Neutrophil gelatinase-associated lipocalin levels are associated with skin thickness and metabolic syndrome features in patients with systemic sclerosis

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Abstract

Objective

Systemic sclerosis (SSc) is a chronic multisystem disease characterised by microcirculatory vascular dysfunction and progressive fibrosis of the skin and internal organs. Neutrophil gelatinase-associated lipocalin (NGAL) is a protein primarily secreted by immune cells and is known to be elevated in inflammatory states. Our study aims to investigate whether serum NGAL levels differ between individuals with SSc and healthy controls, and to explore its relationship with a comprehensive characterisation of disease features in SSc patients.

Methods

A cross-sectional study was conducted that included 81 individuals with SSc and 76 healthy age- and sex-matched controls. A multivariable analysis using linear regression was performed to determine whether NGAL serum levels differ between patients and controls, and to examine the relationship between NGAL levels and disease characteristics.

Results

Serum levels of NGAL do not differ significantly between patients and controls (188±110 vs. 197±147 ng/ml, p=0.67). However, after multivariable analysis, extension of the skin involvement (beta coefficient 5 [95% confidence interval 0.5–10] ng/ml, p=0.030) and disease duration (beta coefficient 6 [95% CI 0.1–12] ng/ml, p=0.045) were significantly associated with higher NGAL levels. Additionally, in patients with SSc, NGAL levels were independently and significantly related to a dyslipidaemia pattern manifested by higher serum levels of several lipid profile markers, such as LDL: HDL cholesterol ratio, apolipoprotein B: apolipoprotein A1 ratio, and atherogenic index. Furthermore, a significant and negative association between NGAL and the Homeostasis Model Assessment of Insulin Sensitivity (HOMA2-S%) was found.

Conclusion

NGAL serum levels in patients with SSc correlate positively and independently with extension of the skin involvement. NGAL levels also correlate with features of metabolic syndrome, such as a dyslipidaemic pattern and reduced insulin sensitivity, which may be associated with an increased risk of atherosclerosis and cardiovascular disease in patients with SSc.

Key words

systemic sclerosis, scleroderma, neutrophil gelatinase-associated lipocalin, lipocalin-2, skin thickness, dyslipidaemia, insulin sensitivity

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Introduction

Systemic sclerosis also called scleroderma (SSc) is a chronic multisystem disease characterised by widespread vascular dysfunction and progressive fibrosis of the skin and internal organs. It is considered a heterogeneous disease, reflected in a wide range of organic manifestations, disease progression and severity, and outcomes (1). SSc is traditionally classified based on the extent of skin involvement and the accompanying pattern of internal organ involvement, as well as the presence of overlapping features with other systemic rheumatic diseases. In this regard, the major subsets of SSc include limited cutaneous, diffuse cutaneous, SSc sine scleroderma, and SSc overlap syndrome (2). Cutaneous manifestations such as thickening and induration, and Raynaud's phenomenon, are almost universal clinical features of SSc. Other characteristics of the disease are the presence of digital ulcers and tissue loss, musculoskeletal manifestations, and gastrointestinal, pulmonary, and cardiac involvement (3, 4). Cardiovascular disease is common in SSc but often unrecognised until late in the disease. Furthermore, SSc patients are at increased risk of atherosclerosis compared with healthy subjects (5).

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein also known as lipocalin-2 that represents a family of lipocalin proteins. It is known to be a syderophoric protein that plays a role in regulating iron activity (6). The molecule of NGAL-containing iron interacts with receptors on the cell surface. Then, it is transported into the cell and releases iron inside. Although it is primarily secreted by immune cells, including neutrophils, macrophages, and dendritic cells, it is also expressed in a wide range of human tissues, such as the kidneys, heart, lungs, liver, stomach, and colon (7). NGAL is believed to be an acute-phase protein and plays a role in inflammation. In this regard, its serum concentrations positively correlated with inflammatory cytokines and increases in inflammatory states (8). Besides its levels rise in acute and chronic kidney and is believed to be a biomarker of kidney injury (9). High serum NGAL concentrations were also observed in heart failure and coronary artery disease, arterial hypertension, obesity, diabetes, and metabolic complications such as insulin resistance (8). To date, the differences in NGAL serum levels between patients with SSc and healthy controls, as well as the relationship of this molecule with clinical and laboratory manifestations in SSc, have not been investigated. In this study, we quantified serum NGAL levels in a well-characterised cohort of SSc patients and healthy subjects. Utilising multivariable analysis, we explored the differential expression of NGAL between patients and controls and its associations with a comprehensive array of disease manifestations.

Methods

Study participants

This was a cross-sectional study that included 81 patients with SSc and 76 sex and age-matched controls. All of them were 18 years or older and SSc patients met the American College of Rheumatology/European League Against Rheumatism 2013 classification criteria for SSc (10). They had been diagnosed by rheumatologists and were periodically followed-up at rheumatology outpatient clinics of our institution. For inclusion in the present study, SSc disease duration needed to be ≥1 year. Patients who had suffered a cardiovascular event were excluded. The patients were also excluded if they had a history of cancer or any other chronic disease, evidence of active infection or a glomerular filtration rate <60 ml/min/1.73m^2. The study protocol was approved by the Institutional Review Committee at Hospital Universitario de Canarias, and all subjects provided informed written consent (Approval code: EscleZ).

Assessments and data collection

Surveys in SSc patients and controls were performed to assess cardiovascular risk factors and medication use. Subjects completed a questionnaire and underwent a physical examination to determine anthropometric measurements and blood pressure. Medical records were reviewed to ascertain spe-

cific diagnoses, medications, and comorbidities. Hypertension was defined as a systolic or a diastolic blood pressure higher than, respectively, 140 and 90 mmHg. Obesity, defined as a body mass index (BMI) equal to or greater than 30 kg/m^2. Disease duration for SSc was defined as the time since the onset of the first SSc-related symptom other than Raynaud's phenomenon. SSc subtypes, limited and diffuse, were determined according to the distribution of skin thickness. The modified Rodnan Skin Score (mRSS) skin score was used to assess skin thickening (11). This score has been commonly used as an outcome measure in clinical trials. It rates the severity of these features from 0 (normal) to 3 (most severe) in 17 distinct areas of the body and shows an acceptable degree of intra-rater variability. Oesophageal involvement was defined as any sign of dysmotility evident on manometry. Articular involvement was determined by clinical evidence of joint swelling, deformity, contractures, and tendon friction rubs. Interstitial lung disease was defined instrumentally by forced vital capacity (FVC) ≤ 80%, forced expiratory volume in one second- FEV1/FVC ≥ 70% and/or diffusing capacity of the lung for carbon monoxide (DLCO) < 80% and interstitial changes on chest high-resolution computed tomography. Nailfold capillaroscopy was performed as previously described (12) and scleroderma patterns were sub-graded as 'early', 'active' and 'late' (12).

The cardiovascular risk score (SCORE2) was calculated according to the 2021 European Society of Cardiology guidelines on cardiovascular disease prevention in clinical practice (13). SCORE2 categorizes risk as low to moderate, high, or very high based on different age groups (<50, 50–69, and ≥70 years). The SCORE2 scoring system is designed to estimate the 10-year risk of both fatal and non-fatal cardiovascular events in individuals between the ages of 40 and 69 years. However, for healthy individuals who are 70 years or older, the SCORE2-OP (older persons) algorithm provides estimates for both 5-year and 10-year risk of fatal and non-fatal cardiovascular events. In patients with SSc, a carotid ultrasound examination was performed to evaluate the thickness of the carotid intima-media wall (cIMT) within the common carotid artery. The objective was to identify any localised plaques in the carotid arteries situated outside the skull (extracranial carotid tree). The measurements were carried out using the EsaoteMylab 70 ultrasound system from Genova, Italy. This system is equipped with a 7–12 MHz linear transducer and employs the Quality Intima Media Thickness in real-time (QIMT) automated software-guided radiofrequency technique developed by Esaote in Maastricht, Holland. The assessment process adhered to the guidelines established in the Mannheim consensus (14), which establishes criteria for identifying plaques within the accessible extracranial carotid arteries. These arteries include the common carotid artery, the bulb, and the internal carotid artery. Plaque criteria were established as the presence of a localised bulge within the arterial lumen, with a measurement of cIMT exceeding >1.5 mm. Additionally, the bulge needed to be at least 50% larger than the adjacent cIMT or result in an arterial lumen reduction of >0.5 mm (14).

Laboratory assessments

Fasting serum samples were collected and frozen at -80°C until analysis. Cholesterol, triglycerides, and HDL-cholesterol were measured using the enzymatic colorimetric assay (Roche). LDL-cholesterol was calculated using the Friedewald formula. Dyslipidaemia was defined if one of the following was present: total cholesterol >200 mg/dl, triglycerides >150 mg/dl, HDL cholesterol <40 in men or <50 mg/dl in women, or LDL cholesterol >130 mg/dl. A standard technique was used to measure high-sensitivity C-reactive protein (CRP). NGAL was measured by electrochemiluminescence immunoassay method (MERCK® MILLIPLEX map Multiplex Detection). Both the intra- and inter-coefficients of variability were <10% for these assays. The homeostatic model assessment (HOMA) method was performed to determine IR. Briefly, the HOMA model enabled an estimate of insulin sensitivity (%S) and β-cell function (%B) from fasting plasma insulin, C peptide, and glucose concentrations. In this study we used HOMA2, the updated-computer HOMA model (15). This model can be used to assess insulin sensitivity and β-cell function from paired fasting plasma glucose and specific insulin, or C peptide, concentrations across a range of 1–2,200 pmol/l for insulin and 1–25 mmol/l for glucose. C peptide better estimates β-cell function since it is a marker of secretion; and insulin data is preferable when calculating %S since HOMA-%S is derived from glucose disposal as a function of insulin concentration. In our study, IR and %S were calculated using insulin serum levels. Otherwise, %B was calculated using C-peptide serum levels. The computer model provided a value for insulin sensitivity expressed as HOMA2-%S (in which 100% is normal). HOMA2-IR (insulin resistance index) is simply the reciprocal of %S. Insulin (Architect Abbott, 2000I) and C peptide (Immulite 2000, Siemens) were determined by chemiluminescent immunometric assays.

Statistical analysis

Demographic and clinical characteristics of patients SSc were presented as mean (standard deviation) or percentages for categorical variables. For continuous variables that did not follow a normal distribution, data were reported as median and interquartile range (IQR). The association between disease-related data and NGAL was examined using multivariable linear regression analysis, with adjustments made for confounding variables. Confounders were selected from demographics if their *p*-values were below 0.20 in the univariable analysis to NGAL. All analyses were conducted using Stata software, v. 17/SE (StataCorp, College Station, TX, USA), with a two-sided significance level set at 5%. A *p*-value less than 0.05 was considered statistically significant.

Results

Demographic, laboratory, and disease-related data

In our study, we included 81 patients with SSc and 76 healthy controls

matched by age and sex. The characteristics of both populations are described in Table I. The subjects with SSc had a significantly lower BMI than the controls, although the effect size of this difference was small. No differences were found in the frequency of hypertension, dyslipidaemia or smoking, but the patients with SSc were less frequently diabetic. Additionally, although the patients with SSc tended to take statins less frequently, they were more often on aspirin treatment. The SCORE2 values did not differ between the groups. In terms of lipid profile, patients with SSc exhibited significantly higher triglyceride levels and Apo B: Apo A1 ratio, but lower apolipoprotein A1 levels. Additionally, C-peptide levels and HOMA2-B%-C-peptide were significantly elevated in patients with SSc compared to controls (Table I).

Eighty-one percent of the patients with SSc had the limited and 19% the diffuse type. The mean age at recruitment was 60±10 years. The disease duration was 8 (IQR 4–11) years. The median mRSS score was 4 (IQR 1–8). The presence of digital ulcers and calcinosis was reported in 15% and 19% of the patients, respectively. At the time the study was conducted, 16% of patients were taking prednisone with a median dose of 5 (IQR 5–7.5) mg/day, and 5% of the patients were taking methotrexate. Additionally, 55 (72%) patients were found to be positive for anti-centromere and 11 (14%) were positive for anti-Scl70. Other features related to the disease are shown in Table I.

Remarkably, NGAL did not differ between controls and patients (188±110 *vs*. 197±147 ng/ml, p=0.67).

Disease related data association with NGAL in patients with SSc

Regarding demographics or cardiovascular comorbidity, in patients with SSc no significant relationships were found between sex, classical cardiovascular risk factors, cardiovascular risk calculators, or the use of statins or aspirin, with serum NGAL levels (Table II). Only age showed a trend toward statistical significance.

With respect to disease characteristics, after adjusting for covariates, both

Table I. Demographics of systemic sclerosis patients and controls.

	Controls (n=76)	Scleroderma (n=81)	*p*
NGAL, ng/ml	188 ± 110	197 ± 147	0.67
Demographics			
Female, n (%)	71 (93)	76 (94)	0.92
Age, years	61 ± 12	60 ± 11	0.44
BMI, kg/m^2	**31 ± 3**	**29 ± 6**	**0.022**
Cardiovascular comorbidity			
Hypertension, n (%)	37 (49)	32 (40)	0.25
Current smoking, n (%)	11 (14)	7 (9)	0.25
Diabetes, n (%)	**19 (26)**	**7 (9)**	**0.006**
Dyslipidaemia, n (%)	63 (83)	72 (89)	0.28
BMI > 30 kg/m^2, n (%)	20 (26)	26 (32)	0.43
Statins, n (%)	29 (38)	20 (25)	0.069
Aspirin, n (%)	6 (20)	22 (27)	**<0.001**
SCORE2 calculator			
SCORE 2, %	5 (2-9)	4 (2-7)	0.15
SCORE2 categories, n (%)			
Low to moderate	38 (50)	45 (56)	
High	30 (39)	27 (33)	0.72
Very high	8 (11)	9 (11)	
Laboratory data			
CRP, mg/dl	2.0 (1.0-4.3)	2.2 (0.8-4.6)	0.51
Cholesterol, mg/dl	205 ± 43	207 ± 37	0.71
Triglycerides, mg/dl	**146 ± 64**	**187 ± 92**	**0.002**
HDL-cholesterol, mg/dl	55 ± 16	52 ± 12	0.16
LDL-cholesterol, mg/dl	120 ± 37	118 ± 33	0.66
LDL: HDL-cholesterol ratio	2.3 ± 0.9	2.4 ± 0.9	0.74
Non-HDL-cholesterol, mg/dl	149 ± 40	155 ± 36	0.35
Lipoprotein A, mg/dl	49 (14-102)	36 (13-91)	0.80
Apolipoprotein A1, mg/dl	**184 ± 40**	**165 ± 27**	**<0.001**
Apolipoprotein B, mg/dl	105 ± 30	105 ± 25	0.88
Apo B: Apo A1 ratio	**0.6 ± 0.2**	**0.7 ± 0.2**	**0.048**
Atherogenic index	3.9 ± 1.2	4.2 ± 1.2	0.21
Insulin resistance indices			
Glucose, mg/dl	105 ± 27	98 ± 22	0.098
Insulin, μU/ml	8.9 (6.4-14.5)	9.4 (4.9-18.0)	0.15
C-peptide, ng/ml	**2.6 ± 1.6**	**4.1 ± 3.2**	**<0.001**
HOMA2-IR	1.2 (0.8-2.0)	1.3 (0.6-2.2)	0.17
HOMA2-S%	91 ± 53	80 (45-160)	0.091
HOMA2-B%-C-peptide	**120 ± 59**	**181 ± 122**	**<0.001**
Systemic sclerosis related data			
SSc type, n (%)			
Limited, n (%)		66 (81)	
Diffuse, n (%)		15 (19)	
Disease duration, years		8 (4-11)	
Modified Rodnan Skin Score, units		4 (0-8)	
Raynaud phenomenon, n (%)		72 (90)	
Digital ulcers, n (%)		12 (15)	
Calcinosis, n (%)		13 (16)	
Arthritis, n (%)		8 (10)	
Gastric reflux, n (%)		41 (51)	
Pathological oesophageal manometry, n (%)		18 (55)	
Nailfold capillaroscopy pattern			
Normal		16 (22)	
Early		24 (33)	
Active		11 (15)	
Late		2 (3)	
Unclassified or not valuable		19 (26)	
Interstitial lung disease, n (%)		13 (17)	
FVC, %		93 ± 18	
FEV1, %		100 ± 18	
DLCO, %		75 ± 20	
Pulmonary hypertension, n (%)		12 (18)	
Anti-centromere antibody positivity, n (%)		55 (72)	
Anti-Scl70 antibody, n (%)		11 (14)	
Therapies			
Current NSAIDs, n (%)		8 (11)	
Current prednisone, n (%)		13 (16)	
Prednisone, mg/day		5 (5-7.5)	
Methotrexate, n (%)		4 (5)	
Chloroquine, n (%)		4 (5)	
Bosentan, n (%)		3 (4)	
Carotid atherosclerosis			
Intima media thickness, mm		663 ± 146	
Plaque		28 (34)	

Data represent mean ± SD or median (IQR) when data were not normally distributed.
NGAL: neutrophil gelatinase-associated lipocalin; Oesophageal manometry assessment was available only for 33 patients; BMI: body mass index; CRP: C reactive protein; SSc: systemic sclerosis; NSAIDs: non-steroidal anti-inflammatory drugs; SCORE2: systematic coronary risk; FVC: forced vital capacity; FEV: forced expiratory volume; DLCO: diffusion capacity of the lung for the carbon monoxide assessment; HOMA2-IR: insulin resistance index through homeostatic model assessment (calculated with glucose and insulin serum levels); HOMA2-S%: insulin sensitivity index through homeostatic model assessment (calculated with glucose and insulin serum levels); HOMA2-B%-C-peptide: β-cell function index through homeostatic model assessment (calculated with glucose and C-peptide serum levels). Significant p-values are depicted in bold.

mRSS (beta coef. 5 [95%CI 0.5–10] ng/ml, p=0.030) and disease duration (beta coef. 6 [95%CI 0.1–12] ng/ml, p=0.045) were significantly associated with higher NGAL levels. Additionally, the use of NSAIDs was significantly linked to elevated NGAL levels in subjects taking these medications (beta coef. 104 [3-205] ng/ml, p=0.043). However, the type of disease (limited or diffuse) or the presence of major organ involvement such as ulcers, calcinosis, pulmonary or cardiovascular disease, and the ANA profile, did not show significant relations to NGAL levels (Table II).

Relationship of lipid profile and insulin resistance indices to NGAL values

The relationship between the lipid profile, insulin resistance indices, and serum NGAL levels in patients with SSc is detailed in Table III. Regarding the lipid profile, after multivariable adjustment for age, smoking, and statin use, a negative and significant associations between NGAL and HDL-cholesterol was found. Furthermore, NGAL levels were independently and significantly related to several lipid profile markers, such as LDL: HDL cholesterol ratio, apolipoprotein B: apolipoprotein A1 ratio, and atherogenic index. Furthermore, in assessing the potential relationship between insulin resistance indices and NGAL, we disclosed a significant and negative association between NGAL and HOMA2-S% (Table III).

Table II. Demographics and disease related data association with NGAL in patients with systemic sclerosis.

	NGAL, ng/ml beta coef. 95% (CI), p			
	Univariable		Multivariable	
Demographics				
Female, n (%)	-36 (-172-101)	0.60		
Age, years	-3 (-6-0.004)	0.050		
BMI, kg/m^2	1 (-5-7)	0.69		
Cardiovascular comorbidity				
Hypertension, n (%)	26 (-42-95)	0.45		
Current smoking, n (%)	74 (-41-190)	0.20		
Diabetes, n (%)	-64 (-179-52)	0.28		
Dyslipidaemia, n (%)	30 (-29-89)	0.32		
BMI >30 kg/m^2, n (%)	33 (-39-104)	0.37		
Statins, n (%)	-0.6 (-77-76)	0.99		
Aspirin, n (%)	41 (-37-118)	0.30		
SCORE2 calculator				
SCORE 2, %	-4 (-12-3)	0.27		
SCORE2 categories, n (%)				
Low to moderate	-	-		
High	3 (-72-78)	0.94		
Very high	-56 (-170-57)	0.33		
Systemic sclerosis related data				
CRP, mg/dl	30 (-29-89)	0.32		
SSc type, n (%)				
Limited	-			
Diffuse	-17 (-102-68)	0.68		
Disease duration, years	5 (-1-11)	0.13	**6 (0.1-12)**	**0.045**
Modified Rodnan Skin Score, units	**6 (0.8-10)**	**0.023**	**5 (0.5-10)**	**0.030**
Raynaud phenomenon, n (%)	57 (-53-167)	0.31		
Digital ulcers, n (%)	47 (-48-143)	0.33		
Calcinosis, n (%)	25 (-67-118)	0.59		
Arthritis, n (%)	-7 (-124-110)	0.91		
Gastric reflux, n (%)	-55 (-123-12)	0.11	-52 (-119-14)	0.12
Pathological oesophageal manometry, n (%)	-75 (-230-80)	0.33		
Nailfold capillaroscopy pattern				
Normal	-			
Pathological	-20 (-103-63)	0.63		
Interstitial lung disease, n (%)	-8 (-104-87)	0.86		
FVC, %	-0.2 (-2-1)	0.80		
FEV1, %	-0.9 (-3-1)	0.34		
DLCO, %	0.2 (-0.9-1)	0.72		
Pulmonary hypertension, n (%)	-18 (-110-74)	0.70		
Anti-centromere antibody positivity, n (%)	20 (-58-98)	0.61		
Anti-Scl70 antibody, n (%)	-44 (-142-54)	0.38		
Therapies				
Current NSAIDs, n (%)	**116 (15-218)**	**0.025**	**104 (3-205)**	**0.043**
Current prednisone, n (%)	54 (-35-143)	0.23		
Prednisone, mg/day	-26 (-58-6)	0.10	-30 (-68-8)	0.11
Methotrexate, n (%)	-51 (-202-101)	0.51		
Chloroquine, n (%)	40 (-121-182)	0.69		
Bosentan, n (%)	-10 (-184-164)	0.91		
Carotid atherosclerosis				
Intima media thickness, mm	-0.7 (-0.3-0.2)	0.53		
Plaque	-11 (-103-81)	0.81		

In this analysis NGAL (neutrophil gelatinase-associated lipocalin) is the dependent variable. Oesophageal manometry assessment was available only for 33 patients.
SSc: systemic sclerosis; BMI: body mass index; CRP: C reactive protein; NSAIDs: non-steroidal anti-inflammatory drugs; SCORE2: systematic coronary risk assessment; FVC: forced vital capacity; FEV: forced expiratory volume; DLCO: diffusion capacity of the lung for the carbon monoxide. Significant p-values are depicted in bold.

Discussion

To our knowledge, no previous studies have analysed NGAL levels in patients with SSc. Based on our results, NGAL serum values do not differ from those in the general population but are associated with specific disease characteristics, disease duration, and metabolic syndrome features such as a deleterious lipid profile and reduced insulin sensitivity.

Regarding SSc features, we disclosed a positive and significant relationship between disease duration and skin thickness, as measured by the modified Rodnan skin score, with NGAL serum levels. This relationship persisted after multivariable adjustment. Skin involvement is a nearly universal feature of SSc. It is also known that in SSc, there is a deposition of adhesion molecules in the skin and infiltration of cells from the monocyte or macrophage lineages (16). This phenomenon could potentially lead to the subsequent overexpression of NGAL in serum, resulting in its upregulation. However, despite this, we did not find a relationship between NGAL and other organ manifestations such as pulmonary, cardiac, or vascular involvement. There were also no differences in NGAL levels between the limited and diffuse types of the disease. We do not have an exact explanation for this finding. Diffuse SSc differs from localised SSc not only in skin involvement but also in the distinct pattern of organ involvement. This difference might account for the findings in our study. The low prevalence of these manifestations in our cohort of patients with SSc may also have influenced the results. Therefore, studies specifically designed to address the values of NGAL in these

Table III. Relationship of lipid profile and insulin resistance indices to NGAL values.

	NGAL, ng/ml Beta coef. 95%(CI), *p*			
Lipid profile				
Cholesterol, mg/dl	0.2 (-0.8-1)	0.74		
Triglycerides, mg/dl	0.3 (-0.1-0.6)	0.19	0.3 (-0.06-0.7)	0.096
HDL-cholesterol, mg/dl	**-3 (-6-(-0.4))**	**0.027**	**-3 (-6-(-0.3))**	**0.036**
LDL-cholesterol, mg/dl	0.2 (-0.8-1)	0.66		
LDL:HDL-cholesterol ratio	**47 (5-89)**	**0.029**	**44 (0.5-87)**	**0.047**
Non-HDL-cholesterol, mg/dl	0.5 (-0.4-2)	0.28		
Lipoprotein A, mg/dl	-0.08 (-0.5-0.4)	0.72		
Apolipoprotein A1, mg/dl	-1 (-2-0.003)	0.051	-1 (-2-0.1)	0.075
Apolipoprotein B, mg/dl	1 (-0.09-3)	0.066	1 (-0.2-3)	0.087
Apo B:Apo A1 ratio	**258 (93-423)**	**0.003**	**238 (74-404)**	**0.005**
Atherogenic index	**38 (9-68)**	**0.011**	**37 (8-66)**	**0.013**
Insulin resistance indices*				
Glucose, mg/dl	0.7 (-2-3)	0.59		
Insulin, µU/ml	0.2 (-2-3)	0.87		
C-peptide, ng/ml	6 (-6-18)	0.34		
HOMA2-IR	2 (-20-24)	0.85		
HOMA2-S%	-0.2 (-0.4-0.06)	0.16	**-0.2 (-0.5-(0.02)**	**0.037**
HOMA2-B%-C-peptide	0.1 (-0.2-0.4)	0.34		

In this analysis NGAL (neutrophil gelatinase-associated lipocalin) is the dependent variable.
Multivariable analysis for lipid profile is adjusted for age, smoking and the use of statins.
*Multivariable analysis for insulin resistance indices is adjusted for age and smoking and is only performed in non-diabetic patients with systemic sclerosis.
HOMA2-IR: Insulin resistance index through homeostatic model assessment (calculated with glucose and insulin serum levels). HOMA2-S%: Insulin sensitivity index through homeostatic model assessment (calculated with glucose and insulin serum levels). HOMA2-B%-C-peptide: β-cell function index through homeostatic model assessment (calculated with glucose and C-peptide serum levels).
HDL: high density lipoprotein; LDL: low density lipoprotein. Significant *p*-values are depicted in bold.

organ manifestations will be needed to clarify this issue.

We found a strong positive association between NGAL and a dyslipidaemic lipid profile in patients with SSc. Specifically, after multivariable adjustment, NGAL was positively associated with HDL:LDL ratio, apolipoprotein B: apolipoprotein A1 ratio, and the atherogenic index, and negatively related to HDL. There was also a trend towards significance with triglycerides, and apolipoproteins B and A1. The observed relationship with multiple lipid profile components underscores the potential role of NGAL in lipid metabolism in patients with SSc. Besides, NGAL was related with lower levels of insulin sensitivity when assessed through HOMA index. In this regard, this negative relationship between NGAL and HOMA2-S implies that higher levels of NGAL are associated with lower insulin sensitivity. This suggests that the molecule might be linked to or contribute to insulin resistance in patients with SSc. Taken together, in clinical or research contexts, this suggests that NGAL may play a role in the development of metabolic syndrome, potentially increasing the risk of cardiovascular disease in patients with SSc. This finding aligns with previous reports on the role of NGAL in metabolic complications. In this regard, the adipose tissue is recognised as a source of NGAL, with elevated concentrations observed in conditions such as obesity, type 2 diabetes mellitus, and non-alcoholic fatty liver disease (17). Furthermore, NGAL is an independent risk factor for insulin resistance (18, 19). In this context, it has been demonstrated that a deficiency in NGAL offers protection against the progression of glucose metabolism disorders (20). Additionally, it has been documented that hyperglycaemia stimulates the synthesis of NGAL (21). We believe that these relationships observed in the general population likely persist in patients with SSc. This would explain the association found in SSc patients between NGAL and lipid profile values and insulin resistance indices.

NGAL concentrations have been reported to increase in patients with cardiovascular complications. This is supported by the expression of NGAL in heart tissue and atherosclerotic plaques (22). Besides, NGAL plasma levels were elevated in coronary artery disease and also in acute and chronic heart failure (23). Despite this, we did not find a relationship between NGAL and the presence of carotid subclinical atherosclerosis or other manifestations such as the presence of digital ulcers or pulmonary hypertension. We hypothesize that since atherosclerosis in patients with immune-mediated diseases differs etiopathogenetically from that in the general population, this could result in a different expression of NGAL as well.

Our data may have several implications in clinical practice or future research. For example, since NGAL is a biomarker associated with inflammation and tissue damage, particularly within the kidneys, regular assessment of NGAL levels might help in the early detection and management of kidney damage in patients with SSc. Besides, we believe studying NGAL levels in SSc patients could lead to insights into the mechanisms of organ involvement beyond the kidneys, as NGAL is also linked to lung and cardiovascular damage.

We acknowledge the limitation of our study's relatively small sample size. However, SSc is not a prevalent disease, which makes recruiting a large number of subjects challenging. Consequently, our results should be considered preliminary and will require confirmation in future studies on this topic. Furthermore, the cross-sectional design of our study does not allow for causal inferences. Longitudinal studies will be necessary to confirm the role that NGAL may play in this disease.

In conclusion, NGAL serum levels do not differ between patients with SSc and controls. However, NGAL is associated with a prominent manifestation of the disease in patients with SSc, specifically extension of the skin involvement. There also appears to be a relationship between NGAL and certain metabolic comorbidities, such as dyslipidaemia and insulin resistance, in SSc.

References

1. DI BATTISTA M, LEPRI G, CODULLO V *et al.*: Systemic sclerosis: one year in review 2023. *Clin Exp Rheumatol* 2023; 41(8): 1567-74. https://doi:10.55563/clinexprheumatol/ki76s5
2. SOBANSKI V, GIOVANNELLI J, ALLANORE Y *et al.*: Phenotypes determined by cluster analysis and their survival in the prospective european scleroderma trials and research cohort of patients with systemic sclerosis. *Arthritis Rheumatol* 2019; 71(9): 1553-70. https://doi.org/10.1002/art.40906
3. ALLANORE Y, SIMMS R, DISTLER O *et al.*:

Systemic sclerosis. *Nat Rev Dis Primers* 2015; 1. https://doi.org/10.1038/nrdp.2015.2
4. ARIAS-NUÑEZ MC, LLORCA J, VAZQUEZ-RODRIGUEZ TR *et al*.: Systemic sclerosis in Northwestern Spain: a 19-year epidemiologic study. *Medicine* 2008; 87(5): 272-80. https://doi.org/10.1097/md.0b013e318189372f
5. AU K, SINGH MK, BODUKAM V *et al*.: Atherosclerosis in systemic sclerosis: A systematic review and meta-analysis. *Arthritis Rheum* 2011; 63(7): 2078-90. https://doi.org/10.1002/art.30380
6. GOETZ DH, HOLMES MA, BORREGAARD N, BLUHM ME, RAYMOND KN, STRONG RK: The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. *Mol Cell* 2002; 10(5): 1033-43. https://doi.org/10.1016/S1097-2765(02)00708-6
7. KJELDSEN L, JOHNSEN AH, SENGELØV H, BORREGAARD N: Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. *J Biol Chem* 1993; 268(14): 10425-32. https://doi.org/10.1016/S0021-9258(18)82217-7
8. ROMEJKO K, MARKOWSKA M, NIEMCZYK S: The review of current knowledge on neutrophil gelatinase-associated lipocalin (NGAL). *Int J Mol Sci* 2023; 24(13): 10470. https://doi.org/10.3390/IJMS241310470
9. MISHRA J, QING MA, PRADA A *et al*.: Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. *J Am Soc Nephrol* 2003; 14(10): 2534-43. https://doi.org/10.1097/01.asn.0000088027.54400.c6
10. VAN DEN HOOGEN F, KHANNA D, FRANSEN J *et al*.: 2013 classification criteria for systemic sclerosis: an American College Of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2013; 72(11): 1747-55. https://doi.org/10.1136/annrheumdis-2013-204424
11. KHANNA D, FURST DE, CLEMENTS PJ *et al*.: Standardization of the modified Rodnan skin score for use in clinical trials of systemic sclerosis. *J Scleroderma Relat Disord* 2017; 2(1): 11-18. https://doi.org/10.5301/jsrd.5000231
12. SMITH V, HERRICK AL, INGEGNOLI F *et al*.: Standardisation of nailfold capillaroscopy for the assessment of patients with Raynaud's phenomenon and systemic sclerosis. *Autoimmun Rev* 2020; 19(3): 102458. https://doi.org/10.1016/j.autrev.2020.102458
13. VISSEREN FLJ, MACH F, SMULDERS YM *et al*.: 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. *Eur Heart J* 2021; 42(34): 3227-337. https://doi.org/10.1093/eurheartj/ehab484
14. TOUBOUL PJ, HENNERICI MG, MEAIRS S *et al*.: Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. *Cerebrovasc Dis* 2012; 34(4): 290-96. https://doi.org/10.1159/000343145
15. WALLACE TM, LEVY JC, MATTHEWS DR: Use and abuse of HOMA modeling. *Diabetes Care* 2004; 27(6): 1487-95. https://doi.org/10.2337/diacare.27.6.1487
16. SOLLBERG S, PELTONEN J, UITTO J, JIMENEZ SA: Elevated expression of beta 1 and beta 2 integrins, intercellular adhesion molecule 1, and endothelial leukocyte adhesion molecule 1 in the skin of patients with systemic sclerosis of recent onset. *Arthritis Rheum* 1992; 35(3): 290-98. https://doi:10.1002/art.1780350307
17. WU G, LI H, ZHOU M *et al*.: Mechanism and clinical evidence of lipocalin-2 and adipocyte fatty acid-binding protein linking obesity and atherosclerosis. *Diabetes Metab Res Rev* 2014; 30(6): 447-56. https://doi.org/10.1002/dmrr.2493
18. YAN QW, YANG Q, MODY N *et al*.: The adipokine lipocalin 2 is regulated by obesity and promotes insulin resistance. *Diabetes* 2007; 56(10): 2533-40. https://doi.org/10.2337/db07-0007
19. LAW IKM, XU A, LAM KSL *et al*.: Lipocalin-2 deficiency attenuates insulin resistance associated with aging and obesity. *Diabetes* 2010; 59(4): 872-82. https://doi.org/10.2337/db09-1541
20. JANG Y, LEE JH, WANG Y, SWEENEY G: Emerging clinical and experimental evidence for the role of lipocalin-2 in metabolic syndrome. *Clin Exp Pharmacol Physiol* 2012; 39(2): 194-99. https://doi.org/10.1111/j.1440-1681.2011.05557.x
21. LIN Y, RAJALA MW, BERGER JP, MOLLER DE, BARZILAI N, SCHERER PE: Hyperglycemia-induced production of acute phase reactants in adipose tissue. *J Biol Chem* 2001; 276(45): 42077-83. https://doi.org/10.1074/jbc.m107101200
22. HEMDAHL AL, GABRIELSEN A, ZHU C *et al*.: Expression of neutrophil gelatinase-associated lipocalin in atherosclerosis and myocardial infarction. *Arterioscler Thromb Vasc Biol* 2006; 26(1): 136-42. https://doi.org/10.1161/01.atv.0000193567.88685.f4
23. CRUZ DN, GAIAO S, MAISEL A, RONCO C, DEVARAJAN P: Neutrophil gelatinase-associated lipocalin as a biomarker of cardiovascular disease: A systematic review. *Clin Chem Lab Med* 2012; 50(9): 1533-45. https://doi.org/10.1515/cclm-2012-0307