Total plaque area and plaque echogenicity are novel measures of subclinical atherosclerosis in patients with systemic lupus erythematosus

Sara C Croca MD, PhD[1]

Maura Griffin MSc, DIC, PhD [2]

Filipa Farinha MD[1]

David A Isenberg FAMS, MD, FRCP[1]

Andrew Nicolaides MS, FRCS, PhD (Hon) [3]

Anisur Rahman PhD, FRCP[1]

[1] Centre for Rheumatology Research, Division of Medicine, University College London, 5 University Street, London WC1E 6JF, UK

[2] Vascular Screening and Diagnostic Centre, London, UK and Vascular Screening and Diagnostic Centre, Nicosia, Cyprus

[3] Department of Surgery, Imperial College, London, UK and University of Nicosia Medical School, Cyprus

Corresponding Author and address for correspondence

Professor Anisur Rahman -

Centre for Rheumatology Research, Division of Medicine, UCL

Room 412, Rayne Building

5 University Street

London WC1E 6JF

Tel 00442034479035 Fax 00442034479278

e-mail anisur.rahman@ucl.ac.uk

ORCID ID http://orcid.org/0000-0003-2346-4484

e-mail addresses of other authors

saracroca@gmail.com, maurabgriffin@googlemail.com, Filipa.mcfarinha@gmail.com, d.isenberg@ucl.ac.uk, anicolaides1@gmail.com

ABSTRACT

Objectives

Patients with systemic lupus erythematosus (SLE) have an increased risk of developing cardiovascular disease (CVD). Multiple studies have shown that these patients have increased numbers of carotid plaques and greater intima-media thickness (IMT) than healthy controls. Measures such as total plaque area (TPA) and plaque echogenicity may be more sensitive and more relevant to cardiovascular risk than presence of plaque and IMT alone. Our objective was to produce the first report of TPA and echogenicity in a population of patients with SLE.

Methods

One hundred patients with SLE and no history of clinical CVD were recruited. Clinical, serological and treatment variables were recorded and serum was tested for antibodies to apolipoprotein A-1 and high-density lipoprotein. Both carotid and both femoral artery bifurcations of each patient were scanned to determine IMT, TPA and echogenicity of plaques. Univariable and multivariable statistical analyses were carried out to define factors associated with each of these outcomes.

Results

Thirty-six patients had carotid and/or femoral plaque. Increasing age was associated with presence of plaque and increased IMT. Triglyceride levels were associated with presence of plaque. Mean (SD) TPA was 60.8 (41. 6)mm^2. Patients taking prednisolone had higher TPA. Most plaques were echolucent but increased echogenicity was associated with prednisolone therapy and persistent disease activity.

Conclusion

TPA and plaque echogenicity in patients with SLE are associated with different factors than those associated with presence of plaque and IMT. Longitudinal studies may show whether these outcome measures add value in the management of cardiovascular risk in SLE.

Keywords

Systemic lupus erythematosus

Atherosclerosis

Cardiovascular disease

Vascular ultrasound

Key messages

1) Total plaque area (TPA) and atherosclerotic plaque echolucency are associated with increased cardiovascular risk in non-SLE populations.

2) In 100 patients with SLE, TPA was higher in those taking prednisolone.

3) Most plaques were echolucent but increased echogenicity was associated with disease activity and taking prednisolone.

Background

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease with a prevalence of about 1 in 1000 in the United Kingdom(1). It is 9 times more common in women than men. Multiple studies have shown that the relative risk of developing atherosclerosis and cardiovascular disease (CVD) is higher in patients with SLE than would be expected. Manzi *et al* showed that relative risk of coronary artery disease (CAD) is raised by 50-fold in women with SLE aged 35-44 compared with women of the same age without SLE(2). CVD events occur at a relatively young age (average = 49 years) in the presence of fewer traditional risk factors than in non-SLE patients(3).

Ultrasound studies have shown that 30-40% of patients with SLE have carotid plaque (4-6). In 217 female patients followed for ten years, progression of carotid plaque occurred in 27% while carotid intima-media thickness (IMT) increased by a mean of 0.011mm/year(7). In 392 women with SLE followed for a mean of 8 years, higher IMT or presence of plaque at baseline predicted future development of CAD or stroke in multivariable analysis(8).

Previously published vascular ultrasound studies in SLE have mainly been confined to the carotids and limited to measuring IMT and presence of plaque(4, 5, 8). No studies have described echogenicity of plaque or total plaque area (TPA). Although femoral plaques are also associated with CVD(9), few previous studies in patients with SLE have reported on femoral plaque(10-12). Scanning both carotid and femoral bifurcations gives a more representative sample of the global burden of subclinical atherosclerosis and avoids missing patients who have only femoral plaques.

Population studies(13) and studies in clinic cohorts at risk of CVD(14) showed that TPA is a better predictor of CAD than IMT. Echogenicity of plaque is also important because more echolucent plaques have a large lipid core with a higher content of inflammatory material and a thin fibrous cap, factors predisposing to rupture causing

CVD events(15). Therefore, the purpose of this study was to report on TPA and echogenicity as well as IMT and presence of plaque in 100 patients with SLE and no previous CVD.

PATIENTS AND METHODS

Patients

We carried out carotid and femoral ultrasound scans on 100 patients from the Lupus Clinic at University College London Hospital who met American College of Rheumatology revised criteria for SLE (16) and had no previous history of CVD. Absence of CVD (defined as CAD, stroke or myocardial infarction with confirmatory evidence from blood tests and/or imaging) was confirmed by analysis of medical records. All patients gave informed consent. The study was approved by the combined UCL/UCLH Research Ethics Committee (Reference 06/Q0505/79)

SLE disease activity was determined by the British Isles Lupus Assessment Group (BILAG)-2004 Index (17). Persistently active disease was defined as having Global BILAG-2004 score >5 on at least two consecutive visits from the previous four visits. Data on therapy and previous serology were obtained from medical records.

Blood results from the day of the scan or nearest clinic visit were obtained either from tests carried out as part of routine clinical practice or by enzyme-linked immunosorbent assays (ELISA) carried out in our laboratory for IgG and IgM anti-apolipoprotein A1 (anti-ApoA-1) and IgG anti-HDL. Previous papers suggested that anti-ApoA-1 and anti-HDL may play a role in promoting development of atherosclerosis in SLE and similar diseases such as rheumatoid arthritis(18-21). Table 1 shows all the parameters studied.

Indirect ELISA to detect IgG anti-ApoA-1 and IgG anti-HDL antibodies

IgG anti-ApoA-1 antibodies were detected by a modification of the indirect ELISA protocol previously described (19, 21, 22). All steps were carried out at 37^0C except where specified. A Nunc-Maxisorb 96-well ELISA plate was divided in half. The test

side was coated with 10µg/mL ApoA-1 (Sigma A0722) in 70% ethanol. The control side was coated with 70% ethanol. After incubation for 90min, the plates were washed and blocked with 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for one hour. Serum samples at 1:50 dilution in 1% BSA-PBS were tested in duplicate; each sample was added to two test wells and two control wells. On each plate, a 7-point dilution of the positive control (pool of six serum samples from patients with high serum IgG anti-apoA-1) was performed starting at 1:25 dilution to create a standard curve. Following incubation for one hour, goat anti-human IgG-alkaline phosphatase conjugate (Sigma A3150) diluted 1:1000 in 1% BSA-PBS was added at room temperature for one hour followed by alkaline phosphatase substrate. Absorbance at 405nm was recorded after 60 minutes. For each, sample net OD was calculated by subtracting the OD in the control well from that in the matching test well to exclude non-specific background binding. The mean net OD from the duplicate samples was converted to absorbance units (AU) by comparison to the positive control standard curve. 100AU = OD for 1:50 dilution of the positive control sample.

IgG anti-HDL were detected using the same method except that the antigen loaded on the plate was 20 μg/mL HDL in 70% ethanol. The plates were kept at 4^0C overnight before adding samples. Samples were diluted 1:100 in 1% BSA-PBS before loading.

Indirect ELISA to detect IgM anti-ApoA-1 antibodies

This was the same as the ELISA for IgG anti-Apo-A1 except for the following points. The positive control was pooled serum from four patients known to have high IgM anti-Apo-A-1. The secondary antibody was goat anti-human IgM-horse radish peroxidase (HRP) conjugate (Sigma A 6907) diluted 1:5000 in 1% BSA-PBS. After one-hour incubation the plate was washed and HRP substrate was added. After five minutes the reaction was stopped with TMB stop solution and the OD was read at 450nm.

Ultrasound scanning

All scans were performed by the same experienced vascular scientist (MG) using the Philips iU22 ultrasound system (Philips Ultrasound, Bothell, USA) with a linear array L9-3 MHz transducer. The methods are described in detail in the Supplementary Data.

Briefly, both carotid and both femoral bifurcations were scanned. IMT was measured on screen and the mean of IMT from both carotids (IMTcc) was used in statistical analysis.

An arterial bifurcation was classified as having plaque if there was a focal thickening of greater than 1.2 mm(23, 24). The ultrasound images of these plaques were stored as DICOM files and transferred to a dedicated PC for application of imaging software program. This programme calculated the TPA and echogenicity. TPA was defined as the sum of the cross-sectional areas of all plaques seen in longitudinal images.

Plaque echogenicity is defined in terms of grey scale. The grey scale of blood (echolucent and thus black on ultrasound) =0, whereas the grey scale of adventitia =190. Thus the echolucency of any plaque can be defined in terms of a grey scale figure between 0 and 190 and the grey scale median (GSM) for all plaques in an individual patient is a measure of overall echogenicity.

Statistical analysis

Initially, we compared demographic, clinical and serological features between patients with and without plaques. For continuous variables we compared groups (plaque and non-plaque) using a t-test if the variable was normally distributed or a Mann-Whitney U test if the distribution was not normal. For categorical variables, we used a Pearson's chi-squared test (Table 1). Subsequently, variables with a skewed distribution were normalized by taking natural logarithms. Those that could not be normalized by using natural logarithms were categorized by using the median as a cut-off point.

The association of clinical and serological features with presence of plaques was then investigated in all 100 patients by using odds ratios in a univariable analysis. Because of the strong association between age and presence of plaques, odds ratios were expressed both unadjusted and adjusted for age. The significant variables after adjustment for age were used as covariates in a logistic regression analysis with plaque presence as the dependent variable (Table 2).

The association between clinical and serological features with IMTcc in all 100 patients was subsequently investigated in a univariable analysis. Unadjusted and age-adjusted odds ratios of features for tertiles of IMTcc were used. The significant features were then used as covariates in a linear regression analysis with IMTcc as a continuous dependent variable (Table 3).

For each patient with plaque, TPA was calculated by adding up the areas of all the plaques present. Also, for each patient the average value of the GSM of all plaques present was calculated. The association of clinical and serological features with TPA and average GSM in the 36 patients with plaques was investigated by dividing them into two groups using the median values for TPA and average GSM. Odds ratios of features for high or low TPA and GSM were calculated respectively (Tables 4 and 5). The significant variables after adjustment for age (adjustment not necessary for GSM) were used as covariates in logistic regression analyses with TPA and GSM as the dependent variables (Tables 4 and 5).Statistical analysis was performed with IBM® SPSS® statistics version 22. Significance was set at 0.05.

RESULTS

Characteristics of patients studied

Of the 100 patients scanned, 95% were women and the overall mean age was 45.2 years (SD 12.4; range 20-66). Mean age at SLE diagnosis was 29.2 years (SD 10.9; range 8-56). Mean follow-up in the lupus clinic was 16 years (SD 10.0; range 2-46).

56 patients were Caucasian, 25 were Afro-Caribbean, 11 were South Asian and 8 patients had other ethnic backgrounds (Chinese or mixed race). Fewer than one

third of the patients (n= 26) had hypertension and only two had diabetes. Although only 11 patients were smokers at the time of the scan, 34 were ever-smokers.

Prevalence of plaques

36 patients were found to have plaque and a total of 85 bifurcations with plaque were identified. Plaques were present in one bifurcation in 9 patients, in two bifurcations in 12 patients, in three in 8 patients and in four in 7 patients. 14 patients had plaque exclusively in the carotids and 7 exclusively in the femorals.

Table 1 shows clinical, demographic, serological, therapy and disease activity characteristics of the 100 subjects who were scanned, comparing the patients with and without plaque.

Patients found to have plaque were significantly older (mean age 53.9; SD 8.76; range 27-66) than those without plaque (mean age 40.0; SD 11.38; range 20-66) ($p<0.001$) but no associations with sex or ethnicity were found. Those with plaque had longer disease duration at the time of the scan than those without plaque (mean 21±12 years versus 13±7 years, $p=0.001$). The systolic blood pressure at the time of the scan was higher in those with plaque (mean 132±15 mmHg versus 123±15 mmHg, $p=0.005$), although 36% of those without plaques and 33% of those with plaque were on antihypertensive therapy. Smoking (current or ever) did not differ significantly between the plaque and no plaque groups. There was no association between increased disease activity or drug treatment and presence of plaque.

Total cholesterol/HDL ratio (mean 3.4±1.0 versus 2.9±0.8, $p=0.005$) and serum triglyceride level (mean 1.3±0.5 mmol/l versus 1.0±0.4 mmol/l, $p=0.001$) were higher in the plaque group. Anti-cardiolipin antibodies had been positive at some point in 44% of patients with plaque and 24% of patients with no plaque ($p=0.033$) but historical positivity for lupus anticoagulant and anti-beta2GPI did not differ between those groups.

Table 2 shows the association of a number of variables with the presence or absence of plaque expressed as crude and age-adjusted odds ratios. Increasing age ($P < 0.001$), total cholesterol/ HDL ratio ($P=0.007$) and triglycerides ($P=0.003$) were associated with plaque presence. The odds ratios of these features remained significant after adjustment for age. In a multivariable logistic regression analysis, only age and triglycerides were independent predictors of plaque presence. This model had a NagelKerke R Squared value of 0.445 and could classify correctly 76% of patients as having plaque present or absent.

IMT Results

The mean IMT (SD) values in cm were 0.053 (0.009) and 0.056 (0.010) for the right and left CCA respectively. Table 3 shows that systolic and diastolic blood pressure, total cholesterol and HDL-Cholesterol were the only factors that were significantly associated with increased common carotid IMT (mean of both sides) after adjustment for age. In a multivariable linear regression analysis, only age and diastolic blood pressure were significant. When the calculated IMT based on this model was plotted against observed IMT (graph not shown), there was a moderate correlation between the two ($r = 0.621$)

TPA Results

Considering the 36 patients with plaque, the mean TPA was 60.8 mm^2 (SD 41.6; range 7.0-166). All the 85 plaque bifurcation territories identified were included in this analysis. The two factors associated with TPA were treatment with prednisolone and level of IgG anti-apoA1 (Table 4). Taking prednisolone, at any dose, and at a dose above 5mg/day was significantly associated with increased risk of above-median TPA after adjustment for age at scan.

Conversely, higher IgG anti-apoA1 levels (expressed as the natural logarithm) were associated with lower TPA. Mean ln IgG anti-apoA1 was 3.64 ± 1.22 in the 18 patients with TPA above the median and 4.57 ± 1.38 in the 18 patients with TPA

below the median. This relationship remained borderline significant after adjustment for age at scan (OR 0.57, P= 0.049).

However, in a multivariable logistic analysis, only age and prednisolone > 5mg were significant whereas the association with ln IgG anti-ApoA-1 lost significance. This model had a NagelKerke R Squared value of 0.410 and could classify correctly 72% of patients as having TPA above or below median.

GSM analysis

The median GSM value for plaque in the 36 plaque-positive patients was 47.6 (IQR 37.6-62.6) with a range of 14-112. Most plaques were heterogeneous and discrete white areas were identified in two thirds. Over 50% of plaques analyzed had GSM < 60. Thus, the majority of plaques were predominantly echolucent.

The factors significantly associated with GSM are summarized in Table 5. Age was not associated with GSM; thus adjustment for age was not necessary. In this analysis, persistently active disease, taking prednisolone (at any dose) and lower C3 level were the only factors associated with increased GSM. Patients taking prednisolone were 4.4 times more likely to have GSM above the median than below it (95% CI 1.0 to 18.6, p=0.046). Patients with persistently active disease were 7.9 times more likely to have GSM above the median than below it (95% CI 1.7 to 37.4, p=0.010).

In a multivariable logistic analysis, both prednisolone at any dose and persistently active disease were significant. Low C3 lost significance, perhaps because it is related to disease activity. This model had a NagelKerke R Squared value of 0.389 and could classify correctly 72% of patients as having GSM above or below the median.

DISCUSSION

This is the first study to describe TPA and echolucency of plaque in patients with SLE. We have shown that the factors influencing these outcomes are different from those associated with the standard outcome measures of presence of plaque and IMT.

A systematic analysis of 80 studies (71 reporting IMT and 44 reporting plaque) by Wu *et al*(25) noted extensive heterogeneity, but concluded that there was significantly increased prevalence of plaque in patients with SLE compared with controls (odds ratio 2.45, 95% CI 2.02 to 2.97). Only treatment with corticosteroids and triglyceride levels were significantly associated with increased risk of plaque. Factors significantly associated with increased carotid IMT were age, duration of disease, ESR, use of corticosteroids, triglyceride level, level of high-density lipoprotein (HDL) and disease activity.

We noted the presence of plaques in 36% of patients. Increasing age was associated with both presence of plaque and increased IMT, whereas raised triglyceride levels were only associated with presence of plaque. The fact that we found no association between disease activity or drug treatment and IMT or presence of plaque may be related to the relatively small study population. Unlike Gustafsson *et al*, we did not find any association between previous lupus nephritis and presence of plaque(26). The proportions of patients with previous nephritis were similar in our plaque (12/36) and no-plaque (24/64) groups. Our results are not directly comparable to those of the Swedish group as their patients were almost all Caucasian whereas 44% of ours are of other ethnicities. They also had a different definition of plaque (>1mm thickening of IMT)(26).

We found no association of plaque or IMT with either anti-ApoA-1 or anti-HDL antibodies. Other authors have shown an association of elevated IgG anti-ApoA-1 with increased risk of CVD events in patients with rheumatoid arthritis(20) and with histological features of plaque vulnerability in patients with no autoimmune disease

who underwent carotid endarterectomy(27). Conversely, in previous studies, we did not find any associations between IgG anti-ApoA-1 and clinical CVD in patients with SLE(19, 21).

Our results on presence of plaque and IMT in patients with SLE are consistent with the work of other groups(4-7, 10, 12, 26). As reviewed by Wu et al (25), factors associated with plaque and IMT typically include standard CVD risk factors such as age and lipid levels. In contrast to plaque and IMT, our study shows that TPA was not associated with levels of any lipid metabolite or with blood pressure.

TPA gives a more comprehensive measure of the total burden of subclinical atherosclerosis than either plaque number or IMT. Spence has reviewed benefits of measuring plaque burden rather than thickness alone, particularly as focal carotid plaques grow along the artery faster than they thicken(28). Perez *et al* showed, in a population of 2035 Argentines with no history of CVD, that adding TPA to a Framingham risk score calculation led to a change in the risk category for 768 patients (491 increased risk and 277 decreased risk)(29). Cardioprotective treatment was intensified in the patients with increased risk. The population studied in that paper had higher Framingham risk profile than ours with mean age 59 years, 57% male, 35% hypertensive, 27% hypercholesterolemic and 14% diabetic. Use of TPA could be even more important in identifying accurately the higher-risk individuals in a population of patients with SLE, where Framingham scores are typically low. Our study shows that TPA can be readily measured in such patients and that increased TPA is associated with taking prednisolone, but not associated with disease activity. Longitudinal studies to investigate relationship between TPA and cardiovascular outcomes in patients with SLE are needed to define whether measuring TPA could be used to modify treatment in these patients.

We found that most plaques in these patients were echolucent (GSM <60). Although it has been postulated that systemic inflammation promotes increased echolucency, we found the opposite; persistently active disease was associated with more

echogenic plaque. Actually, the evidence supporting the theory that systemic inflammation promotes plaque echolucency is sparse and of limited relevance to patients with SLE. Yamagami *et al* studied 246 patients and found that low plaque echogenicity was associated with serum interleukin-6 levels but there was only a borderline association with C-reactive protein level(30). In 5434 subjects from the Tromso study, there was no association between plaque echogenicity and either fibrinogen or C-reactive protein(31)

Plaque echolucency may predict increased risk of future cardiovascular events. A study comparing histology of plaques removed by carotid endarterectomy with pre-operative scans showed that lipid-rich plaques had lower echogenicity(32). It has been proposed that these lipid-rich plaques are more unstable and prone to rupture and thus that low echogenicity of plaque predicts increased risk of cardiovascular events(30, 31, 33). The Tromso study showed significant increased risk of myocardial infarction in women with lower plaque echogenicity even after multivariable analysis(13).

Increased plaque echolucency was associated with increased risk of subsequent ipsilateral ischemic stroke in both symptomatic(34) and asymptomatic(33) patients with >50% stenosis of the carotid artery and with increased risk of coronary events in 357 Japanese patients with chronic CAD on statins(35). These results, however, may be of limited relevance to our patients, none of whom had CAD or carotid stenosis >50%. The patients in the ipsilateral stroke studies cited above were primarily men and over 65 years old(33, 34).

Limitations of our study include the absence of data on cumulative corticosteroid dose, which could be more relevant to build-up of atherosclerosis over time. There were very few men, diabetics or current smokers so we could have missed potential effects of these variables on ultrasound outcomes. We did not have data on Body Mass Index as height of subjects was not measured. The study does not include a healthy control group and we did not confirm the findings in a separate cohort of

patients with SLE. It is possible that associations of TPA and echogenicity with variables such as disease activity and lipid levels were not apparent because they were only measured at a single time point.

It will be important to carry out longitudinal studies in these patients to assess whether higher TPA and echolucency of plaques are predictive factors for CVD events. We are analysing repeat scans in the patients approximately five years after the initial scans to measure changes in TPA, IMT, number, location and echogenicity of plaques and factors that influence progression/regression of those outcome measures.

DECLARATIONS

COMPETING INTERESTS

Professor A Nicolaides is a consultant to LifeQ Medical Ltd. None of the other authors has any conflicts of interest to disclose.

FUNDING

This research was supported by the Rosetrees Trust and LUPUS UK. It was carried out at a center supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre.

AUTHORSHIP CONTRIBUTIONS

SC and AR designed the study. SC recruited the subjects and carried out the laboratory assays. SC, AR, FF and DI collected and analysed clinical, demographic and disease activity data. SC and MG carried out the scanning. MG, SC and AN analysed the scan results. FF carried out statistical analysis. AR wrote the final manuscript. All authors contributed to manuscript preparation and approved the final manuscript.

References

1. Rees F, Doherty M, Grainge M, Davenport G, Lanyon P, Zhang W. The incidence and prevalence of systemic lupus erythematosus in the UK, 1999-2012. Annals of the rheumatic diseases. 2016;75(1):136-41.

2. Manzi S, Meilahn EN, Rairie JE, Conte CG, Medsger TA, Jr., Jansen-McWilliams L, et al. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. Am J Epidemiol. 1997;145(5):408-15.

3. Elliott JR, Manzi S, Edmundowicz D. The role of preventive cardiology in systemic lupus erythematosus. Curr Rheumatol Rep. 2007;9(2):125-30.

4. Roman MJ, Shanker BA, Davis A, Lockshin MD, Sammaritano L, Simantov R, et al. Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. The New England journal of medicine. 2003;349(25):2399-406.

5. Manzi S, Selzer F, Sutton-Tyrrell K, Fitzgerald SG, Rairie JE, Tracy RP, et al. Prevalence and risk factors of carotid plaque in women with systemic lupus erythematosus. Arthritis Rheum. 1999;42(1):51-60.

6. Farzaneh-Far A, Roman MJ, Lockshin MD, Devereux RB, Paget SA, Crow MK, et al. Relationship of antiphospholipid antibodies to cardiovascular manifestations of systemic lupus erythematosus. Arthritis Rheum. 2006;54(12):3918-25.

7. Thompson T, Sutton-Tyrrell K, Wildman RP, Kao A, Fitzgerald SG, Shook B, et al. Progression of carotid intima-media thickness and plaque in women with systemic lupus erythematosus. Arthritis Rheum. 2008;58(3):835-42.

8. Kao AH, Lertratanakul A, Elliott JR, Sattar A, Santelices L, Shaw P, et al. Relation of carotid intima-media thickness and plaque with incident cardiovascular events in women with systemic lupus erythematosus. The American journal of cardiology. 2013;112(7):1025-32.

9. Khoury Z, Schwartz R, Gottlieb S, Chenzbraun A, Stern S, Keren A. Relation of coronary artery disease to atherosclerotic disease in the aorta, carotid, and femoral arteries evaluated by ultrasound. The American journal of cardiology. 1997;80(11):1429-33.

10. Tektonidou MG, Kravvariti E, Konstantonis G, Tentolouris N, Sfikakis PP, Protogerou A. Subclinical atherosclerosis in Systemic Lupus Erythematosus:

Comparable risk with Diabetes Mellitus and Rheumatoid Arthritis. Autoimmun Rev. 2017;16(3):308-12.

11. Kisiel B, Kruszewski R, Juszkiewicz A, Raczkiewicz A, Bachta A, Klos K, et al. Systemic lupus erythematosus: the influence of disease-related and classical risk factors on intima media thickness and prevalence of atherosclerotic plaques--a preliminary report. Beneficial effect of immunosuppressive treatment on carotid intima media thickness. Acta Cardiol. 2015;70(2):169-75.

12. Theodorou E, Nezos A, Antypa E, Ioakeimidis D, Koutsilieris M, Tektonidou M, et al. B-cell activating factor and related genetic variants in lupus related atherosclerosis. Journal of autoimmunity. 2018;92:87-92.

13. Johnsen SH, Mathiesen EB, Joakimsen O, Stensland E, Wilsgaard T, Lochen ML, et al. Carotid atherosclerosis is a stronger predictor of myocardial infarction in women than in men: a 6-year follow-up study of 6226 persons: the Tromso Study. Stroke; a journal of cerebral circulation. 2007;38(11):2873-80.

14. Spence JD, Eliasziw M, DiCicco M, Hackam DG, Galil R, Lohmann T. Carotid plaque area: a tool for targeting and evaluating vascular preventive therapy. Stroke; a journal of cerebral circulation. 2002;33(12):2916-22.

15. Johnsen SH, Mathiesen EB. Carotid plaque compared with intima-media thickness as a predictor of coronary and cerebrovascular disease. Curr Cardiol Rep. 2009;11(1):21-7.

16. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40(9):1725.

17. Isenberg DA, Rahman A, Allen E, Farewell V, Akil M, Bruce IN, et al. BILAG 2004. Development and initial validation of an updated version of the British Isles Lupus Assessment Group's disease activity index for patients with systemic lupus erythematosus. Rheumatology. 2005;44(7):902-6.

18. Batuca JR, Ames PR, Amaral M, Favas C, Isenberg DA, Delgado Alves J. Anti-atherogenic and anti-inflammatory properties of high-density lipoprotein are affected by specific antibodies in systemic lupus erythematosus. Rheumatology (Oxford, England). 2009;48(1):26-31.

19. O'Neill SG, Giles I, Lambrianides A, Manson J, D'Cruz D, Schrieber L, et al. Antibodies to apolipoprotein A-I, high-density lipoprotein, and C-reactive protein are

associated with disease activity in patients with systemic lupus erythematosus. Arthritis Rheum. 2010;62(3):845-54.

20. Vuilleumier N, Bas S, Pagano S, Montecucco F, Guerne PA, Finckh A, et al. Anti-apolipoprotein A-1 IgG predicts major cardiovascular events in patients with rheumatoid arthritis. Arthritis Rheum. 2010;62(9):2640-50.

21. Croca S, Bassett P, Chambers S, Davari M, Alber KF, Leach O, et al. IgG anti-apolipoprotein A-1 antibodies in patients with systemic lupus erythematosus are associated with disease activity and corticosteroid therapy: an observational study. Arthritis Res Ther. 2015;17:26.

22. Delgado Alves J, Kumar S, Isenberg DA. Cross-reactivity between anti-cardiolipin, anti-high-density lipoprotein and anti-apolipoprotein A-I IgG antibodies in patients with systemic lupus erythematosus and primary antiphospholipid syndrome. Rheumatology (Oxford, England). 2003;42(7):893-9.

23. Ebrahim S, Papacosta O, Whincup P, Wannamethee G, Walker M, Nicolaides AN, et al. Carotid plaque, intima media thickness, cardiovascular risk factors, and prevalent cardiovascular disease in men and women: the British Regional Heart Study. Stroke; a journal of cerebral circulation. 1999;30(4):841-50.

24. Griffin M, Nicolaides AN, Belcaro G, Shah E. Cardiovascular risk assessment using ultrasound: the value of arterial wall changes including the presence, severity and character of plaques. Pathophysiol Haemost Thromb. 2002;32(5-6):367-70.

25. Wu GC, Liu HR, Leng RX, Li XP, Li XM, Pan HF, et al. Subclinical atherosclerosis in patients with systemic lupus erythematosus: A systemic review and meta-analysis. Autoimmun Rev. 2016;15(1):22-37.

26. Gustafsson JT, Herlitz Lindberg M, Gunnarsson I, Pettersson S, Elvin K, Ohrvik J, et al. Excess atherosclerosis in systemic lupus erythematosus,-A matter of renal involvement: Case control study of 281 SLE patients and 281 individually matched population controls. PLoS One. 2017;12(4):e0174572.

27. Montecucco F, Vuilleumier N, Pagano S, Lenglet S, Bertolotto M, Braunersreuther V, et al. Anti-Apolipoprotein A-1 auto-antibodies are active mediators of atherosclerotic plaque vulnerability. European heart journal. 2011;32(4):412-21.

28. Spence JD. Determinants of carotid plaque burden. Atherosclerosis. 2016;255:122-3.

29. Perez HA, Garcia NH, Spence JD, Armando LJ. Adding carotid total plaque area to the Framingham risk score improves cardiovascular risk classification. Arch Med Sci. 2016;12(3):513-20.

30. Yamagami H, Kitagawa K, Nagai Y, Hougaku H, Sakaguchi M, Kuwabara K, et al. Higher levels of interleukin-6 are associated with lower echogenicity of carotid artery plaques. Stroke; a journal of cerebral circulation. 2004;35(3):677-81.

31. Halvorsen DS, Johnsen SH, Mathiesen EB, Njolstad I. The association between inflammatory markers and carotid atherosclerosis is sex dependent: the Tromso Study. Cerebrovasc Dis. 2009;27(4):392-7.

32. Waki H, Masuyama T, Mori H, Maeda T, Kitade K, Moriyasu K, et al. Ultrasonic tissue characterization of the atherosclerotic carotid artery: histological correlates or carotid integrated backscatter. Circ J. 2003;67(12):1013-6.

33. Huibers A, de Borst GJ, Bulbulia R, Pan H, Halliday A, group A-c. Plaque Echolucency and the Risk of Ischaemic Stroke in Patients with Asymptomatic Carotid Stenosis Within the First Asymptomatic Carotid Surgery Trial (ACST-1). Eur J Vasc Endovasc Surg. 2016;51(5):616-21.

34. Gronholdt ML, Nordestgaard BG, Schroeder TV, Vorstrup S, Sillesen H. Ultrasonic echolucent carotid plaques predict future strokes. Circulation. 2001;104(1):68-73.

35. Uematsu M, Nakamura T, Sugamata W, Kitta Y, Fujioka D, Saito Y, et al. Echolucency of carotid plaque is useful for assessment of residual cardiovascular risk in patients with chronic coronary artery disease who achieve LDL-C goals on statin therapy. Circ J. 2014;78(1):151-8.

Table 1

	Plaque (*n*= 36)	**No plaque (*n*= 64)**	**p**
Sex, N (F : M)	33 : 3	62 : 2	0.251
Ethnicity, N (%)			
Asian	2 (6)	9 (14)	0.508
Afro-Caribbean	8 (22)	17 (26)	
Caucasian	23 (64)	33 (52)	
Other	3 (8)	5 (8)	
Age at scan (years), mean ± SD	54 ± 9	40 ± 11	**<0.001**
Age at SLE diagnosis (years), mean ± SD	33 ± 11	27 ± 11	**0.014**
Disease duration at scan (years), mean ± SD	21 ± 12	13 ± 7	**0.001**
Blood Pressure (mmHg)			
Systolic, mean ± SD	132 ± 15	123 ± 15	**0.005**
Diastolic, mean ± SD	75 ± 10	76 ± 10	0.651
Intima - Media Thickness (cm)			
Common carotid artery, median (IQR)	0.058 (0.010)	0.050 (0.010)	**<0.001**
Overall, median (IQR)	0.123 (0.048)	0.065 (0.016)	**<0.001**
Total plaque area (cm^2), mean ± SD	60.79 ± 41.58	N/A	N/A
Total length of plaque (cm), mean ± SD	3.20 ± 2.05	N/A	N/A
Total plaque thickness (cm), mean ± SD	0.563 ± 0.289	N/A	N/A
Global GSM, median (IQR)	47.6 (25.0)	N/A	N/A
Common carotid GSM, median (IQR)	44.0 (30.4)	N/A	N/A
Global BILAG score at scan[a], median (IQR)	1 (6)	2 (8)	0.094
Persistently active disease, N (%)	22 (61)	26 (42)	0.067
Damage score at scan, median (IQR)	1 (2)	0 (1)	**0.048**
History of lupus nephritis, N (%)	12 (42)	24 (38)	0.682

Lipid profile (mmol/L)			
Total cholesterol, mean ± SD	5.1 ± 0.9	4.7 ± 1.1	0.087
HDL, mean ± SD	1.6 ± 0.5	1.7 ± 0.5	0.309
LDL, mean ± SD	2.8 ± 0.8	2.5 ± 0.9	0.063
Total cholesterol/ HDL ratio, mean ± SD	3.4 ± 1.0	2.9 ± 0.8	**0.005**
Triglycerides, mean ± SD	1.3 ± 0.5	1.0 ± 0.4	**0.001**
Smoking status, N (%)			
Current smoker	6 (20)	5 (8)	0.111
Ever smoker	11 (41)	23 (44)	0.766
Diabetes, N (%)	1 (3)	1 (2)	0.677
Treatment regimen at scan			
Hydroxychloroquine, N (%)	23 (64)	42 (66)	0.861
Immunosuppression, N (%)	13 (36)	32 (50)	0.180
Azathioprine, N (%)	5 (14)	12 (19)	0.534
MMF, N (%)	6 (17)	14 (22)	0.532
Others, N (%)	2 (6)	8 (13)	0.267
Prednisolone at any dose, N (%)	22 (61)	42 (66)	0.652
Prednisolone>5mg, N (%)	6 (17)	18 (28)	0.198
Prednisolone dose at scan(mg), median (IQR)	4.1 (5.0)	5.0 (6.8)	0.336
B-cell depletion (ever), N (%)	11 (31)	20 (31)	0.943
ACE inhibitors, N (%)	12 (33)	23 (36)	0.793
Aspirin, N (%)	7 (19)	7 (11)	0.239
Statins, N (%)	7 (19)	6 (9)	0.151
Blood results at scan or nearest clinic visit			
Homocysteine[b] (µmol/L), median (IQR)	16 (4)	13 (5)	0.053
Serum urea (µmol/L), median (IQR)	5.7 (3.2)	5.1 (2.9)	**0.039**
Serum creatinine (µmol/L), median (IQR)	68 (23)	66 (19)	0.309

Serum vitamin D (nmol/L), median (IQR)	51 (43)	60 (39)	0.615
Serum albumin (g/L), median (IQR)	44 (6)	43 (4)	0.770
ESR (mm/h), median (IQR)	14 (21)	16 (16)	0.826
CRP (mg/dL), median (IQR)	2.2 (3.9)	1.5 (3.4)	0.257
C3 (g/l), mean ± SD	1.04 ± 0.22	1.02 ± 0.24	0.548
Anti-dsDNA (IU/L), median (IQR)	13 (56)	23 (65)	0.191
Anti-ApoA1 IgG (AU), median (IQR)	62 (193)	68 (113)	0.281
Anti-ApoA1 IgM (AU), median (IQR)	20 (54)	13 (115)	0.846
Anti-HDL IgG (AU), median (IQR)	10.7 (39.2)	7.4 (19.9)	0.081
Historical serological profile (ever positive)			
Anti-C1q*, N (%)	14 (29)	7 (25)	0.695
Anti-cardiolipin (IgG and/or IgM), N (%)	16 (44)	15 (24)	**0.033**
Anti-β2 GP1, N (%)	3 (9)	2 (3)	0.279
Lupus anticoagulant, N (%)	7 (19)	12 (19)	0.962

[a]BILAG-2004 Score calculated using the formula A=12, B=8, C=1, D=0, E=0.

[b]Homocysteine levels were available only for 36% (n=13) of patients with plaque and 45% (n=29) of patients without plaque. Data on anti-C1q is available only for 28 patients with plaque and 48 patients without plaque.

Legend

Comparison of demographic, clinical and serological features between patients with and without plaque

Table 2

UNIVARIABLE ANALYSIS				
Feature	**Crude Odds Ratio (95% CI)**	**P**	**Odds Ratio (95% CI) Adjusted for age at scan**	**P Adjusted for age**
Age at scan (years)	**1.133 (1.075 to 1.195)**	**< 0.001**	**----------**	
Disease duration (years)	**1.091 (1.041 to 1.143)**	**< 0.001**	1.033 (0.979 to 1.091)	0.239
Age at diagnosis (years)	**1.049 (1.008 to 1.090)**	**0.017**	0.963 (0.912 to 1.017)	0.178
SBP (mmHg)	**1.040 (1.011 to 1.070)**	**0.007**	1.009 (0.977 to 1.042)	0.587
DBP (mmHg)	0.991 (0.952 to 1.031)	0.645	0.983 (0.939 to 1.029)	0.459
Persistently active disease present	2.176 (0.941 to 5.034)	0.069	1.191 (0.445 to 3.184)	0.728
Damage score at scan	1.335 (0.953 to 1.870)	0.093	1.326 (0.362 to 4.854)	0.670
History of Lupus nephritis present	1.190 (0.517 to 2.740	0.682	1.644 (0.592 to 4.572)	0.341
Total Cholesterol (mmol/dL)	1.413 (0.948 to 2.105)	0.090	1.058 (0.387 to 2.888)	0.913
HDL-Cholesterol (mmol/dL)	0.654 (0.289 to 1.476)	0.306	0.485 (0.179 to 1.316)	0.156
LDL-Cholesterol (mmol/dL)	1.576 (0.970 to 2.550)	0.066	1.158 (0.652 to 2.055)	0.617
Non-HDL Cholesterol mmol/dL	**1.771 (1.117 to 2.805)**	**0.015**	1.325 (0.773 to 2.272)	0.306

T-Chol./HDL ratio	**1.992 (1.208 to 3.286)**	**0.007**	**1.859 (1.024 to 3.374)**	**0.041**
Triglycerides (mmol/dL)	**4.499 (1.696 to 11.937)**	**0.003**	**3.652 (1.177 to 11.32)**	**0.025**
Smoking at scan	2.750 (0.765 to 9.891)	0.121	3.648 (0.848 to 15.70)	0.082
Smoking - ever	0.847 (0.317 to 2.262)	0.741	0.638 (0.204 to 1.994)	0.638
HCQ therapy	0.972 (0.395 to 2.176)	0.861	1.367 (0.475 to 3.938)	0.562
Immunosuppression	0.565 (0.244 to 1.3070	0.182	0.888 (0.322 to 2.454)	0.819
Prednisolone-any dose	0.746 (0.298 to 1.864)	0.530	1.106 (0.408 to 3.001)	0.843
Prednisolone > 5 mg/day	0.511 (0.182 to 1.435)	0.202	0.955 (0.249 to 3.669)	0.947
B-Cell depletion	0.968 (0.400 to 2.344)	0.943	1.048 (0.694 to 6.544)	0.186
ACE Inhibitors	0.891 (0.377 to 2.108)	0.793	1.048 (0.375 to 2.932)	0.929
Aspirin	1.996 (0.629 to 6.140)	0.245	2.576 (0.640 to 10.36)	0.183
Statin	2.333 (0.718 to 7.578)	0.159	1.345 (0.333 to 5.498)	0.672
Urea	**1.176 (1.010 to 1.369)**	**0.037**	1.057 (0.884 to 1.264)	0.543
lnCreatinine	1.810 (0.544 to 6.025)	0.334	1.476 (0.346 to 6.298)	0.599
Vitamin D	0.995 (0.979 to 1.011)	0.530	0.980 (0.960 to 1.000)	0.050
Albumin	1.007 (0.926 to 1.096)	0.864	1.003 (0.905 to 1.112)	0.955

lnESR	1.156 (0.696 to 1.919)	0.576	0.890 (0.487 to 1.626)	0.705
lnCRP	1.341 (0.846 to 2.126)	0.212	0.934 (0.533 to 1.637)	0.812
C3 at scan	1.758 (0.284 to 10.89)	0.544	0.467 (0.048 to 4.494)	0.510
lnAnti-dsDNA	0.884 (0.685 to 1.140)	0.342	0.929 (0.686 to 1.2590	0.635
lnAntiApoA1IgG	0.857 (0.622 to 1.180)	0.344	1.025 (0.701 to 1.488)	0.913
lnAntiApoA1IgM	1.016 (0.836 to 1.234)	0.876	1.038 (0.821 to 1.312)	0.753
AntiHDLIgG (above median)	1.690 (0.740 to 3.857)	0.231	1.191 (0.445 to 3.184)	0.728
Anticardiolipin ever	2.613 (0.797 to 8.573)	0.113	1.397 (0.294 to 6.644)	0.675
APL ever	1.667 (0.724 to 3.841)	0.230	1.058 (0.387 to 2.888)	0.913
Lupus Anticoagulant	**3.564 (1.022 - 12.43)**	**0.046**	2.293 (0.508 to 10.34	0.229

MULTIVARIABLE LOGISTIC REGRESSION

Feature	**Odds Ratio (Exp (B))**	**P**	**95% CI of Odds Ratio**
Age at scan	**1.128**	**< 0.001**	**1.069 to 1.190**
Triglycerides	**3.652**	**0.025**	**1.177 to 11.32**

Legend

Association of clinical and serological features with odds ratio for presence (N=36) versus absence (N = 64) of plaques

Table 3

		UNIVARIABLE ANALYSIS			
IMTcc (mm) Tertiles	**Feature**	**Prevalence**	**Crude Odds Ratio (95% CI)**	**Odds Ratio (95% CI) Adjusted for age at scan**	**P Unadjusted for age**
	Age at scan (years)				
1st			**1**	**----------**	
2nd			**1.049 (1.006 to 1.095)**	**----------**	**0.026**
3rd			**1.125 (1.061 to 1.192)**	**----------**	**< 0.001**
	Disease duration (years)				
1st			1	1	
2nd			1.053 (0.999 to 1.110)	1.024 (0.960 to 1.093)	0.055
3rd			1.052 (0.982 to 1.084)	0.952 (0.890 to 1.017)	0.210
	Age at diagnosis (years)				
1st			1	1	
2nd			1.015 (0.967 to 1.065)	0.960 (0.898 to 1.027)	0.556
3rd			**1.105 (1.045 to 1.169)**	1.049 (0.981 to 1.120)	**< 0.001**
	SBP (mmHg)				
1st			1	1	
2nd			**1.039 (1.003 to 1.075)**	1.040 (0.995 to 1.087)	**0.031**
3rd			**1.078 (1.034 to 1.124)**	**1.096 (1.034 to 1.162)**	**< 0.001**

	DBP (mmHg)				
1st			1	1	
2nd			1.050 (0.998 to 1.105)	**1.062 (1.005 to 1.122)**	0.058
3rd			**1.075 (1.013 to 1.140)**	**1.098 (1.022 to 1.180)**	**0.016**
	Persistently active disease present				
1st		54%	1		
2nd		52%	0.925 (0.357 to 2.399)		0.873
3rd		39%	0.559 (0.211 to 1.483)		0.241
	Damage score at scan				
1st			1		
2nd			1.406 (0.945 to 2.091)		0.093
3rd			1.153 (0.742 to 1.791)		0.527
	History of Lupus nephritis present				
1st		40%	1		
2nd		50%	1.471 (0.572 to 3.781)		0.423
3rd		25%	0.490 (0.171 to 1.407)		0.181
	Total Cholesterol (mmol/dL)				
1st			1	1	
2nd			**1.910 (1.161 to 3.139)**	**1.732 (1.037 to 2.893)**	**0.011**

3rd		**2.248 (1.287 to 3.926)**	**1.924 (1.038 to 3.567)**	**0.004**
	HDL-Cholesterol (mmol/dL)			
1st		1	1	
2nd		**2.917 (1.122 to 7.587)**	**2.890 (1.080 to 7.736)**	**0.028**
3rd		1.794 (0.645 to 4.980)	2.775 (0.763 to 10.10)	0.263
	LDL-Cholesterol (mmol/dL)			
1st		**1**	**1**	
2nd		1.548 (0.882 to 2.715)	1.381 (0.769 to 2.479)	0.128
3rd		**2.381 (1.251 to 4.532)**	1.933 (0.925 to 4.042)	**0.008**
	Non-HDL Cholesterol mmol/dL			
1st		**1**	**1**	
2nd		1.626 (0.952 to 2.779)	1.420 (0.808 to 2.494)	0.075
3rd		**2.119 (1.193 to 3.766)**	1.636 (0.848 to 3.158)	**0.010**
	T-Chol./HDL ratio			
1st		1		
2nd		0.863 (0.492 to 1.515)		0.609
3rd		1.275 (0.757 to 2.148)		0.361
	Triglycerides (mmol/dL)			
1st		1		

2nd			2.441 (0.864 to 6.898)		0.092
3rd			1.693 (0.577 to 4.966)		0.338
	Smoking at scan				
1st		10%	1		
2nd		21%	2.217 (0.563 to 8.738)		0.247
3rd		4%	0.386 (0.040 to 3.688)		0.394
	Smoking – ever				
1st		32%	1		
2nd		55%	2.730 (0.894 to 8.338)		0.075
3rd		25%	0.700 (0.213 to 2.305)		0.557
	HCQ therapy				
1st		72%	1		
2nd		57%	0.523 (0.195 to 1.400)		0.195
3rd		64%	0.720 (0.259 to 2.002)		0.528
	Immunosuppression				
1st		**59%**	**1**	1	
2nd		**37%**	**0.394 (0.150 to 1.033)**	0.465 (0.171 to 1.265)	**0.056**
3rd		**32%**	**0.322 (0.118 to 0.879)**	0.428 (0.131 to 1.399)	**0.025**
	Prednisolone-any dose				
1st		67%	1		
2nd		57%	0.654 (0.249 to 1.718)		0.388

3rd		68%	1.152 (0.416 to 3.188)		0.785
	Prednisolone > 5 mg/day				
1st		26%	1		
2nd		17%	0.564 (0.173 to 1.836)		0.338
3rd		31%	1.351 (0.483 to 4.033)		0.538
	B-Cell depletion				
1st		33%	1		
2nd		17%	0.857 (0.312 to 2.355)		0.765
3rd		31%	0.800 (0.282 to 2.266)		0.674
	ACE Inhibitors				
1st		38%	1		
2nd		37%	0.941 (0.357 to 2.480)		0.902
3rd		29%	0.650 (0.232 to 1.820)		0.411
	Aspirin				
1st		9%	1	1	
2nd		27%	3.455 (0.932 to 12.80)	2.974 (0.770 to 11.48)	0.054
3rd		7%	0.731 (0.125 to 4.287)	0.605 (0.083 to 4.430)	0.727
	Statin				
1st		12%	1		

2nd		20%	1.850 (0.508 to 6.742)	0.347
3rd		7%	0.569 (0.102 to 3.162)	0.413
	Urea			
1st			1	
2nd			1.037 (0.897 to 1.200)	0.621
3rd			0.947 (0.789 to 1.137)	0.558
	lnCreatinine			
1st			1	
2nd			1.307 (0.362 to 4.723)	0.683
3rd			0.408 (0.078 to 2.137)	0.289
	Vitamin D			
1st			1	
2nd			1.003 (0.984 to 1.023)	0.731
3rd			0.996 (0.997 to 1.016)	0.705
	Albumin			
1st			1	
2nd			0.957 (0.849 to 1.078)	0.471
3rd			0.927 (0.598 to 1.023)	0.131
	lnESR			
1st			1	
2nd			1.224 (0.661 to 2.266)	0.520

3rd		1.052 (0.598 to 1.850)	0.861
	lnCRP		
1st		1	
2nd		0.771 (0.441 to 1.349)	0.362
3rd		1.244 (0.737 to 2.099)	0.413
	C3		
1st		1	
2nd		0.563 (0.062 to 5.148)	0.611
3rd		1.676 (0.206 to 13.63)	0.629
	lnAnti-dsDNA		
1st		1	
2nd		1.079 (0.802 to 1.452)	0.617
3rd		1.131 90.843 to 1.517)	0.412
	lnAntiApoA1IgG		
1st		1	
2nd		1.122 (0.737 to 1.709)	0.591
3rd		0.863 (0.596 to 1.248)	0.433
	lnAntiApoA1IgM		
1st		1	
2nd		1.099 (0.885 to 1.365)	0.392
3rd		0.896 (0.695 to 1.155)	0.396

	AntiHDLIgG (above median)				
1st		52%	1		
2nd		50%	0.909 (0.356 to 2.321)		0.842
3rd		46%	0.788 (0.302 to 2.054)		0.626
	Anticardiolipin ever				
1st		12%	1		
2nd		15%	1.217 (0.295 to 5.017)		0.785
3rd		15%	1.217 (0.295 to 5.017)		0.785
	APL ever				
1st		36%	1		
2nd		55%	2.215 (0.843 to 5.823)		0.107
3rd		29%	0.720 (0.256 to 2.026)		0.533
	Lupus Anticoagulant				
1st		8%	1	1	
2nd		11%	1.522 (0.282 to 8.202)	0.878 (0.134 to 5.760)	0.623
2rd		23%	3.500 (0.788 to 15.54)	3.817 (0.636 to 22.92)	0.086

MULTIVARIABLE LINEAR REGRESSION (not logistic) (only significant features shown)

Feature	Beta (Standardised)	t	P
Age at scan	0.559	6.791	< 0.001
Diastolic BP	0.229	2.866	< 0.001

Legend

Association of clinical and serological features with increasing IMT

The 100 patients were divided into tertiles on the basis of IMT. The range for the 1st tertile was 0.040-0.050cm, the second tertile was 0.050-0.0575cm and the third tertile was 0.0575-0.0875cm). For each variable the table shows the value for patients in the highest or middle tertile of IMTcc compared to the 1st tertile. Significant or nearly significant features were adjusted for age.

Table 4

	UNIVARIABLE ANALYSIS			
Feature	**Crude Odds Ratio (95% CI)**	**P**	**Odds Ratio (95% CI) Adjusted for age at scan**	**P Adjusted for age**
Age at scan (years)	1.059 (0.974 to 1.153)	0.180	----------	
Disease duration (years)	1.004 (0.949 to 1.092)	0.897	0.982 (0.920 to 1.047)	0.573
Age at diagnosis (years)	1.027 (0.967 to 1.092)	0.383	1.014 (0.951 to 1.083)	0.666
SBP (mmHg)	1.025 (0.980 to 1.072)	0.280	1.014 (0.965 to 1.066)	0.586
DBP (mmHg)	0.977 (0.917 to 1.042)	0.488	0.975 (0.913 to 1.042)	0.457
Persistently active disease present	1.000 (0.262 to 3.820)	1.000	1.412 (0.330 to 6.035)	0.642
Damage score at scan	1.142 (0.686 to 1.899)	0.610	1.110 (0.664 to 1.854)	0.691
History of Lupus nephritis present	0.795 (0.211 to 3.000)	0.735	0.955 (0.238 to 3.826)	0.948
Total Cholesterol (mmol/dL)	0.484 (0.213 to 1.099)	0.083	0.445 (0.185 to 1.066)	0.069
HDL-Cholesterol (mmol/dL)	0.416 (0.101 to 1.707)	0.223	0.421 (0.098 to 1.806)	0.244
LDL-Cholesterol (mmol/dL)	0.546 (0.235 to 1.269)	0.160	0.485 (0.200 to 1.175)	0.109
Non-HDL Cholesterol (mmol/dL)	0.637 (0.292 to 1.388)	0.256	0.597 (0.267 to 1.332)	0.208
T-Chol./HDL ratio	0.886 (0.448 to 1.754)	0.729	0.853 (0.424 to 1.714)	0.654

Triglycerides (mmol/dL)	1.121 (0.297 to 4.232)	0.866	1.348 (0.343 to 5.303	0.669
Smoking at scan	1.182 (0.197 to 7.082)	0.855	1.914 (0.241 to 15.211)	0.539
Smoking – ever	0.900 (0.177 to 4.564)	0.899	0.875 (0.170 to 4.502)	0.873
HCQ therapy	0.786 (0.201 to 3.071)	0.301	0.930 (0.223 to 3.882)	0.921
Immunosuppression	2.080 (0.519 to 8.339)	0.301	2.369 (0.547 to 10.25)	0.249
Prednisolone-any dose	**4.375 (1.027 to 18.63)**	**0.046**	**4.895 (1.086 to 22.06)**	**0.039**
Prednisolone > 5 mg/day	6.538 (0.679 to 62.99)	0.104	**48.73 (1.218 to 1949.7)**	**0.039**
B-Cell depletion	2.227 (0.517 to 9.549)	0.283	5.999 (0.938 to 38.36)	0.581
ACE Inhibitors	1.000 (0.250 to 3.998)	1.000	1.457 (0.318 to 6.678)	0.628
Aspirin	0.700 (0.132 to 3.699)	0.675	0.789 (0.143 to 4.278)	0.781
Statin	3.077 (0.511 to 18.53)	0.220	2.954 (0.414 to 16.23)	0.308
Urea	1.092 (0.892 to 1.337)	0.393	1.078 (0.874 to 1.329)	0.481
lnCreatinine	1.624 (0.267 to 9.882)	0.599	2.172 (0.316 to 14.93)	0.430
Vitamin D	1.017 (0.989 to 1.045)	0.233	1.013 (0.984 to 1.042)	0.387
Albumin	1.070 (0.930 to 1.230)	0.345	1.050 (0.905 to 1.218)	0.521
lnESR	0.655 (0.303 to 1.417)	0.282	0.674 (0.306 to 1.486)	0.328

lnCRP	1.954 (0.925 to 4.125)	0.079	1.904 (0.888 to 4.084)	0.098
C3 at scan	3.555 (0.155 to 81.55)	0.428	2.368 (0.094 to 59.52)	0.600
lnAnti-dsDNA	0.888 (0.612 to 1.287)	0.530	0.922 (0.625 to 1.360)	0.683
lnAntiApoA1IgG	**0.571 (0.330 to 0.993)**	**0.047**	**0.572 (0.329 to 0.995)**	**0.049**
lnAntiApoA1IgM	0.929 (0.598 to 1.150)	0.262	0.831 (0.591 to 1.170)	0.290
AntiHDLIgG (above median)	3.250 (0.811 to 13.03)	0.096	2.743 (0.650 to 11.58)	0.170
Anticardiolipin ever	0.692 (0.128 to 3.752)	0.670	0.408 (0.062 to 2.702)	0.353
APL ever	0.800 (0.216 to 2.967)	0.739	0.555 (0.132 to 2.331)	0.421
Lupus Anticoagulant	0.480 (0.076 to 3.029)	0.435	0.338 (0.047 to 2.446)	0.283

MULTIVARIABLE LOGISTIC REGRESSION (only significant features shown)

Feature	**Odds Ratio (Exp (B))**	**P**	**95% CI**
Age at scan (years)	**1.137**	**0.031**	**1.012 to 1.278**
Prednisolone > 5 mg	**48.73**	**0.039**	**1.218 to 1949.7**

Legend

Association of clinical and serological features with TPA in the 36 patients with atherosclerotic plaques

The population of 36 patients with plaque was divided into two groups; those above and those below the median TPA. For each variable, the table shows the values for patients in the above-median TPA group compared to those in the below-median TPA group expressed as odds ratios.

Table 5

	UNIVARIABLE ANALYSIS	
Feature	**Odds Ratio (95% CI)**	**P**
Age at scan (years)	0.989 (0.917 to 1.066)	0.775
Disease duration (years)	0.982 (0.928 to 1.040)	0.537
Age at diagnosis (years)	1.010 (0.952 to 1.072)	0.736
SBP (mmHg)	0.983 (0.941 to 1.027)	0.448
DBP (mmHg)	0.933 (0.868 to 1.002)	0.055
Persistently active disease present	**7.857 (1.651 to 37.40)**	**0.010**
Damage score at scan	1.223 (0.728 to 2.053)	0.447
History of Lupus nephritis present	2.000 (0.520 to 7.691)	0.313
Total Cholesterol (mmol/dL)	0.792 (0.380 to 1.649)	0.533
HDL-Cholesterol (mmol/dL)	0.672 (0.180 to 2.513)	0.555
LDL-Cholesterol (mmol/dL)	0.832 (0.372 to 1.860)	0.654
Non-HDL Cholesterol mmol/dL	0.889 (0.419 to 1.887)	0.760
T-Chol./HDL ratio	1.275 (0.639 to 2.545)	0.490
Triglycerides (mmol/dL)	0.977 (0.259 to 3.682)	0.973

Smoking at scan	0.423 (0.065 to 2.766)	0.369
Smoking – ever	0.444 (0.083 to 2.388)	0.345
HCQ therapy	2.080 (0.519 to 8.339)	0.301
Immunosuppression	3.500 (0.825 to 14.85)	0.089
Prednisolone-any dose	**4.375 (1.027 to 18.63)**	**0.046**
Prednisolone > 5 mg/day	6.538 (0.679 to 62.99)	0.104
B-Cell depletion	1.300 (0.313 to 5.393)	0.718
ACE Inhibitors	2.800 (0.658 to 11.92)	0.164
Aspirin	1.429 (0.270 to 7.549)	0.675
Statin	1.429 (0.270 to 7.549)	0.675
Urea	1.161 (0.927 to 1.455)	0.194
lnCreatinine	10.25 (0.994 to 104.7)	0.051
Vitamin D	0.990 (0.964 to 1.017)	0.476
Albumin	0.879 (0.754 to 1.026)	0.102
lnESR	1.138 (0.538 to 2.406)	0.735
lnCRP	0.963 (0.487 to 1.904)	0.913

C3 at scan	**0.020 (0.001 to 0.777)**	**0.036**
lnAnti-dsDNA	1.006 (0.999 to 1.012)	0.078
lnAntiApoA1IgG	0.727 (0.437 to 1.207)	0.218
lnAntiApoA1IgM	0.889 (0.648 to 1.220)	0.467
AntiHDLIgG (above median)	1.031 (0.999 to 1.064)	0.059
Anticardiolipin ever	0.369 (0.060 to 2.274)	0.283
APL ever	0.318 (0.081 to 1.244)	0.728
Lupus Anticoagulant	0.480 (0.076 to 3.029)	0.435

MULTIVARIABLE LOGISTIC REGRESSION (only significant features shown)

Feature	**Odds Ratio (Exp (B))**	**P**	**95% CI for Odds Ratio**
Persistently active disease present	**9.661**	**0.010**	**1.724 to 54.126**
Prednisolone-any dose	**5.664**	**0.044**	**1.050 to 30.554**

Legend

Association of clinical and serological features with GSM in 36 patients with atherosclerotic plaques

The population of 36 patients with plaque was divided into two groups; those above and those below the median GSM. For each variable, the table shows the values for patients in the above-median GSM group compared to those in the below-median GSM group expressed as odds ratios. GSM was not associated with age, so there was no need to adjust for age.