News Release

Title

Development of a novel invasive test using leukocyte-derived proteins in urine for the diagnosis of glomerulonephritis

Key Points

○ Because histological examination for the diagnosis of glomerulonephritis, which induces renal dysfunction and accounts for 20% of cases of end-stage kidney failure, is a great burden to patients, alternative noninvasive tests are needed.

○ We observed accumulation of leukocytes expressing CD11b, a component of the cell adhesion molecule Mac-1, in glomeruli with lupus nephritis complicated with systemic lupus erhythematosus and vasculitis and found a correlation with the levels of CD11b in the urine of patients from Nagoya University and its affiliated hospitals. These findings were further confirmed in urine samples from Gunma University and Saitama Medical University.

○ A comparative analysis among urinary CD11b, two other leukocyte-derived molecules (CD163 and CD16b), urinary protein, and conventional parameters for renal dysfunction demonstrated the superior diagnostic performance of urinary CD11b to predict active lupus nephritis. Therefore, measurement of urinary CD11b was suggested as a useful diagnostic test and alternative to histological examination.

Summary

A joint research team composed of the former graduate student Akimitsu Kitagawa, professor Shoichi Maruyama, their collaborators at the Department of Nephrology at Nagoya University Graduate School of Medicine (Dean: Kadomatsu Kenji, MD, PhD), and associate professor Naotake Tsuboi at the Department of Nephrology at Fujita Health University (Professor: Yukio Yuzawa; Dean: Nakao Iwata, MD, PhD) demonstrated significant elevation of surface molecules expressed on leukocytes, which play major roles in renal inflammation, in urine samples collected at Nagoya University, Gunma University, and Saitama Medical University from patients with glomerulonephritis.

Glomerulonephritis, which accounts for 20% of cases of end-stage kidney dysfunction, is characterized by leukocyte accumulation and inflammation in glomerulus, a small filtering unit of the kidney. However, because kidney biopsy essential for diagnosis is associated with risk of bleeding and requires hospitalization of patients, repeated examinations for the evaluation of therapeutic efficacy and disease recurrence are not encouraged.

The joint research team hypothesized that surface molecules on inflammatory glomerular leukocytes leak into urine. Accordingly, these leukocytes were analyzed in 272 urine samples from patients with kidney disease. The study revealed significant elevation of urinary CD11b with increased numbers of glomerular leukocytes expressing CD11b in patients with lupus nephritis, a major kidney complication of systemic lupus erhythematosus, and in patients with vasculitis.

Moreover, the result was confirmed in urine samples from patients with lupus nephritis enrolled in the joint research study with Gunma University and Saitama Medical University, and further analysis of the diagnostic performance for predicting active lupus nephritis demonstrated marked superiority of urinary CD11b compared with two other leukocyte-derived molecules (CD163 and CD16b), urinary protein, and conventional parameters for renal dysfunction.

The current study demonstrated that urinary CD11b reflects glomerular accumulation of inflammatory leukocytes in lupus nephritis and vasculitis-related glomerulonephritis. Therefore, the research team expects that measurement of urinary CD11b may be a useful noninvasive alternative to kidney biopsy to facilitate the diagnosis, therapeutic efficacy assessment, and disease recurrence prediction in patients with lupus nephritis and vasculitis-related glomerulonephritis.

The current research results were published in *Kidney International*, the official journal of the International Society of Nephrology. (Epub 31. January. 2019).

Research Background

Kidney biopsy is essential for the diagnosis, monitoring, and treatment of patients with glomerulonephritis, which induces renal dysfunction and accounts for 20% of cases of end-stage kidney dysfunction. Kidney biopsy is beneficial for evaluating the type, activity, and prognosis of glomerulonephritis, but is invasive for patients and is associated with the risk of bleeding and the need for hospitalization. Therefore, a noninvasive alternative test is needed for evaluation of therapeutic efficacy and prediction of disease recurrence in outpatient care and for the diagnosis of patients with poor general health or in the elderly.

Leukocytes infiltrate into the glomerulus, a small filtration unit in kidney, and induce inflammation at the active stage of glomerulonephritis. In particular, neutrophils and macrophages have been shown to play central roles in acute inflammation. Neutrophils and macrophages express Mac-1, an adhesion molecule for leukocytes to the vascular endothelium, composed of CD11b and CD18, on their surfaces.

In this study, the researchers tested their hypothesis that CD11b expressed on leukocytes leaks into the urine and that measurements of CD11b could help predict glomerular leukocyte accumulation in the active stage of glomerulonephritis.

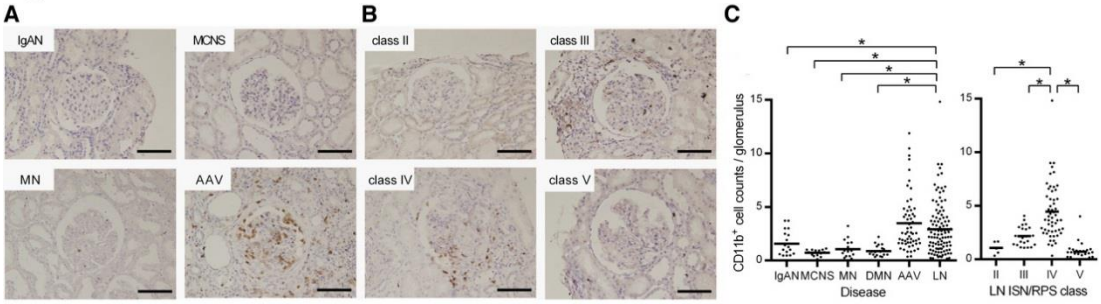
Research Results

The researchers' observations of kidney specimens from 272 patients with various types of kidney glomerular diseases, collected at Nagoya University and its affiliate hospitals, revealed a significant increase in glomerular accumulation of leukocytes expressing CD11b in patients with systemic lupus erhythematosus (lupus nephritis)-related and vasculitis-related glomerulonephritis, particularly in 118 cases with class III and IV lupus nephritis, who often require intensive treatment due to high disease activity (Figure 1). Urinary CD11b levels at the time of biopsy were significantly increased in patients with class III and IV lupus nephritis and were strongly correlated with accumulation of glomerular CD11b-positive leukocytes (Figure 2). Urinary CD11b levels were also increased in urine samples collected from patients with lupus at Gunma University and Saitama Medical University and were

decreased after treatment. Moreover, a comparative analysis to evaluate the diagnostic performance of urinary CD11b for class III and IV lupus nephritis demonstrated its higher sensitivity and specificity than other leukocyte surface molecules (CD163 and CD16b), the inflammatory mediator monocyte chemotactic protein-1, and conventional indices in kidney function, such as serum creatinine and urinary protein (Figure 3). Additionally, the results from human patient samples were further corroborated with those from an animal disease model of glomerulonephritis, and experiments using cultured cells suggested that CD11b was released from the cell surface when leukocytes recognized immune complexes, pathogenic inducers for glomerulonephritis, deposited on the glomerulus or during the process of cell transmigration from the circulation to extracapillary spaces under inflammatory conditions.

Taken together, these results indicated that U-CD11b was a useful tool for the diagnosis of lupus nephritis and vasculitis-related glomerulonephritis and for evaluation of therapeutic efficacy and the prediction of disease recurrence. In addition, urinary CD11b measurement may be a noninvasive diagnostic approach with applications in developing countries with insufficient medical environments in which patients may experience end-stage renal failure without a specific diagnosis on histological assessment.

Figure 1



Glomerular accumulations of CD11b positive cells in various kidney diseases and lupus nephritis (LN)

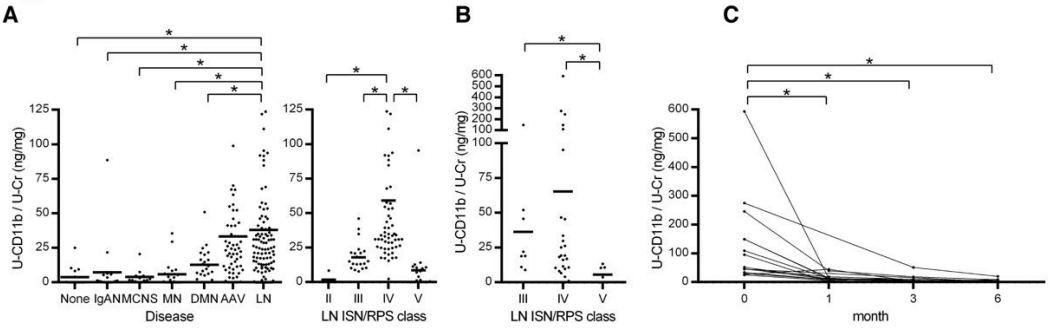
A, B: Microscopic observation of glomerular $CD11b^+$ cells (brown) in various kidney diseases (A) and class II, III, IV, and V LN (B).

C: Quantitative analysis of $CD11b^+$ cell numbers in glomeruli from patients with various kidney diseases and class II, III, IV, and V LN.

Each patient is represented by a dot, and the mean of each group is shown as a horizontal bar. *P < 0.05.

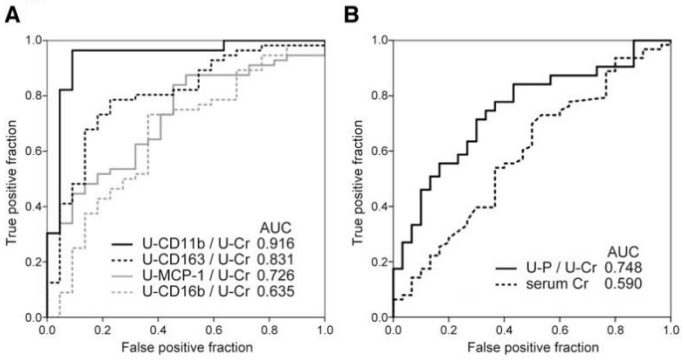
IgAN: IgA nephropathy, MCNS: minimal change nephrotic syndrome, MN: membranous nephropathy, DMN: diabetic nephropathy, AAV:ANCA-related vasculitis

Figure 2



Elevations of urinary CD11b in various kidney diseases and LN
A: CD11b was measured in urine samples from patients with indicated kidney diseases (left) and class II, III, IV, and V LN (right) in N-KDR cohort.
B: Elevations of urinary CD11b (U-CD11b) were confirmed in active class III and IV LN patients from Gunma/Saitama cohort.
Each patient is represented by a dot, and the mean of each group is shown as a horizontal bar. *P < 0.05.
C: Kinetics of U-CD11b in 15 identical patients with class III or IV LN from the Gunma/Saitama cohort.
Longitudinal urine sampling for 6 months was conducted in patients administered remission induction therapy.
Each line represents an identical patient. *P < 0.05.

Figure 3



Comparison of the diagnostic ability for active LN among urinary excretion of CD11b and other molecules.
A: ROC curves of U-CD11b, U-CD16b, U-CD163, and U-MCP-1 to predict ISN/RPS class III and IV LN subpopulations.
Urinary concentrations of each molecule were corrected by U-Cr in patients with LN.
B: ROC curves of proteinuria (U-P/U-Cr) and serum Cr (sCr) to predict ISN/RPS class III and IV LN subpopulations are shown.
The corresponding AUCs for indicated biomarkers are also displayed in each panel.
AUC: Area Under the Curve. The more close to 1 of AUC, biomarkers perform greater specificity and sensitvity.

Research Summary and Future Perspective

Biomarkers enabling diagnosis from blood or urine samples are highly valuable in the clinical setting. Racial factors, as well as sex, genetic background, and environmental factors, affect the onset and activity of lupus nephritis. Because the current study was performed using biological samples from a Japanese patient population, the research team has initiated an international joint study with Chinese and Mexican institutions to evaluate the clinical significance of U-CD11b among different ethnicities and is planning a large-scale scientific study involving expansion of collaboration to other countries. Because ACNA-associated vasculitis often manifests as rapidly progressive kidney dysfunction, the research team will analyze U-CD163 in biosamples collected at Nagoya University and Progressive Renal Diseases Research, Research on Intractable Diseases, from the Ministry of

Health, Labour and Welfare of Japan. For the practical application of current biomarkers, the research team will approach companies to develop diagnostic reagents that are easy to use and accurate in the clinical setting. The final goal of the current study is to contribute to improving the quality of treatment options for patients with glomerulonephritis based on enhancement of the accuracy of diagnosis based on the global standard established by this study.

Publication

Urinary levels of the leukocyte surface molecule CD11b associate with glomerular inflammation in lupus nephritis

Akimitsu Kitagawa,[*] Naotake Tsuboi,[*, †, ††] Yuki Yokoe,[*] Takayuki Katsuno,[‡] Hidekazu Ikeuchi,[§, ¶] Hiroshi Kajiyama,[‖, ¶] Nobuhide Endo,[*] Yuriko Sawa,[*] Junya Suwa,[§] Yutaka Sugiyama,[*] Asaka Hachiya,[*] Toshihide Mimura,[‖, **] Keiju Hiromura,[§, **] and Shoichi Maruyama[*]

**Department of Nephrology, Internal Medicine, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan*

[†]Department of Nephrology, School of Medicine, Fujita Health University, Toyoake, Aichi, Japan

[‡]Department of Nephrology and Rheumatology, Aichi Medical University, Nagakute, Aichi, Japan

[§]Department of Nephrology and Rheumatology, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan

[‖]Department of Rheumatology and Applied Immunology, Faculty of Medicine, Saitama Medical University, Iruma, Saitama, Japan

Kidney International, published online on January 31, 2019.

DOI：10.1016/j.kint.2018.10.025

Japanese ver.

https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Kidney_I_20190201.pdf