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Prevalence of subclinical atherosclerosis is increased in systemic sclerosis and is associated with serum proteins: a cross-sectional, controlled study of carotid ultrasound

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Abstract

Objectives. SSc is associated with an increased prevalence of atherosclerosis (ATS). This study assessed the prevalence of subclinical ATS as measured by carotid US and explored serum proteins to identify potential biomarkers of SSc-ATS.

Methods. Forty-six SSc female patients and 46 age- and ethnicity-matched controls underwent carotid US to assess the presence of plaque and carotid intima media thickness (CIMT). Abstracted data included demographics, ATS risk factors and serum measurements [cholesterol, proinflammatory high-density lipoprotein (piHDL), CRP, lipoproteins]. Serum cytokines/proteins analyses included circulating type I IFN activity by quantifying IFN-inducible genes, soluble junctional adhesion molecule A (sJAM-A) and 100 serum proteins by using a microplate-based multiplex platform. Proteins significant at $P < 0.05$ on bivariate analyses for the presence of plaque were used to develop a composite measure.

Results. Patients with SSc had more plaque (45.6% *vs* 19.5%, $P = 0.01$) but similar CIMT compared with controls. Multiplex analysis detected significant associations between serum proteins of inflammation, vasculopathy and fibrosis with ATS in SSc, including IL-2, IL-6, CRP, keratinocyte growth factor, intercellular adhesion molecule 1, endoglin, plasminogen activator inhibitor 1 and insulin-like growth factor binding protein 3 associated with carotid plaque. Myeloid progenitor inhibitory factor 1, serum amyloid A, thrombomodulin, *N*-terminal pro-brain natriuretic peptide (BNP), and Clara cell secretory protein 16 kD correlated with CIMT. The median composite score for the plaque group was 6 and for the no plaque group it was 2 ($P < 0.0001$).

Conclusion. Patients with SSc have a higher prevalence of carotid plaque than matched controls, and patients with SSc-plaque *vs* patients without plaque have elevated serum proteins implicated in both vasculopathy and fibrosis. Further studies are needed to evaluate the role of these proteins in SSc compared with healthy controls.

Key words: systemic sclerosis, atherosclerosis, serum proteins, endothelial dysfunction, type I interferon, carotid intima media thickness.

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Introduction

SSc is a connective tissue disease characterized by immune activation, fibrotic processes and widespread vasculopathy. The association between CTDs and atherosclerosis (ATS) has been described in SLE and RA [1, 2]. However, the mechanisms of CTD-ATS remain elusive and may include inflammation burden, dyslipidaemia and disease-specific immune dysregulations [3–7].

The initial event in the pathogenesis of SSc is thought to be vascular injury. Mechanisms include immune-mediated endothelial injury [8, 9] and impaired angiogenesis in response to repeated ischaemia-reperfusion injury [10]. The SSc-related microvascular damage is well characterized and recent data suggest that there is increased carotid intima media thickness (CIMT) in patients with SSc compared with controls [11, 12]. Recent population-based data from Australian and UK registries also suggest increased prevalence of hard cardiac events compared with the general population [13, 14]. With these recent observations, the identification and utility of surrogate markers for SSc-ATS damage and the role of contributing factors to accelerated ATS in SSc still need to be studied.

Therefore we conducted a cross-sectional study to (i) evaluate the prevalence of subclinical atherosclerotic plaque and CIMT in patients with SSc and age- and ethnicity-matched healthy controls, and (ii) explore serum cytokines/proteins associated with carotid plaque and CIMT in patients with SSc.

Methods

Study subjects

Forty-six women who met the 1980 American Rheumatism Association criteria for SSc [15] were recruited. Control subjects were randomly selected from a cohort of 167 controls (initially recruited for the Biomarkers of ATS in SLE Cohort Study, which were women, self-reportedly healthy and without clinical evidence of SLE [13]) and matched to SSc subjects for age and ethnicity. All subjects were at least 18 years of age and provided institution-approved [University of California at Los Angeles (UCLA)] informed consent. Exclusion criteria (obtained directly from the subjects) included history of pre-existing cardiovascular disease (CVD) (myocardial infarction, stroke, peripheral vascular disease), uncontrolled hypertension, uncontrolled diabetes, current pregnancy and current or previous use of statin therapy within 6 months [because statins can reduce proinflammatory high-density lipoprotein (piHDL)] [16].

Clinical data

Sociodemographic information (age, ethnicity), height and weight, along with CVD risk factors including smoking, hypertension, diabetes mellitus and family history of CVD were collected. These data were obtained from the clinic charts and also from a self-administered health history questionnaire, which also assessed the medication. We assessed SSc disease duration (defined as the first non-RP symptom) and scleroderma subtype: limited cutaneous or diffuse cutaneous.

Carotid US

Carotid US was performed using a 5-mHz linear array transducer on a Toshiba 140 US (Toshiba, Tustin, CA, USA). Sonographers measured CIMT and plaque in the right and left common carotid arteries, carotid bulb and the first 1.5 cm of the internal and external carotid arteries. Plaque was defined as a focal projection within the intima media layer $\geqslant$50% of the adjacent CIMT. The carotid USs were read by a single blinded reader (N.R.).

Serum analysis

Serum analysis (drawn the morning of the US after overnight fasting) included CRP, ESR, lipid profile [total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides] and homocysteine. The serum of patients with SSc was further analysed for apolipoprotein A-1 (ApoA-1), apolipoprotein B100 (ApoB100) and lipoprotein.

Proinflammatory high-density lipoprotein

Normal HDL prevents oxidation of LDL and therefore the oxidation of dichlorofluorescein (DCFH), which releases a fluorochrome upon oxidation. To determine the functional properties of our subjects' HDL, we measured the change in fluorescence intensity resulting from oxidation of DCFH by LDL in the presence or absence of test HDL. Twenty microlitres of LDL solution from normal plasma (final concentration of 50 μg/ml) and 90 μl of test HDL (at a final concentration of 10 μg/ml cholesterol) were incubated in 96-well plates for 1 h. Ten microlitres of DCFH solution (0.2 mg/ml) was then added to each well and incubated for 2 h. Fluorescence was determined with a plate reader (Spectra Max, Gemini XS; Molecular Devices, Sunnyvale, CA, USA). Values of DCFH activated by LDL in the absence of HDL were normalized to 1.0 as the positive control. The piHDL levels were reported as mean (S.D.).

Soluble junctional adhesion molecule A

Plasma levels of soluble junctional adhesion molecule A (sJAM-A) were measured using a sandwich ELISA [9]. Reagents used include goat anti-human JAM-A (R&D Systems, Minneapolis, MN, USA) for the capture antibody and mouse anti-JAM-A antibody (Santa Cruz Biotechnology, Dallas, TX, USA) for the primary antibody. A standard curve was produced on each plate using recombinant sJAM-A conjugated to the Fc portion of IgG (R&D Systems). Nanomolar concentrations of IgG/Fc were used as a negative control on each plate to ensure specificity.

Type I IFN activity

Type I IFN activity was quantified as previously described [17]. HeLa cells were cultured in DIFCO/10% FBS/non-essential amino acids/10 mM Hepes at 37°C in

5% CO_2, plated at 2 × 105 cells/well in a 24-well plate and exposed to 50% scleroderma or control sera, or recombinant IFN-α (1 kU/well, used as positive control) or recombinant IFN-γ (200 ng/well, used as negative control for type I IFN-inducible genes; Peprotech, Rocky Hill, NJ, USA) for 6 h. TriPure was added and cells were stored at −70°C until RNA extraction. cDNA was made with Superscript II reverse transcriptase (Invitrogen). Real-time PCRs were run on an ABI PRISM 7900HT in duplicate using 2× SYBR GREEN PCR master mix (Applied Biosystems, Foster City, CA, USA) and primers previously described [17], at a concentration of 2.5 μM. The type I IFN-inducible genes quantified by this assay were IFN-induced protein-44 (IFI44), myxovirus resistance-1, IFN-induced protein with tetratricopeptide repeats 1 and double-stranded RNA-activated protein kinase. Samples were normalized to media alone after normalization to the housekeeping gene *HPRT-1*, and results were reported as fold induction/media (more details are available in Ansell *et al.* [16]).

Exploratory proteins

We utilized a microplate-based multiplex platform (SearchLight, Aushon Biosystems, Billerica, MA, USA) to quantify 100 proteins in SSc serum samples. The proteins were empirically selected by the authors to reflect processes of vasculopathy, inflammation and fibrosis; some of the proteins are still being explored, as they are involved in more than one pathway (i.e. IL-6). Each well of the microtitre plate was coated with analyte-specific antibodies, followed by washing and detection with a horseradish peroxidase (HRP)-based enzyme assay. Proteins were run in duplicate and the mean is reported (see supplementary Table S1, available at *Rheumatology* Online).

Statistical analysis

We compared the SSc patients to the controls using paired *t*-tests, Pearson's correlation, Wilcoxon matched-pairs signed-rank test and Spearman's correlation for parametric data.

Wilcoxon rank-sum tests and Spearman's correlations were used to assess the association of protein values with plaque and average CIMT and $P < 0.05$ was considered significant.

Development of a composite score

The proteins were examined in bivariate analyses with the presence or absence of plaque using Student's *t*-tests or Wilcoxon rank-sum tests. Proteins significant at the $\alpha = 5\%$ level were selected for receiver operating characteristic (ROC) analysis with the presence or absence of plaque as the outcome. The optimal cut point was determined for each protein using the criterion of maximum accuracy. Subjects were then classified as positive or negative on each protein. A composite score was determined by summing the number of positives among the selected candidate proteins (score 0–8). The discriminative predicting ability was assessed using ROC analysis.

For IFN analysis, we analysed the IFN signatures for association with plaque and CIMT using three methods: (i) We compared high *vs* low IFN producers based upon the 95th percentile control cut-offs on at least two IFN-inducible genes. (ii) We performed a principal component analysis (PCA). a separate composite score for each one-, two- and three-component PCA solution was formed by taking the Euclidean distance of the projected points in each case. These three PCA composite scores were then compared between the no-plaque and plaque groups using *t*-tests or Wilcoxon rank-sum tests. (iii) We also assessed IFN gene expression values by rescaling so that the maximum value for the gene was 1.0. These normalized values were summed across the set of genes to obtain a score for each subject. The composite score was then compared between the no-plaque and plaque groups. Spearman's correlation coefficient and resulting *P*-values were computed for the correlation between the above composite scores and (i) average CIMT and (ii) maximum CIMT.

Results

Forty-six females with SSc and 46 matched controls were enrolled (Table 1). Mean (S.D.) disease duration from the first non-RP symptom was 6.5 (5.2) years and 23 patients had limited and 23 had diffuse SSc. The SSc patients and controls had similar BMIs (Table 1).

A higher proportion of patients with SSc were reformed smokers, whereas a higher proportion of controls were current smokers ($P < 0.001$; Table 1). Patients with SSc had a greater family history of CVD compared with controls ($P < 0.05$; Table 1). Patients with SSc had higher triglycerides and inflammatory markers (ESR) but lower HDL compared with controls (Table 1). Among patients with SSc with and without plaque, there were no differences in inflammatory markers or levels of various lipoproteins (Table 2).

Carotid US

Patients with SSc had significantly more plaque than the controls ($P = 0.01$; Table 1). Even though patients with SSc had increased right CIMT, the average CIMT was not different between the groups (Table 1). There was no difference between plaque and CIMT in patients with limited *vs* diffuse SSc (see supplementary Table S1, available at *Rheumatology* Online).

Serum proteins analysis

Multiplex analysis

We explored the associations between various proteins and the presence/absence of plaque and CIMT in SSc (see supplementary Table S2, available at *Rheumatology* Online). Table 3 summarizes proteins significant at $P < 0.05$ with the presence/absence of plaque or CIMT. We divided the proteins into the following categories: fibrosis, inflammation and vasculopathy. The fibrosis group of proteins was associated with CIMT while the proteins implicated in vasculopathy were mostly associated with plaque. The inflammation proteins were associated with both plaque and CIMT. Among the 100 proteins studied,

Table 1 Demographics, CVD risk factors, piHDL and carotid US findings in SSc patients *vs* controls

	SSc ($n = 46$)		Controls ($n = 46$)		
	n	Mean (S.D.)	*n*	Mean (S.D.)	*P*-value
Age, years		48.6 (13.3)		48.6 (13.3)	—
Race, *n* (%)		—		—	—
Caucasian	35 (76.1)		35 (76.1)		
Black	4 (8.7)		4 (8.7)		
Asian	7 (15.2)		7 (15.2)		
Ethnicity, *n* (%)[a]					
Hispanic	5 (10.9)		5 (10.9)		
Non-Hispanic	30 (65.2)		30 (65.2)		
Risk factors, *n* (%)		—		—	
HTN	12/41 (29.2)		9/41 (21.9)		0.43
DM	1/41 (2.4)		3/41 (7.3)		0.15
Smoking					
Current	1/41 (2.4)		10/41 (24.4)		<0.001*
Past	14/41 (34.1)		0 (0)		<0.001*
Family history of CVD	25/40 (62.5)		10/40 (25)		<0.001*
BMI	35	24.8 (4.7)	35	23.9 (4.4)	0.51
Total cholesterol, mg/dl	45	198.4 (37.5)	45	196.8 (56.7)	0.60
LDL, mg/dl	45	117.2 (32.6)	45	113.2 (52.5)	0.22
HDL, mg/dl	45	52.8 (13.2)	45	60.1 (14.3)	<0.001*
Triglyceride, mg/dl	45	159.3 (119.6)	45	115.7 (63.3)	0.01*
ESR, mm/h	32	23.8 (18.2)	32	11.3 (11.2)	<0.001*
Homocysteine, mg/dl	22	10.2 (3.3)	22	8.6 (2.8)	0.07
piHDL, FU	30	0.88 (0.92)	30	0.91 (0.54)	0.20
Plaque, *n* (%)	21 (45.6)	—	9 (19.5)	—	0.01*
Right CIMT, mm	44	0.6 (0.15)	44	0.56 (0.13)	0.03*
Left CIMT, mm	44	0.57 (0.12)	44	0.56 (0.14)	0.21
Average CIMT, mm	44	0.59 (0.13)	44	0.56 (0.13)	0.07

All patients and controls were women. [a]Eleven patients did not report their ethnicity. FU: fluorescence units; HTN: hypertension; DM: diabetes mellitus; CVD: cardiovascular disease; LDL: low-density lipoprotein; HDL: high-density lipoprotein; piHDL: proinflammatory high-density lipoprotein; CIMT: carotid intima media thickness. *P-values <0.05.

only 2 were present in both SSc-plaque and SSc-CIMT: *N* terminal pro-brain natriuretic peptide (NT pro-BNP) and IL-6 (Table 3).

We found that eight proteins were associated with the presence of plaque ($P < 0.05$): CRP, insulin-like growth factor binding protein 3 (IGFBP-3), keratinocyte growth factor (KGF), IL-2, endoglin, intercellular adhesion molecule 1 (ICAM-1), plasminogen activator inhibitor 1 active (PAI-1) and IL-6 (Fig. 1A). Interestingly, five different proteins were associated with increased CIMT ($P < 0.05$): myeloid progenitor inhibitory factor 1 (MPIF-1), serum amyloid A (A-SAA), NT pro-BNP, Clara cell secretory protein 16 kD (CC16) and thrombomodulin (Fig. 1B). IGFBP-3 and PAI-1 were lower in patients with SSc-plaque, while the rest of the proteins were significantly higher (Fig. 1A). There were no differences in the piHDL, type I IFN and sJAM-A in SSc subjects *vs* controls (Table 2 and supplementary Tables S1and S2, available at *Rheumatology* Online).

Association of plaque with multiplex proteins

We modelled the predictive value of the eight proteins associated with plaque at $P < 0.05$ (Table 3) by deriving a composite score. The median (25th–75th percentile) composite score for the plaque group was 6 (5–6) and for the no plaque group 2 (1–4), $P < 0.0001$ (Fig. 2A). The ROC analysis showed that the optimal cut point of the composite score for predicting plaque is five (Fig. 2B). Specifically, the presence of more than five of the eight proteins is associated with a sensitivity/specificity of 0.90/0.84 for having SSc-plaque.

Discussion

The association with ATS is well described in other CTDs, but remains controversial in SSc. The mortality due to macrovascular disease is not sufficiently understood [18], although a recent meta-analysis suggests increased CIMT and reduced flow-mediated dilatation (both associated with increased risk of CVD) in SSc [12]. In addition, a recent population-based cohort study using the Health Improvement Network from the UK found that in patients with SSc, the risk of incident MI and stroke were increased 2-fold, while the risk for peripheral vascular disease was increased 4-fold compared with patients without SSc [14]. A similar cross-sectional cohort study

Table 2 Age, CVD risk factors, cholesterol, CIMT and apolipoproteins in SSc patients with and without plaque

	Plaque ($n = 25$)		No plaque ($n = 21$)		
	n	Mean (S.D.)	*n*	Mean (S.D.)	*P*-value
Age, years		47.2 (13.8)		50.2 (12.8)	0.44
Risk factors, *n* (%)					
HTN	21	6 (28.5)	20	6 (30)	1
DM	21	1 (4.7)	20	0 (0.0)	1
Smoking					
Current	21	0 (0.0)	20	1 (5.0)	0.48
Past	21	4 (19.0)	20	10 (50.0)	0.51
Family history of CVD	21	10 (47.6)	19	15 (78.9)	0.55
Total cholesterol, mg/dl	21	196.8 (34.9)	25	196.6 (42.5)	0.98
LDL, mg/dl	21	112.7 (32.4)	25	118.4 (34.9)	0.57
HDL, mg/dl	21	52.4 (11.5)	25	52.9 (14.5)	0.89
Triglycerides, mg/dl	21	194.2 (158)	25	126.6 (59.4)	0.14
Average CIMT, mm	20	50.2 (12.8)	24	0.57 (0.13)	0.35
ESR, mm/h	21	28 (16.5)	25	21.68 (20.38)	0.13
CRP, mg/dl	21	0.9 (0.7)	25	0.6 (0.46)	0.09
Homocysteine, mg/dl	20	11.2 (5.7)	21	8.9 (2.32)	0.13
ApoA-1, ng/ml	20	156.6 (32.1)	24	151.7 (22.1)	0.62
ApoB100, ng/ml	20	93.9 (22.2)	24	96.7 (22.8)	0.68
Lipoprotein, ng/ml	19	67.9 (75.6)	24	94.7 (96.6)	0.99

HTN: hypertension; DM: diabetes mellitus; CVD: cardiovascular disease; LDL: low-density lipoprotein; HDL: high-density lipoprotein; CIMT: carotid intima media thickness; ApoA-1: apolipoprotein A-1; ApoB100: apolipoprotein B100.

Table 3 Associations of serum proteins ($P < 0.05$) with the presence/absence of plaque and CIMT in SSc

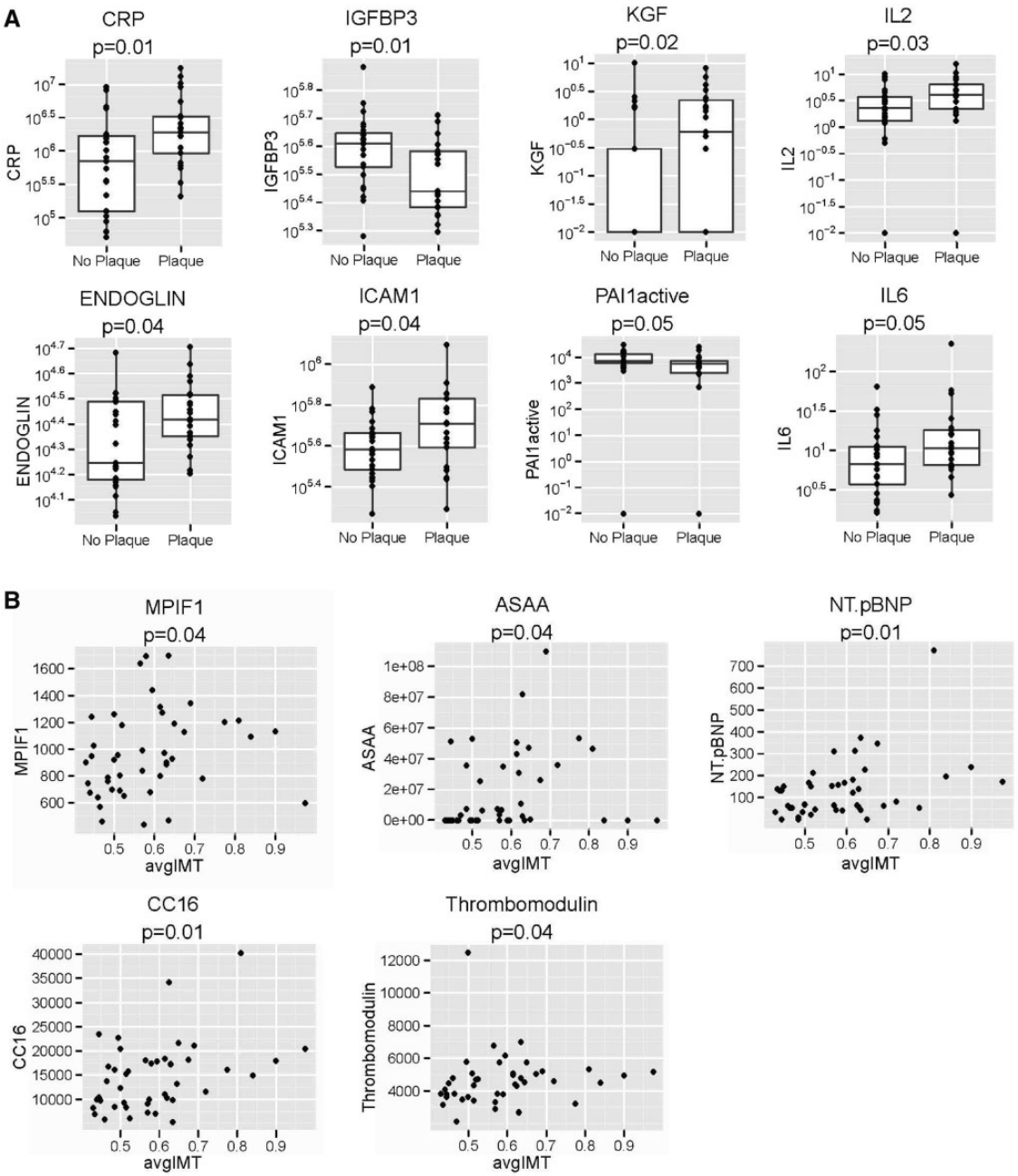
Biomarker (pg/ml)	No plaque, mean (S.D.) ($n = 25$)	Plaque, mean (S.D.) ($n = 21$)	*P*-value	CIMT ($n = 44$)	
				Correlation coefficients	*P*-value
Fibrosis					
MPIF-1	901.2 (143.7)	265.8 (223.3)	0.17	0.31	0.04*
CC16	14 492.7 (6631.9)	14 845.4 (7555.1)	0.93	0.37	0.01*
Inflammation					
IL-2	3.0 (2.6)	5.0 (3.8)	0.02*	−0.17	0.25
CRP	1 627 772.0 (2 437 838.6)	3 856 899.5 (4 734 651.2)	0.00*	0.14	0.35
IL-6	11.0 (13.5)	25.2 (48.5)	0.04*	0.28	0.06*
A-SAA	18 790 613.4 (30 238 222.3)	23 431 668.8 (30 070 001.2)	0.43	0.31	0.04*
Vasculopathy					
KGF	0.8 (2.1)	1.6 (2.1)	0.01*	0.19	0.22
ICAM-1	403 423.3 (131 159.9)	539 501.8 (242 768.3)	0.04*	0.16	0.29
PAI-1	9607.8 (6527.1)	6680.8 (5898.0)	0.04*	−0.10	0.29
Endoglin	22 444.1 (9253.0)	8860.8 (0.0394)	0.03*	0.07	0.51
IGFBP-3	404 848.8 (116 838.8)	322 117.3 (93 709.0)	0.00*	0.13	0.66
TM	4826.3 (1865.1) ($n = 24$)	4412.1 (1262.7)	0.00	0.32	0.03*

CIMT: carotid intima media thickness; MPIF-1: myeloid progenitor inhibitory factor 1; CC16: Clara cell secretory protein 16 kDa; A-SAA: serum amyloid A; KGF: keratinocyte growth factor; ICAM-1: intercellular adhesion molecule 1; PAI-1: plasminogen activator inhibitor 1; IGFBP-3: insulin-like growth factor binding protein 3; TM: thrombomodulin. *P-values <0.05.

involving patients from the Australian Scleroderma Cohort Study showed that the odds ratio of coronary heart disease was 3.2 when adjusted for cardiovascular risk factors [13]. These recent observations have kindled interest in assessing the surrogate markers that are associated or play a role in the pathogenesis of SSc-ATS.

Our study demonstrates that subclinical ATS, as measured by carotid plaque, is significantly higher in patients

FIG. 1 (**A**) Proteins significantly associated with the presence/absence of carotid plaque; (**B**) proteins significantly associated with increased CIMT

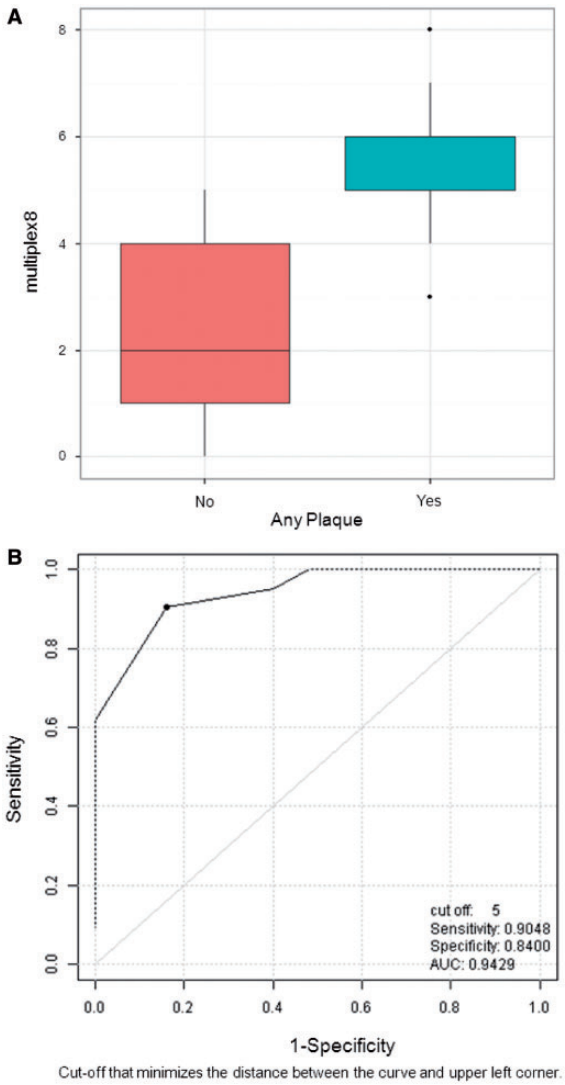


with SSc compared with controls. We also found that certain novel proteins are independently associated with carotid plaque and CIMT and we have developed a composite score that discriminates patients with and without plaque.

SSc-ATS has been described in coronary arteries [19, 20] and the cerebrovascular vasculature [20], but the most robust data are derived from carotid US [12]. We elected to utilize carotid US for our study because it is a non-invasive measurement [21] and because it is a surrogate marker of ATS in the general population [22] and rheumatic diseases [11].

Carotid artery plaque burden is a strong predictor of atherosclerotic-related mortality, as evidenced by longitudinal and population studies [23]. Coronary angiography and the coronary calcium score in patients with SSc [24, 25] are not well studied. However, there has been discussion about the contribution of CIMT as an

FIG. 2 (**A**) Composite score to model the predictive value of the eight proteins associated with plaque; (**B**) ROC analysis showing the ability of the five of eight proteins to predict the presence of plaque



independent risk factor for CVD: analysis from the Rotterdam study showed the additional predictive value of CIMT for CVD risk is small [26]. A recent report addressing the impact of untreated hypertension in a cohort of treatment-naive patients showed that CIMT is increased starting at an early level of blood pressure (BP) elevation and that it further correlates with higher levels of BP [27]. Based on these reports, the CIMT measurement may reflect an intrinsic vascular reaction (such as media thickness) in response to hypertension rather than incipient plaque.

In our bivariate analysis, we found that the four proteins grouped under fibrosis were associated with increased CIMT in SSc, while the vasculopathy markers had greater associations with plaque (Table 3). The function and studies supporting the role of each protein in ATS are summarized in supplementary Table S3, available at *Rheumatology* Online.

Our analyses showed associations of serum cytokines that have been previously described in human ATS and RA, such as IL-6, TNF-α and CRP [28, 29]. We also discovered new associations of serum proteins that have not been described in CTD-ATS and may play a unique mechanistic role in SSc. MPIF-1 is a specific inhibitor of myeloid progenitor cells and is the most potent activator of monocytes [30], with a monomeric structure that makes MPIF-1 a more attractive therapeutic target [31]. MPIF-1 plays a role in the angiogenesis process and is involved in human ATS via up-regulation of MMP-2 [31, 32]. Vascular endothelial cells, when exposed to MPIF-1 *in vitro*, showed markedly enhanced migration ability [31]. CC16 is found in airway secretions and serum and has been described as a highly sensitive biomarker of altered lung epithelial permeability [33] and a potential marker for active SSc-related pulmonary fibrosis [34]. We also found significant associations with SSc-plaque and KGF, an epithelial growth factor that controls epithelial cell differentiation and enhances T cell immune reconstitution in murine models of allogeneic umbilical cord blood transplant [35]. It has been linked to angiogenesis and fibroblast biology [36], which makes it an interesting target in T cell–mediated SSc manifestations. The role of these novel findings need to be explored. It is yet unknown whether these proteins are associated with SSc-vasculopathy or play a role in SSc-ATS.

It is unclear why we found that patients with SSc-plaque had lower levels of PAI-1 active and IGFBP-3 (Fig. 1A). PAI-1 is the main inhibitor of the plasminogen activator and has been found to be elevated in ATS, RA and SLE [37]. A possible explanation could be the fact that aside from a circadian variation, PAI-1 active spontaneously converts to latent forms [37]. IGFBP-3 has been found to be elevated in both ATS [38] and SSc [39], but no data about SSc-ATS exists.

We explored a composite index that can differentiate patients with plaque *vs* no plaque. The advantage of a composite index is that it can increase accuracy and reduce the variability associated with individual markers [40]. The eight-protein index is the first step in exploring the association with carotid plaque and has been successful in recent work by Farina *et al.* [40], where the four-gene biomarker was found to have a higher association with skin score than individual genes. Future studies need to assess if the eight-protein index has a higher association or is predictive of SSc-ATS as assessed by the presence of plaque and hard events.

Abnormally functioning piHDL has been associated with ATS in the general population, in subjects with SLE or RA [41, 42] and in a small SSc study [24]. piHDL may increase the atherosclerotic risk by potentiating LDL oxidation [43]. In our study there was no difference in piHDL relative to plaque or CIMT ($P = 0.09$), which might be explained by the heterogeneity of SSc and by the small sample size.

Recent evidence indicates that type I IFNs could play important roles in the development of vasculopathy and premature ATS in SLE [13, 17, 44]. IFN-α promotes an imbalance of endothelial damage and repair by interfering with proper vasculogenesis [17], promoting foam cell formation [13] and platelet activation [10] in patients with SLE. Type I IFNs may be associated to the development of ATS in humans and murine models in the absence of overt autoimmune disease [14, 45] and has been reported in other CTDs to correlate well with disease activity [46]. In our relatively small study, type I IFN serum activity did not correlate with CIMT or plaque in SSc. It is still possible that type I IFNs may contribute to aberrant vascular repair in SSc.

sJAM-A is a novel vascular biomarker that has been shown to be increased in SSc compared with controls [47]. sJAM-A plays an important role in the inflammatory thrombosis leading to atherogenesis [48] and correlates with the severity of coronary artery disease [49]. In this study there was no association between CIMT or plaque, which questions the role of sJAM-A in SSc-ATS.

Strengths and limitations

Our study is the first to look at correlations between subclinical SSc-ATS and serum proteins. We were able to identify a distinct set of proteins that could predict the presence of atherosclerotic plaque in patients with SSc.

Our study is not without limitations. First, it is limited by the lack of a control group for the serum proteins study (we only compared the piHDL and type I IFN in both cases and controls). In addition, some of the observed differences in the protein levels might be secondary to confounders such as age, ethnicity or treatment regimens that were not accounted for in the present study. Another major limitation of our study is our sample size. Although we matched for age and race, we found some differences in the CVD risk factors (i.e. smoking, history of diabetes). It is well known that SSc is phenotypically heterogeneous, and our small sample size did not allow for subclassification of the disease. Although we used carotid US to measure subclinical ATS, our study did not include measures of endothelial function such as flow-mediated dilatation or capture hard events such as myocardial infarction or cerebrovascular events. Although the role of steroid exposure and aspirin use are well defined in the pathophysiology of ATS, our study did not collect data on a cohort of patients large enough to permit further analysis. We recorded the presence/absence of diabetes mellitus but did not include glycosylated haemoglobin or microalbuminuria. Lastly, our study has the limitations of a cross-sectional design. The relationship between progression of atherosclerotic plaque and various serum proteins found to have significant associations still needs to be studied prospectively.

Conclusions

In conclusion, our study suggests that patients with SSc have a higher prevalence of carotid plaque than matched controls, and patients with SSc-ATS (as defined by presence of carotid plaque) have elevated ATS serum proteins that are implicated in both vasculopathy and fibrosis. Further prospective studies are needed to validate our preliminary data and evaluate the pathophysiological role of ATS-associated cytokines in SSc-related vasculopathy.

Rheumatology key messages

- Scleroderma patients have increased carotid plaque compared with age- and race-matched controls.
- Certain serum proteins implicated in vasculopathy/fibrosis are higher in SSc-plaque.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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