

Evaluation of Endothelial Cell Adhesion Molecules and Anti-C1q Antibody in Discriminating between Active and Non-Active Systemic Lupus Erythematosus

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Submitted: 8 Dec 2015

Accepted: 9 Apr 2016

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Abstract

Background: Detecting the active state of systemic lupus erythematosus (SLE) is important but challenging. This study aimed to determine the diagnostic accuracy of serum endothelial cell adhesion molecules (ICAM-1 and VCAM-1) and anti-C1q antibody in discriminating between active and non-active SLE.

Methods: Using SELENA-SLE disease activity index (SLEDAI), 95 SLE patients (45 active and 50 non-active) were assessed. A score above five was considered indicative of active SLE. The blood samples were tested for serum ICAM-1, VCAM-1 and anti-C1q antibody using enzyme-linked immunosorbent assay (ELISA).

Results: The levels of serum VCAM-1 and anti-C1q antibody were significantly higher in active SLE patients. Both VCAM-1 and anti-C1q were able to discriminate between active and non-active SLE (p -value < 0.001 and 0.005, respectively). From the receiver operating characteristic curves (ROCs) constructed, the optimal cut-off values for VCAM-1 and anti-C1q antibody in discriminating between active and non-active SLE were 30.5 ng/mL (69.0% sensitivity, 60.0% specificity, PPV 58.5%, NPV 66.7%) and 7.86 U/mL (75.6% sensitivity, 80% specificity, PPV 77.3%, NPV 78.4%), respectively. However, serum ICAM-1 level was unable to discriminate between the two groups (p -value = 0.193).

Conclusion: Anti-C1q antibody demonstrated the best diagnostic accuracy in discriminating between active and non-active SLE patients.

Keywords: cell adhesion molecules, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), anti-C1q antibody, systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with unpredictable episodes of flares along its chronic course. It is important to be able to detect flares (active SLE) from progressive-permanent changes that have occurred due to the disease itself and to distinguish between active SLE and other acute illnesses (1). Apart from clinical presentations, laboratory tests are helpful in detecting SLE

flares. Increased anti-dsDNA antibody titre and reduced serum C3 and C4 levels are among the indicators of active SLE (2). However, as there is no single measure or laboratory test that can determine the disease activity status in all SLE patients, scoring systems which comprise a variety of clinical and laboratory parameters have been created. SLEDAI is one of them, considered by many as the easiest assessment tool and practical for research classification and clinical use (3, 4). Several forms of SELENA-SLE disease activity

index (SLEDAI) modifications exists, including the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) trial (SELENA-SLEDAI) which has several different definitions in order to improve the clarification of individual items to better capture changes in disease activity (5, 6). SELENA-SLEDAI was one of three internationally accepted indices included in the SLE Responder Index (SRI), a composite disease activity assessment to assess the therapeutic efficacy in clinical trials (7).

Anti-dsDNA antibody, serum C3 and serum C4 levels are among the laboratory parameters included in the SELENA-SLEDAI. However, a substantial percentage of SLE patients are persistently negative for anti-dsDNA antibody and can be as high as 20% (8). The associations of complement proteins, including both the native molecules and activation products with SLE disease activity also showed inconsistent results. Therefore, it is necessary to find other reliable biomarkers which can at least complement the readily available laboratory tests for detecting flares in SLE patients (9). The biomarkers that have been studied include inflammatory markers and autoantibodies such as anti-nucleosome antibody, which was also studied in our centre. Anti-nucleosome antibody was shown to be sensitive and specific for SLE diagnosis and correlated well with disease activity (10). This study however, was looking at the potential of new biomarkers to detect flares in SLE patients. This was determined by establishing a new cut-off level which can best discriminate between active and non-active SLE patients.

Endothelial cell adhesion molecules (ECAMs), including ICAM-1 and VCAM-1 are transmembrane proteins grouped under the immunoglobulin superfamily (11, 12). They are important for immune cells transmigration from circulation into tissues undergoing inflammatory processes. An *in vitro* study demonstrated that VCAM-1 is not expressed by naïve endothelial cells while ICAM-1 is lowly expressed on the surface of endothelial cells and antigen presenting cells (APCs). However, their expressions are greatly increased by pro-inflammatory cytokines such as IL-1, IFN- γ and TNF- α . Studies of these circulating soluble adhesion molecules in SLE have yielded contradictory results (13–15). Furthermore, there is disagreement as to whether soluble adhesion molecules are an accurate reflection of membrane-bound proteins. ICAM-1 has been studied mainly in disease activity and organ-specific involvement in SLE. Multiple lines

of evidence have reported that ICAM-1 showed statistically significant elevations in SLE patients compared to healthy controls (13) while in some other studies, these significant elevations were seen in active SLE but not non-active SLE patients (15–17). The serum level of VCAM-1 is sustained for two to three hours following cytokine induction and gradually diminishes over several days (11). A previous study evaluated soluble VCAM-1 in patients with SLE and found it elevated in active patients compared to normal controls, and it was associated with the disease activity. In addition, Spronk et al. (28) documented an elevation of soluble VCAM-1 levels with disease activity and a decline in clinical remission in SLE, but found no significant difference in ICAM-1 levels between SLE patients and healthy controls.

The complement system is activated during SLE flares. C1q is the first component of the classical pathway and it is activated by immune complexes. The presence of anti-C1q antibody resulted in delayed clearance of immune complexes in some conditions including SLE (18). The anti-C1q antibody is strongly associated with lupus nephritis, one of the most serious complications of SLE (19, 20). However, some studies have shown that anti-C1q antibody was not specifically associated with lupus nephritis activity but the overall SLE disease activity (21, 22). This study compared ICAM-1, VCAM-1 and anti-C1q antibody levels in active SLE patients with non-active SLE patients in order, to evaluate their potential to discriminate between the two groups and to determine the best cut-off levels if they are able to do so.

Materials and Methods

Subjects and data collection

This was a cross-sectional study involving SLE patients from two tertiary hospitals in the east of Peninsular Malaysia over a 15-month period. Included in this study were 95 SLE patients (45 active and 50 non-active SLE) who fulfilled the revised American College of Rheumatology (ACR) classification criteria and who consented to participate. The non-active SLE cases were selected from outpatient clinics by a simple random sampling method. Due to the difficulty in obtaining active SLE cases, a universal sampling method was applied for this group. The disease activity was evaluated during blood sampling using the SELENA-SLEDAI score system. Patients with a SELENA-SLEDAI score

above five were identified as having active SLE (10, 23). Relevant clinical data were gathered from the patients' medical records. Approval for this study was obtained from the Human Research Ethics Committee, Universiti Sains Malaysia (USM/KK/PPP/JEPeM [251.3.(6)]) and the Medical Research and Ethics Committee (MREC) of the Ministry of Health, Malaysia (NMRR-12-1060-12717).

Blood sampling and assays

Blood samples were collected from the peripheral vein and allowed to clot prior to separation. Serum obtained was aliquot and stored at -80 degrees Celsius until test analysis. Serum ICAM-1 and VCAM-1 levels were quantitatively measured using ELISA kits from Cusabio, China. The anti-C1q antibody levels were determined using an ELISA kit from Orgentec, Germany that quantitatively measures the IgG subclass of antibodies against C1q. Anti-nuclear antibody (ANA) titre was measured by indirect immunofluorescence technique using Hep-2 cell substrate (MBL, Japan). ANA with titres 1:80 and above were considered positive. Anti-dsDNA antibody was semi-quantitatively determined using the *Crithidia luciliae* indirect immunofluorescence test (CLIFT) method (MBL, Japan). Anti-dsDNA with titres 1:10 were considered positive and the results were reported in titres from 1:10 to 1:160. Serum C3 and C4 level analyses were done using quantitative determination by immunonephelometry (BN-ProSpec, Siemens, USA). Serum C3 and C4 levels were considered low at levels less than 0.66g/L and 0.20g/L, respectively. All analyses were carried out according to manufacturers' protocols.

Statistical analysis

Data entry and analysis was performed using Statistical Package for Social Sciences (SPSS) version 22. The Shapiro-Wilk's test was used to evaluate the data distribution and the median with interquartile range (IQR) of serum ICAM-1, VCAM-1 and anti-C1q antibody were determined. The median differences between ICAM-1, VCAM-1 and anti-C1q antibody levels in active and non-active SLE patients were performed using the Mann-Whitney test. Comparisons were considered significant if the p -value < 0.05. The area under the receiver operating characteristic curve (AUC-ROC) analysis was performed to determine the ICAM-1, VCAM-1 and anti-C1q

antibody optimal cut-off values and their accuracy (sensitivity and specificity) in discriminating between active and non-active SLE.

Results

Demographic and clinical data

Ninety-one (95.8%) of the SLE patients were female and 90 (94.7%) were of Malay ethnicity. The majority of the patients were young adults with more than half aged between 20 and 35 years old. Of the 95 SLE patients, 55 (57.9%) patients had lupus nephritis. Four active SLE patients were not on treatment. Two were newly diagnosed cases awaiting treatment commencement, while the other two were defaulters. Two non-active SLE patients were stable and did not require treatment. The demographic and clinical data for the patients is summarised in Table 1.

Prevalence of ANA, anti-dsDNA, serum C3 and C4 in SLE patients

ANA was positive in 36 (68.9%) of active SLE and 35 (70.0%) of non-active SLE patients. Anti-dsDNA antibody was positive in 29 (64.4%) of active SLE and 12 (24.0%) of non-active SLE patients. Twenty-six (57.8%) of active and six (12%) of non-active SLE patients had low serum C3 levels. Low serum C4 levels in active SLE and non-active SLE patients were 33 (73.3%) and 24 (48%), respectively.

Serum VCAM-1 and anti-C1q levels were higher in active SLE patients

No significant difference was observed between the levels of serum ICAM-1 in active and non-active SLE patients (p -value = 0.193). However, the median of serum VCAM-1 level differed significantly between the active and non-active SLE groups (p -value = 0.005). Serum VCAM-1 level was higher in active SLE patients (34.53, IQR 25.83 ng/mL) compared to non-active SLE patients (27.75, IQR 20.76 ng/mL). Serum anti-C1q level was significantly higher in active SLE than non-active SLE patients (19.67, IQR 43.59 U/mL vs 2.75, IQR 5.69 U/mL; p -value < 0.001). A summary of the comparisons between levels of serum markers in active SLE and non-active SLE groups is shown in Table 2.

Table 1: Demographic and clinical variables of active SLE and non-active SLE patients

Variables	Active SLE <i>n</i> = 45; <i>n</i> (%)	Non-active SLE <i>n</i> = 50; <i>n</i> (%)
Age (years)	26.00 (8.57) ^a	31.32 (8.63) ^a
< 20	12 (26.7)	6 (12.0)
20–35	26 (57.8)	27 (54.0)
> 35	7 (15.5)	17 (34.0)
Sex		
Female	42 (93.3)	49 (98)
Race		
Malay	44 (97.8)	46 (92.0)
Others	1 (2.2)	4 (8.0)
Disease duration (years)	1.00 (2.75)	6.50 (7.50)
Clinical features		
CNS	14 (31.1)	0 (0.0)
Vasculitis	6 (13.3)	0 (0.0)
Musculoskeletal	8 (17.8)	0 (0.0)
Renal	36 (80.0)	10 (20.0)
Mucocutaneous	25 (55.6)	1 (2.0)
Serositis	3 (6.7)	0 (0.0)
Fever	13 (28.9)	0 (0.0)
Haematological	15 (33.3)	2 (4.0)
Medications	41 (91.1)	48 (96.0)
Corticosteroids	41 (91.1)	39 (78.0)
Antimalarials	27 (60.0)	30 (60.0)
Immunosuppressants	18 (40.0)	16 (32.0)
Azathioprine	15 (33.3)	10 (20.0)
Cyclophosphamide	2 (4.4)	4 (8.0)
Mycophenolate mofetil (MMF)	2 (4.4)	3 (6.0)

^aMean (SD)**Table 2:** Summary of comparison between levels of serum markers in active SLE and non-active SLE groups

Variables	Active SLE (<i>n</i> = 45) Median (IQR)	Non-active SLE (<i>n</i> = 50) Median (IQR)	Z statistic ^a	<i>p</i> -value ^a
ICAM-1 (ng/mL)	194.41 (330.48)	146.62 (88.11)	-1.301	0.193
VCAM-1 (ng/mL)	34.53 ^a (25.83)	27.75 ^a (20.76)	-2.840	0.005
Anti-C1q (U/mL)	19.67 ^a (43.59)	2.75 ^a (5.69)	-4.625	< 0.001

^aMann-Whitney test, *p*-value < 0.05 is significant. IQR, interquartile range

Determination of VCAM-1 and anti-C1q cut-off levels

The AUC for ICAM-1, VCAM-1 and anti-C1q antibody were determined using ROC methodology. The AUC of the three markers are shown in Table 3. All possible combinations of sensitivity versus1-specificity that could be achieved by changing the threshold value were summarised using the AUC-ROC. VCAM-1 and anti-C1q antibody significantly discriminate between active SLE and non-active SLE patients (Figure 1). The optimal cut-off values for VCAM-1 and anti-C1q antibody in discriminating active SLE and non-active SLE were 30.5 ng/mL (69.0% sensitivity, 60.0% specificity, PPV 58.5%, NPV 66.7%) and 7.86 U/mL (75.6% sensitivity, 80% specificity, PPV 77.3%, NPV 78.4%), respectively (Table 4).

Discussion

Current routine tests for determining SLE disease activity include anti-dsDNA antibody, serum C3 and C4 levels, which are also listed in the SELENA-SLEDAI criteria. The prevalence of anti-dsDNA antibody in SLE patients ranged from 36 to 69% (24). In this study, the anti-dsDNA antibody levels were positive in 41 (43.2%) SLE patients with no false positive results based on the revised ACR classification criteria. This result

is comparable to the gold standard FARR-RIA technique, and in agreement with other studies using the CLIFT method (25). Serum complement levels were low in SLE with a higher percentage seen in serum C4 (73.3%) than in serum C3 (57.8%). However, nearly half of non-active SLE patients also had low serum C4, indicating that it was not useful in discriminating between active and non-active SLE. Serum C3 was better at reflecting disease activity as it was found to be low in only 12.0% of non-active SLE patients.

In this study the levels of ICAM-1, VCAM-1 and anti-C1q antibody were higher in active than non-active SLE patients. However, the differences were only significant for VCAM-1 and anti-C1q antibody. The finding on ICAM-1 was in accordance with some previous studies (26, 27), even though other studies have reported that ICAM-1 showed a statistically significant elevation in active SLE patients (15–17). The binding of the functionally active soluble ICAM-1 to their respective ligands on activated leukocytes might explain the insignificant increased of ICAM-1 in active compared to non-active SLE (28). One study found that a circadian variation exists for ICAM-1. Therefore, inconsistent blood-sampling times might contribute to different results produced in different ICAM-1 studies (29). The range of serum ICAM-1 in this study differed from those reported in previous ICAM-1 studies. This difference was most likely due to the use of immunoassay from

Table 3: The AUC-ROC of ICAM-1, VCAM-1 and anti-C1q antibody

Variables	ROC		
	AUC	p-value	95% Confidence interval
ICAM-1 (ng/mL)	0.578	0.193	-
VCAM-1 (ng/mL)	0.669	0.005	0.56, 0.78
Anti-C1q (U/mL)	0.766	< 0.001	0.68, 0.87

AUC is significantly different at $p < 0.05$

Table 4: Cut-off value and diagnostic accuracy of VCAM-1 and anti-C1q antibody in discriminating between active and non-active SLE

Variables	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
VCAM-1	30.5 ng/mL	69.0	60.0	58.5	66.7
Anti-C1q	7.86 U/mL	75.6	80.0	77.3	78.4

PPV-positive predictive value; NPV-negative predictive value

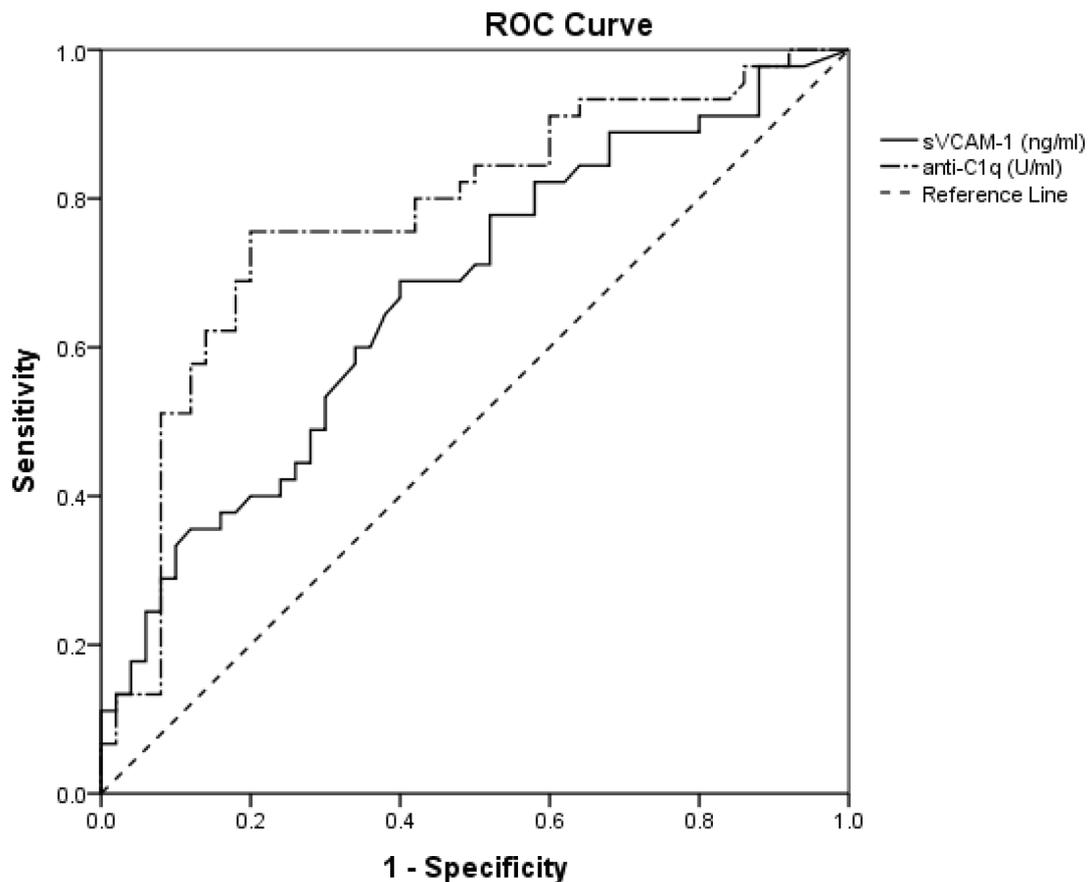


Figure 1: ROC curves obtained for VCAM-1 and anti-C1q antibody

different manufacturers. The non-standardised immunoassays available for serum ICAM-1 were reflected in the different range of readings and reported units in different studies (13, 15, 17).

Serum VCAM-1 levels in this study were significantly higher in active SLE than non-active SLE patients. This finding agreed with other studies which had collectively shown that VCAM-1 elevations were consistently observed during SLE flares (13, 28, 30, 31). However, no significant difference was found in VCAM-1 levels between SLE patients with and without lupus nephritis. The range of VCAM-1 values in this study was basically lower than reported in previous studies. One study in the United States found that the levels of circulating endothelial cell adhesion molecules were significantly lower in blacks compared to whites (32). Another study of random nuclear families in Russia showed significant familial, genetic and environmental effects regarding the variation of VCAM-1 (33). These studies suggested that VCAM-1 baseline levels differed in different

study populations. Therefore, the lower range of serum VCAM-1 level in this study might be due to the different study population where the majority of the subjects were of Malay ethnicity.

Despite having been widely researched, the role of anti-C1q antibody in SLE remains uncertain. In this study, anti-C1q antibody levels differed significantly between active and non-active SLE patients. According to the cut-off value given by the manufacturer (10 U/mL), it was found that 30 (66.7%) active SLE patients had high anti-C1q antibody levels compared to only nine (18.0%) non-active SLE. These findings were similar to other studies that found a higher prevalence of anti-C1 antibody in active SLE than in non-active SLE (21, 34, 35). In this study, the prevalence of anti-dsDNA and anti-C1q antibodies in active SLE were comparable (64.4% vs 66.7%). Twenty-one (46.7%) of active SLE patients were positive for both autoantibodies, while eight (17.8%) were positive for either one. These findings showed that anti-C1q antibody can potentially provide

additional information in SLE patients with negative anti-dsDNA antibodies. The prevalence of anti-C1q antibody in this study was 41.1%, which was in accordance with studies done in Brazil (39.5%) and India (58.3%). The variations found might be due to the studied populations, as well as the different ELISA kits used (23). Anti-C1q antibody was also found in healthy populations, with the prevalence ranging between 2.0 and 8.0% (36). Many previous studies have found that anti-C1q antibody is a useful marker for SLE with renal involvement (lupus nephritis) (20, 37). However, other studies proved that anti-C1q antibody was not significantly associated with lupus nephritis (21, 22, 35). This current study showed that there was no significant difference between anti-C1q antibody levels in lupus nephritis and non-lupus nephritis patients. A meta-analysis study on anti-C1q antibody in lupus nephritis concluded that it should be used as part of a panel of serological tests, rather than as an independent assay. Anti-C1q assay also needed further refinement, so that a single assay with greater sensitivity and specificity can be adopted by diagnostic laboratories for routine clinical use (38, 39).

The ROC curves were constructed to see the ability of each marker to discriminate between active and non-active SLE patients. Both VCAM-1 and anti-C1q antibody were able to discriminate between active and non-active SLE patients. The optimal cut-off values for VCAM-1 and anti-C1q were determined according to the left shoulder tip of their respective ROC curves. Based on this study, the VCAM-1 cut-off value of 30.5 ng/mL provided 69.0% sensitivity, 60.0% specificity, 58.5% PPV and 66.7% NPV. Anti-C1q antibody cut-off value of 7.86 U/mL provided 75.6% sensitivity, 80.0% specificity, 77.3% PPV and 78.4% NPV for this purpose. Based on this study, anti-C1 antibody was superior to VCAM-1 in differentiating between active and non-active SLE cases. Despite many studies suggesting anti-C1q antibody as a specific marker for detecting lupus nephritis flare, this finding showed its potential as a global SLE flare marker as well.

ROC curves constructed for serum C3 and serum C4 in this study showed neither parameters was useful in discriminating between active and non-active SLE. The ROC curve for anti-dsDNA antibody in this study was unable to give a reliable result for comparison in view of its semi-quantitative results (titre).

Conclusion

The serum levels of VCAM-1 and anti-C1q antibody were significantly higher in active SLE than non-active SLE patients. This study also supported the ability of serum VCAM-1 and anti-C1q antibody to discriminate between active SLE from non-active SLE. Anti-C1q antibody was better at discriminating between the two groups and provided additional information, particularly in active SLE patients with negative anti-dsDNA antibodies. Therefore, it may be included as one of the laboratory test panels in determining whether an SLE patient is in an active or non-active state.

Acknowledgements

Special thanks to the staff of the Department of Immunology, School of Medical Sciences, Universiti Sains Malaysia.

Conflict of Interest

None.

Funds

This study was funded by the Universiti Sains Malaysia, Short Term Grant 304/PPSP/661312079.

Authors' Contributions

Conception and design : WZWAH, HM
Analysis and interpretation of the data: HM
Drafting of the article: HM
Critical revision of the article for important intellectual content: NKY, WZWAH
Final approval of the article: WZWAH
Provision of study materials or patients: WSWG, AMI
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