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Renal Inflammation Assessment using Complement Activation and CD163

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Abstract: Lupus nephritis (LN) and renal ANCA-associated vasculitis (rAAV) are autoimmune kidney diseases that can lead to kidney failure, cardiovascular complications, and death. Kidney biopsy remains the gold-standard diagnostic, an invasive procedure with risks. Commonly used non-invasive surrogates, including urine protein-creatinine ratios (UPCR), hematuria, and autoantibody levels, lack sensitivity and specificity for active inflammation. Our pilot study evaluated urine-soluble CD163 (usCD163), urine complement activation products (uCAPs: C3a, C5a, sC5b-9), and SIGLEC-1, as candidate non-invasive kidney inflammation biomarkers. Patients with biopsy-proven LN or rAAV were enrolled, and urine samples were collected from -14 days to +238 days relative to biopsy. Biomarker levels were measured using ELISA or MesoScale-Discovery assays. UsCD163 and UPCR were significantly elevated in LN and rAAV versus healthy controls. usCD163 and uCAPs correlated strongly with UPCR, and the biomarker panel distinguished complete renal remission (CRR) from non-remission. Validation in a larger, longitudinal cohort is underway.

Introduction

Lupus nephritis (LN) and renal ANCA-associated vasculitis (rAAV) are severe autoimmune kidney diseases with significant morbidity and mortality.(1–3) Patients with LN and rAAV are at risk of kidney failure, infection, cardiovascular events, and death.(2,4) While both conditions present similarly clinically, they arise from different immunological mechanisms, therefore requiring accurate diagnosis.(5) Currently, the kidney biopsy remains the gold standard for diagnosis, histologic classification, and assessment of renal inflammation.(6) However, it is invasive with risks of bleeding and infection, and provides modest prognostic information of highly dynamic diseases, making it unfeasible for repeated long-term monitoring.(6,7)

Non-invasive markers, including urine protein-creatinine ratio (UPCR), hematuria, serum creatinine, and autoantibodies, are commonly used as part of routine care.(6,7) However, these markers are often absent in early disease, lag behind flares, and are insufficiently sensitive and specific for active renal inflammation.(7–9) Further, many traditional markers reflect chronic structural damage rather than underlying immune activity.(9,10) This leaves a gap for biomarkers that distinguish active renal inflammation from chronic damage and can be repeatedly measured. For this reason, urine biomarkers have been increasingly examined as an alternative to better capture intrarenal immune activation

Study Objectives

This pilot study evaluated several urine biomarkers: urine-soluble CD163 (usCD163), urine complement activation products (uCAPs: C3a, C5a, sC5b-9), and sialic acid-binding Ig-like lectin 1 (SIGLEC-1). These markers represent distinct biologic pathways relevant to kidney autoimmunity. CD163 is a receptor expressed by macrophages, which is cleaved off their surface upon activation.(11) As macrophages represent a dominant cell type involved in renal inflammation, usCD163 is a promising marker.(12,13) uCAPs reflect activation of the complement cascade, which is involved in immune complex clearance, inflammation, and tissue injury.(14,15) Complement dysregulation is a key component in autoimmunity, with different cascades implicated in LN or rAAV.(14,15) SIGLEC-1 is associated with type I interferon (T1FN) signaling, a pathway strongly implicated in LN.(16,17) As components of T1FN are difficult to measure due to their short half-life and fluctuations, SIGLEC-1 has been proposed to be a more stable surrogate.(16) While promising, these candidates are insufficiently validated.

The objectives of this pilot study were to evaluate these biomarkers' capacity to distinguish LN and rAAV from healthy controls, their correlation to UPCR, and their relationship to complete renal remission (CRR). These aims address the key limitations of current non-invasive markers, which poorly reflect active inflammation and remission.

Methods

Patients with biopsy-proven LN or rAAV were enrolled from the Biobank for Molecular Classification of Kidney Disease (REB15-1026). Healthy controls were recruited from the University of Calgary. Urine samples from LN and rAAV patients were collected -14 to +238 days relative to biopsy.

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Biopsy classification and patient demographics were collected (Table 1). Biomarker levels were quantified using enzyme-linked immunosorbent assay (ELISA: CD163, sC5b-9, SIGLEC-1) and MesoScale-Discovery assays (C3a, C5a). All values were normalized to urine creatinine to account for differences in urine concentration.

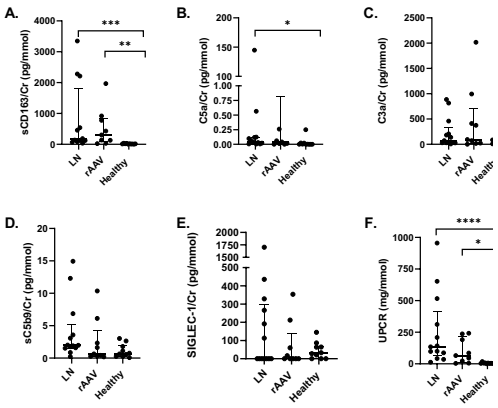
Median concentrations of biomarkers were compared through Kruskal-Wallis tests with multiple comparisons. Spearman's rank correlation coefficient was evaluated between each biomarker and UPCR. Receiver operator characteristic (ROC) curves were used to assess the performance of the biomarkers in distinguishing CRR from non-CRR. P-values were adjusted with the Bonferroni Correction.

Results

This study included 13 LN patients, 9 with rAAV, and 10 healthy controls (Table 1). Female patients comprised 12 of 13 (92.3%) in LN, 5 of 9 (55.6%) in rAAV, and 7 of 10 (70%) in healthy controls. Nearly half of LN patients had class III+V (6/13:46.2%) biopsy findings. All rAAV patients were ANCA-positive (100%), 7 of which were MPO-positive (77.8%), and 2 of which PR3-positive (22.2%). Based on Berden classification, 4 patients (44.4%) were focal, 2 (22.2%) were mixed, and 3 (33.3%) were sclerotic, while none were crescentic.

Median levels of usCD163 and UPCR were significantly elevated in patients with either LN or rAAV compared to the healthy controls (Figure 1). Median levels of C5a were significantly elevated in LN compared to healthy controls, but not rAAV vs. healthy controls. No significant between-group differences were observed for C3a, sC5b-9, or SIGLEC-1. No biomarker differed between LN and rAAV.

Figure 1: *Median Concentrations of Urinary Biomarkers*

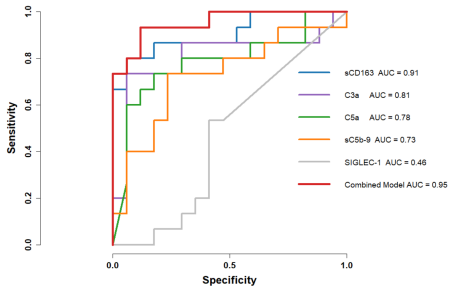


Urinary sCD163(A), uCAPs (C5a[B], C3a[C], sC5b-9[D]), SIGLEC-1(E), and UPCR(F) levels were measured and normalized to urine creatinine. Each point represents individual samples (LN=13, rAAV=9, Healthy controls=10). Bars indicate Median±IQR. Groups were compared using the Kruskal-Wallis non-parametric test with Dunn's multiple comparisons, with Bonferroni Correction. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Spearman analysis showed that usCD163 was strongly correlated with UPCR (rho=0.9087, p<0.0001; Figure 2). The uCAPs had varying degrees of correlation, with C5a being the strongest (rho=0.6793, p<0.0001) followed by C3a (rho=0.5987, p=0.0003) and sC5b-9 (rho=0.5535, p=0.001). SIGLEC-1 had no significant correlation (rho=0.2384, p=0.1889) with UPCR.

CRR was defined as a UPCR of less than 300mg/g. ROC analysis showed the biomarkers could distinguish CRR from non-CRR (Figure 3) with usCD163 showing the strongest individual performance (AUC=0.91), followed by C3a (AUC=0.81), C5a (AUC=0.78), and sC5b-9 (AUC=0.73). SIGLEC-1 (AUC=0.46) was excluded from the combined model as it did not correlate with UPCR, the clinical measure used to define CRR. The combined model performed best overall (AUC=0.95).

Figure 3: *Urinary Biomarker Panel Distinguishing CRR from non-CRR*



ROC curves were generated for each biomarker in relation to CRR status (UPCR<300mg/g). The combined model, excluding SIGLEC-1 (AUC=0.46), demonstrated the greatest discriminatory performance (AUC=0.95), followed by usCD163 (AUC=0.91), C3a (AUC=0.81), C5a (AUC=0.78), and sC5b-9 (AUC=0.73).

Discussion

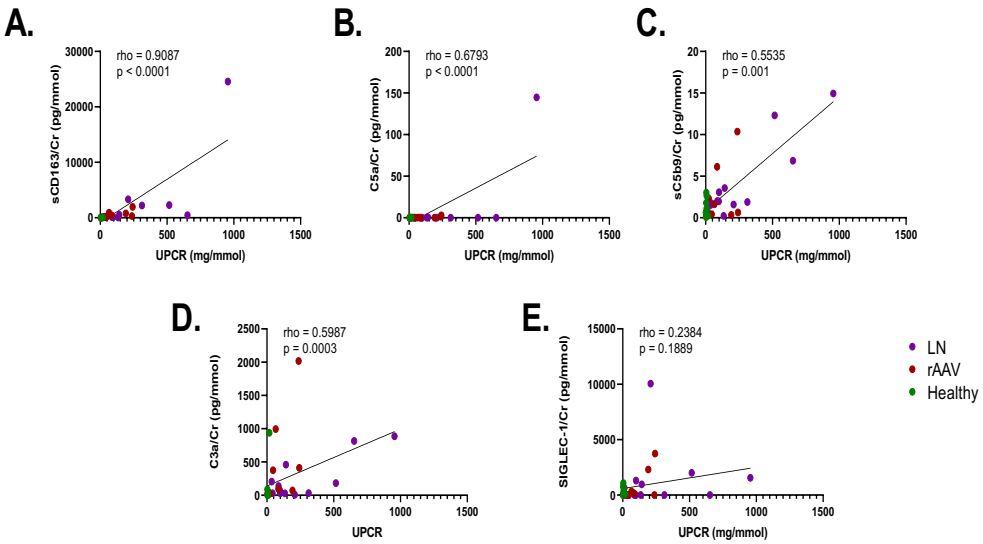
The findings of this pilot study support the potential of four novel urinary biomarkers for the detection of LN and rAAV. usCD163 showed the strongest overall performance, and in combination with uCAPs, we could predict renal remission with a high AUC of 0.95. Their elevation in both LN and rAAV compared to healthy controls suggests that it reflects active macrophage infiltration involved in both LN and rAAV, potentially providing a direct indication of intrarenal immune activity.

A crucial point in interpreting these findings is the role of UPCR itself. While widely used in clinical practice as a marker of kidney involvement, it is not a direct measure of active inflammation. Proteinuria can persist despite inflammatory resolution due to chronic structural damage to the

kidney.(9,10,18) For that reason, while correlation with UPCR is helpful, it does not reflect ongoing inflammation. A biomarker that correlates with UPCR may track overall renal burden, but one that does not may still provide information about other pathophysiologic processes.

Due to the small sample size, the findings are less generalizable and more vulnerable to outlier effects. The cross-sectional design did not allow us to assess if these biomarkers track dynamic changes in disease activity within individual patients. As the urine samples were collected over a wide window, this could have introduced variability as biomarker levels may change with treatment, flare, or remission. Despite these limitations, this study provided encouraging evidence that urinary biomarkers can improve non-invasive assessments of autoimmune kidney diseases. These findings support continued investigation in a larger, longitudinal study, now underway.

Figure 2: *Correlations of Urinary Biomarkers to UPCR*



Spearman correlations between UPCR and urinary sCD163(A), uCAPs (C5a[B], C3a[C], sC5b-9[D]), and SIGLEC-1(E), all normalized to urine creatinine levels. Each point represents an individual sample (LN=13[purple], rAAV=9[red], Healthy controls=10[green]); line of best fit shown. P-values corrected with Bonferroni Correction.

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Table 1: *Patient Demographics and Histological Classification*

	LN				rAAV		Healthy Controls
Number of Patients	13				9		10
Mean Age ± standard deviation (years)	37.1 ± 12.3				61.7 ± 20.8		34 ± 16.2
Female Patients	12 (92.3%)				5 (55.6)		7(70)
Autoantibodies	N/A				*ANCA +*	9 (100)	N/A
					MPO +	7 (77.8)	
					PR3 +	2 (22.2)	
Renal Biopsy Classification	*LN Class*	*I*	0 (0)	*Berden Class*	*Focal*	4 (44.4)	N/A
		II	1 (7.8)		*Crescentic*	0 (0)	
		III	1 (7.8)		*Mixed*	2 (22.2)	
		IV	3 (23.1)		*Sclerotic*	3 (33.3)	
		V	1 (7.8)				
		III + V	6 (46.2)				
		IV + V	1 (7.8)				

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