Soluble Urine ALCAM Reflects Renal Disease Activity in Lupus Nephritis

POSTER 0353

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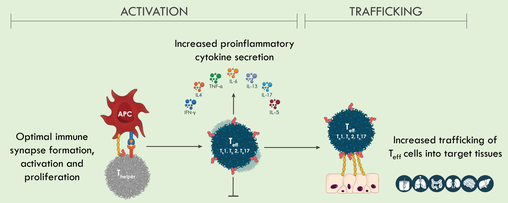
Introduction

Lupus nephritis (LN) is a leading cause of morbidity and mortality in systemic lupus erythematosus (SLE) patients. While LN pathogenesis has yet to be fully elucidated, T cells have been strongly implicated in the pathology of the disease.

CD6 is a co-stimulatory receptor on T cells that binds to activated leukocyte cell adhesion molecule (ALCAM), a ligand expressed on antigen presenting cells (APCs) and various epithelial and endothelial tissues. The CD6-ALCAM pathway plays an integral role in modulating T cell activation and trafficking and is central to immune-mediated inflammation. Animal studies in models of SLE/LN have shown that blockade of the CD6-ALCAM pathway was able to reduce disease manifestations and improve survival. In humans, we previously reported that soluble urine ALCAM is a potential biomarker of disease in LN (Stanley et al., 2016). Elucidating the potential for ALCAM and/or CD6 to be biomarkers of disease severity may provide insight into predicting patient response to targeted treatments.



To study the role of ALCAM and CD6 in LN, we tested samples from the Accelerated Medicines Partnership (AMP), a multi-center public-private partnership working towards the development of new diagnostics and treatments for several diseases including SLE/LN. In this study, we evaluated the cross-sectional and longitudinal correlations of serum and urine ALCAM and CD6 with measures of LN severity to further support the development of serum and urine biomarkers for this population of patients with limited approved therapies.

Methods

Patient samples were acquired from subjects that qualified for the AMP Phase 2 study of SLE/LN (Paul et al., 2020). Serum and urine samples were obtained from patients with biopsy proven LN and healthy controls without kidney disease across multiple sites. Follow-up longitudinal sampling (0, 3, 6, and 12 months) was available for a subset of subjects. Soluble ALCAM levels were quantified by ELISA (Sigma Aldrich, RAB0013-1KT), while CD6 levels were quantified by an electrochemiluminescent assay. Urine creatinine levels were measured using the improved Jaffe method from BioAssay Systems (DICT015). Urine ALCAM and CD6 levels were normalized to urine creatinine from the same sample. Subject demographics and characteristics, including urine protein creatinine ratio (UPCR), were collected by each site and provided by AMP in a collated dataset.

For statistical analysis, all observations that lie 3xIQR (interquartile range) below the first quartile, or 3xIQR above the third quartile, are considered outliers and not included in subsequent analyses. Pairwise comparisons between groups were assessed using the Wilcoxon test which does not assume that the data is normally distributed. A mixed model for repeated measures (MMRM) was used to assess how UPCR changed over the course of the one-year study. The MMRM model included fixed effects for visit, sex, age, race, ISN, baseline normalized urine ALCAM, baseline normalized urine CD6, dsDNA, C3, and C4 to assess their associations with UPCR. An unstructured covariance matrix was utilized for model fitting. All statistical analyses were performed using SAS® version 9.4.

Results

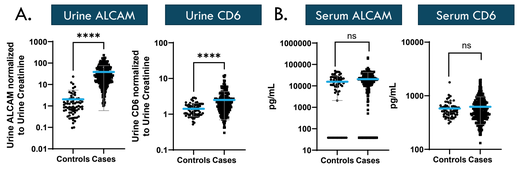
TABLE 1. BASELINE DEMOGRAPHICS FOR SUBJECTS INCLUDED IN ANALYSES

Demographics shown as either sample size (percentage of total) [N (%)] or mean ± standard deviation. Cross-sectional analyses performed for baseline values. Subjects with a baseline and at least two follow-up visits were included in the longitudinal modeling.

	Healthy Controls* (N = 70)	LN Cases* (N = 270)	Subjects with at least three visits** (N = 124)
Sex			
Male	30 (42.86%)	36 (13.33%)	18 (14.52%)
Female	40 (57.14%)	232 (85.93%)	106 (85.48%)
Missing	0	2 (0.74%)	0
Age	n = 70 46.64 (12.218)	n = 268 36.99 (11.981)	n = 124 36.02 (11.048)
Race			
White	64 (91.43%)	84 (31.11%)	37 (29.84%)
Non-white	6 (8.57%)	186 (68.89%)	87 (70.16%)
ISN lupus nephritis class			
No LN, I, II		33 (12.22%)	2 (1.61%)
III		46 (17.04%)	24 (19.35%)
IV		42 (15.56%)	22 (17.74%)
Mixed (III+V, IV+V)	NA	58 (21.48%)	36 (29.03%)
V		61 (22.59%)	38 (30.65%)
VI		11 (4.07%)	1 (0.81%)
Missing		19 (7.04%)	1 (0.81%)
dsDNA			
Positive		165 (61.11%)	84 (67.74%)
Negative	NA	79 (29.26%)	37 (29.84%)
Missing		26 (9.63%)	3 (2.42%)
Serum C3*** (mg/dl)	NA	n = 257 80.06 (35.777)	n = 122 72.88 (35.218)
Serum C4*** (mg/dl)	NA	n = 255 15.80 (9.849)	n = 122 14.22 (9.120)
UPCR*** (ratio)	NA	n = 262 17.83 (242.202)	n = 121 2.76 (2.796)
SELENA-SLEDAI	NA	n = 270 10.94 (6.037)	n = 124 12.16 (5.934)
Renal SLEDAI***	NA	n = 270 6.65 (3.604)	n = 124 7.16 (3.432)
Medications [1]			
None		38 (14.07%)	9 (7.26%)
Azathioprine		29 (10.74%)	17 (13.71%)
Calcineurin inhibitor		15 (5.56%)	9 (7.26%)
Cyclophosphamide	NA	5 (1.85%)	1 (0.81%)
Mycophenolate		130 (48.15%)	67 (54.03%)
Steroid		178 (65.93%)	87 (70.16%)
Other [2]		10 (3.70%)	3 (2.42%)

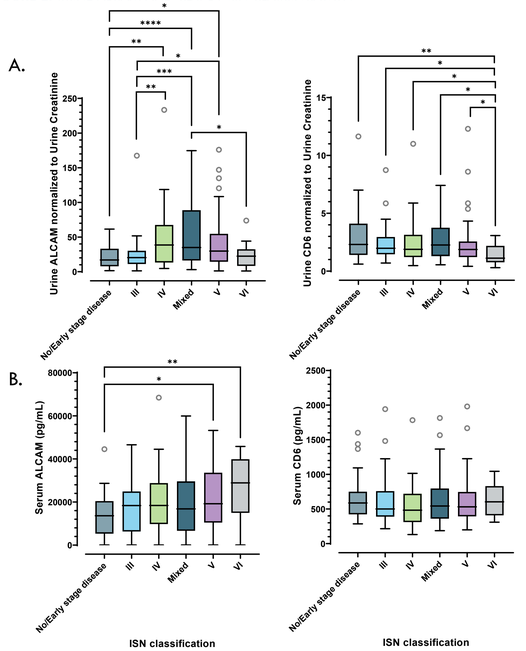
*Subjects with baseline value for urine ALCAM
**Subjects with baseline and at least two follow-up values for urine ALCAM
***Abbreviations – complement components C3 and C4, urine protein creatinine ratio (UPCR), renal SLE Disease Activity Index (SLEDAI) is a score used to assess kidney disease activity and consists of the four kidney-related parameters: hematuria, pyuria, proteinuria, and urinary casts
[1] Subjects receiving more than one medication are included under each medication being received and are counted more than once under the medications category. A total of 270 subjects is used as the denominator to calculate percentage of total.
[2] Other: novel agent, random mAb, sirolimus, study treatment

FIGURE 1. URINE ALCAM AND CD6 ARE ELEVATED IN LN



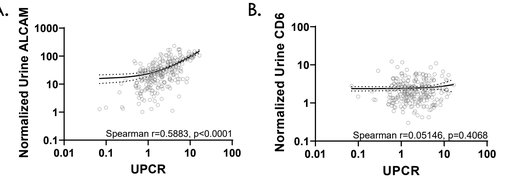
(A) Normalized urine ALCAM and CD6 is significantly elevated in all LN cases (N=270) compared to healthy controls (N=70) at the first visit (V0). No significant differences in (B) serum ALCAM or CD6 between cases (N=251) and controls (N=63). **** p<0.0001

FIGURE 2. URINE ALCAM AND CD6 BY ISN CLASSIFICATION



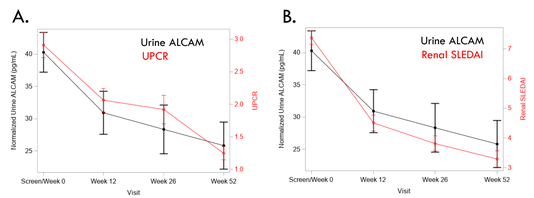
Cross-sectional analysis of (A) normalized urine ALCAM (right) and CD6 (left) show significantly elevated levels of ALCAM, but not CD6, in subjects classified to have proliferative (III and IV) and membranous (V) LN disease. While significant comparisons are observed with urine CD6, all of the comparisons are with class VI which, in this study, has a small sample size (N=11) and may lose significance with a larger sample size. (B) Serum ALCAM (left) is elevated in class V compared to the no/early stage disease group but no significant differences were observed with serum CD6 (right).

FIGURE 3. URINE ALCAM CORRELATES WITH UPCR



(A) Normalized urine ALCAM significantly correlates with UPCR (p<0.0001) while (B) urine CD6 does not which suggests that while both proteins are elevated in the urine of LN subjects, only urine ALCAM changes with disease severity.

FIGURE 4. URINE ALCAM TRENDS WITH DISEASE SEVERITY



Urine ALCAM trends with UPCR and renal SLEDAI. Mean plots with standard error bars for (A) normalized urine ALCAM and UPCR and (B) normalized urine ALCAM and renal SLEDAI.

FIGURE 5. MMRM MODELING FOR UPCR

Mixed Model for Repeated Measures for UPCR as the Dependent Variable						
Effect	Estimate	DF	T Value	Pr > |t|	Lower	Upper
Week 0	0	.	.	.	.	.
Week 12	-0.82	144	-5.01	<.0001	-1.15	-0.50
Week 26	-0.79	167	-2.85	0.0049	-1.34	-0.24
Week 52	-1.51	165	-7.50	<.0001	-1.91	-1.11
Baseline Normalized Urine ALCAM	0.019	153	6.45	<.0001	0.013	0.024
Baseline Normalized Urine CD6	-0.027	152	-0.46	0.65	-0.14	0.089
dsDNA	0.47	326	2.23	0.027	0.055	0.89
Serum C3	-0.012	272	-3.02	0.0028	-0.019	-0.0041
Serum C4	0.038	242	2.73	0.0068	0.010	0.065

MMRM was used to observe the patterns of change in UPCR from baseline over time and their associations with fixed effects. The fit of the model revealed that there was a statistically significant reduction in mean UPCR at post-baseline visits, which is most likely driven by the medications that the subjects were receiving. In addition to the significant effect of post-baseline visits, dsDNA (p=0.027), C3 (p=0.0028), C4 (p=0.0068), and baseline normalized urine ALCAM (p<0.0001) were significantly associated with UPCR. Baseline normalized urine CD6, ISN classification, age, sex, and race were not significantly associated with UPCR and therefore, did not influence UPCR.

Conclusions

In this large multi-center cohort of LN subjects, we expand upon previous studies and provide additional evidence for the role of urine ALCAM as an LN biomarker.

Both urine ALCAM and CD6 are significantly elevated in LN cases compared to controls, suggesting aberrant activity of the CD6-ALCAM pathway. While both proteins are elevated in LN cases, only urine ALCAM is correlated with changes in measures of disease severity, suggesting that the changes observed with urine ALCAM are due to the disease severity rather than nonspecific proteinuric changes. Importantly, longitudinal modeling shows that urine ALCAM is associated with UPCR across the one-year study.

This study is the first to show that over time, urine ALCAM changes with measures of LN disease severity such as UPCR. Follow-up studies and analyses are in progress to evaluate the utility of urine ALCAM as a biomarker to monitor disease and treatment response over time.

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References

1. Stanley, Samantha et al. "Comprehensive aptamer-based screening identifies a spectrum of urinary biomarkers of lupus nephritis across ethnicities." Nature communications vol. 11,1 2197. 4 May. 2020, doi:10.1038/s41467-020-15986-3
2. Hoover, Paul et al. "Accelerating Medicines Partnership: Organizational Structure and Preliminary Data From the Phase 1 Studies of Lupus Nephritis." Arthritis care & research vol. 72,2 (2020): 233-242. doi:10.1002/acr.24066

Disclosures

The study was funded by Equillium, Inc. The presenting author (**DC**) declares the following conflicts of interest during the past 24 months in relationship to this presentation: Current employee, stockholder and owns intellectual property/patents of Equillium. **NS** has no conflicts of interest to declare. **JA** is an employee and stockholder of Equillium. **JG** has no conflicts of interest to declare. **JJ** has had consultancy roles with Progentec Diagnostics. **JB** has had consultancy roles with Glaxo Smith Kline, Janssen, Ventus, and Equillium and was an advisor for Bristol Myers Squibb. **NJ** has no conflicts of interest to declare. **SC** is an employee, officer, and stockholder of Equillium. **MF** is an employee and stockholder of Equillium and was an employee of Arena. **CN** is an employee and stockholder of Equillium. **CM** has no conflicts of interest to declare. **CP** has had consultancy roles and received grant/research support with Equillium and has consultancy roles with Kidneycure and Progentec.

Additional Information

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