News Release

Title

Development of a urine test for the diagnosis and prediction of renal outcomes of rapidly progressive glomerulonephritis

-Testing method using leukocyte-derived molecules in urine-

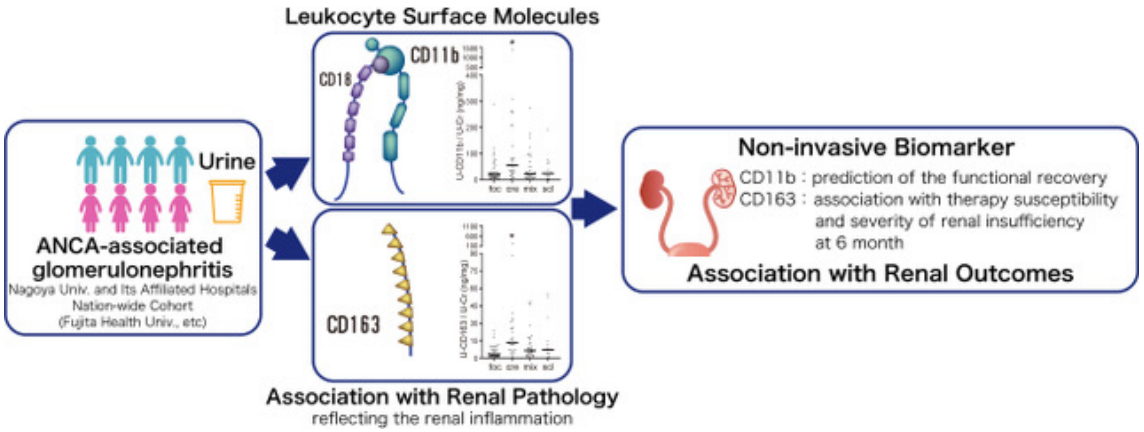
Key Points

・Glomerular crescent is a representative histological finding used to predict unfavorable renal outcomes in patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis (GN), an incurable renal disease that involves a rapid decline of renal function and requires long-term use of renal replacement therapy lasting weeks to months after disease onset (rapidly progressive glomerulonephritis; RPGN). Although renal biopsy is a useful diagnostic tool for analyzing disease activity that helps decide the treatment strategy for ANCA-GN, it is cumbersome for patients. Therefore, alternative noninvasive tests are needed.

・We observed the elevation of urinary leukocyte surface molecules CD11b (U-CD11b) and CD163 (U-CD163) and their association with glomerular crescent formation in ANCA-GN patients from Nagoya University, its affiliated hospitals, and some more core hospitals, including university hospitals, in Japan.

・The U-CD163 level at diagnosis was increased in patient groups with a low response to immunosuppressive therapy or renal function impairment after 6 months. On the contrary, the U-CD11b level at diagnosis was associated with renal recovery following immunosuppressive therapy.

・Although both U-CD11b and U-CD163 reflect inflammatory cell accumulation in the glomeruli of ANCA-GN, their clinical significance is different. The combined analysis of U-CD11b and U-CD163 at diagnosis may be useful, not only for evaluating renal inflammatory status, but also as a biomarker of the response to immunosuppressive treatment and kidney functional outcomes that can help decide the treatment regimen.



Summary

A joint research team comprising former graduate student Yuki Yokoe, Professor Shoichi Maruyama, their collaborators at the Department of Nephrology at Nagoya University Graduate School of Medicine (Dean: Kenji Kadomatsu, MD, PhD) and Professor Naotake Tsuboi at the Department of Nephrology at Fujita Health University (Dean: Nakao Iwata, MD, PhD) revealed significant elevations in the surface molecules U-CD11b and U-CD163 expressed on leukocytes, which cause renal inflammation, and their associations with renal outcomes following immunosuppressive therapy in ANCA-GN urine samples collected by Nagoya University and Japan Research Committee of the Ministry of Health, Labour, and Welfare for Intractable Vasculitis and for Intractable Renal Disease.

ANCA-GN is an intractable renal disease that clinically presents with rapid renal functional impairment and histologically demonstrates inflammation characterized by leukocyte accumulation and glomerular crescent. Therefore, histological investigation of renal biopsy specimens is used for evaluating disease activity in ANCA-GN. However, ANCA-GN patients are required to be hospitalized for monitoring biopsy-related renal bleeding considering that histological examinations are not as frequently performed as blood and urine examination.

The joint research team has proved the urinary leakage of leukocyte-derived molecules, which play major roles in renal inflammation, in glomerulonephritis. The current study was conducted using ANCA-GN samples collected by Nagoya University and its affiliated hospitals and by core hospitals, including university hospitals, in Japan;

it revealed a significant accumulation of leukocytes expressing CD11b, which plays a role in leukocyte adhesion to the vascular endothelium, and a scavenger receptor CD163 in the glomerulus, particularly in glomerular crescents susceptible to immunosuppressive therapy, and the significant elevation of U-CD11b and U-CD163. Furthermore, when the association of both these urinary molecules with renal outcomes was analyzed, the U-CD163 level at diagnosis was observed to increase in patients with unachieved remission or with more severe renal impairment following 6 months of immunosuppressive treatment. Thus, the U-CD11b level was used to predict the average recovery rate of renal function up to 2 years after disease onset.

Although some biomarkers can predict unfavorable renal outcomes in ANCA-GN, this was the first report indicating markers reflecting renal leukocyte accumulation and its association with treatment response (U-CD163) and renal recovery (U-CD11b). Thus, measuring U-CD11b and U-CD163 instead of performing histological examinations may be a useful diagnosis tool for evaluating therapeutic efficacy and predicting disease recurrence in ANCA-GN.

The current research results were published in *Nephrology Dialysis Transplantation (NDT)*—an official journal of the ERA-EDTA (European Renal Association-European Dialysis and Transplant Association) (Epub 8.July. 2020).

Research Background

Anti-neutrophil cytoplasmic antibody (ANCA)–associated glomerulonephritis (ANCA-GN) is defined as the renal involvement of ANCA-associated vasculitis (AAV), which is an intractable disease characterized by rapid kidney function decline and a histopathology of glomerular crescent formation following destruction of glomerular capillaries in the kidney. Histological evaluations are essential for the diagnosis, monitoring, treatment-related decision-making, and prediction of the prognosis in ANCA-GN; however, histological evaluations require invasive kidney biopsies for which patients require hospitalization. Moreover, patients with ANCA-GN are older adults and their procedure-related risk of renal bleeding is high, often involving vascular inflammation. Therefore, a noninvasive alternative test is required to monitor the disease activity in outpatient care and for diagnosing ANCA-GN in patients who are unable to undergo invasive testing due to poor general health or complications.

The glomerulus is a small organ inside the kidneys that is involved in blood filtration. Glomerular-infiltrating leukocytes initiate and promote renal inflammation in ANCA-GN. Specific types of leukocytes, such as neutrophils and macrophages, are established as leukocyte subsets that play major roles in acute inflammation. Neutrophils and macrophages express CD11b, a component of the adhesion molecule Mac-1, which allows their adhesion to the vascular endothelium; macrophages express CD163, a scavenger receptor. Although the joint research team has revealed the urinary leakage of CD11b and CD163, the clinical significance of these urinary molecules needs clarification. With the hypothesis that the levels of U-CD11b and U-CD163 at diagnosis predict glomerular leukocyte accumulation, response to therapy, and renal outcomes, the research team analyzed the clinical significance of U-CD11b and U-CD163 in ANCA-GN biosamples collected by Nagoya University and its affiliated hospitals and core Japanese hospitals affiliated with the Japan Research Committee of the Ministry of Health, Labor, and Welfare for Intractable Vasculitis and for Intractable Renal Disease.

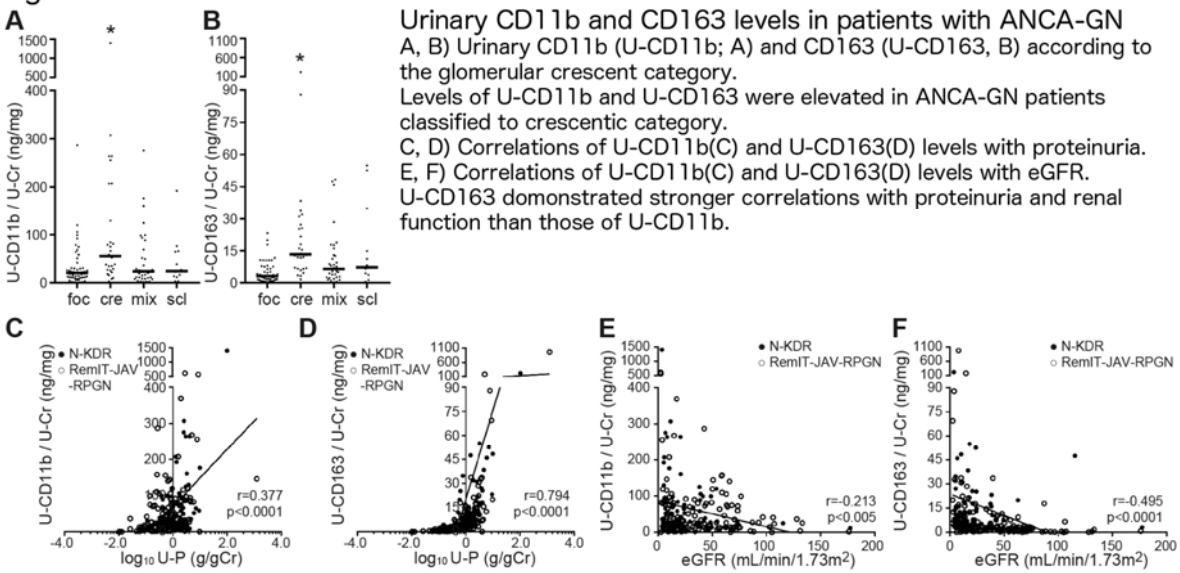
Research Results

The research team measured levels of CD11b and CD163 at histological diagnosis in 226 ANCA-GN urine samples collected by Nagoya University and its affiliated hospitals (88 cases) and core Japanese hospitals affiliated with the Japan Research Committee of the Ministry of Health, Labour, and Welfare for Intractable Vasculitis and for Intractable Renal Disease (138 cases), and analyzed their associations with histological classification of kidney glomerulus, degree of proteinuria, and renal function (Figure 1). The results demonstrated the significant elevation of U-CD11b and U-CD163 in ANCA-GN patients histologically classified to the crescentic category, in which crescents were formed in more than half total number of glomeruli and less than half the total number of glomeruli were abolished. Moreover, correlations of urinary molecules that were positive for proteinuria and negative for renal function were stronger in U-CD163.

Histological analyses focusing on the distributions of CD11b+ and CD163+ leukocyte subsets in diseased glomeruli demonstrated a more dominant distribution of CD11b+ cells in the undisrupted area than in the glomerular crescent; this was in contrast with the global distribution of CD163+ cells in diseased glomeruli. In addition, the U-CD11b and U-CD163 levels significantly correlated with the rate of formation of glomerular crescents with CD11b+ cells and the rate of formation of glomerular crescents

with CD163+ cells, respectively (Figure. 2). Further, when the association of both urinary molecules with post-treatment renal outcomes were analyzed at 6 months after the treatment, U-CD163 levels were found to be significantly reduced and those at the time of diagnosis were found to be high in patients who failed to achieve remission or progressed to severe renal dysfunction (Figure. 3). Although these associations were not found for U-CD11b, the analyses to determine the association of the two urinary molecules and other clinical parameters with yearly impairment of renal function over a 24-month observation period demonstrated the level of U-CD11b, but not that of U-CD163, at diagnosis as an independent factor for predicting renal recovery despite the age, renal dysfunction, and degree of proteinuria as factors contributing to the unfavorable renal outcome (Figure. 4).

Figure 1

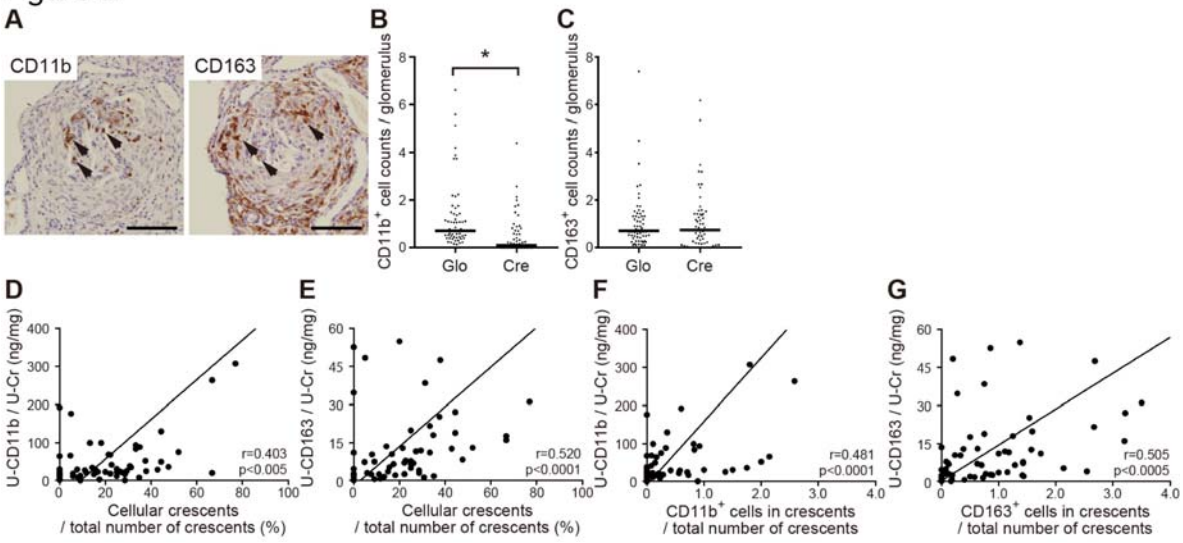


Urinary CD11b and CD163 levels in patients with ANCA-GN

A, B) Urinary CD11b (U-CD11b; A) and CD163 (U-CD163, B) according to the glomerular crescent category.
Levels of U-CD11b and U-CD163 were elevated in ANCA-GN patients classified to crescentic category.
C, D) Correlations of U-CD11b(C) and U-CD163(D) levels with proteinuria.
E, F) Correlations of U-CD11b(C) and U-CD163(D) levels with eGFR.
U-CD163 domonstrated stronger correlations with proteinuria and renal function than those of U-CD11b.

foc; focal, cre; crescentic, mix; mixed, sci; sclerotic, U-P; proteinuria, U-Cr; urinary creatinine, eGFR; estimated glomerular filtration rate, N-KDR; Nagoya Kidney Disease Registry, RemIT-JAV-RPGN; nationwide inception cohort study of remission induction therapy in Japanese patients with AAV and RPGN.

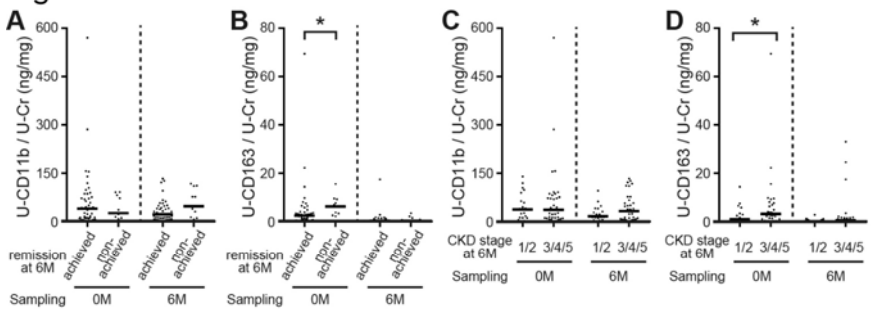
Figure 2



Intraglomerular distributions of CD11b+ and CD163+ cells in ANCA-GN

A) CD11b+ cells (left) and CD163+ cells (right) in a crescentic glomerulus of human ANCA-GN kidney. Arrows represents cells expressing indicated leukocyte markers.
B) Histological quantification of CD11b+ cells (B) and CD163+ cells (C) in glomerular tufts (Glo, left) and in a crescent (Cre, right) in ANCA-GN.
CD11b+ cells ditributed dominantly in glomerular tufts, CD163+ cells uniformly accumulated both in glomerular tufts and crescents.
D, E) Correlations of U-CD11b (D) and U-CD163 (E) with the formation rate of glomerular cellular crescents on ANCA-GN biosamples.
F, G) Correlations of U-CD11b (F) and U-CD163 (G) with leukocytes expressing the respective molecules in glomerular crescents on ANCA-GN biosamples.

Figure 3



Elevations of U-CD11b and U-CD163 levels according to renal outcomes in ANCA-GN patients

A, B) Associations of U-CD11b (A) and U-CD163 (B) levels at 0M and 6M in patients with renal remission status at 6M following the therapy.
U-CD163 levels at the diagnosis were elevated in non-achieved patients to remission at 6M.
C, D) Associations of U-CD11b (C) and U-CD163 (D) levels at 0M and 6M in patients with CKD stages (1/2 and 3/4/5) at 6M following the therapy.
U-CD163 levels at the diagnosis were elevated in severe CKD patients at 6M.

Table 1

	Age (years old)	Sex (male)	eGFR (mL/min/1.73m2)	U-P/U-Cr (g/g)	U-CD11b/U-Cr (ng/mg)	U-CD163/U-Cr (ng/mg)
Coefficient (95% CI)	-0.23 (-0.36 - -0.10)	+1.93 (-1.4 - +5.25)	-0.50 (-0.56 - -0.43)	-6.4 (-11.2 - -1.6)	**+4.3** (+1.0 - +7.7)	+2.8 (-1.6 - +7.2)
p value	0.001	0.25	<0.001	0.010	**0.011**	0.21

Factors associating with yearly eGFR slope in ANCA-GN patients
Multivariable linear regression models is shown.
Baseline eGFR, U-P, and U-CD11b, but not U-CD163, were independently associated with yearly eGFR slope following treatment.
Most importantly, U-CD11b reflected the increase in eGFR, and in contrast, baseline eGFR and U-P predicted the decrease in eGFR.
U-P; proteinuria, U-Cr; urinary creatinine, eGFR; estimated glomerular filtration rate

Research Summary and Future Perspectives

The results indicated that both U-CD11b and U-CD163 are useful markers to reflect glomerular inflammation in ANCA-GN, particularly that caused by leukocyte accumulation. Moreover, the U-CD11b level at diagnosis, which predicts functional recovery after immunosuppressive treatment, provides useful information for deciding the regimen and strength of immunosuppressive therapy in ANCA-GN patients. Because ACNA-associated vasculitis often manifests as rapidly progressive kidney dysfunction, histological findings obtained by rapid and non-invasive urinary tests are clinically useful for patients hesitating to undergo renal biopsies, including elderly patients, those with poor general condition or bleeding risk, and those in developing countries with non-sophisticated diagnostic systems.

The current study was conducted using biological samples from a Japanese patient population. However, there are large differences in the disease subtypes of AAV. To evaluate the clinical significance of U-CD11b in ANCA-GN for different ethnicities, the

research team is planning an international joint study with the European research institutes. Moreover, it will approach companies to develop diagnostic reagents for the practical application of the identified biomarkers. Based on the accurate diagnosis by non-invasive testing, the research team aims to improve the outcomes for ANCA-GN patients treated with the appropriate immunosuppressive treatment.

Publication

Clinical Impact of Urinary CD11b and CD163 on the Renal Outcomes of Anti-neutrophil Cytoplasmic Antibody-Associated Glomerulonephritis

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