Microvascular C5b-9 deposition in non-lesional skin in patients with SLE and its correlation with active lupus nephritis: a prospective observational study

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ABSTRACT

Objective Tissue damage in lupus nephritis (LN) is mediated by activation of the classical complement pathway. Complement-mediated upregulation of endothelial cell adhesion molecules is seen in dermal blood vessels of non-lesional skin of patients with active lupus. In diseases with systemic complement activation, extensive microvascular C5b-9 deposition is seen in non-lesional skin. In this study, we assess the presence of systemic complement pathway activation as determined by non-lesional skin microvascular C5b-9 deposition in patients with LN.

Methods Eight patients with active LN and eight patients without active LN underwent non-lesional skin biopsies. Using a diaminobenzidine technique, specimens were evaluated for microvascular C5b-9 consistent with systemic complement pathway activation.

Results Five of eight patients with active LN and one of eight patients without active LN demonstrated positive C5b-9 staining in non-lesional skin (p=0.04). Positive non-lesional C5b-9 staining has greater specificity, 87.5%, for active LN than pyuria, low complements, elevated double-stranded DNA (dsDNA) and proteinuria. Urine protein creatinine ratio was significantly higher in patients with positive non-lesional C5b-9 deposition (5.18 vs 1.20; p=0.04). C5b-9 deposition was not associated with a higher NIH Activity Index, interstitial fibrosis, dsDNA or lower complements.

Conclusion This is the first study to demonstrate evidence in non-lesional skin of microvascular C5b-9 indicative of systemic complement pathway activation in LN. C5b-9 deposition is statistically more common and demonstrated greater specificity than most historical biomarkers for active LN. The findings support a potential role for microvascular C5b-9 assessment in non-lesional skin as a biomarker for LN activity.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Quantitative assessment of microvascular C5b-9 deposition in non-lesional skin can identify complement dysregulation.

WHAT THIS STUDY ADDS

⇒ This is the first study to demonstrate evidence in non-lesional skin of microvascular C5b-9 deposition indicative of systemic complement pathway activation in patients with lupus nephritis (LN).

⇒ Non-lesional skin C5b-9 deposition demonstrated greater specificity for active LN than pyuria, proteinuria, elevated double-stranded DNA and hypocomplementemia.

⇒ Non-lesional skin microvascular C5b-9 deposition is associated with greater proteinuria in LN and is statistically more common in active LN than inactive LN.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Findings support a potential role for microvascular C5b-9 assessment in non-lesional skin as a biomarker for LN activity.

INTRODUCTION

Tissue damage in lupus nephritis (LN) is mediated by the deposition of immune complexes and activation of the complement pathway.[1] The presence of antibodies to double-stranded DNA (dsDNA) generates complement fixing immune complexes which can activate the classical cascade, resulting in the cleavage of C5 into C5b with subsequent activation of terminal complement components (C5b-9), also known as membrane attack complex (MAC). The alternative complement pathway has also been shown to play a pathological role in SLE by amplifying the generation of C3 and C5 activation products.[2] In one study of LN, renal C5b-9 deposition was present in 45.5% of non-responders compared with 13% without C5b-9 deposition (OR=5.4; CI 95% 0.8–36.4).[3] Wilson *et al* reviewed 57 renal biopsies with LN and found that those with LN ISN/RPS Class III or IV, who were non-responders, had significantly more intense capillary wall C5b-9 staining (p=0.01).[4]

Additionally, local inflammatory reactions in the skin can provide clues regarding vascular injury in SLE. Complement-mediated upregulation of endothelial cell adhesion molecules

and of nitric oxide is seen in the dermal blood vessels of non-lesional, non-sun-exposed skin from patients with active lupus.[5 6] Endothelial protein C receptor, a biomarker for poor response in LN, is also increased in non-lesional, non-sun-exposed skin.[7] In diseases with systemic complement activation, such as atypical haemolytic uraemic syndrome (aHUS), a subset of patients with thrombotic thrombocytopenic purpura, post-transplant thrombotic microangiopathy and catastrophic severe/critical COVID-19, extensive C5b-9 deposition is also seen in non-lesional skin of the deltoid and suggests the critical role of complement-mediated endothelial cell injury in the evolution of the thrombotic microangiopathy.[8–12] These earlier studies support the hypothesis that non-lesional C5b-9 deposition in the skin can be used as a biomarker for disease activity and evidence of systemic complement activation in LN.

The goal of this study is to investigate the correlation between C5b-9 deposition in non-lesional skin with LN disease activity.

PATIENTS AND METHODS

This is an observational prospective study of 16 patients seen between November 2022 and April 2023 at either Bellevue Hospital or Tisch Hospital and their respective outpatient clinics. All gave informed consent prior to taking part in this study. All patients fulfilled the criteria for SLE as defined by the American College of Rheumatology 1997 revised classification criteria and had biopsy-proven LN.[13]

Patients consented to 4-mm-non-lesional deltoid skin punch biopsies, regardless of disease activity. All patients were >18 years of age, had no evidence of thrombotic microangiopathic haemolytic anaemia (TMHA) and had either a history of biopsy-proven LN or were undergoing their first clinically indicated renal biopsy. In those undergoing renal biopsy, a skin biopsy was performed within 7 days. The formalin-fixed, paraffin-embedded specimens were assessed for C5b-9 deposition via a diaminobenzidine technique. Our immunohistochemical protocol for the assessment of MAC has previously been described and used Leica Microsystems for C5b-9.[8 14] The dermatopathologist reviewing skin biopsies is very experienced in assessing microvascular C5b-9 of skin, having published papers addressing the utility of the normal deltoid skin in assessing for systemic complement pathway activation. The pathologist was blinded to clinical history. C5b-9 within the epidermal basement membrane zone (BMZ), the BMZ of the eccrine coil, the elastic fibres in the dermis and the elastic lamina of vessels were considered non-specific staining patterns. In order for C5b-9 staining to be classified as positive for evidence of systemic complement pathway activation, 10 or greater dermal and or subcutaneous vessels had to exhibit subendothelial or endothelial based staining as outlined in a previously published study.[8 11] The positive microvessels encompassed capillaries, venules, arterioles and small arteries.

Classification as active LN required an Activity Index (AI) of >1 on biopsy or persistent urine protein creatinine ratio (uPCR) >0.7 and albumin <3.7 g/dL. Inactive LN required an AI of 0 on renal biopsy or uPCR ≤0.7, albumin ≥3.7 g/dL and no escalation of care by the treating physician.[15 16] Serological activity was defined as having a low C3 or C4, or elevated dsDNA according to the testing laboratory's reference range.

Chart review was performed at the time of specimen collection to obtain demographics (age, sex, self-reported race and ethnicity), clinical data (SELENA-SLEDAI (Safety of Estrogens in Systemic Lupus Erythematous National Assessment SLE Disease Activity Index) and disease duration), current and prior anti-malarial, glucocorticosteroid and immunosuppression (eg, azathioprine, methotrexate, belimumab, rituximab, cyclophosphamide, mycophenolate mofetil, mychophenolic acid, anifrolumab, leflunomide, voclosporin, tacrolimus) use, and prior renal biopsy reports (ie, ISN/RPS LN classification, AI, Chronicity Index CI), interstitial fibrosis tubular atrophy (IFTA) percentage, as well as immunofluorescence and electron microscopy findings). Laboratory data included urinalysis, uPCR, serum complement levels, dsDNA antibodies by ELISA, creatinine, estimated glomerular filtrate rate, white cell count, platelets count, albumin and absolute lymphocyte count.

Clinical, demographic and laboratory parameters were compared between individuals based on the presence or absence of C5b-9 deposition consistent with systemic complement pathway activation.

Clinical, demographic, laboratory and histopathological parameters between participants with and without significant C5b-9 staining were compared using the independent samples median test for continuous variables and the Fisher's exact test for categorical variables using IBM SPSS V.28.0.1.1. A two-sided $p < 0.05$ was considered statistically significant with Bonferroni adjustment for multiple comparisons.

Patient involvement

Patients were not involved in the design of this research. Patients will be informed of the results at their follow-up clinic appointments.

RESULTS

The patients' age ranged from 22 to 58 years. The median age was 34.5 years, of which C5b-9 negative patients on average were numerically older (respectively, 35 year vs 29.5 year) but this difference was not statistically significant (p=0.61) (table 1). Nine of the 16 patients had renal biopsies either on the day of the skin biopsy or within 7 days of having had the skin biopsy. These nine cases did not show evidence of a thrombotic microangiopathy (TMA) and eight had evidence of active LN on renal biopsy. The other seven patients had a remote history of a kidney biopsy showing active LN. Two patients had

Table 1 Patient demographics and disease characteristics

	C5b-9 positive (n=6)	C5b-9 negative (n=10)	P value
Female	4 (67)	8 (80)	0.60
Age, years	29.5 (17)	35 (21)	0.61
Ethnicity			
Hispanic	3 (50)	2 (20)	0.30
Race			
Asian	2 (33)	0	0.5
Black	0	4 (40)	0.23
White	0	2 (20)	0.50
Other	4 (66)	4 (40)	0.60
ISN/RPS LN Class*			0.58
III	3 (50)	2 (20)	
IV	2 (33)	2 (20)	
III/V	0	1 (10)	
IV/V	1 (17)	2 (20)	
V	0	2 (20)	
Secondary APS	0	2 (20)	0.50
Asymptomatic aPL	2 (33)	3 (30)	1.0
Disease duration, years	5.5 (6)	10 (16.25)	0.12
SELENA SLEDAI	13 (11)	6 (12)	0.12
Non-renal SELENA SLEDAI	3 (4.5)	3 (3.5)	1.0
Serologically active	6 (100)	7 (70)	0.25
Active LN	5 (83)	3 (30)	0.04
NIH Activity Index†	7 (5)	7 (12)	1.0
NIH Chronicity Index†	3 (5)	2 (3)	1.0
IFTA (%)†	20 (21.9)	10 (25)	1.0
Laboratory tests			
Albumin, g/dL	2.65 (1.1)	3.5 (1.3)	0.1
Adjusted dsDNA‡	4.95 (5.5)	2.5 (12.3)	0.32
C3, mg/dL	69.5 (63.8)	63 (48)	1.0
C4, mg/dL	18.5 (15.8)	20 (13.2)	1.0
Urine studies§			
Red blood cells >5/hpf	4 (67)	3 (30)	0.30
uPCR	5.18 (5.1)	1.2 (2.06)	0.04
Active LN			
	C5b-9 positive (n=5)	C5b-9 negative (n=3)	
uPCR	5.18 (5.11)	0.8 (2.17)	0.001

Data are n (%) or median (IQR).
P values are evaluated using SPSS with t-tests or χ^2 tests of independence.
*One person LN Class missing.
†Three people without light microscopy details available.
‡Adjusted dsDNA calculated by dividing value by reference upper limit of normal.
§One person unable to provide urine due to being anuric.
dsDNA, double-stranded DNA; IFTA, interstitial fibrosis tubular atrophy; ISN/RPS, International Society of Nephrology and Renal Pathology Society; LN, lupus nephritis; NIH, National Institute of Health; SELENA SLEDAI, Safety of Estrogens in Systemic Lupus Erythematous National Assessment SLE Disease Activity Index; uPCR, urine protein creatinine ratio.

secondary anti-phospholipid syndrome (APS) and five had asymptomatic anti-phospholipid antibodies (aPL).

The majority of patients were female (12 of 16). Five (31.2%) were Hispanic. Two were Asian, four black, two white and eight other. Patients without C5b-9 positivity tended to have had lupus for longer than those with C5b-9 positivity (respectively, 10 years vs 5.5 years; p=0.12). All ISN/RPS LN classes, excluding I and II, were present in the C5b-9 negative group but only classes III, IV and IV/V were represented in the C5b-9 positive group, of which

class III was the most common (50%; p=0.58) (table 1). Light microscopy results from prior renal biopsies were not available for three patients with inactive LN, and therefore, LN class was unknown. Eight (50%) of the patients had active LN. Six of the total 16 patients (37.5%) were positive for endothelial and or subendothelial C5b-9 deposition at levels consistent with systemic complement pathway activation (figure 1A).

In those patients with positive C5b-9 deposition as defined in the Patients and methods section, 5 of 6 (83%) had active LN, and of those negative for C5b-9 deposition, 3 of 10 (30%) had active LN (table 1). In those patients with active LN, five of eight (62.5%) had positive C5b-9 deposition consistent with systemic complement pathway activation, and in those with inactive LN, one of eight (12.5%) demonstrated positive C5b-9 (p=0.04) (table 2). These findings demonstrate that systemic complement activation is present in LN and is significantly more common in active disease. Among the eight patients with active LN, C5b-9 positivity correlated with a significantly higher uPCR (5.18 vs 0.8; p=0.001). C5b-9 deposition positivity was not associated with a higher AI, CI, IFTA, dsDNA, presence of aPL or APS, or hypocomplementemia (table 1).

Cutaneous evidence of systemic complement pathway activation as demonstrated by quantitative C5b-9 assessment in microvessels has a greater specificity, 87.5%, for active LN than pyuria (62.5%), hypocomplementemia (57.1%), elevated dsDNA (42.8%) and proteinuria (57.1%) (table 3). In contrast, haematuria had a specificity of 100% for active LN. Despite a high specificity for active LN, C5b-9 deposition had the lowest sensitivity (62.5%) for active LN.

One patient with inactive LN who was negative for non-lesional C5b-9 was taking a complement inhibitor for a remote history of post-transplant TMA (table 4). Most patients were on background hydroxychloroquine (87.5%) and glucocorticosteroids (75%). Five of the eight patients with active LN had received high-dose steroids at the time of skin biopsy, three of which had non-lesional C5b-9 deposition. A similar number of patients with and without C5b-9 deposition were taking biologics, mycophenolate and/or calcineurin inhibitors at the time of skin biopsy (p>0.05).
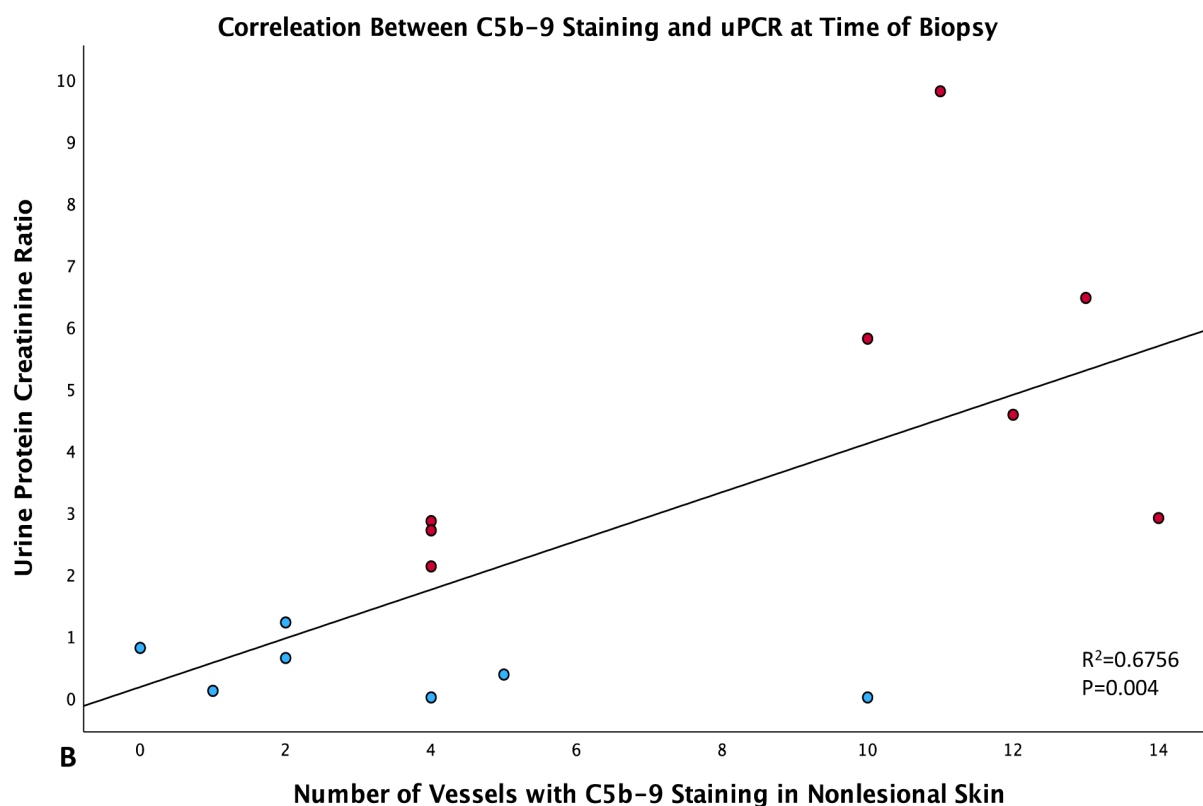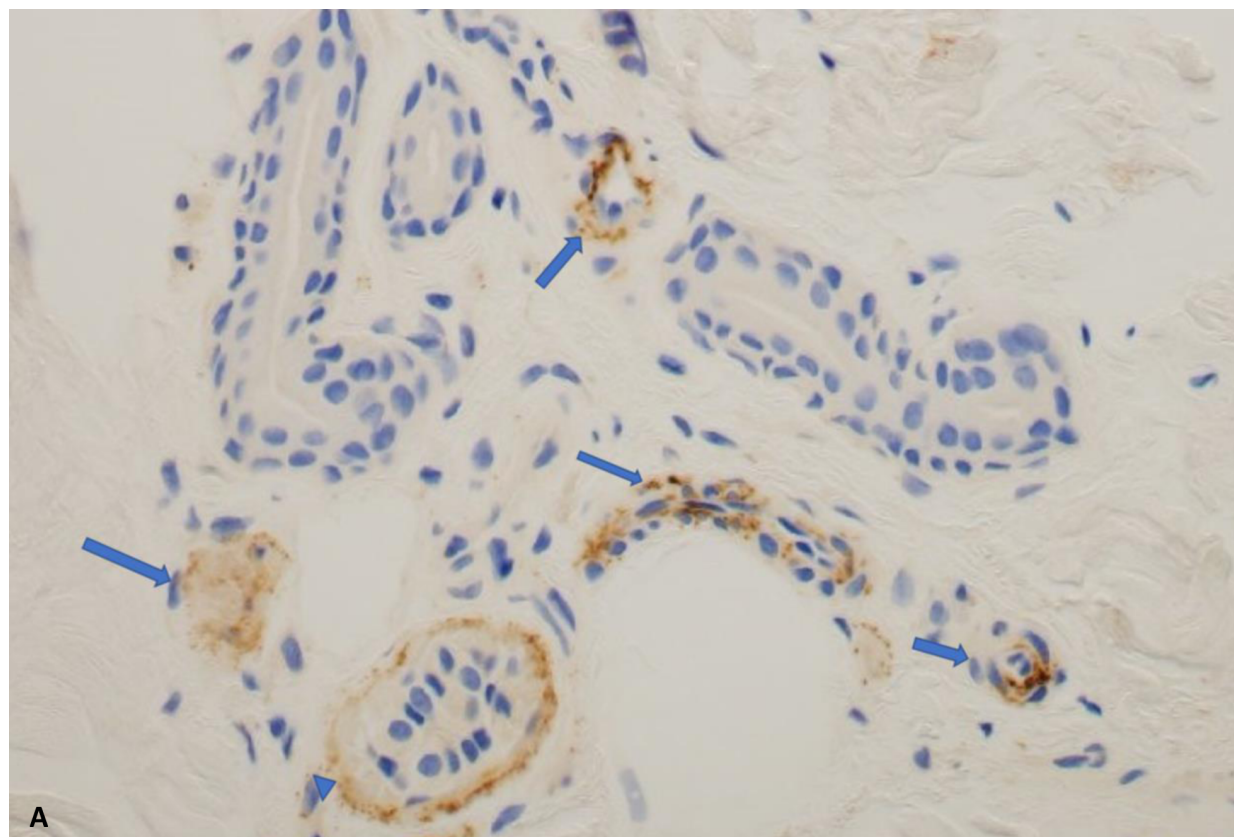
DISCUSSION

SLE is a chronic autoimmune disease with a relapsing and remitting course. LN is a major predictor of morbidity and mortality in SLE, and within the first 10 years of SLE onset, LN is present in 50%–60% of patients.[17] Despite considerable advances in treatment options for LN, renal survival rates have plateaued since the mid-1990s; up to 30% of patients with LN progress to end-stage kidney disease (ESKD).[18] Determining ongoing LN activity is often difficult as other comorbidities, such as diabetes or hypertension, can lead to proteinuria in the absence of ongoing SLE activity. Furthermore, chronic damage from

prior LN activity can result in irreversible injury that is accompanied by persistent proteinuria absent ongoing immune-mediated activity. Therefore, a repeat renal biopsy maybe required to accurately assess LN disease activity state, but this is an invasive procedure that carries risks. One study recently showed that despite reaching a perceived clinical remission by laboratory tests, 19.6% of patients had persistent activity on repeat renal biopsy.[19] This demonstrates the importance of finding alternative biomarkers of LN activity that can readily be used by physicians in everyday practice.

SLE flares are associated with complement pathway activation; however, hypocomplementemia does not always accompany active disease. In our study, the presence of low complement had a sensitivity of 75% for active LN, thereby not accounting for 25% of patients. Discordance between SLE activity and serum complement levels has been largely attributed to the fact that serum complement levels are a static appraisal of a dynamic process involving activation, consumption, catabolism and synthesis of complement proteins, leading to a wide range of normal complement levels. Complements are also an acute phase reactant and therefore synthesis is increased during times of inflammation, such as SLE activity, therefore counterbalancing the increased consumption.[20] Only haematuria was more specific and sensitive than non-lesional C5b-9 deposition for active LN. Evaluation of haematuria by urinalysis is a cost-effective and readily available test; however, haematuria can be confounded by menses in females of reproductive age. Biomarkers that directly detect activation of the terminal complement pathway are more reliable measures of ongoing pathologic systemic complement activation in SLE. Microvascular C5b-9 is ubiquitous and present in non-lesional skin of individuals without systemic complement activation but at intensity below our definition of positivity that is meaningful and consistent with systemic complement pathway activation. The key in establishing pathogenetically significant systemic complement pathway activation is the identification of at least 10 positive staining vessels and avoiding false positive results as might occur if one erroneously counts a vessel showing only elastic fibre as a positive vessel or if a larger sample of skin is used.[11]

In our study, 6 out of 16 patients had evidence of systemic complement pathway activation as demonstrated by quantitative assessment of C5b-9 deposition. Of those six patients, five had active LN demonstrating the association of cutaneous microvascular C5b-9 with LN disease activity. Most patients with cutaneous vascular C5b-9 deposition had nephrotic range proteinuria in contrast to those without significant vascular C5b-9 deposition who had subnephrotic range proteinuria (table 2, figure 1B). A statistically significant finding that persisted when compared among only those with active LN (table 1). This is consistent with previous studies that found renal C5b-9 deposition was associated with both a higher IFTA and proteinuria at the time of biopsy.[21] This association suggests that systemic complement pathway activation

Figure 1 (A) Image demonstrates extensive fine granular deposits of C5b–9 involving the endoluminal aspect of the microvasculature, specifically capillaries and venules. Evidence of systemic complement pathway activation is based on counting the number of microvessels encompassing capillaries, venules, arterioles and small arteries showing endothelial and subendothelial deposition of C5b-9 (arrows). Note the non-specific staining pattern within the basement membrane zone of the eccrine gland. Epithelial basement membrane zone and elastic fibre staining is not of any diagnostic significance (arrow head). (B) Red data point: active LN; blue data points: inactive LN. LN, lupus nephritis; uPCR, urine protein creatinine ratio.

Table 2 Individual case characteristics

Case	Age/sex	LN status	C5b-9 staining	LN class	Simultaneous skin/renal biopsy	AI/CI score	IFTA (%)	uPCR	Low C3 or C4	Elevated dsDNA
1	35F	Active	Negative	III/V	Yes	2/2	10	2.12	Yes	Yes
2	29F	Inactive	Positive	III	No	6/2	10	0	No	Yes
3*	44F	Inactive	Negative	IV	No			0.37	Yes	No
4	46M	Active	Positive	IV	Yes	8/2	15	5.8	Yes	Yes
5	25F	Inactive	Negative	III	No	6/6	10	0.64	No	Yes
6*	28F	Inactive	Negative	V	No			0.11	No	No
7*†	57F	Inactive	Negative		No					
8	43F	Inactive	Negative	III	No	7/2	5	0	No	No
9	22M	Active	Positive	IV/V	Yes	6/4	30	2.9	No	Yes
10	39F	Active	Positive	IV	Yes	12/6	35	4.6	Yes	Yes
11	58M	Inactive	Negative	IV/V	No	14/2	30	0.7	Yes	Yes
12	34F	Inactive	Negative	V	Yes	0/1	5	1.2	Yes	Yes
13	25F	Active	Positive	III	No	5/6	25	6.46	No	Yes
14	30F	Active	Negative	IV/V	Yes	13/5	30	2.76	Yes	Yes
15	51M	Active	Negative	IV	Yes	15/5	40	2.70	Yes	Yes
16	30F	Active	Positive	III	Yes	10/0	7.5	9.80	Yes	Yes

*Renal biopsy interpretation not available in electronic health record.
†Anuric and did not provide labs at visit.
AI, Activity Index; CI, Chronicity Index; dsDNA, double-stranded DNA; IFTA, interstitial fibrosis tubular atrophy; LN, lupus nephritis; uPCR, urine protein creatinine ratio.

plays an important role in tubulointerstitial injury and can be identified via non-lesional skin biopsies. Tubulointerstitial injury, specifically IFTA, strongly associates with poor renal outcomes and is a reliable predictor of progression to ESKD.[22] These studies support the hypothesis that C5b-9 deposition is a potential biomarker for more intense disease and poor response to traditional treatment.

If non-lesional skin microvascular C5b-9 deposition is a reflection of renal tubular C5b-9 in LN, then the

Table 3 Sensitivity and specificity of significant microvascular C5b-9 (ie, 10 or more positive staining vessels) in non-lesional skin biopsy compared with traditional biomarkers of LN disease activity

	Sensitivity (%)	Specificity (%)
Positive C5b-9 deposition	62.5	87.5
Elevated dsDNA	100	42.8
Low complements	75	57.1
Pyuria	87.5	62.5
Haematuria	87.5	100
uPCR >0.5	100	57.1

Haematuria greater than 5 red blood cell/hpf. Pyuria greater than 5 white blood cell/hpf. Hypocomplementemia and dsDNA determined by testing laboratory's reference values.
dsDNA, double-stranded DNA; LN, lupus nephritis; uPCR, urine protein creatinine ratio.

absence of C5b-9 in three patients with active LN may be a biomarker of those that will have a good response to standard LN treatment. In those with inactive LN, only one patient was positive for C5b-9 deposition despite a uPCR <0.01 and normal complement levels. Hypothetically, the presence of significant cutaneous microvascular C5b-9 in inactive LN may be a predictor for future flares or relapse with immunosuppression tapering. At this time, our paper does not have the necessary follow-up to assess the relationship between C5b-9 deposition and subsequent outcomes and this remains an area of needed future study. Serial monitoring microvascular C5b-9 deposition in non-lesional skin as a means of monitoring treatment response has previously been shown to be of no diagnostic value as Cb5-9 can persist for several months following its deposition despite treatment with eculizumab, an inhibitor of C5 (18). However, given the strong association of non-lesional C5b-9 positivity with active LN seen in our study, non-lesional C5b-9 could serve as a biomarker for active LN in those individuals lacking hypocomplementemia or rising dsDNA but have persistent proteinuria.

Cutaneous microvascular C5b-9 deposition has previously been identified as a biomarker for systemic complement pathway activation in diseases associated with TMA.[8 12] TMA is characterised by thrombocytopenia and microangiopathic haemolytic anaemia (MAHA) with microvascular thrombosis resulting in systemic organ damage. One form of TMA is complement-mediated TMA (CM-TMA), of which aHUS and TMA associated

Table 4 Current and prior SLE specific medication use at the time of non-lesional skin biopsy

	C5b-9 positive (n=6)	C5b-9 negative (n=10)	P value
Current medications, N (%)			
Hydroxychloroquine	5 (83)	9 (90)	0.63
Glucocorticoid steroids	6 (100)	6 (60)	0.23
Prednisone >40 mg*	2 (33)	3 (30)	0.17
Mycophenolate mofetil†	3 (50)	4 (40)	0.55
Mycophenolate mofetil >2 g	1 (17)	1 (10)	0.89
Calcineurin inhibitor	1 (17)	2 (20)	0.69
Belimumab	1 (17)	1 (10)	0.89
Ravulizumab	0	1 (10)	0.63
Other‡	2 (33)	1 (10)	0.60
Prior medications			
Belimumab	0	1 (10)	0.56
Rituximab	1 (17)	3 (30)	0.56
Glucocorticoid steroids	6 (100)	4 (40)	0.23
Cyclophosphamide	1 (17)	5 (50)	0.31
Azathioprine	2 (33)	4 (40)	0.61
Mycophenolate mofetil†	1 (17)	6 (60)	0.15
Calcineurin inhibitor	1 (17)	1 (10)	0.62

*Prednisone equivalent dosing.
†Includes mycophenolic acid.
‡Azathioprine or methotrexate.

LN are subgroups.[23] A pauci-inflammatory thrombogenic vasculopathy, the histological hallmark of TMA, is observed in 8%–17% of LN biopsies[24 25] and is associated with a poor renal response.[23 26] In a retrospective study of 79 patients with LN-associated TMA on renal biopsy and 79 without TMA-associated LN, TMA-associated LN was associated with a higher incidence of acute haemodialysis (35% vs 5%; p<0.002), higher IFTA (43% vs 13%, p<0.001) and inferior 3-year renal survival rates (68% vs 89%, p=0.002).[27] Case series report that refractory TMA associated LN is highly responsive to treatment with the complement inhibitor eculizumab, with one study showing renal recovery in 80% of patients.[23 26] In unpublished data at our institution, we found seven of nine (77%) patients with TMHA who were subsequently treated with either eculizumab or ravulizumab, also had a non-lesional skin biopsy positive for significant microvascular C5b-9 deposition. Of those seven, four had renal TMA on biopsy as well. In this study, all the patients had undergone a kidney biopsy at some point in their clinical course including nine patients who had a recent kidney biopsy that was temporally associated with the skin biopsy. None of the patients had evidence of TMA on renal biopsy nor was there laboratory evidence of MAHA at the time of skin biopsy including cases that showed evidence of systemic complement pathway activation on the skin biopsy. Hence, levels of non-lesional microvascular C5b-9 indicative of systemic complement pathway activation can be seen in settings other than systemic or intrarenal TMA.

Unlike catastrophic APS, asymptomatic aPL and APS are not associated with systemic complement pathway activation, therefore we did not exclude SLE patients with asymptomatic aPL or APS from our study. This is supported by our finding that neither of the two patients with secondary APS had C5b-9 positivity and that there was no statistical difference between the presence of asymptomatic aPL in patients with and without C5b-9 positivity (2 vs 3, respectively; p=1.0) (table 1).

In patients with active LN, the probable basis is one of the classic complement pathway activations driven by immune complexes. While complement inhibition is a standard treatment in TMA syndromes and has proven to be efficacious in other complement-driven diseases such as neuromyelitis optica[28] and cold agglutin disease,[29] the efficacy of complement inhibition in the setting of LN without thrombotic angiopathy has not been established, although clinical trials with ravulizumab, a long-acting monoclonal antibody inhibitor of C5, and vemircopan, an oral inhibitor of Factor D, are currently in progress. Lupus prone mice treated with a monoclonal antibody specific for C5, effectively blocking the formation of C5b-9 resulted in a delayed onset of proteinuria, prolonged survival and improved renal pathologic changes, implicating a role for complement inhibitors in the treatment of LN irrespective of TMA.[30] There are ongoing phase II studies of complement inhibitors such as eculizumab in LN to assess efficacy and safety. Stratifying results by the presence or absence

of evidence of systemic complement pathway activation like non-lesional C5b-9 would be of interest. Additionally, future studies can provide data to determine which patients with LN benefit from complement inhibition in relation to following: intrarenal TMA, systemic TMA, intrarenal C5b-9 or non-lesional C5b-9.

Our study is limited by the small sample size, limiting our ability to capture true clinical differences between groups. Additionally, the absence of recent renal biopsies in patients with inactive disease prevents description of the renal histology at the time of skin biopsy. Finally, the short period of follow-up in patients with active LN precluded studying the predictive value related to outcome or treatment response in relation to C5b-9 deposition.

This is the first study to demonstrate evidence in non-lesional skin of levels of microvascular C5b-9 indicative of systemic complement pathway activation in patients with LN. C5b-9 deposition consistent with systemic complement pathway activation is statistically more common and demonstrated greater specificity than most historical biomarkers in active LN. Our findings support a potential role for microvascular C5b-9 assessment in non-lesional skin as a biomarker for LN activity and subsequently decreasing the need for more invasive testing like repeat renal biopsy. Longer term follow-up is needed to assess if non-lesional skin C5b-9 is associated with unsatisfactory treatment response and/or poor outcomes in active LN. Furthermore, larger studies that are adequately powered to assess the association between renal histological activity index and tubulointerstitial changes on long-term outcomes are also needed.

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