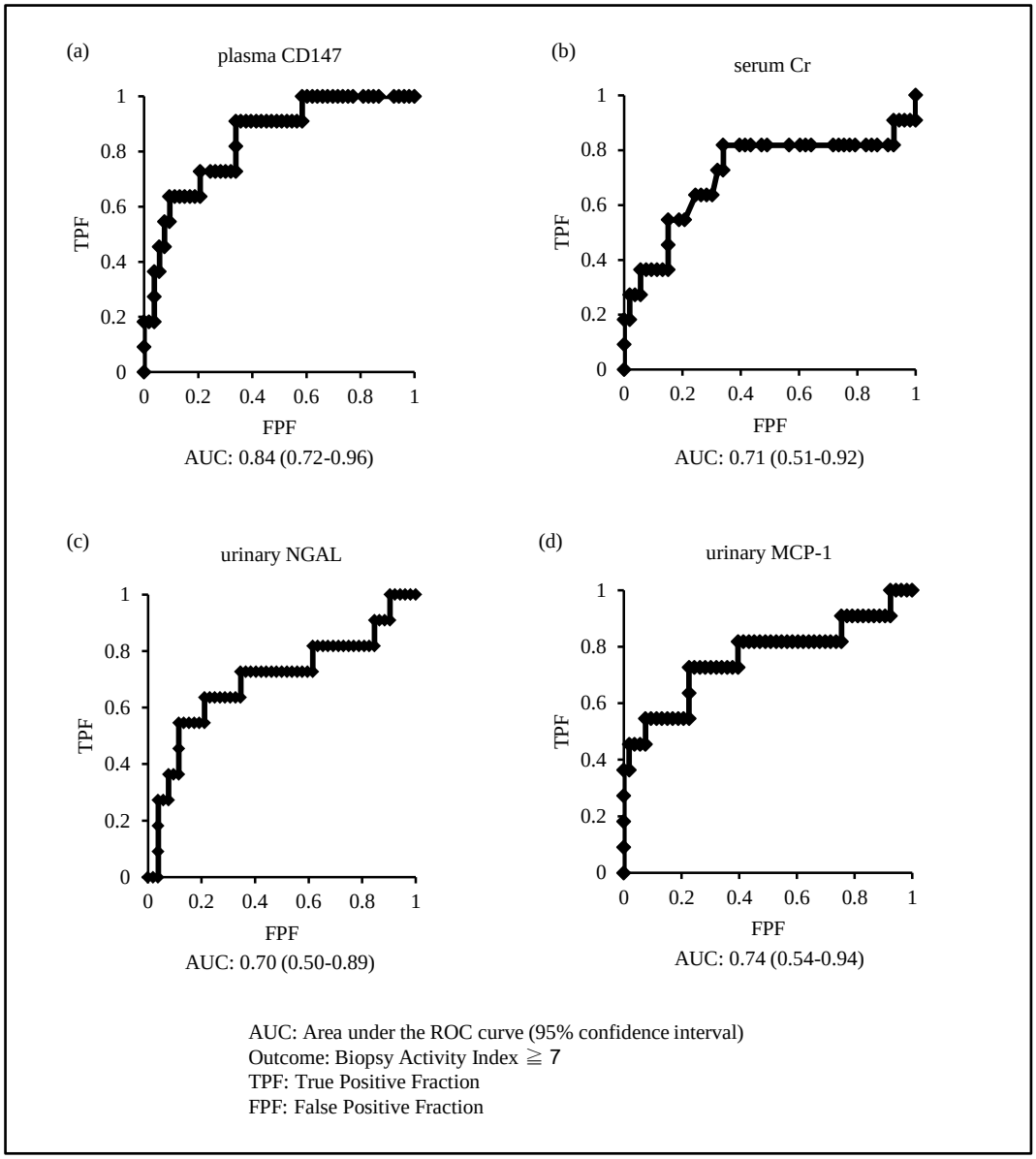

Figure 4. Comparison of ROC curves of plasma CD147, serum Cr, urinary NGAL and MCP-1

ROC curve analysis of each biomarker shows the relationship with higher BAI (BAI≥7), which indicates active LN with severe inflammation. (a) Plasma CD147. (b) Serum Cr. (c) Urinary NGAL. (d) Urinary MCP-1. The AUC of plasma CD147 tends to be higher than those of the other biomarkers, but not significantly.

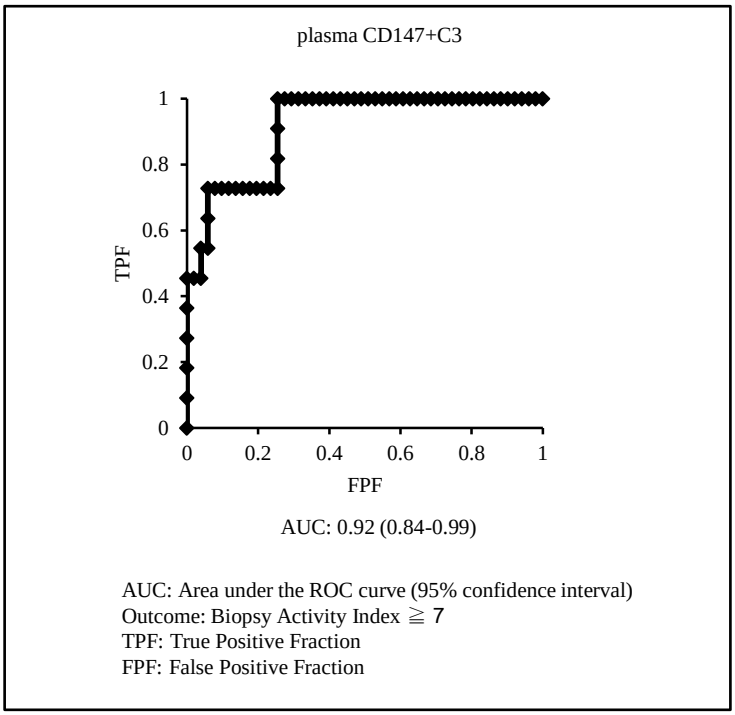

Figure 5. ROC curve of the combination biomarker

ROC curve analysis of plasma CD147+component C3 indicates the relationship with higher BAI (BAI≥7). The area under the ROC curve (AUC) score was 0.92. The optimal biomarker would be needed to achieve higher diagnostic accuracy (AUC>0.90) for the guidance of LN. The AUC score of this combination reached at a reliable level for estimating pathologically LN activity.

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