

# A PANEL OF LUPUS BIOMARKERS FOR THE MONITORING OF SYSTEMIC LUPUS ERYTHEMATOSUS: PERFORMANCE CHARACTERISTICS IN DISTINCT SLE COHORTS

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# ABSTRACT

## PURPOSE

Antibody titers to double stranded DNA (anti-dsDNA) and complement C3 and C4 proteins have clinical utility in the routine monitoring of systemic lupus erythematosus (SLE). We evaluated the performance characteristics of antibody titers to C1q (anti-C1q), and complement activation products (C4d bound to erythrocytes, EC4d) as additional biomarkers to monitor SLE disease activity.

## METHODS

SLE patients (total 124 patients, mean age 42 years, 97% females) were enrolled from three different cohorts in the United States. The first cohort enrolled 37 subjects, all selected for active disease in the presence of complement activation. The second and third cohorts enrolled 64 and 24 SLE subjects, respectively with a range of severity and complement activation. Disease activity was assessed longitudinally using the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) SLE disease activity index (SLEDAI) without anti-dsDNA and complement components; this modification is referred to as the clinical SELENA-SLEDAI. Also, the Physician's Global Assessment of disease activity (PGA: 0-3-point scale) was collected. Antibody titers to dsDNA and C1q and serum C3 and C4 were determined using standard immunoassays (low C3/C4 was defined as either C3 or C4 below normal). EC4d levels were determined using quantitative flow cytometry and expressed as net mean fluorescence intensity (MFI). The relationships between fluctuations in SLE disease activity and biomarkers were analyzed using Spearman rank test and linear mixed effect models with the clinical SELENA-SLEDAI and PGA as the dependent variables and the antibodies and complement measures as independent variables (autoantibody titers and EC4d levels were log normalized for the analysis). Marginal R2 was calculated to evaluate the proportion of variance explained by independent variables.

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## RESULTS

624 study visits were collected in the 124 patients. At baseline, both clinical SELENA-SLEDAI (average 6.0 points) and PGA scores (average 1.0 point) correlated with EC4d levels C3/C4 status, anti-dsDNA and anti-C1q titers (p<0.01). Linear mixed effect models revealed that changes in EC4d, C3/C4 status anti-dsDNA and anti-C1q titers were all significantly associated with fluctuations in disease activity as assessed using the clinical SELENA-SLEDAI and the PGA (p<0.01)(Table I). Of the 124 subjects, 97 of them presented with either chronically low C3/C4 (n=40) or normal C3/C4 status at all visits (n=57). In this subset, changes in EC4d levels were associated with fluctuations in SELENA-SLEDAI (estimate 1.2±0.3, marginal R2=8%) and PGA (estimate 0.2±0.1, marginal R2=9%).

## CONCLUSION

These data indicate that anti-dsDNA, antiC1q, low complement and EC4d are associated with disease fluctuations in SLE. EC4d correlates with disease activity among subjects with chronically low or normal complement C3 or C4.

## OBJECTIVE

Evaluate the performance characteristics of C4d bound to erythrocytes (EC4d), anti-C1q, anti-ds DNA, and complement C3/C4 as biomarker of disease activity in systemic lupus erythematosus.

## METHODS

This retrospective analysis was conducted using data from 3 SLE cohorts.

Study	Design	N	Reference
1	Flare Study, selected subjects with abnormal CB-CAPs and active disease	37	Reduction in erythrocyte-bound complement activation products and titres of anti-C1q antibodies associate with clinical improvement in systemic lupus erythematosus. Buyon, et al., Lupus Sci Med. 2016 Sep 30;3(1):e00016
2	Consecutive subjects from the OMRF Lupus Cohort	64	The Deposition of Complement C4d Split Product on Platelets and Erythrocytes Correlate with Disease Activity and Improvement in Systemic Lupus Erythematosus. Merrill et al., ACR 2014
3	Consecutive subjects from the Johns Hopkins Cohort, all under MTX and HCQ	23	Complement C4d Split Products on Erythrocytes Are Associated with Composite Measure of Disease Activity in Systemic Lupus Erythematosus Subjects Receiving Methotrexate and Hydroxychloroquine. Petri et al., ACR 2016

## Biomarkers

- EC4d (elevated: >14 net mean fluorescent intensity[MFI])
- Anti-C1q (positive: > 20 units)
- Anti-dsDNA by chemiluminescence (positive: >35 Units/ml)
- Low complement C3 (<81 mg/dL) and/or Ca (<12.9 mg/dL)

## Outcome Variables

- Non-immuological (clinical) hybrid SELENA-SLEDAI or PGA.

## Statistical Analysis

- Spearman rank test at baseline
- Longitudinal data by linear mixed effect models.

# BASELINE CHARACTERISTICS

Overall, a total of 624 study visits were collected in the 124 patients.

	Study [1]	Study [2]	Study [3]	Combined
Number of patients	37	64	23	124
Age	34	42	56	42
Gender [Percent Females]	95%	97%	100%	97%
PGA [0-3] Average (avg)	1.6	1.4	0.6	1.3
Study Visit (avg nb)	10.5	2.0	4.7	5.0
PGA [0-3] Avg	1.6	1.4	0.6	1.3
Clinical SELENA-SLEDAI avg	8.1	6.3	1.4	6.0
Anti-dsDNA (>35 Units)	91%	38%	4%	46%
Anti-C1q (>20 Units)	62%	20%	13%	31%
Low C3 or C4	73%	39%	22%	46%
EC4d (>14 net MFI)	86%	39%	14%	49%

# BIOMARKERS AND DISEASE ACTIVITY

At baseline, both clinical SELENA-SLEDAI and PGA scores correlated with EC4d levels C3/C4 status, anti-dsDNA and anti-C1q titers (p<0.01).

	Spearman R	P Value
EC4d [Log Net MFI]	0.434	<0.01
Low Complement	0.251	<0.01
Anti-dsDNA by CIA [Log Units]	0.371	<0.01
Anti-C1q [Log Units]	0.405	<0.01

Linear mixed effect models revealed that changes in EC4d, C3/C4 status anti-dsDNA and anti-C1q titers were all significantly associated with fluctuations in disease activity as assessed using the clinical SELENA-SLEDAI and the PGA (p<0.01)

SLE marker	Clinical SELENA SLEDAI Slope Estimate ±SEM	PGA Slope estimate ±SEM
EC4d (Log Net MFI)	1.2±0.3 (6%) (p<0.01)	0.17±0.04 (6%) (p<0.01)
Low Complement	1.7±0.4 (4%) (p<0.01)	0.30±0.06 (6%) (p<0.01)
Anti-dsDNA (log U/ml)	1.1±0.2 (18%) (p<0.01)	0.10±0.02 (8%) (p<0.01)
Anti-C1q (Log U)	1.2±0.2 (10%) (p<0.01)	0.15±0.03 (8%) (p<0.01)

Of the 124 subjects, 97 of them presented with either chronically low C3/C4 (n=40) or normal C3/C4 status at all visits (n=57). In this subset, changes in EC4d levels were associated with fluctuations in SELENA-SLEDAI and PGA

Clinical SELENA-SLEDAI	N/study Visits	EC4d Slope Estimate ±SEM	P value	R²
All subjects	124/612	1.2±0.3	<0.001	6%
Low Complement all visits	40/208	0.8±0.5	0.097	3%
Normal Complement all visits	57/193	1.6±0.4	0.001	9%
Low or normal complement, all visits	97/401	1.2±0.3	<0.001	8%

PGA	N/study Visits	EC4d Slope Estimate ±SEM	P value	R²
All subjects	124/619	0.2±0.1	<0.001	6%
Low Complement all visits	40/210	0.2±0.1	0.02	5%
Normal Complement all visits	57/197	0.2±0.1	0.01	5%
Low or normal complement, all visits	97/407	0.2±0.1	<0.001	9%

## CONCLUSION

EC4d is a marker of SLE disease fluctuations (PGA and clinical SELENA-SLEDAI) vent when complement C3/C4 remains constant over time.