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Original Article

ORIGINAL ARTICLE

Urinary RANTES and MCP-1 as noninvasive biomarkers for differential diagnosis and prediction of treatment response in acute interstitial nephritis

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ABSTRACT

Background. Although kidney biopsy is definitive for the diagnosis of acute interstitial nephritis (AIN) and acute tubular necrosis (ATN), its invasiveness limits its use. We aimed to identify urine biomarkers for differentiating AIN and ATN and to predict the response of patients with AIN to steroid treatment.

Methods. In this prospective cohort study, biopsy-proven ATN ($n = 34$) and AIN ($n = 55$) were included. Urinary cytokine/chemokine [interleukin-9, monocyte chemoattractant protein-1 (MCP-1), regulated on activation, normal T cell expressed and secreted (RANTES), tumor necrosis factor-α, tumor growth factor-β and vascular endothelial growth factor] levels and the proportion of immune cells [expressing cluster of differentiation (CD)45, CD3, CD20] and proliferating tubular cells (expressing Ki-67) were analyzed by immunohistochemistry. Cytokine/chemokine levels and intrarenal immunohistochemistry data according to the response to steroid treatment in the AIN patients were also analyzed.

Results. The urinary RANTES/creatinine ratio and the percentages of intrarenal CD45-, CD3-, CD20- and Ki-67-positive cells were significantly higher in the AIN group than in the ATN group ($P < .05$ for all). Among steroid-administered patients with AIN, renal function improved significantly in the steroid responder group. These patients had higher urinary MCP-1/creatinine and intrarenal CD45 and Ki-67 levels than those in the non-responder group.

Conclusions. The potential of the urinary RANTES/creatinine ratio as a noninvasive biomarker for differentiating AIN from ATN is highlighted. Urinary MCP-1/creatinine levels and the proportion of total intrarenal leukocytes and proliferating tubular cells may serve as indicators for predicting the response of patients with AIN to steroid treatment.

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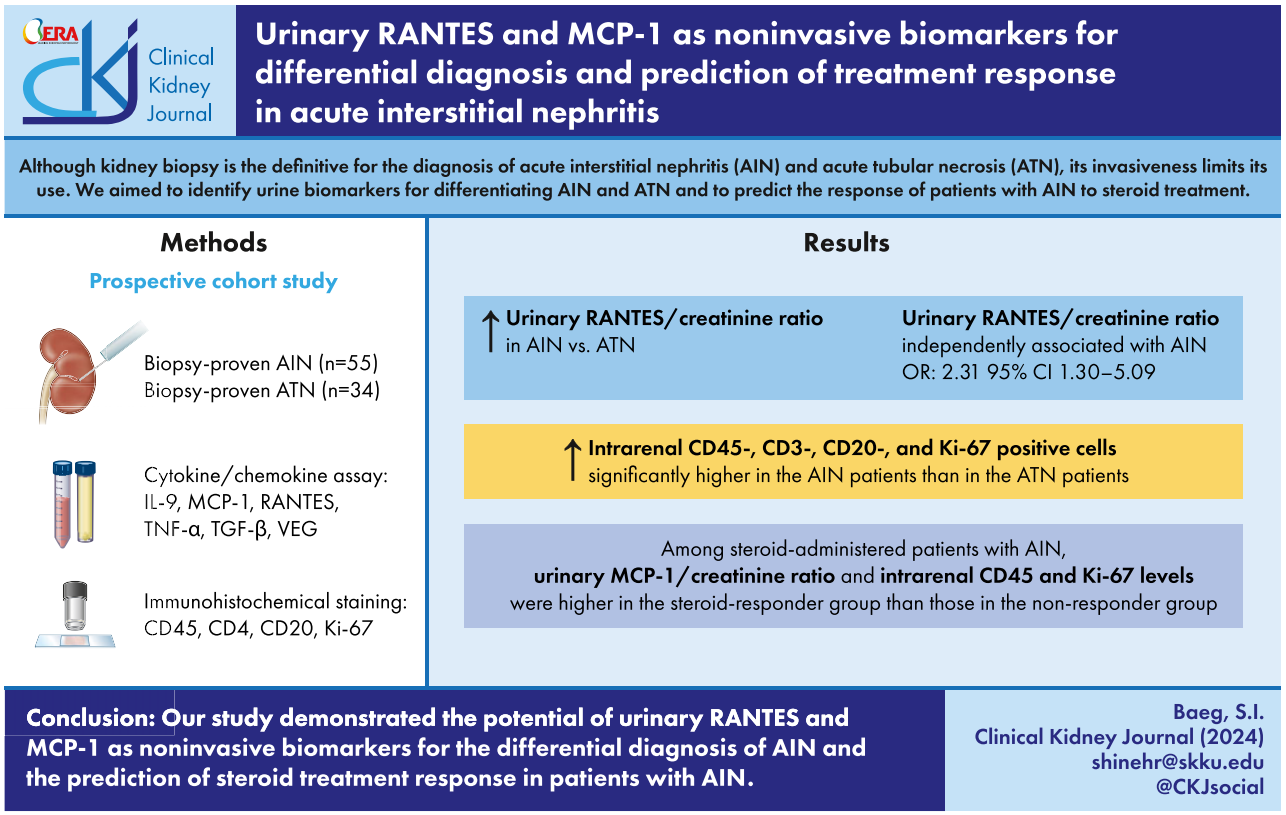
GRAPHICAL ABSTRACT



Keywords: acute interstitial nephritis, acute kidney injury, acute tubular necrosis, chemokines, cytokines

KEY LEARNING POINTS

What was known:

- Cell-mediated injury and delayed hypersensitivity reactions are the primary pathophysiology of acute interstitial nephritis (AIN).
- Urinary tumor necrosis factor-α and interleukin-9 have been reported as novel biomarkers for differentiating AIN from other kidney diseases.

This study adds:

- In a prospective cohort of biopsy-proven acute tubular necrosis and AIN, urinary RANTES/creatinine ratio showed and independent association with AIN and demonstrated good diagnostic performance.
- Among patients with AIN who were treated with steroids, urinary monocyte chemoattractant protein-1/creatinine ratio and intrarenal CD45 and Ki-67 were significantly higher in the steroid-responder group than in the non-responder group.

Potential impact:

- The findings from this study may contribute to the early diagnosis and clinical management of AIN.

INTRODUCTION

Acute interstitial nephritis (AIN) is a significant contributor to acute kidney injury (AKI), accounting for 10%–35% of such cases and diagnosed in roughly 2.8% of all kidney biopsies [1]. Histologically, AIN presents with interstitial edema and infiltration of lymphocytes, macrophages and eosinophils, consequences of hypersensitivity reactions to various antigens [1, 2]. Among hospitalized patients, acute tubular necrosis (ATN) is the leading cause of AKI [3]. Ischemic or toxic insults to renal tubular epithelial cells induce cell death or detachment from the basement membrane, leading to tubular dysfunction [4]. In both AIN and ATN, incomplete recovery occurs in over 30% and 20% of patients, respectively, potentially progressing to end-stage kidney disease [4, 5].

Early diagnosis and prompt intervention, including withdrawal of offending agents and appropriate conservative treatment, are crucial for the successful management of both AIN and ATN [1, 4]. While ATN diagnosis often relies on clinical findings, differentiating between ATN and AIN can be challenging, often necessitating invasive kidney biopsy for a definitive diagnosis of AIN [2, 4]. However, kidney biopsy carries the risk of bleeding and may not be feasible for all patients, leading to potential delays in diagnosis [6]. This highlights the critical need for noninvasive biomarkers to facilitate timely and accurate diagnosis of AIN, ultimately improving patient outcomes [7].

Despite the common practice of high-dose steroid therapy for AIN, its effectiveness remains debatable [1, 8] with several adverse effects limiting its widespread application [9, 10]. Therefore, identifying reliable urine biomarkers to predict response to steroid therapy is crucial for selecting patients who will benefit most [9]. This study aimed to identify such biomarkers for differentiating AIN from ATN and predicting the successful steroid response in patients with AIN.

MATERIALS AND METHODS

Study design and patient selection

This prospective cohort study included adult patients (≥18 years) with biopsy-proven ATN or acute-to-subacute interstitial nephritis (AIN) at Samsung Medical Center between 2001 and 2020. Patients with a history of kidney transplantation or other concomitant histologic diagnoses, such as glomerulonephritis, diabetic nephropathy, or hypertensive or benign nephrosclerosis, were excluded. Patients with only ATN or AIN were included in the final analysis. All included patients met the criteria of acute kidney disease proposed by Kidney Disease: Improving Global Outcomes. This study was approved by the Institutional Review Board of the Samsung Medical Center in compliance with the Declaration of Helsinki (number: 2020-12-122).

Data collection and outcomes

Clinical data regarding age, sex, body weight, comorbidities and causes of AKI were extracted from electronic medical records. Laboratory data including baseline total white blood cell count; levels of hemoglobin, blood eosinophil count, blood urea nitrogen and serum creatinine; estimated glomerular filtration rate (eGFR), levels of electrolytes, albumin; urinalysis with microscopic evaluation, spot urine protein to creatinine ratio (uPCR), spot urine albumin to creatinine ratio (uACR), 24-h urine protein or albumin levels, and kidney biopsy results were collected.

Patients who received high-dose steroid (methylprednisolone, prednisolone or deflazacort) within 3 months after kidney biopsy were classified into the steroid treatment group. Steroid treatment was carefully determined considering each patient's age, underlying medical condition, baseline kidney function, and the degree of tubular atrophy and interstitial fibrosis in the biopsy results. In most cases, intravenous methylprednisolone was administered first, followed by a switch to an oral formulation. Steroid treatment was usually continued for 8–12 weeks unless steroid-associated serious adverse events developed. The data on duration of steroid treatment and eGFR were collected up to 6 months after the biopsy.

The primary outcome was a change in renal function. Secondary outcomes included differences in serum and urinary cytokine/chemokine levels, and the proportion of cluster of differentiation (CD)45-, CD3-, CD20- and Ki-67-positive cells identified by immunohistochemical staining, depending on the histological diagnosis. In patients with AIN, the 6-month eGFR after renal biopsy was analyzed based on steroid treatment. A responder was defined as a patient whose eGFR after 6 months of biopsy increased by at least 30% compared with their baseline eGFR.

Sample collection and multiple cytokine/chemokine assays

Both serum and urine samples were collected on the day of kidney biopsy, before initiation of specific treatment from the patients who consented to additional blood and urine collection. Serum and urine samples were collected from seven healthy individuals as the control group. Urine samples were centrifuged at 2500 rpm for 15 min under –4°C. The supernatant of each sample was then aliquoted with 1.4 mL and 1.0 mL aliquots for urine and serum, respectively. After adding 1/100 volume of protease inhibitor and 0.1 M PMSF (Sigma Aldrich, St Louis, MO, USA) to each sample, all samples were stored at –70°C until analysis.

Six cytokines/chemokines previously reported to play a significant role in the pathophysiology and differential diagnosis of AIN and ATN, as well as the prognosis of kidney disease, were included for the following reasons [7, 11–14]. Tumor necrosis factor (TNF)-α and interleukin (IL)-9 reflect tubulointerstitial injury; monocyte chemoattractant protein (MCP)-1, and regulated on activation, normal T cell expressed and secreted (RANTES, CCL5) are important C-C chemokines that induce inflammation by stimulating T cells, monocyte and eosinophils; transforming growth factor (TGF)-β1 induces acute tubular injury through mitochondiral oxidative damage and inflammatory cell infiltration and renal tissue fibrosis; and vascular endothelial growth factor (VEGF) is essential for the differentiation of endothelial cells and angiogenesis. TNF-α was measured using a TNF-α ELISA kit R&D Systems (Novus Biologicals, Centennial, CO, USA). IL-9 and TGF-β1 were measured using DuoSet ELISA kits (R&D Systems, Minneapolis, MN, USA). RANTES, MCP-1 and VEGF levels were measured using a Quantikine ELISA kit (R&D Systems) according to the manufacturer's instructions. Urinary cytokine/chemokine levels were adjusted for urinary creatinine concentrations.

Semiquantitative analyses of immunohistochemical staining

Formalin-fixed renal tissue was immunohistochemically stained for CD45 (leukocyte common antigen), CD3 (surface marker of T cells), CD20 (transmembrane protein of the B cell surface) and Ki-67 (cellular marker of proliferation). Four-micrometer thick sections were deparaffinized in xylene, rehydrated through a graded alcohol series, and placed in a citrate buffer solution (pH 6.0). Antigen retrieval was enhanced by heating the slides in a pressure cooker and microwave for 10 min. Endogenous peroxidases were blocked by immersing the cooled slides in hydrogen peroxide solution (Dako, Carpinteria, CA, USA) for 30 min. After overnight incubation at 4°C with a serum-free protein block (Dako), the slides were incubated for 1 h at 25°C with the following antibodies at 1:100 dilution: mouse anti-human antibodies CD45, CD20 and

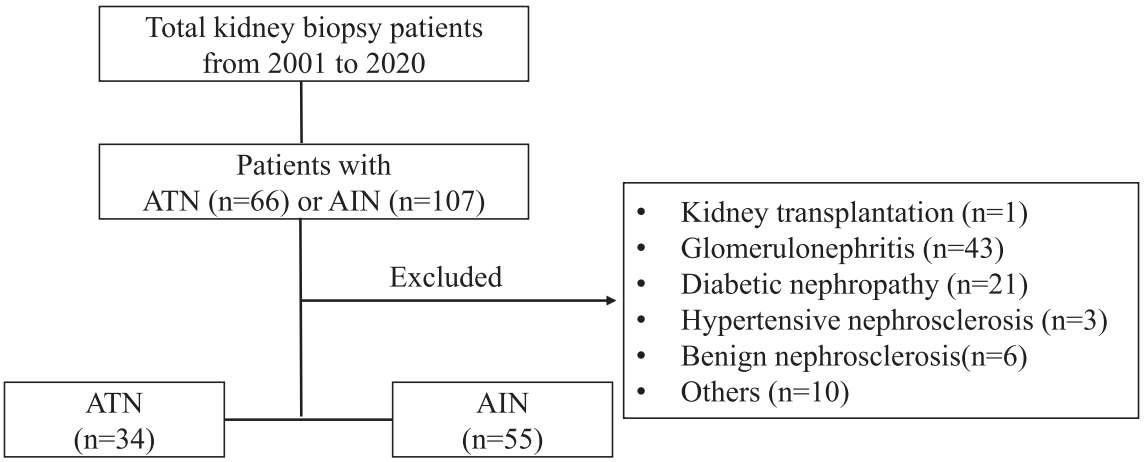


Figure 1: Study flow. Among all patients who underwent kidney biopsy at Samsung Medical Center between 2001 and 2020, 66 were diagnosed with ATN and 107 with acute to subacute interstitial nephritis (AIN). After excluding patients with coexisting histological diagnoses, 34 and 55 patients were assigned to the ATN and AIN groups, respectively. Serum and urine cytokine and chemokine levels were measured in 37 patients.

Ki-67, and polyclonal rabbit anti-human CD3 (Dako). Next, the slides were incubated for 30 min at 25°C with a mixed solution of dextran coupled with peroxidase and goat secondary antibodies (Dako). Finally, the slides were developed with 3,3′-diaminobenzidine tetrahydrochloride (Dako) for a brown color and counterstained with Mayer's hematoxylin (Dako). QuPath (version 0.2.3; https://qupath.github.io; Queen's University, Belfast, Northern Ireland) was used to analyze the percentages of CD45-, CD3-, CD20- and Ki-67-positive cells in renal tissues [15, 16].

Statistical analysis

Demographic and baseline clinical data are presented as median (interquartile range) or number (percentage), as appropriate. Continuous variables were compared using the Mann–Whitney U test and Kruskall–Wallis test, while categorical variables were compared using the chi-square test or Fisher's exact test. Univariate and multivariate logistic regression analyses were performed to test the independent associations of cytokines/chemokines with ATN or AIN. Cytokines/chemokines used as variables in the logistic regression analysis were log-transformed. The area under the receiver operating characteristic curve (AUROC) was analyzed to evaluate the cytokine/chemokines' discriminatory ability. All statistical analyses were performed using IBM SPSS Statistics 25 (IBM Corporation, Armonk, NY, USA) and R software (version 4.1.2; www.r-project.org; R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was set at P-value $<.05$.

RESULTS

Study flow and patient characteristics

From 2001 to 2020, 66 and 107 patients were diagnosed with ATN and AIN, respectively. After the exclusion of other concomitant histologic diagnoses, 34 patients were classified into the ATN group, and 55 were classified into the AIN group (Fig. 1).

Table 1 summarizes the baseline patient characteristics. The median age was 61 years, with comparable comorbidities between both groups. The median eGFR was 18.4 mL/min/1.73 m^2. The median uPCR and uACR were 910.0 mg/gCr and 189.8 mg/gCr, respectively. Some 47.1% of patients with ATN and 69.1% of patients with AIN showed a pattern with tubular proteinuria (uACR/uPCR ratio <0.4). The proportion of stage 1, 2 and 3 AKI in all patients were 47.2%, 6.7% and 46.1%, respectively. The most common cause of AKI was herbal medication, followed by medications including antibiotics and non-steroidal anti-inflammatory drugs.

Renal outcome

The median eGFR at 6 months after kidney biopsy was 41.0 [interquartile range (IQR) 27.1–64.6] mL/min/1.73 m^2 in the ATN group and 43.2 (IQR 33.5–59.4) mL/min/1.73 m^2 in the AIN group (Fig. 2A). Changes in eGFR did not show statistically significant differences between the two groups.

Among 55 patients with AIN, 44 patients (79%) received steroid treatment for a mean duration of 9.8 weeks. The demographics and laboratory results according to steroid treatment were compared in Supplementary data, Table S1. Notably, the baseline eGFR was lower in the responder group than the non-responder group. In the non-responder group, the median eGFR at 6 months [44.3 (IQR 38.5–45.5) mL/min/1.73 m^2] increased by 2.5 mL/min/1.73 m^2 compared with the baseline level. In contrast, the median value of 6-month eGFR in the responder group [39.7 (IQR 32.3–51.0) mL/min/1.73 m^2] significantly increased by 22.5 mL/min/1.73 m^2 compared with the baseline level (Fig. 2B). The demographics and laboratory results of these patients were shown in Supplementary data, Table S2. The median time between kidney biopsy and the initiation of steroid therapy was 2 days (IQR 1–4) and was not different between the non-responder and the responder groups.

Cytokine/chemokine analysis

Six cytokines/chemokines were measured in the blood and urine of 37 patients and 7 normal controls. Compared with the control group, urinary cytokines/chemokines except TGF-β1/creatinine were significantly different among three groups (Table 2). Urinary RANTES/creatinine level was significantly higher in the AIN group than in the ATN group [ATN vs AIN, 12.0 (0–23.3) vs

Table 1: Demographics and laboratory results at the time of kidney biopsy.

Variables	Overall ($n = 89$)	ATN ($n = 34$)	AIN ($n = 55$)	P-value
Age, years	61 (50–67)	56 (41–66)	63 (52–69)	.25
Male, *n* (%)	43 (51.8)	18 (41.9)	25 (58.1)	.26
Body weight, kg	61.9 (55.0–68.3)	64.6 (55.4–77.0)	60.7 (55.0–66.5)	.11
Comorbid condition, *n* (%)				
Diabetes mellitus	24 (28.9)	7 (23.3)	17 (32.1)	.40
Hypertension	39 (47.0)	13 (43.3)	26 (49.1)	.62
Ischemic heart disease	9 (10.8)	6 (20.0)	3 (5.7)	.07
Heart failure	7 (8.4)	5 (16.7)	2 (3.8)	.09
Cerebrovascular disease	5 (6.0)	2 (6.7)	3 (5.7)	1.00
Laboratory variables				
Hb, g/dL	9.9 (9.0–11.5)	9.9 (8.9–11.4)	10.0 (9.2–11.5)	.77
WBC, $\times 10^3/\mu$L	7.48 (5.79–10.0)	6.89 (5.02–8.87)	8.0 (6.0–10.7)	.14
Blood eosinophil count,/μL	118.5 (40.4–258.5)	102.3 (27.2–219.3)	122.9 (47.6–303.2)	.25
BUN, mg/dL	30.3 (21.4–45.0)	32.6 (21.1–53.5)	29.1 (21.9–44.6)	.53
Serum creatinine, mg/dL	3.05 (1.80–4.62)	3.56 (2.01–6.36)	2.78 (1.71–4.47)	.17
eGFR, mL/min/1.73 m^2	18.4 (11.2–33.5)	17.1 (8.1–32.8)	21.4 (12.4–34.7)	.27
Albumin, g/dL	3.8 (3.3–4.2)	3.9 (3.4–4.2)	3.8 (3.3–4.2)	.90
Urinalysis and proteinuria				
Blood, ≥2+, *n* (%)	34 (38.2)	11 (32.4)	23 (41.8)	.37
Protein, ≥2+, *n* (%)	19 (21.3)	8 (23.5)	11 (20.0)	.69
WBC ≥5/HPF, *n* (%)	31 (34.5)	5 (14.7)	26 (47.3)	.02
RBC ≥5/HPF, *n* (%)	25 (28.1)	7 (20.6)	18 (32.7)	.22
uPCR, mg/gCr	910.0 (330.0–1592.5)	905.0 (292.5–3102.5)	910.0 (337.5–1412.5)	.37
uACR, mg/gCr	189.8 (90.2–1086.3)	488.01 (97.70–1421.06)	169.1 (85.5–574.4)	.35
uACR/uPCR ratio	0.25 (0.15–0.56)	0.39 (0.17–0.59)	0.23 (0.14–0.47)	.94
24-h urine protein, mg/day	451.7 (226.3–808.5)	315.6 (157.0–892.7)	511.0 (316.8–766.8)	.20
24-h urine microalbumin, mg/day	101.9 (49.8–195.1)	103.0 (36.5–251.4)	101.5 (54.2–184.9)	.86
AKI stage, *n* (%)				
Stage 1	42 (47.2)	13 (38.2)	29 (52.7)	.34
Stage 2	6 (6.7)	2 (5.9)	4 (7.3)	
Stage 3	41 (46.1)	19 (55.9)	22 (40.0)	
AKI cause, *n* (%)				
Herbal medication	32 (36.0)	15 (44.1)	17 (30.9)	.18
Medications (antibiotics, NSAIDs, others)	28 (31.5)	8 (23.5)	18 (32.7)	
Infections	7 (7.9)	3 (8.8)	4 (7.3)	
Others	9 (10.1)	6 (17.6)	3 (5.5)	
Unknown	15 (16.9)	2 (5.9)	13 (23.6)	

Data are presented as median (IQR) or *n* (%).
BUN, blood urea nitrogen; Cr, creatinine; Hb, hemoglobin; NSAIDs, non-steroidal anti-inflammatory drug; RBC, red blood cell; WBC, white blood cell.

64.0 (16.0–303.0) pg/mg, $P < .01$]. Urinary MCP-1/creatinine ratio also tended to be higher in the AIN group than in the ATN group. Serum levels of these cytokines and chemokines were comparable between the ATN and AIN groups (Supplementary data, Table S3).

Multivariable analysis revealed that urinary RANTES/creatinine ratio showed an independent association with AIN [odds ratio (OR) 2.31, 95% confidence interval (CI) 1.30–5.09, $P = .01$, Table 3]. Similarly, the urinary MCP-1/creatinine ratio showed a high OR for AIN, although it was not statistically significant. The AUROC of the urinary RANTES/creatinine ratio in predicting AIN was 0.776 (95% CI 0.623–0.930, $P < .01$) (Fig. 3).

Semiquantitative analysis of intrarenal immune cells and proliferating tubular cells

Immunohistochemical staining for CD45, CD3, CD20 and Ki-67 was performed on kidney biopsy specimens from 89 patients (Fig. 4A). Subsequent semiquantitative analysis revealed that the proportion of cells positive for CD45 [ATN vs AIN, 11.0% (7.4–18.3) vs 30.9% (17.5–41.0), $P < .01$], CD3 [12.6% (9.6–16.2) vs 36.4% (23.2–46.5), $P < .01$] and CD20 [4.2% (3.5–7.0) vs 8.0% (5.1–9.8), $P < .01$] were significantly higher in the AIN group than in the ATN group (Fig. 4B). Additionally, the number of proliferating tubular cells expressing Ki-67 was significantly higher in the AIN group than in the ATN group [0.8% (0.3–1.5) vs 1.3% (0.7–2.2), $P = .02$].

Analyses of cytokine/chemokine levels and intrarenal immunohistochemistry data according to the response to steroid treatment in the AIN group

Table 4 presents the cytokine/chemokine and intrarenal immunohistochemistry data for patients with AIN who received steroid treatment. Notably, urinary MCP-1/creatinine was significantly higher in the responder group than in the non-responder group [non-responder vs responder, 592.5 (242.3–1268.8) vs 2141.0 (1586.8–4084.5) pg/mg, $P = .01$]. Furthermore, the proportion of cells positive for CD45 and Ki-67 was significantly higher in the responder group than in the non-responder group.

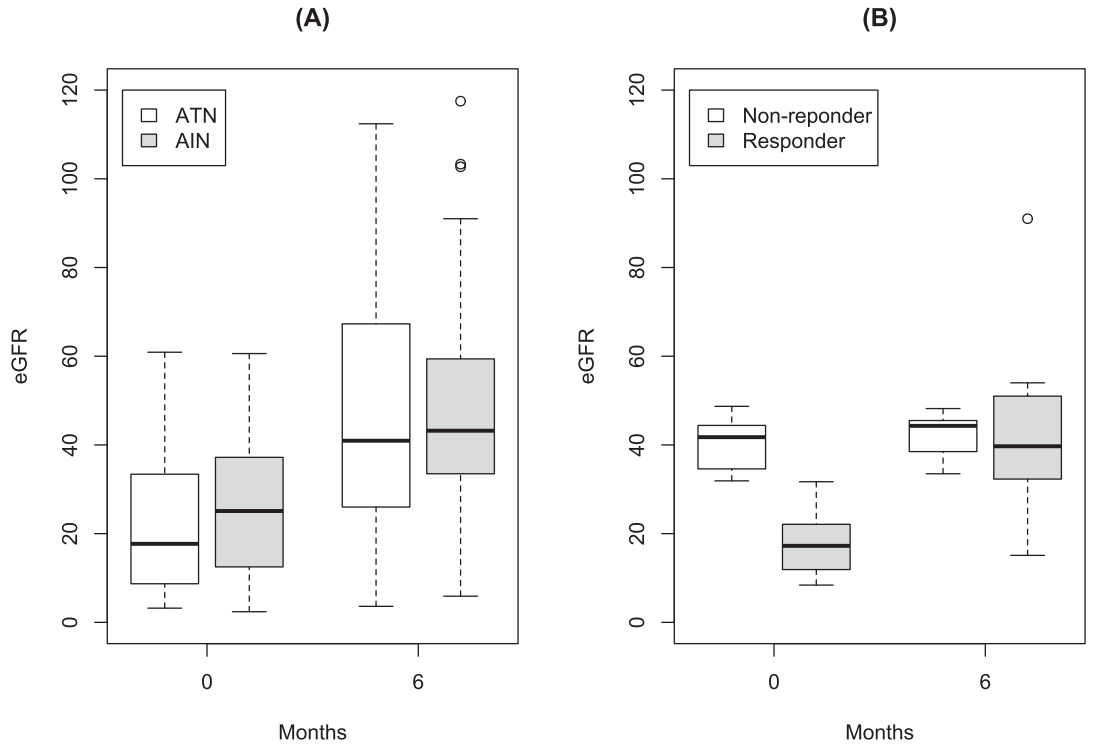


Figure 2: Changes in renal function. (**A**) Median eGFR was comparable in the ATN and AIN groups at 6 months post-biopsy. (**B**) Changes in median eGFR over 6 months in patients with AIN treated with steroids. The responder group showed a significant increase in mean eGFR. Boxplots represent the median eGFR, with the upper and lower borders of the box representing the 25th and 75th percentiles, respectively. The whiskers extend to 1.5 times the IQR, and the dots indicate outliers.

Table 2: Urinary cytokine/chemokine analysis results.

Cytokines/chemokines	ATN ($n = 14$)	AIN ($n = 23$)	Control ($n = 7$)	*P*-value
TNF-α/Cr (pg/mg)	1.0 (0–3.8)	3.0 (1.0–9.0)	0.0 (0.0–0.0)	.01
IL-9/Cr (pg/mg)	65.0 (0.0–129.5)	73.0 (17.0–156.0)	0.0 (0.0–0.0)	.02
TGF-β1/Cr (ng/mg)	8.5 (0.0–129.5)	12.0 (0.0–58.0)	0.0 (0.0–0.0)	.17
RANTES/Cr (pg/mg)	12.0 (0.0–23.3)	64.0 (16.0–303.0)	0.1 (0.1–0.1)	.001
VEGF/Cr (pg/mg)	325.5 (90.0–2040.0)	358.0 (193.0–1291.0)	0.1 (0.1–0.1)	<.001
MCP-1/Cr (pg/mg)	1390.0 (292.0–3001.3)	1871.0 (86.0–4459.0)	5.6 (3.1–6.2)	<.001

Data are presented as median (IQR) or *n* (%).
Cr, creatinine.

Table 3: Association of blood eosinophil counts, leukocyturia and urinary cytokine/chemokine levels with acute interstitial nephritis.

	Univariable analysis		Multivariable analysis	
Variables	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Blood eosinophil count	0.96 (0.60–1.45)	.84	0.82 (0.41–1.54)	.54
Leukocyturia	5.5 (1.16–40.71)	.05	5.00 (0.56–71.08)	.17
uPCR	0.76 (0.37–1.47)	.41	0.14 (0.02–0.55)	.02
Urine TNF-α/Cr	1.40 (0.84–2.65)	.24		
Urine IL-9/Cr	1.22 (0.88–1.71)	.23		
Urine TGF-β1/Cr	1.00 (0.71–1.41)	.99		
Urine RANTES/Cr	1.62 (1.14–2.49)	.01	2.31 (1.30–5.09)	.01
Urine VEGF/Cr	1.08 (0.68–1.79)	.74		
Urine MCP-1/Cr	1.42 (0.89–2.45)	.16	1.62 (0.86–3.55)	.16

Cr, creatinine.

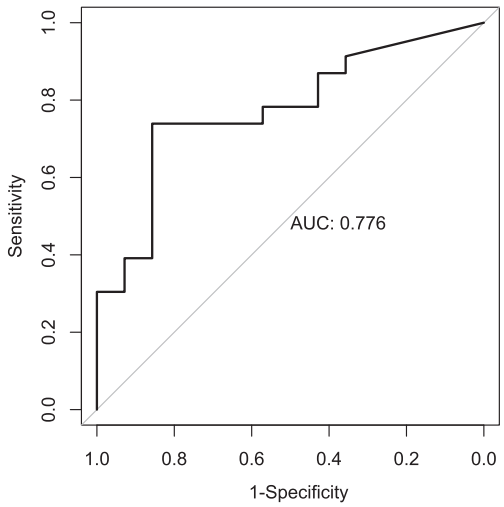


Figure 3: The area under the receiver operating characteristic curve analysis of urinary RANTES/creatinine. The AUROC of urinary RANTES/creatinine in predicting acute interstitial nephritis was 0.776 (95% CI 0.623–0.930, $P < .01$). The optimal cut-off value for urinary RANTES/creatinine was 24.3 pg/mg. Sensitivity and specificity were 73.9% and 85.7%, respectively. Positive predictive value and negative predictive value were 33.3% and 10.5%, respectively. AUC, area under the curve.

DISCUSSION

In this study, we investigated serum and urinary cytokines/chemokines as potential biomarkers for noninvasive differential diagnosis of ATN and AIN. Additionally, we aimed to analyze intrarenal leukocyte infiltration and proliferating tubular cells to predict the response to steroid treatment in the AIN group.

The urinary RANTES/creatinine ratio was significantly higher in the AIN group, demonstrating an independent association with AIN in the multivariable analysis. Urinary MCP-1/creatinine also tended to be higher in the AIN group. Additionally, urinary RANTES/creatinine and MCP-1/creatinine were significantly higher in the ATN and AIN groups compared with the control group. Notably, the number of total intrarenal leukocytes, T cells, B cells and Ki-67-expressing proliferating tubular cells was significantly higher in the AIN group than in the ATN group. Furthermore, our study revealed significantly higher levels of urinary MCP-1/creatinine, total intrarenal leukocytes and proliferating tubular cells in the responder group of patients with AIN treated with steroids than in the non-responder group.

RANTES acts as a chemoattractant for memory T cells, monocytes and eosinophils, thereby driving interstitial inflammation and disease progression [17, 18]. A previous study

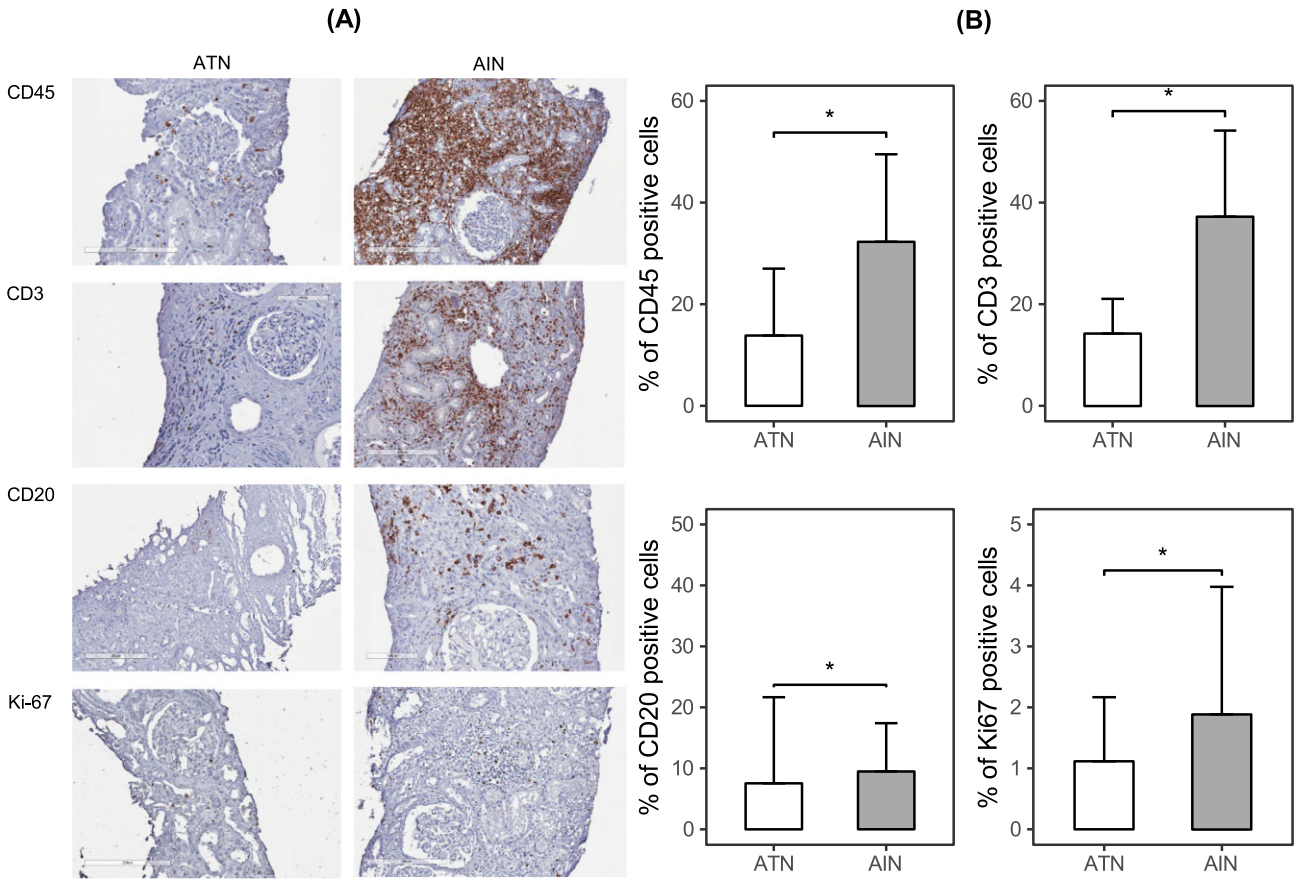


Figure 4: Semiquantitative analysis of immunohistochemistry of intrarenal CD45-, CD3-, CD20- and Ki-67-positive cells. (**A**) Immunohistochemical staining was performed for CD45 (leukocyte common antigen), CD3 (surface marker of T cells), CD20 (transmembrane protein of B cell surface) and Ki-67 (cellular marker of proliferation). Positive cells for each marker appear brown. (**B**) Intrarenal immune cells expressing CD45, CD3 and CD20, as well as proliferating tubular cells expressing Ki-67, were compared using semiquantitative analysis with QuPath. The AIN group showed significantly higher infiltration of total leukocytes, T cells and B cells than did the ATN group. Additionally, the proportion of proliferating tubular cells expressing Ki-67 was significantly higher in the AIN group than in the ATN group. $^{*}P < .05$, compared with the ATN group.

Table 4: Cytokine/chemokine levels and intrarenal immunohistochemistry results for immune cells and proliferating tubular cells in patients with AIN treated using steroids.

Variables	Non-responder ($n = 6$)	Responder ($n = 8$)	P-value
Serum			
TGF-β1 (ng/mL)	5.5 (4.0–6.3)	6.5 (4.0–9.5)	.47
RANTES (ng/mL)	57.5 (26.5–70.5)	45.0 (29.3–89.3)	.75
VEGF (pg/mL)	303.5 (256.5–609.8)	261.5 (175.8–749.8)	.52
MCP-1 (pg/mL)	136.1 (105.2–160.4)	282.9 (138.0–348.8)	.07
Urine			
TNF-α/Cr (pg/mg)	2.0 (0.8–16.0)	3.0 (0.5–7.8)	.60
IL-9/Cr (pg/mg)	9.0 (0–112.3)	94.0 (37.8–467.0)	.07
TGF-β1/Cr (ng/mg)	9.5 (1.5–29.8)	6.5 (0–113.8)	.90
RANTES/Cr (pg/mg)	27.0 (14.3–78.8)	119.0 (9.0–308.5)	.30
VEGF/Cr (pg/mg)	249.0 (79.0–651.5)	492.0 (215.0–1387.8)	.12
MCP-1/Cr (pg/mg)	592.5 (242.3–1268.8)	2141.0 (1586.8–4084.5)	.01
Intrarenal immune cells			
CD45-positive rate	15.0 (10.5–21.0)	27.0 (23.3–35.0)	.03
CD3-positive rate	23.0 (19.0–39.3)	32.5 (19.8–40.5)	.52
CD20-positive rate	6.5 (3.8–8.8)	8.0 (5.5–10.0)	.47
Ki-67-positive rate	0 (0–0.3)	1.0 (1.0–3.3)	.01

Data are presented as median (IQR).
Cr, creatinine.

investigating the distribution of CCR5, a receptor for RANTES, found a correlation between CCR5-positive cells and CD3+ T cells, specifically within the tubular epithelium and tubulitis areas in AIN. Additionally, interstitial CCR5-positive cells increased alongside rising serum creatinine levels [19]. Consistent with these findings, our study revealed significantly higher urinary RANTES/creatinine ratios in the AIN group than in the ATN group, supporting RANTES' key role in interstitial inflammation pathogenesis. Furthermore, urinary RANTES/creatinine levels demonstrated an independent association with AIN and displayed good performance in the AUROC analysis. This finding aligns with the significantly higher number of intrarenal T-cells expressing CD3 in the AIN group than in the ATN group, further supporting the critical role of T-cell-mediated injury in AIN pathophysiology [20, 21].

Potential urine biomarkers have been investigated for AIN diagnosis [22–24]. MCP-1 has been shown to induce monocyte recruitment and activate T cells, natural killer cells and basophils, ultimately leading to tubulointerstitial injury [12]. Urine biomarker analyses in healthy adults and patients with AIN have identified significantly higher urinary MCP-1 levels in the AIN group [22, 24], further correlating with the severity of inflammatory cell infiltration [22]. Although we observed a trend of higher urinary MCP-1/creatinine ratio in the AIN group, this finding must be further validated. Nevertheless, urinary RANTES and MCP-1 hold promise as potential noninvasive biomarkers for AIN diagnosis.

Urinary TNF-α and IL-9 have also been reported as valuable biomarkers for differentiating AIN from other kidney diseases [23, 25, 26]. Moreover, a recent study investigating AKI associated with immune checkpoint inhibitors (AKI-ICI) confirmed AIN in all biopsy specimens and demonstrated the strong discriminatory power of urinary TNF-α in diagnosing AKI-ICI [27]. Primarily secreted by T cells, TNF-α and IL-9 ultimately contribute to tubulointerstitial injury [7, 28–30]. While we observed comparable levels of urinary TNF-α and IL-9 in the ATN and AIN groups, urinary IL-9 showed a trend of being higher in the steroid-responder group of patients with AIN. Further studies with larger cohorts are needed to establish the clinical relevance of TNF-α and IL-9 as biomarkers.

Our study also employed semi-quantitative analysis of total intrarenal leukocytes, T cells, B cells and Ki-67-positive proliferating tubular cells. The AIN group exhibited a predominance of interstitial infiltration by total leukocytes, T cells and B cells compared with their prevalence in the ATN group. Notably, T cells expressing CD3 constituted the largest population (36.4%) in the AIN group. These findings align with previous reports demonstrating T cells as the most prevalent immune cell infiltrating the renal interstitium in AIN [27, 31, 32]. CD45, the leukocyte common antigen, is known to play a crucial role in T cell activation via T cell receptor–CD3 complex and CD4/CD8 [33]. In our study, CD45 and CD3 expression levels were significantly positively correlated, further supporting the notion that T cell-mediated inflammation is the primary pathophysiological mechanism underlying AIN [31]. Although B cells are typically rare in the AIN renal interstitium [34], our study revealed a considerable intrarenal infiltration of B cells, reaching up to 8% of total nucleated cells in the AIN group. Notably, previous reports have documented diffuse interstitial B cell infiltration in AIN, and a positive correlation has been observed between the total areas of CD3(+) T cells and CD20(+) B cells, suggesting a potential role for B cells in interstitial injury through secreting cytokines/chemokines [35].

The primary treatment approach for drug-induced AIN involves discontinuation of the offending medication. However, the role of steroid therapy in AIN remains controversial [2, 8, 36, 37]. Despite steroid treatment, some patients experience insufficient renal function recovery and progression to chronic kidney disease [36, 38]. A longer interval between discontinuing the offending drugs and initiating steroid treatment has been associated with incomplete renal recovery [8, 38]. Therefore, early diagnosis alongside the identification of patients expected to respond favorably to steroid treatment are critical aspects in AIN management [39]. Interstitial fibrosis has been linked with poor response to steroid treatment in AIN [8, 40], while neutrophilic infiltration in renal tissue has been associated with a positive

response [40]. In our study, 61% of patients receiving steroids displayed an eGFR increase of ≥30% compared with baseline levels. In these steroid responders, urinary MCP-1/creatinine levels and the number of intrarenal cells positive for CD45 and Ki-67 were significantly higher than those in non-responders. Notably, MCP-1 has been implicated in the activity of inflammation in various renal diseases, including renal vasculitis, and immunoglobulin A (IgA) nephropathy [41]. Additionally, the interstitial proliferation index, assessed using Ki-67 positivity, has shown a significant correlation with disease activity in systemic vasculitis and IgA nephropathy [42]. High urinary MCP-1 levels and the proportion of intrarenal total leukocytes and proliferating tubular cells may reflect active intrarenal inflammation and anticipate a favorable response to steroid treatment in AIN.

Our study has several limitations. First, despite the prospective design, blood and urine samples could not be collected from all patients. However, we believe that the analyses of serum and urine samples collected on the day of renal biopsy were reliable for identifying novel biomarkers. Second, changes in cytokine/chemokine levels after the biopsy were not evaluated. However, measuring cytokines/chemokines at the time of biopsy was the most appropriate approach, as our study focused on the differential diagnosis of ATN and AIN. Third, the follow-up period for a significant portion of patients with AIN was relatively short, with less than 1 year of data available. Fourth, it was difficult to determine the exact onset and duration of AKI. Despite these limitations, our study's strengths lie in its prospective design and inclusion of patients definitively diagnosed with ATN and AIN, excluding other histologic findings.

In conclusion, our study demonstrates the potential of urinary RANTES and MCP-1 as noninvasive biomarkers for the differential diagnosis of AIN and the prediction of steroid treatment response in patients with AIN, respectively. Further studies with larger patient cohorts are required to validate and standardize the use of these biomarkers for AIN.

SUPPLEMENTARY DATA

Supplementary data are available at *Clinical Kidney Journal* online.

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AUTHORS' CONTRIBUTIONS

Conceptualization: S.I.B., J.E.L., G.Y.K., W.H. and H.R.J.; formal analysis: S.I.B. and H.R.J.; methodology: S.I.B., J.E.L., W.H. and H.R.J.; writing—original draft: S.I.B. and H.R.J.; writing—review & editing: S.I.B., K.L., J.J., J.E.L., G.Y.K., W.H. and H.R.J.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

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