THE PREVALANCE OF ANTI-C1q ANTIBODIES IN BLACK SOUTH AFRICANS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND THEIR CLINICAL SIGNIFICANCE

Mohamed Amin Makda

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DECLARATION

I, Mohamed Amin Makda declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Internal Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

.....

.....day of.....2012

For my family – my inspiration, my world.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS PROJECT

Oral Presentations:

Makda, MA., Tikly, M., Skattum, L. The prevelance of anti-C1q antibodies in Black South Africans with systemic lupus erythematosus ant their clinical significance. South African Arthritis and Rheumatism Association congress, Muldersdrift, September 2011.

ABSTRACT

INTRODUCTION: Several studies have shown an association of anti-C1q antibodies (abs) with systemic lupus erythematosus (SLE) nephritis and disease activity. The aim of this study is to determine the relevance of the anti-C1q abs and the C1q levels, in Black South Africans with SLE and their relevance to disease activity and/or organ damage, specifically renal disease.

METHODS: Serum anti-C1q abs and C1q levels were measured in 96 SLE patients who were also assessed for disease activity, using the SELENA Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), and organ damage as measured by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC) Damage Index. Furthermore patients were assessed for the presence of an active urine sediment as evidenced by otherwise unexplained proteinuria, haematuria or cellular casts. Serum anti-C1q abs was measured by a commercial Elisa kit and serum C1q by immunoelectrophoresis.

RESULTS: Of the 96 patients; the majority, 87 were female (90.65%), with a mean (SD) age and disease duration (SD) of 38.1 (13.0) years and 4.2 (4.4) years respectively. An active urine sediment was found in 21 (21.88%) patients. Elevated anti-C1q abs were present in 12 (12.50%) of the patients and 7 (14.29%) of the patients with renal involvement. Serum anti-C1q abs levels correlated significantly with SELENA SLEDAI scores ($p=0.004$, $r=0.41$).Anti-C1q abs levels were significantly higher in patients with an active urine sediment ($p= 0.007$). C1q levels were decreased in 17/96 (17.71%) patients and 11/49 (22.45%) patients with renal involvement. No associations with any other clinical features were observed.

CONCLUSION: The findings indicate that in Black South Africans with SLE, although elevated anti-C1q abs levels were present in only a small minority of patients, the abs were associated with SLE global activity as determined by the SELENA SLEDAI and to the presence of an active urine sediment. These findings suggest that anti-C1q abs are a potential bio-marker of disease activity, especially active renal disease.

ACKNOWLEDGEMENTS

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Lastly, to my family, the centre of my universe – you guys make everything worthwhile.

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NOMENCLATURE

Abs	Antibodies
AC	Apoptotic Cells
ACR	American College of Rheumatology
ANA	Anti-nuclear antibodies
Anti-C1q abs	Anti complement factor 1q antibodies
BSA	Black South African
C1q	Complement factor 1q
dsDNA	Double stranded deoxyribonucleic acid
RNP	Ribonucleoprotein
SLE	Systemic lupus erythematosus
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
SLICC-DI	Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index

Chapter 1. INTRODUCTION

1.1 History of systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a systemic autoimmune disorder, which is associated with an excess of autoantibodies. The disease is the result of a complex interaction of genetic factors, autoantibodies, hormones and environmental factors (Isenberg, 1997) . Tissues and cells are damaged by the deposition of pathogenic autoantibodies and immune complexes, any organ system may be affected and the disease may be easily confused with a variety of infectious, inflammatory, malignant or metabolic disorders

Although SLE is widely recognized today, initial descriptions probably go back as far as Hippocrates (~400BC) and Paracelcus (~1500AD). The word lupus (Latin for wolf) itself was first used to describe certain skin lesions which had the appearance of having been caused by the gnawing of a wolf, after the fall of the Roman Empire. One of the earliest reports is from 916 A.D. in the description of Eraclius, Bishop of Liege, at the shrine of St. Martin in Tours (Smith and Cyr, 1988). Subsequently the term 'Lupus Erythemateux' was coined in the 1850s by the Parisian physicians Cazenave and Clausit in Paris, at the Saint Louis Hospital for skin diseases (Wallace and Lyon, 1999).

Moriz Kaposi was the first physician to recognize more than just the skin disease among patients with SLE, when in a 1872 article he stated that “grave and even dangerous constitutional symptoms may be intimately associated with the process in question (lupus erythematosus), and that death may result”.

The first mention of SLE as we know it was by Sir William Osler in 1895, when he described 29 young ladies with skin rashes and chest pain from inflammation of the lining of the lung (pleurisy) or heart (pericarditis). These patients also had kidney disease,

strokes and brain involvement severe enough to be fatal, so that the majority had succumbed to their disease within two years (Osler, 1976).

During the passage of the twentieth century, several revolutionary immunological phenomena were described in SLE, sparked by Hargraves' discovery of the LE cell in 1948, which set the stage for the study of serology among SLE patients. This culminated in the discovery of the lupus anticoagulant by Conley and Hartman in 1952, and antinuclear antibodies (ANA) by Miescher and Fauconnet in 1954 (Wallace and Hahn, 2002). All this has led to the description of the disease as we know it today, yet the diagnosis of SLE can still be as challenging as ever.

1.2 Epidemiology

1.2.1 Global overview of SLE

SLE is predominately a disease of women in their reproductive years; it has a peak age of onset between the late teens and early 40s, and a female to male ratio of 9:1 (Wallace and Hahn, 2002). The disease occurs worldwide in all population groups, however the prevalence varies between racial groups and between countries, with most studies indicating that the incidence is highest in individuals with African or Asian ancestry.

The incidence of SLE in the general population varies between 3.3 and 8.7/100 000 people (see Table 1) depending on the characteristics of the population being studied (i.e., age, gender, race, ethnic/national origin or period of time studied).

Table 1.1 – Incidence of SLE in selected countries

Location	Incidence*
Iceland (Gudmundsson and Steinsson, 1990)	3.3
United Kingdom (Hopkinson et al., 1993)	3.7
Brazil (Vilar and Sato, 2002)	8.7

* Incidence rates per 100 000 persons per year, including men and women.

Prevalence studies in the general population have also shown a marked variation from 12 to 254 per 100 000 people (Wallace and Hahn, 2002). This variability may be due to the factors listed above, or from methodological differences in case ascertainment and socioeconomic factors. However, true geographical differences cannot be dismissed and may result from disparities in genetic or environmental factors.

Not only are there marked differences in the incidence and prevalence rates, but there appears to be true inter-ethnic variances in the clinical expression of SLE. Patients of African origin have a higher prevalence of discoid lupus and lupus nephritis (Cooper et al., 2002).

1.2.2 SLE in Africa

There is currently no published data on the occurrence rates of SLE in South Africa. It was previously thought to occur less frequently in sub-Saharan African, due to the high prevalence of tropical infectious diseases, particularly malaria. Further confounding issues are the extremely poor access to skilled medical care, resulting in the under diagnosis and

under reporting in most of sub-Saharan Africa. Nevertheless, several studies have shown that patients of African extraction are at a greater risk of developing severe organ involvement, especially renal (Alarcon et al., 2001, Barr et al., 2003, Korbet et al., 2007). A study looking at mainly Black South African (BSA) at Chris Hani Baragwanath Hospital, showed that renal involvement was the only independent predictor for a fatal outcome in South African SLE patients (Wadee et al., 2007).

1.3 Aetiology and pathophysiology

The exact aetiology of SLE remains unknown. SLE occurs in a genetically susceptible individual, who is exposed to a combination of environmental and hormonal factors. The genetic susceptibility has been evident from the familial clustering of cases and the high monozygotic twin concordance rate; whilst increased oestrogenic activity likely plays a role, hence the higher rates in women (Watts et al., 2009). The most consistent environmental component is the exposure to ultra violet light, which is known to worsen both cutaneous and systemic disease (Watts et al., 2009). Furthermore, exposure to a large number of viral infections has been investigated as infectious triggers, although none have been conclusively implicated.

What is known with certainty is that SLE is a multi-systemic autoimmune disease categorized by the production of various autoantibodies and immune complex deposition. A long standing plausible explanation for the autoantibodies found in SLE has been the aptly named ‘waste-disposal hypothesis’ (Pickering et al., 2000). This hypothesis is based on the observation that many of the auto antigens found in SLE, are found on the surface of apoptotic cells (AC) and that impaired clearance of AC is observed in experimental models of SLE. It is this inefficient clearance of AC that provides the almost limitless

antigenic potential for the development of autoantibodies. AC are usually cleared away by the complement system (Lu et al., 2008), which is a complex protein cascade serving a role in the host defence as well as clearance of immune complexes and AC.

1.3.1 Autoantibodies in SLE

The clinical diverse presentation of SLE is only surpassed by the number of autoantibodies described in this condition. There are well over a 100 autoantibodies described, the number steadily increasing each year. ANA are antibodies (abs) developed against nuclear self-antigens and are almost universal in SLE, their absence making the diagnosis doubtful (Zwart et al.). Autoantibodies in SLE can be broadly divided into 3 groups, dependent on the antigen they are directed against; those against nuclear antigens like anti-double stranded DNA (dsDNA), those against serum proteins like anti-complement factor 1q (anti-C1q), and those against membrane phospholipids, like anticardiolipin abs. Apart from the diagnostic value of the ANA, which is positive in over 95% of patients with SLE (Fernando and Isenberg, 2005), several of the abs aid in evaluating organ specific SLE disease.

Anti-dsDNA abs are present in about 60% of patients (Fernando and Isenberg, 2005), are highly specific for SLE and are considered to be the best predictors of a renal flare (Bootsma et al., 1997, Linnik et al., 2005), while several other abs are present in significantly lower numbers of patients (see Table 1.2).

Table 1.2 – Significances of antibodies in SLE (Watts et al., 2009).

Antibody Target	Prevalence in SLE	Clinical Association
Smith (Sm)	~5%(Caucasian) ~40%(Afro-Caribbean)	Vasculitis, CNS lupus.
Ro	~40%	Photosensitivity, rashes &congenital heart block.
La	~15	Sjogren's
U1 RNP	~30%	Raynaud's phenomenon

What has also been clearly shown, is that the prevalence of these autoantibodies has an ethnic variability. A study done at our centre showed that BSA with SLE had a higher prevalence of anti-Sm and anti- ribonucleoprotein (RNP) abs, a finding similar to that found in African-Americans and Afro-Caribbeans (Tikly et al., 1996).

1.3.2 The Complement System

The complement system is a group of over 20 biologically active proteins, mostly produced in the liver, named after its function to "complement" abs in the defense against microbial infection. Consequently, it serves an important function in host defense; however uncontrolled activation can result in tissue injury, as is the case in autoimmune diseases like SLE.

The complement system can be activated via 3 separate pathways namely, the classic pathway, the alternative pathway and the lectin pathway. The classical pathway is predominantly activated via the binding of immune complexes to complement factor 1q (C1q) (Reid, 1986). This binding causes sequential activation and amplification of other complement factors ultimately leading to the formation of a membrane attack complex; the function of which is to create a pore in the microbial cell wall causing lysis (Fig 1.1).

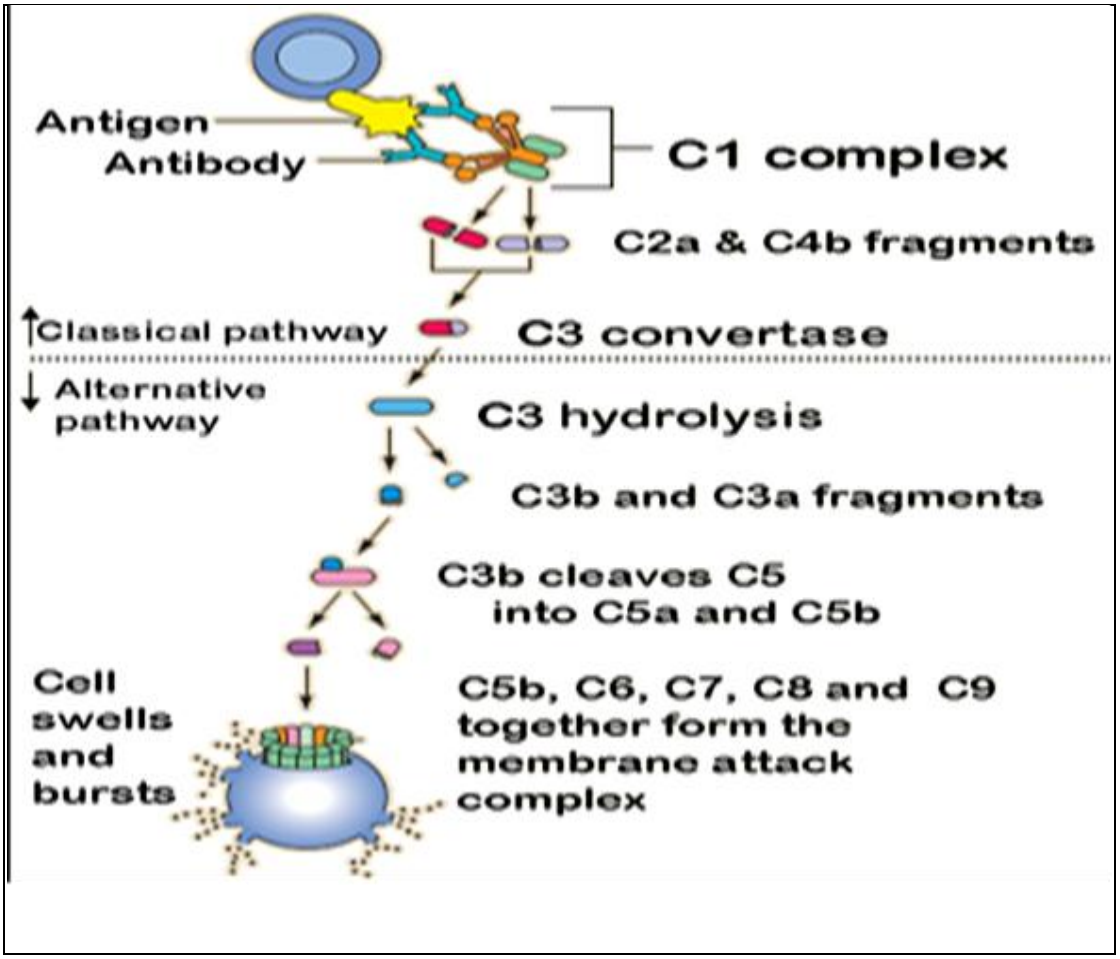

Figure 1.1 The Complement Pathway. (Source, Wikipedia - http://en.wikipedia.org/wiki/Alternative_complement_pathway)

1.3.3 Structure and function of C1q

C1q is a 460 kDa molecule that comprises of six subunits. Each subunit comprises of three polypeptide chains – A, B, and C, each chain in turn comprising of 3segments; a collagen like region, a triple helix region and a carboxy-terminal globular head. The six subunits are brought together into a 3 dimensional structure that resembles a bunch of tulips (Fig 1.2).

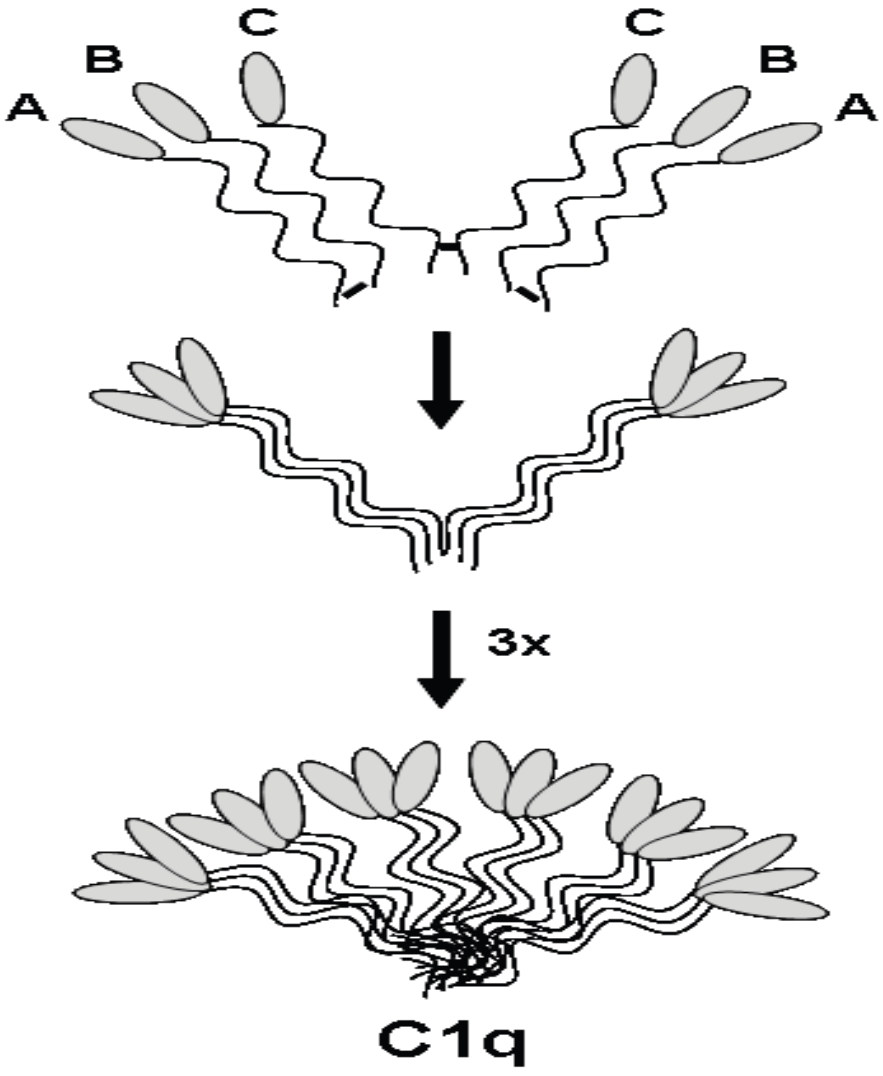

Figure 1.2 Structure of C1q (Lu et al., 2008).

Under normal physiological conditions, free C1q is not found. Rather, it exists as part of a larger complex – the C1qrs complex; consisting of 1 C1q molecule and 2 molecules each of the serine esterases C1r and C1s. The initial activation step is the binding of immune complexes to two or more of the globular head regions of C1q within the C1qrs complex. This results in a conformational change in the C1qrs complex, leading to the activation of the serine esterases C1r and C1s and subsequently activation of the rest of the cascade. Furthermore, C1q is instrumental in the removal of AC. This can be accomplished via 2 mechanisms; directly or indirectly via complement activation (Lu et al., 2008). C1q binds to calreticulin in the surface blebs on AC, but not onto normal cells, thus opsonizing the AC for enhanced phagocytosis (Korb and Ahearn, 1997). Via this mechanism C1q directly opsonizes the AC, without the activation of complement.

Alternatively, C1q can cause opsonization via complement activation. This occurs when polyclonal IgM binds to AC and then recruits C1q, with the subsequent activation of the classic complement pathway (Zwart et al., 2004). This may also occur with C-reactive protein binding to phosphorylcholine on AC, then recruiting C1q and the subsequent complement activation (Kim et al., 2003).

1.3.4 History of anti-C1q antibodies

During the 1970s, several tests were developed to measure immune complexes in diseases in which it was thought they played an important role. Of importance in the field of SLE, one of these tests was the solid phase C1q-binding assay; done by incubating the sera of SLE patients in plates coated with C1q (Agnello et al., 1971). In addition to binding immune complexes, it was subsequently shown that non-complexed IgG from the serum of SLE patients could also bind to the solid-phase C1q (Antes et al., 1988). This discovery

paved the way for the test to be specifically adapted to measure the levels of serum anti-C1q abs. As simple assays to detect anti-C1q abs have become available, they have been used in SLE and several other autoimmune conditions.

Anti-C1q abs have been detected in Felty's syndromre, rheumatoid vasculitis and hypocomplementeamic urticarial vasculitis syndrome (Pickering and Botto, 2010). Thus anti-C1q abs have shown a low specificity for a particular disease.

In SLE, several different sub-classes of IgG anti-C1q abs have been found, although no clinical differences between the classes have so far been reported (Schaller et al., 2009). Anti-C1q abs have been found in renal autopsy specimens from SLE patients with proliferative renal disease, at up to 50 fold greater concentrations than serum concentrations (Mannik and Wener, 1997). They have been shown to correlate with active proliferative lupus nephritis and most studies suggest that severe lupus nephritis is improbable in the absence of anti-C1q abs (Trendelenburg et al., 2006, Trendelenburg et al., 1999). Furthermore, anti-C1q abs are associated with hypocomplementaemia, especially with low levels of C1q, C3 and C4 (Siegert et al., 1991). Finally, hereditary C1q deficiency has been reported to be the greatest hereditary risk factor for the development of SLE (Schejbel et al., 2011).

1.3.5 Pathogenicity of anti-C1q antibodies

The mechanisms that cause immunogenicity of self antigens has not been fully elucidated. The most plausible explanation for the autoantibodies found in SLE has been the aptly named waste-disposal hypothesis (refer to pathogenesis). Taking into consideration this hypothesis, serum C1q would bind to early AC. Once bound, a conformational change

would occur in the C1q molecule, exposing a neoepitope. In a susceptible host, the impaired clearance of the C1q bound apoptotic cell, would lead to uptake of both into an antigen presenting cell. This process leads to the generation of autoantibodies against the apoptotic cell surface antigens, including C1q (Pickering and Botto, 2010). If this is superimposed onto the kidney during renal inflammation, immune complexes and apoptotic renal cells provide the binding sites for C1q. Once bound, C1q then attracts anti-C1q abs that amplify the complement pathway resulting in tissue damage and renal injury.

1.4 Aim of the study

The primary objective of the study was to determine the prevalence of the anti-C1q abs in BSA with SLE and the secondary objectives was to determine their clinical utility by examining their relationship to SLE disease activity and organ damage, specifically as a possible indicator for renal disease.

Chapter 2. PATIENTS AND METHODS

2.1 Study population

The study population comprised of 96 consecutive consenting BSA patients with SLE who were enrolled from the Connective Tissue Disease Clinic of the Chris Hani Baragwanath Hospital, Soweto. All patients were on optimal medical therapy. The patients were matched 1:1 for renal versus non-renal SLE involvement. Renal involvement was defined as fulfilling the renal criteria of the 1997 American College of Rheumatology (ACR) classification for SLE (Hochberg, 1997) at any point in the patient disease course. All patients were older than 18 years of age at disease onset and met the 1997 American College of Rheumatology (ACR) criteria for the classification of SLE (APPENDIX A). Informed consent was obtained from every subject. This study was approved by the University of the Witwatersrand Human Research Ethics Committee (APPENDIX B).

2.2 Clinical assessment

Clinical features were defined according to the 1997 ACR criteria; while autoantibody serology and complement levels were extracted from the clinical notes. An active urine was defined as the presence of an otherwise unexplained active urine sediment (cellular cast – red cell, haemoglobin, granular, tubular or mixed cast) or proteinuria (ACR., 2006).

All patients were assessed for disease activity, as measured by the SELENA Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (Petri et al., 1999); and organ damage as measured by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC) Damage Index (Gladman et al., 1996) (APPENDIX C and D). The SLEDAI and SLICC are 2 validated instruments used in the monitoring of patients with SLE (Griffiths et al., 2005).

2.3 Laboratory investigations

2.3.1 Serum Analysis

Serum was separated from a 5ml clotted blood sample and was stored at -20 degrees Celsius. All samples were then tested for anti-C1q abs by a commercial available enzyme linked immunoassay (ELISA) (Orgentec, Mainz, Deutschland). Concentrations of greater than or equal to 19 U/mL was considered positive. Samples that were found to be positive by ELISA were confirmed by Western blot using the complete C1q molecule under reducing conditions (Martensson et al., 1992). Serum C1q was measured as a percentage of normal by immunoelectrophoresis, accepting 78 to 131% as the normal; using an in-house rabbit-anti-C1q antiserum. The above tests were performed at the Clinical Immunology and Transfusion Medicine Laboratory, Laboratory Medicine Skåne, in Lund, Sweden.

2.4 Statistical Methods

Data was captured onto a Microsoft Excel spread sheet and then analysed using GraphPad InStat (version 3) software and Statistica Version 10.To compare continuous variables between groups, the 2 tail Student's t-test was used for normally distributed data and the Mann Whitney U test for non-normally distributed data, while the Spearman's correlation test was used to test the correlation of continuous variables. Categorical variables were analysed by the Chi-square test or the two-tailed Fisher's exact test where indicated.
A p-value of <0.05 was considered significant.

Chapter 3. RESULTS

3.1 Demographic and clinical features of SLE patients

As shown in Table 1, 90% of the patients were females with a mean age and disease duration of 38.1 and 4.2 years, respectively. The commonest extra-renal features in the overall group were arthritis in 59.4% and discoid lupus in 55.2% of patients. All patients were ANA positive and the most common lupus specific autoantibody was the anti-Sm abs in 52.1% of patients.

There were no significant differences in the clinical features between the renal and non-renal groups. However, the renal group had a higher prevalence of anti-dsDNA abs and C3/C4 hypocomplementaemia (either or both C3 or C4, at the closest time point to study enrolment), as well as higher mean SLEDAI scores, driven largely by the presence of an active urine sediment. As expected when correlating the SLEDAI scores and SLE disease duration, a correlation was found ($p<0.0001$) as shown in figure 3.1.

Of the 49 patients with renal disease, 41 patients had renal histology available. As shown in table 3.2, the most common histological subtype was Class V lupus nephritis (Weening et al., 2004) occurring in almost 35% of patients. There was no statistical difference in C1q or anti-C1q abs levels between histological subtypes.

Table 3.1 Clinical and serological features of renal versus non-renal groups.

Feature	Total(n=96)	Renal (n=49)	Non-Renal (n=47)	p value
Female sex	87 (90.7%)	44 (89.8%)	43 (91.5%)	NS
Age (years)-mean (SD)	38.1 (13.0)	35.7 (13.0)	40.7 (12.7)	NS
Disease (years) -mean (SD)	4.2(4.4)	4.5 (4.6)	4.0 (4.4)	NS
Malar rash	37 (38.5%)	17 (34.7%)	20 (42.6%)	NS
Discoid lupus	53 (55.2%)	25 (51.0%)	28 (59.6%)	NS
Oral ulcers	21 (21.9%)	10 (20.0%)	11 (23.4%)	NS
Photosensitivity	38 (39.6%)	19 (38.8%)	19 (40.4%)	NS
Arthritis	57 (59.4%)	25 (51.0%)	32 (68.1%)	NS
Serositis	11 (11.5%)	7 (14.3%)	4 (8.5%)	NS
Neurological disease	8 (8.3%)	4 (8.2%)	4 (8.5%)	NS
ANA positive	96 (100%)	49 (100%)	47 (100%)	NS
Anti-dsDNA abs positive	30 (31.3%)	22 (44.9%)	8 (17.0%)	0.01
Anti-Sm abs positive	50 (52.1%)	24 (49%)	26 (55.3%)	NS
Anticardiolipin abs positive	14 (14.6%)	9 (18.4%)	5 (10.6%)	NS
C3/C4 Hypocomplementaemia	17 (17.7%)	11 (22.5%)	3 (6.4%)	0.02
SLEDAI - mean (SD)	2.7 (5.6)	4.6 (7.2)	0.8 (1.8)	0.0006
SLICC - mean (SD)	0.7 (0.8)	0.7 (0.9)	0.6 (0.8)	NS
Active Urine Sediment - no. of patient (%)	21 (21.9%)	21 (42.9%)	0	

NS = not significant.

n= number of individuals with feature.

Table 3.2 Histological classes of lupus nephritis.

Class of Lupus Nephritis (Weening et al., 2004)	Total(n=49)
Class I	0
Class II	4 (8.2%)
Class II/V	3 (6.1%)
Class III	12 (24.5%)
Class III/V	2 (4.1%)
Class IV	3 (6.1%)
Class V	17 (34.7%)
Class VI	0
No Data	8 (16.3%)

Table 3.3 Serum analysis of renal versus non-renal groups.

Feature	Total(n=96)	Renal (n=49)	Non-Renal (n=47)	p value
Anti-C1q Abs positive -no. of patients (%)	12(12.5%)	7(14.3%)	5(10.6%)	NS
Anti-C1q (U/mL)- mean (SD)	10.7(14.2)	12.4(16.5)	9.0(11.1)	NS
Low C1q Level - no. of patients (%)	17(17.7%)	10(20.4%)	7(14.9%)	NS
C1q (% of normal) -mean (SD)	109.8%(41.4)	106.0%(56.1)	106.8%(30.1)	NS

NS = not significant.

n= number of individuals with feature

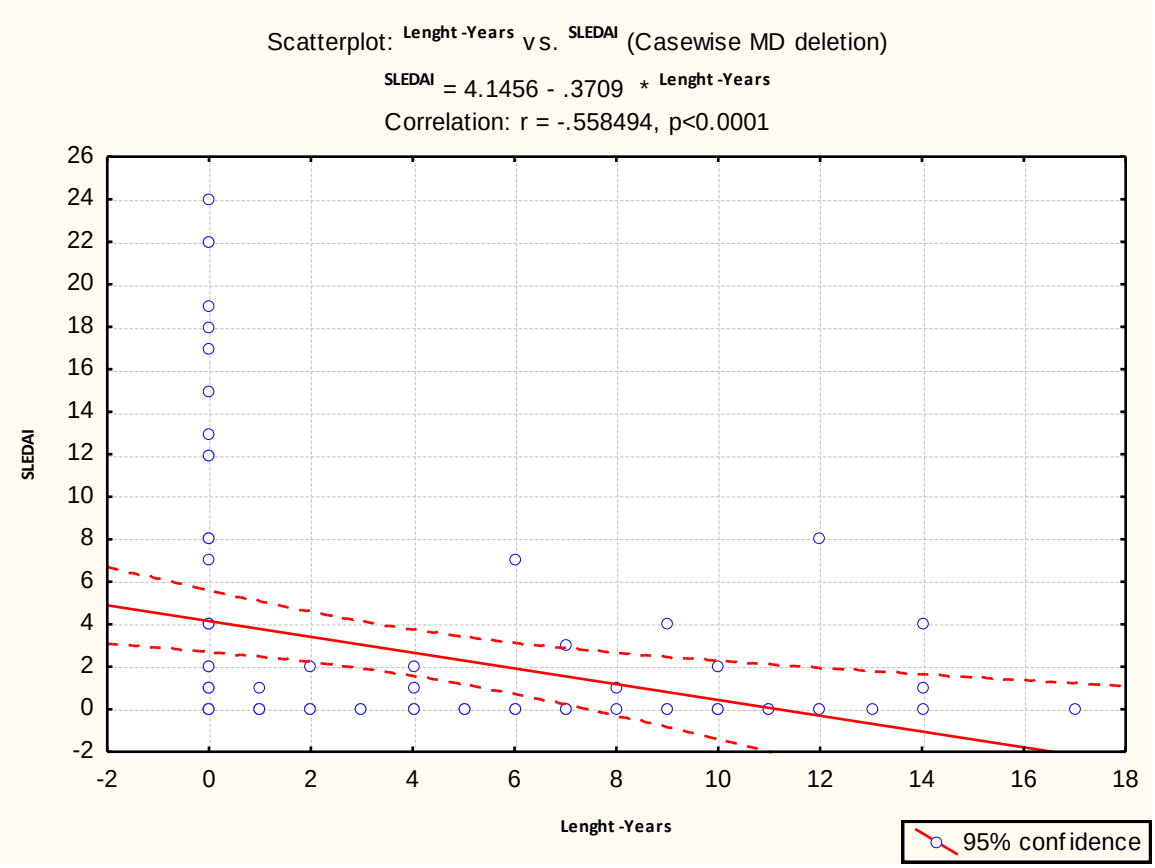

Figure 3.1 Scatterplot of SLEDAI score versus disease duration.

3.2 Clinical and laboratory correlates with anti-C1q abs

3.2.1 Comparison between serum anti-C1q abs levels and urine sediment

There was no significant difference in either anti-C1q abs positivity or titres between the renal and non-renal groups (see Table 3.3). Importantly however, the subgroup of renal patients with an active urine sediment had significant higher abs titres compared to the rest of the patients (mean (SD) = 18.37 (22.77) U/mL in the active urine sediment group versus a mean (SD) = 8.54 (9.79) U/mL in the remainder of the patients, p=0.007, see fig 3.2). Similarly the frequency of the abs was significantly higher in the active urine sediment group (5/21 in the active urine sediment group versus 7/75 in the remainder of the patients, p=0.045).

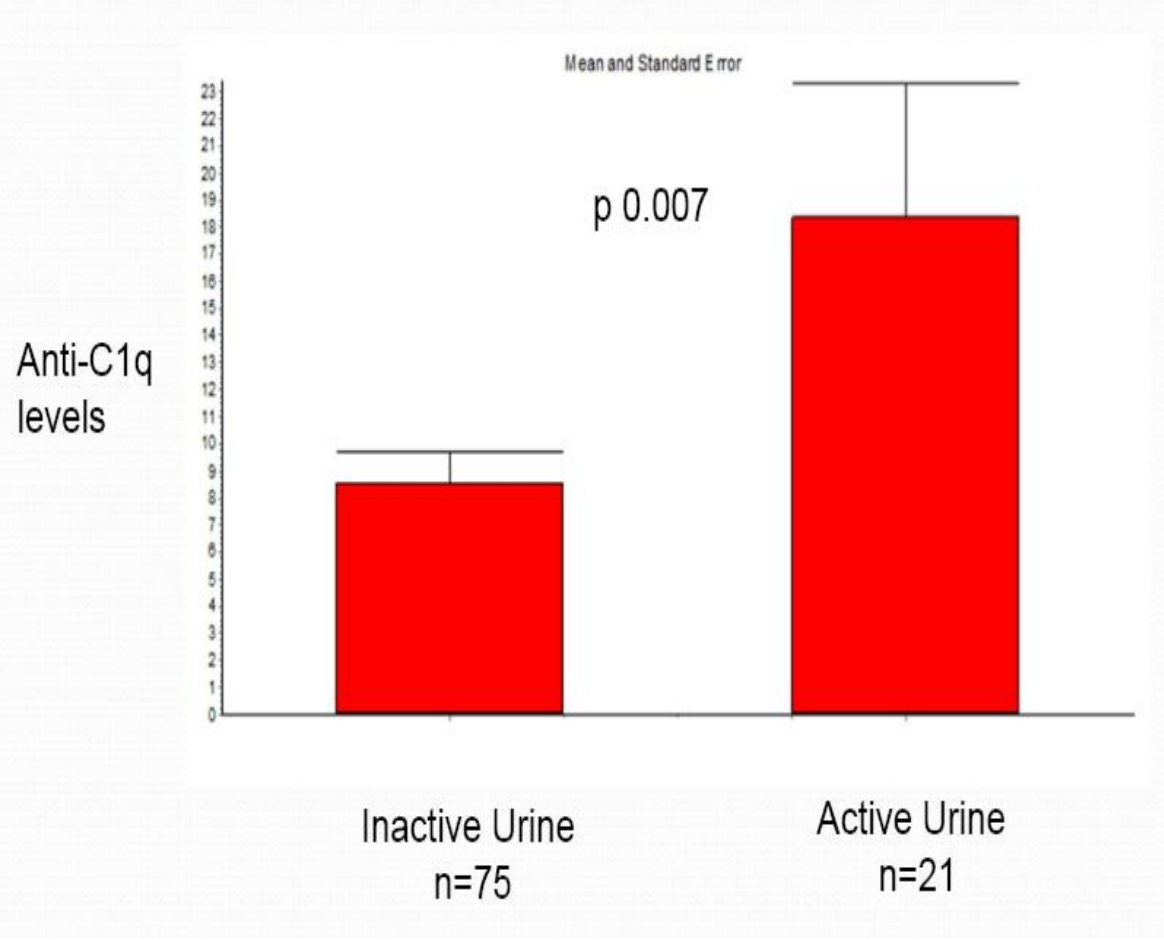

Figure 3.2 Anti-C1q abs levels versus urine sediment.

3.2.2 Correlation between anti-C1q abs and serum C1q levels

Overall C1q levels were low in 17.7% of the patients, with no apparent differences between the groups (see Table 3.3). Moreover, C1q levels were not significantly different between patients with an active urine sediment and the rest of the patients (mean (SD) = 114.76 (65.11) % in the active urine sediment group versus a mean (SD) = 108.07 (31.25) % in the remainder of the patients, p=0.51). No significant clinical or serological correlations, except for the weak correlation with anti-C1q abs (fig3.3), were observed with serum C1q levels.

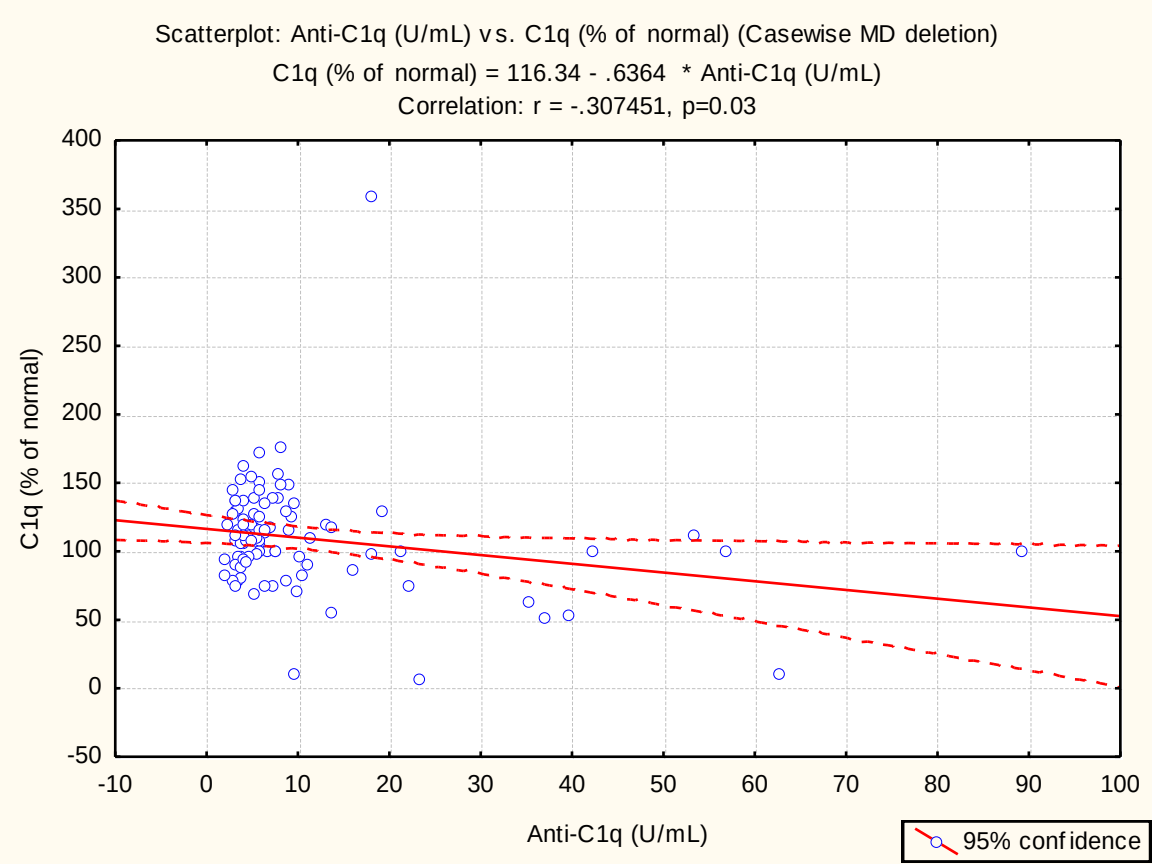

Figure 3.3 Scatterplot of C1q levels versus anti-C1q abs levels.

3.2.3 Correlation between serum anti-C1q abs levels and SLEDAI scores

The anti-C1q abs levels correlated significantly with the SLEDAI scores (fig 3.4). There were no other significant correlations with any other clinical (except urine sediment) or serological features; or with the SLICC-DI and the anti-C1q abs levels.

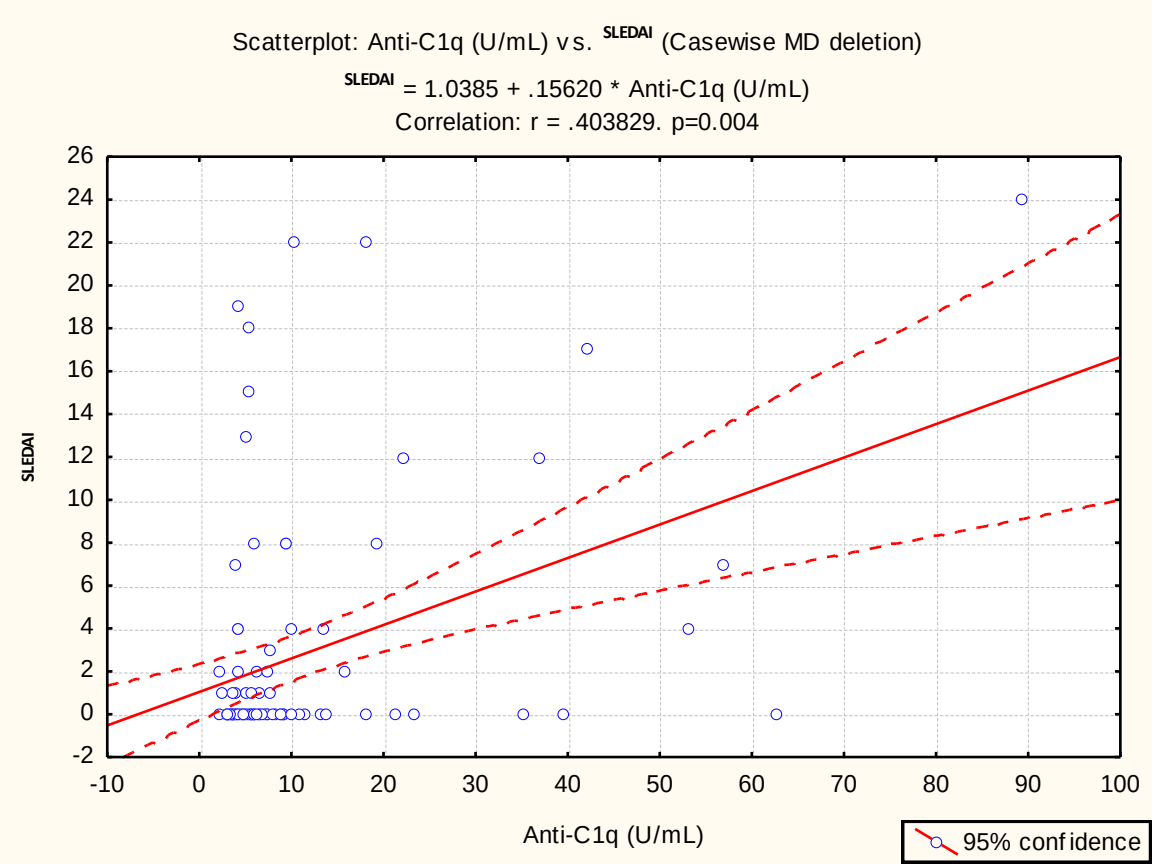

Figure 3.4 Scatterplot of anti-C1q abs levels versus SLEDAI scores.

Chapter 4. DISCUSSION

In this cross sectional study, anti-C1q abs were studied for their role in BSA with SLE, both for disease activity; in particular renal involvement, and disease damage.

The patients that were included in the present study were similar demographically, clinically and serologically as those in a large retrospective study of SLE at the same institution (Wadee et al., 2007). The sex distribution of 9:1 is similar to that seen elsewhere in the world (Wallace and Hahn, 2002).

The commonest clinical features were arthritis, occurring in almost 60% of patients, followed by discoid lupus in 55.2% of patients (see Table 3.1). Several studies have shown that discoid lupus is more common in Black Africans than in other population groups , in which it occurs in about 20% of patients (Wadee et al., 2007) (Cooper et al., 2002).

Since renal disease was a specific inclusion criterion in this study for half the patients, it is not possible to comment on its frequency in BSA based on the present study. However, nephropathy is a frequent complication occurring in up to 40% (Wadee et al., 2007) of patients in this population. That C3/C4 hypocomplementaemia and raised anti-dsDNA abs were commoner in the renal group (see Table 3.1), is in keeping with the notion that lupus nephritis is due to immune complex deposition, of which anti-dsDNA abs are an important constituent, with complement consumption (Bootsma et al., 1997, Fernando and Isenberg, 2005).

The inverse relationship of the SLEDAI scores with disease duration is of interest. It is well known that with time, in most cases disease active declines, partly because of therapy but also due to the natural course of the disease.

Overall, raised anti-C1q abs were found in only 14.2% of the 96 patients, with no statistical difference between the renal and the non-renal groups. The figure of 14.2% is numerically lower than reported in other studies, which have showed between a third to half of patients having raised anti-C1q abs levels (Pickering and Botto, 2010). There was also no difference in the absolute level of the anti-C1q abs between the two groups. Furthermore as expected, Western blot of anti-C1q abs done on the anti-C1q abs positive ELISA samples, were all are negative by Western blot; that is, do not bind any of separated C1q chains A, B or C (Martensson et al., 1992).

Furthermore, it was also confirmed that the level of anti-C1q abs correlated with the overall level of disease activity as measured by the SLEDAI score, and more specifically with the presence of an active urine sediment (Figure 3.2), leading to an association with currently active renal disease. A study published recently has shown similar results, that the anti-C1q abs levels are a good marker of a renal flare (Akhter et al., 2011).

As expected there was an inverse relationship between the level of anti-C1q abs and serum levels of C1q, which can easily be explained by the binding of the antibody to C1q with the subsequent consumption of C1q and deposition in the tissues. This is in keeping with previous reports (Siegert et al., 1991).

Importantly, no correlation was found between the level of anti-C1q abs and other measured variables, especially the level of C3/C4 as previously reported (Isenberg et al., 1997) (Cai et al., 2010). This may have been due to the type of test employed at our centre or a type II error because of the small sample size.

Finally, there was a complete lack of association between C1q levels and disease activity.

4.1 Limitations of the study

The cross sectional nature of the study did not allow for reporting on fluctuations in anti-C1q abs levels and its relationship to disease activity over time. The reference values for both C1q and anti-C1q abs levels are based on those from healthy European blood donors, which may be different from our population of BSA (Gould et al., 2006).

Although most of the patients with renal involvement had a biopsy, this was not available in all cases, furthermore due to factors beyond our control the complement levels and C1q and anti-C1q abs levels with not all taken at precisely the same point in time.

Finally, we did not look at the relationship of abs levels with corticosteroid / immunosuppressive therapy, which may have had an influence on the levels.

Chapter 5. CONCLUSION

The findings of this study are further evidence the anti-C1q abs may have an important role in the management of SLE patients. Although, elevated anti-C1q abs levels were present in only a small minority of patients, the findings indicate that in Black South Africans with SLE, anti-C1q abs were associated with SLE global activity as determined by the SELENA SLEDAI and more importantly, to the presence of an active urine sediment. These findings suggest that anti-C1q abs are a bio-marker of disease activity, especially active renal disease.

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APPENDIX A - 1997 American College of Rheumatology (ACR) classification for SLE (Hochberg, 1997)

Criterion	Definition
1. Malar Rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by physician
5. Nonerosive Arthritis	Involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Pleuritis or Pericarditis	1. Pleuritis--convincing history of pleuritic pain or rubbing heard by a physician or evidence of pleural effusion 1. *OR* 2. Pericarditis--documented by electrocardigram or rub or evidence of pericardial effusion
7. Renal Disorder	1. Persistent proteinuria > 0.5 grams per day or > than 3+ if quantitation not performed 1. *OR* 2. Cellular casts--may be red cell, hemoglobin, granular, tubular, or mixed
8. Neurologic Disorder	1. Seizures--in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance 1. *OR* 2. Psychosis--in the absence of offending drugs or known metabolic derangements, e.g., uremia, ketoacidosis, or electrolyte imbalance
9. Hematologic Disorder	1. Hemolytic anemia--with reticulocytosis 1. *OR* 2. Leukopenia--< 4,000/mm^3 on ≥ 2 occasions 1. *OR* 3. Lyphopenia--< 1,500/ mm^3 on ≥ 2 occasions 1. *OR* 4. Thrombocytopenia--<100,000/ mm^3 in the absence of offending drugs
10. Immunologic Disorder	1. Anti-DNA: antibody to native DNA in abnormal titer 1. *OR* 2. Anti-Sm: presence of antibody to Sm nuclear antigen 1. *OR* 3. Positive finding of antiphospholipid antibodies on: 1. 1. an abnormal serum level of IgG or IgM anticardiolipin antibodies, 2. 2. a positive test result for lupus anticoagulant using a standard method, or 3. 3. a false-positive test result for at least 6 months confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test
11. Positive Antinuclear Antibody	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs

APPENDIX B – Ethics Committee Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Makda

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M080237**
PROJECT	The Prevalence of Anti-C1q antibodies in Black South Africans with Systemic Lupus Erythematosis & Clinical significance
INVESTIGATORS	Dr MA Makda
DEPARTMENT	Rheumatology
DATE CONSIDERED	08.02.29
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 08.05.20 **CHAIRPERSON** .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof M Tikly

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX C - SELENA Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (Petri et al., 1999)

SELENA SLEDAI WORKSHEET

Rater Initials: ____ ____ ____

Visit:_____

Date of assessment: _____

Not Present	Present	Descriptor	Definition
☐ 0	☐ 8	Seizure	Recent onset (last 10 days). Exclude metabolic, infectious or drug cause, or seizure due to past irreversible CNS damage.
☐ 0	☐ 8	Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized, or catatonic behavior. Exclude uremia and drug causes.
☐ 0	☐ 8	Organic Brain Syndrome	Altered mental function with impaired orientation, memory or other intellectual function, with rapid onset and fluctuating clinical features. Include clouding of consciousness with reduced capacity to focus and inability to sustain attention to environment, plus at least two of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious of drug causes.
☐ 0	☐ 8	Visual Disturbance	Retinal and eye changes of SLE. Include cytoid bodies, retinal hemorrhages, serous exudate or hemorrhages in the choroid, optic neuritis, scleritis or episcleritis. Exclude hypertension, infection or drug causes.
☐ 0	☐ 8	Cranial Nerve Disorder	New onset of sensory or motor neuropathy involving cranial nerves. Include vertigo due to lupus.
☐ 0	☐ 8	Lupus Headache	Severe persistent headache: may be migrainous, but must be non-responsive to narcotic analgesia.
☐ 0	☐ 8	CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis or hypertensive causes.
☐ 0	☐ 8	Vasculitis	Ulceration, gangrene, tender finger nodules, periungual infarction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis.
☐ 0	☐ 4	Arthritis	More than 2 joints with pain and signs of inflammation (ie., tenderness, swelling, or effusion).
☐ 0	☐ 4	Myositis	Proximal muscle aching/weakness associated with elevated creatine phosphokinase/aldolase or electromyogram changes or a biopsy showing myositis.
☐ 0	☐ 4	Urinary Casts	Heme-granular or red blood cell casts.
☐ 0	☐ 4	Hematuria	> 5 red blood cells/high power field. Exclude stone, infection or other causes.
☐ 0	☐ 4	Proteinuria	New onset or recent increase of more than 0.5 gm/24 hours.
☐ 0	☐ 4	Pyuria	> 5 white blood cells/high power field. Exclude infection.

SELENA SLEDAI WORKSHEET(continued)

Rater Initials: ____ ____ ____

Visit:_____

Date of assessment: _____

Indicate if descriptor is not present or present at the time of visit or in the preceding 10 days.

Not Present	Present	Descriptor	Definition
☐ 0	☐ 2	Rash	Ongoing inflammatory lupus rash.
☐ 0	☐ 2	Alopecia	Ongoing abnormal, patchy or diffuse loss of hair due to active lupus.
☐ 0	☐ 2	Mucosal Ulcers	Ongoing, oral or nasal ulcerations due to active lupus.
☐ 0	☐ 2	Pleurisy	Classic and severe pleuritic chest pain or pleural rub or effusion or new pleural thickening due to lupus.
☐ 0	☐ 2	Pericarditis	Classic and severe pericardial pain or rub or effusion, or electrocardiogram confirmation.
☐ 0	☐ 2	Low Complement	Decrease in CH50, C3, or C4 below the lower limit of normal for testing laboratory.
☐ 0	☐ 1	Increased DNA binding	> 25% binding by Farr assay or above normal range for testing laboratory.
☐ 0	☐ 1	Fever	> 38°C. Exclude infectious cause.
☐ 0	☐ 1	Thrombocytopenia	< 100,000 platelets/mm^3.
☐ 0	☐ 1	Leukopenia	< 3,000 White blood cells/mm^3. Exclude drug causes.

_____ SELENA-SLEDAI Score

Please indicate Flare Status: ☐ No Flare ☐ Mild or Moderate Flare ☐ Severe Flare

Mild or Moderate Flare (Requires one of the criteria below. Check all that apply.)	**Severe Flare** (Requires one of the criteria below. Check all that apply.)
☐ Change in SLEDAI ≥ 3 points	☐ Change in SLEDAI > 12
New / Worse: ☐ Discoid, photosensitive, profundus, cutaneous vasculitis, bullous lupus ☐ Nasopharyngeal ulcers ☐ Pleuritis ☐ Pericarditis ☐ Arthritis ☐ Fever (SLE)	New / Worse: (Requiring: doubled prednisone OR Prednisone > 0.5 mg/kg/day) ☐ CNS-SLE ☐ Vasculitis ☐ Nephritis ☐ Myositis ☐ Platelet > 60,000/mm^3 mg/dL ☐ Hemolytic anemia: Hb < 7 mg/dL or decrease in Hb > 3 mg/dL ☐ Hospitalization for SLE
☐ Increase in Prednisone, to ≤ 0.5 mg/kg/day.	☐ Prednisone > 0.5 mg/kg/day
☐ Added NSAID or Plaquenil for disease activity.	☐ New cyclophosphamide, azathioprine, methotrexate, mycophenolate mofetil.
☐ ≥ 1.0 increase in Physician's Global Assessment.	☐ Increase in Physician's Global Assessment to > 2.5.

APPENDIX D - Systemic Lupus International Collaborating Clinics/American

College of Rheumatology (SLICC) Damage Index (Gladman et al., 1996)

SLICC/ACR WORKSHEET

Rater Initials: ____ ____ ____

Visit:_____

Date of assessment: _____

Note: Damage (nonreversible change, not related to active inflammation) occurring since onset of lupus, ascertained by clinical assessment and present for at least 6 months unless otherwise stated. Repeat episodes must occur 6 months apart to score 2. The same lesion cannot be scored twice.

Rater Initials: ☐☐☐ Date of Assessment: ___/___/___ (Mo / Day / Yr)

Item		Score	
		Not Present	Present
Ocular (either eye, by clinical assessment)	Any cataract ever	☐ 0	☐ 1
	Retinal change or optic atrophy	☐ 0	☐ 1
Neuropsychiatric	Cognitive impairment (e.g., memory deficit, difficulty with calculation, poor concentration, difficulty in spoken or written language, impaired performance levels) or major psychosis	☐ 0	☐ 1
	Seizures requiring therapy for 6 months	☐ 0	☐ 1
	Cerebrovascular accident (CVA) ever (score 2 > 1 CVA)	☐ 0	☐ 1 ☐ 2
	Cranial or peripheral neuropathy (excluding optic)	☐ 0	☐ 1
	Transverse myelitis	☐ 0	☐ 1
Renal	Estimated or measured glomerular filtration rate < 50 mL/min	☐ 0	☐ 1
	Proteinuria ≥ 3.5 gm/24 hours	☐ 0	☐ 1
	OR		
	End-stage renal disease (regardless of dialysis or transplantation)	☐ 0	☐ 3
Pulmonary	Pulmonary hypertension (right ventricular prominence, or loud P2)	☐ 0	☐ 1
	Pulmonary fibrosis (physical and radiograph)	☐ 0	☐ 1
	Shrinking lung (radiograph)	☐ 0	☐ 1
	Pleural fibrosis (radiograph)	☐ 0	☐ 1
	Pulmonary infarction (radiograph)	☐ 0	☐ 1
Cardiovascular	Angina or coronary artery bypass	☐ 0	☐ 1
	Myocardial infarction (MI) ever (score 2 if > 1 MI)	☐ 0	☐ 1 ☐ 2
	Cardiomyopathy (ventricular dysfunction)	☐ 0	☐ 1
	Valvular disease (diastolic murmur, systolic murmur > 3/6)	☐ 0	☐ 1
	Pericarditis for 6 months, or pericardiectomy	☐ 0	☐ 1

SLICC/ACR WORKSHEET

Rater Initials: ____ ____ ____

Visit:_____

Date of assessment: _____

Note: Damage (nonreversible change, not related to active inflammation) occurring since onset of lupus, ascertained by clinical assessment and present for at least 6 months unless otherwise stated. Repeat episodes must occur 6 months apart to score 2. The same lesion cannot be scored twice.

Item		Score	
		Not Present	Present
Peripheral Vascular	Claudication for 6 months	☐ 0	☐ 1
	Minor tissue loss (pulp space)	☐ 0	☐ 1
	Significant tissue loss ever (e.g., loss of digit or limb) (score 2 if > 1 site)	☐ 0	☐ 1 ☐ 2
	Venous thrombosis with swelling, ulceration, or venous stasis	☐ 0	☐ 1
Gastrointestinal	Infarction or resection of bowel below duodenum spleen, liver or gall bladder ever, for any cause (score 2 if > 1 site)	☐ 0	☐ 1 ☐ 2
	Mesenteric insufficiency	☐ 0	☐ 1
	Chronic peritonitis	☐ 0	☐ 1
	Stricture or upper gastrointestinal tract surgery ever	☐ 0	☐ 1
	Pancreatic insufficiency requiring enzyme replacement or with pseudocyst	☐ 0	☐ 1
Musculoskeletal	Muscle atrophy or weakness	☐ 0	☐ 1
	Deforming or erosive arthritis (including reducible deformities, excluding avascular necrosis)	☐ 0	☐ 1
	Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)	☐ 0	☐ 1
	Avascular necrosis (AVN) (score 2 if > 1 AVN)	☐ 0	☐ 1 ☐ 2
	Osteomyelitis	☐ 0	☐ 1
	Ruptured tendons	☐ 0	☐ 1
Skin	Scarring chronic alopecia	☐ 0	☐ 1
	Extensive scarring of panniculum other than scalp and pulp space	☐ 0	☐ 1
	Skin ulceration (excluding thrombosis) for > 6 months	☐ 0	☐ 1
Other	Premature gonadal failure	☐ 0	☐ 1
	Diabetes (regardless of treatment)	☐ 0	☐ 1
	Malignancy (exclude dysplasia) (score 2 if > 1 site)	☐ 0	☐ 1 ☐ 2