Urine ALCAM is a Strong Predictor of Lupus Nephritis

POSTER 1478

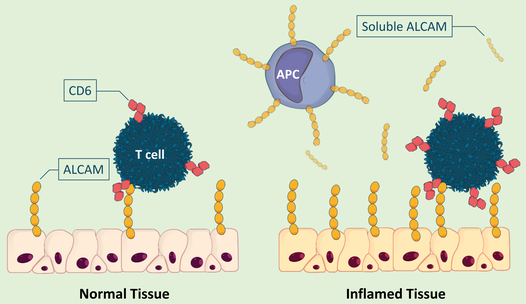
Chu, D.[1], Schwartz, N.[2], Ampudia, J.[1], Guthridge, J.[3], James, J.[3], Buyon, J. P.[4], Jordan, N.[2], Connelly, S.[1], Fung, M.[1], Ng, C. T.[1], AMP SLE/RA consortium, Petri, M.[5], Mohan, C.[4], Putterman, C.[2, 6, 7]

[1]Equillium, La Jolla, CA, USA [2]Albert Einstein College of Medicine, Bronx, NY, USA [3]Oklahoma Medical Research Foundation, Oklahoma City, OK, USA [4]Department of Medicine, Division of Rheumatology, New York University School of Medicine, New York, NY, USA
[5]John Hopkins Medicine, Baltimore, MD, USA [6]Azrieli Faculty of Medicine of Bar-Ilan University, Safed, Israel [7]Galilee Research Institute, Nahariya, Israel

Introduction

T cells play a critical role in the pathogenicity of system lupus erythematosus (SLE) and lupus nephritis (LN). Hence, identifying T cell co-stimulatory pathways and mediators that correlate with disease activity may significantly aid in the development of biomarkers applicable for clinical use, and help identify novel therapeutic targets.

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 tissues, including renal tubular cells. The CD6-ALCAM pathway plays an integral role in modulating T cell activation and trafficking and is central to immune-mediated inflammation. Previous studies have reported that expression of CD6 and ALCAM is upregulated in the kidneys in LN mouse models (Chalmers et al., 2022). Furthermore, blockade of this pathway using an anti-CD6 monoclonal antibody significantly reduced the severity of kidney involvement, implicating an important role for the CD6-ALCAM pathway in the development of LN (Chalmers et al., 2022).



In humans, it has been previously reported that soluble urine ALCAM is significantly elevated in patients with LN and is a potential biomarker of disease (Ding et al., 2020, Parodis et al., 2020, Stanley et al., 2020). Here, we expand upon the previous cross-sectional and longitudinal analyses by evaluating urine ALCAM as a predictor of nephritis progression and/or resolution.

Methods

Patient samples were obtained from subjects that qualified for the AMP Phase 2 study of SLE/LN (Paul et al., 2020). Serum and urine samples were acquired from 270 patients with biopsy proven LN and 70 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 (N=124).

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 measured from the same sample on a pg/mg basis. Subject demographics and characteristics, including urine protein creatinine ratio (UPCR), renal-SLEDAI scores, C3 and C4 protein measurements, and response rate 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, were considered outliers and not included in subsequent analyses. Pairwise comparisons between groups were assessed using the Wilcoxon rank sum which does not assume that the data is normally distributed, and a Spearman correlation was used to measure the strength of association between two ranked variables. To assess changes in urine ALCAM, urine CD6, and UPCR as it relates to response rate, the change in these values was calculated between baseline/week 0 and week 26; week 26 was chosen because this timepoint had the most reported response rate for complete responses (CR), partial responses (PR), and non-responders (NR).

Results

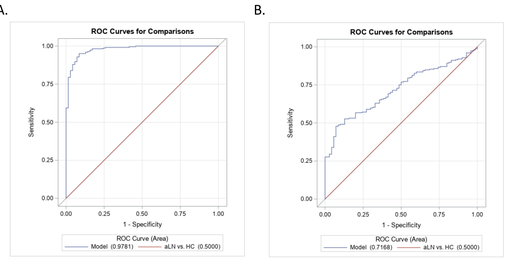
Table 1. Baseline demographics for subjects included in analyses

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 analyses.

	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%)
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 = 251 2.50 (2.017)	n = 116 2.35 (1.908)
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	NA	15 (5.56%)	9 (7.26%)
Cyclophosphamide		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

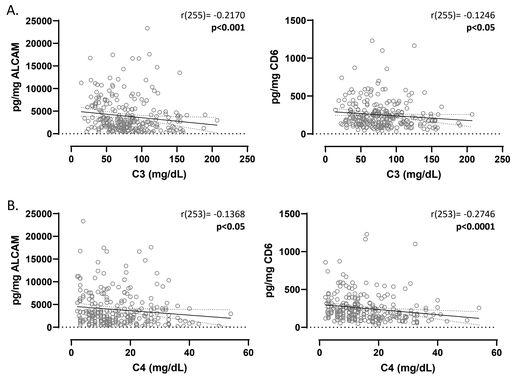
Figure 1. Urine ALCAM is a more sensitive and specific biomarker of LN



The sensitivity and specificity of urine ALCAM and urine CD6 at baseline to distinguish between healthy controls and active LN patients (defined by having renal biopsy-proven LN with a SLEDAI ≥ 6 and renal SLEDAI ≥ 4) was assessed using a receiver operating characteristic (ROC) curve.

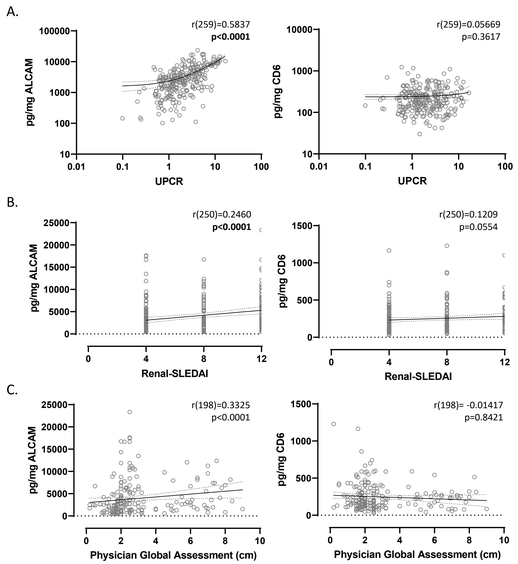
(A) Creatinine normalized urine ALCAM levels at baseline is a better predictor of active LN compared to (B) creatinine normalized urine CD6 with an area under the curve (AUC) of 0.9781 compared to 0.7168.

Figure 2. Urine ALCAM and CD6 negatively correlates with C3 and C4 complement



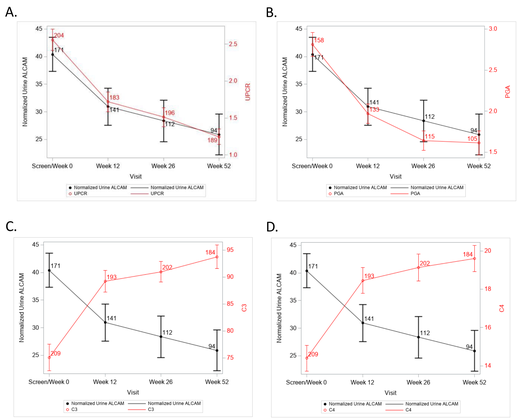
C3 and C4 are commonly measured complement proteins used to monitor individuals with an autoimmune disorder, such as SLE and LN. During an inflammatory flare, the complement system is turned on and complement is consumed, resulting in a decrease in the levels of complement proteins. Both urine ALCAM and urine CD6 negatively correlates with serum (A) C3 and (B) C4 levels. The relationship between urine ALCAM and urine CD6 with C3 and C4 suggests that activity of the CD6-ALCAM pathway trends with SLE and LN severity.

Figure 3. Urine ALCAM significantly correlates with UPCR, renal-SLEDAI, and PGA



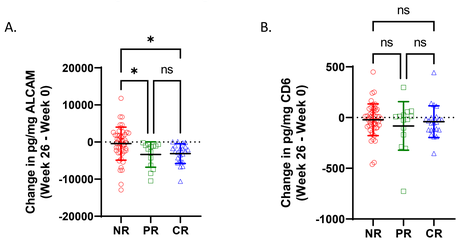
Unlike the relationship between urine ALCAM and urine CD6 with serum C3 and C4 levels, urine ALCAM significantly correlates with (A, left) UPCR, (B, left) renal SLEDAI, and (C, left) Physician Global Assessment whereas no significant correlation was detected with (A-C, right) urine CD6. This suggests that ALCAM could potentially be used as a urinary biomarker to monitor LN disease and response to treatment.

Figure 4. Urine ALCAM trends longitudinally with measures of LN disease



Longitudinally, urine ALCAM trends with both (A) UPCR and (B) PGA. Furthermore, there is an inverse trend between urine ALCAM and (C) C3 and (D) C4 complement. Number of available subjects indicated above each follow-up visit.

Figure 5. Partial and complete responders experience a significant decrease in urine ALCAM while no significant change is observed with urine CD6



Response status at week 26 was used to evaluate how changes in urine ALCAM and CD6 levels may reflect response rate. (A) Partial and complete responders experienced a significant decrease in urine ALCAM levels compared to non-responders (p<0.05). In contrast, (B) no significant changes in urine CD6 levels were detected across non-, partial, nor complete responders.

Conclusions

Lupus nephritis is a leading cause of morbidity and mortality in SLE patients. Currently, a kidney biopsy is required to diagnose disease. However, this process is burdensome to the patient. Hence, there is an unmet need for less invasive biomarkers, such as urine biomarkers, that can either diagnose LN disease and/or predict the course of disease.

- Here, we show that urine ALCAM is a biomarker of disease severity in LN that could be indicative of disease progression and/or response to treatment.
- While both urine ALCAM and CD6 are elevated in patients with LN, only urine ALCAM correlates with currently accepted measures of LN.
- This suggests that while the activity of the CD6-ALCAM pathway is upregulated in LN patients, **urine ALCAM reflects changes in LN and could potentially be used in combination with other measures to inform the clinician of a patient's disease early on, without invasive measures**, so that a treatment plan can be initiated.

Acknowledgments

We would like to thank the Accelerated Medicines Partnership team who lead the sample acquisition and database entry to make this project possible.

We would also like to thank Valeria Marrocco, Phoi Tiet and Nicolette Zahradnikova for help with sample analyses and Wenxin Lu and Nuwan Nanayakkara for their support with the statistical analyses.

References

1. Chalmers, Samantha et al. "The CD6/ALCAM pathway promotes lupus nephritis via T cell-mediated responses." Journal of Clinical Immunology vol. 132,1 (2022).
2. Ding, Huihua et al. "Urinary activated leukocyte cell adhesion molecule as a novel biomarker of lupus nephritis histology." Arthritis Research & Therapy vol. 22,122 (2020).
3. Parodis, Ioannis et al. "ALCAM and VCAM-1 as urine biomarkers of activity and long-term renal outcome in systemic lupus erythematosus." Rheumatology (Oxford) vol. 59,9 (2020).
4. Stanley, Samantha et al. "Comprehensive aptamer-based screening identifies a spectrum of urinary biomarkers of lupus nephritis across ethnicities." nature communications vol. 11,1 (2020).
5. 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).

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.

To request permission or to ask any questions about the poster, please contact medinfo@equilliumbio.com.

© Equillium Inc.