

Serum Lipid Profile in Diffuse versus Limited Systemic Sclerosis

Data from the SASS cohort

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Data about lipoprotein changes and their link with cardiovascular disease and atherosclerosis in systemic sclerosis (SSc) are still challenging. We aimed to evaluate serum lipid profile of patients with SSc and to identify potential relation with different disease specific characteristics (clinical, serological, inflammatory tests) in a cross-sectional study. Standard assessments comprised SSc-related parameters (disease subtype, clinical spectrum, immunological tests) and lipid metabolism (total cholesterol and fractions, triglycerides). Impaired lipid profile (low serum HDL- and high LDL-cholesterol, increased serum triglycerides, slightly modification in total cholesterol level) significantly correlated with diffuse SSc, activity (EUSTAR) and severity (MEDSGER), as well as seropositivity for specific antibodies (anti-centromere and anti-topoisomerase 1). The dyslipidemic profile might represent a pathobiological pathway for atherosclerosis in SSc.

Keywords: systemic sclerosis, lipid metabolism abnormalities; cardiovascular burden

Systemic Sclerosis (SSc) is a rare autoimmune multisystem disease characterized by a broad spectrum of clinical findings as a result of key pathobiological events such as peripheral vasculopathy and excessive skin and visceral fibrosis with subsequent organ damage, occurring on a dysregulated immune background [1, 2, 3]. Based on the extent of skin involvement, diffuse and limited SSc are actually recognized as two main settings of the disease, characterized by different clinical, immunological, therapeutic as well as prognostic features [1, 2].

While microvascular abnormalities and Raynaud's phenomenon are major contributors of vascular pathology in SSc, data about the magnitude and etiology of macrovascular disease and accelerated atherosclerosis in SSc patients are still controversial [1-12].

Furthermore, multifactorial determinants of cardiovascular disease in SSc are already suggested, including classic cardio-vascular risk factors (diabetes, hypertension, dyslipidemia, smoking), endothelial damage and vascular dysfunction, in addition to disease-specific factors (SSc activity, severity, antibodies, medication) [1-19].

Traditional cardio-vascular risk factors seem to be equally distributed among SSc patients and their controls in the majority of studies in the literature [1, 2, 13, 14]; however, their prevalence was not extensively evaluated in such population [1, 13, 14].

Although various papers demonstrated an aberrant lipid metabolism (comprising but not limited to altered serum cholesterol and its fractions, triglycerides, lipoproteins) with significant impact on both subclinical and definite atherosclerosis as well as cardiovascular disease in

autoimmune rheumatic conditions [1, 4, 12, 15, 16], records about lipid changes and their particular relevance in defining cardiovascular burden in SSc remain debatable [18-25].

The objective of this study was to evaluate serum lipid profile of patients with systemic sclerosis and to identify potential relation with different disease specific characteristics (clinical, serological, inflammatory tests).

Experimental part

Material and method

We performed a cross-sectional observational study in consecutive SSc patients (fulfilling either 1980 ACR diagnostic criteria or new 2013 ACR/EULAR classification criteria) enrolled in the SSAS (Early Accelerated Atherosclerosis in Systemic Sclerosis) cohort, attending at least once the outpatient Rheumatology Department in the 162 EUSTAR Center of Iasi, Romania).

Standard assessments collected during the routine monitoring visit in our clinic included:

- *general data about SSc*, such as disease subtype (limited or diffuse cutaneous SSc; 1988 LeRoy classification), clinical spectrum, autoantibodies (total antinuclear antibodies - ANA, anti-topoisomerase-1, anti-centromere) and complement levels (C3 and C4 fractions), disease activity (EUSTAR) and severity (MEDSGER), treatment with synthetic anti-rheumatic drugs;

- *lipid profile*, comprising serum total cholesterol (TC), low- and high-density lipoprotein cholesterol fractions (LDL-C, HDL-C), triglycerides (TG) as well as the atherogenic plasma index (AI).

Lipids were determined as fasting levels using our local laboratory, and lipid levels of risk were classified in accordance with latest NCEP and AHA/ACC recommendations, as follows: plasma total cholesterol more than 200 mg/dL, HDL-cholesterol more than 40 mg/dL, LDL-cholesterol more than 130 mg/dL, while triglycerides more than 200 mg/dL, respectively.

None of enrolled patients was taking concomitant lipid lowering drugs (statins), corticosteroids, beta-blockers or diuretic, given the possible influence on lipid concentrations.

Moreover, we excluded those with comorbidities known to potentially alter lipid profile, such as diabetes, coronary artery disease, hypertension, thyroid disease.

All participants have signed a written informed consent before their enrollment and the study received Ethics Committee approval.

Statistical analysis was done in IBM SPSS-19 software, $p < 0.05$, with a subgroup analysis based on skin extent (diffuse, limited skin involvement) and serology profile (seropositive or seronegative patients). Multivariable regression analysis was done to evaluate association between lipid metabolism parameters and different SSc settings.

Results and discussions

SSc-related parameters

A total of 86 SSc were enrolled in the study, with a slight predominance of those presenting with the limited SSc (lcSSc) (48 cases, 55.81%) vs. diffuse SSc (dcSSc) subtype. Patients were mainly women (76 cases, 88.37%), in their fourth decade, with a mean age 45.3 (23-65) years and mean disease duration of 67 (16-127) months.

We described a wide clinical spectrum in our SSc, including both visceral and non-visceral involvement as follows: all patients presented Raynaud phenomenon, with digital pitting scars in 38 cases (44.18%), active digital ulcers in 25 cases (29.07%) and critical ischemia in only three cases (3.48%) at the time of the study; both non-erosive and erosive arthritis were described in 60 cases (69.76%), with consistent hand disability in up to one third of patients (30 cases, 34.88%) and subsequent altered quality of life (as appreciated by using a Health Assessment Questionnaire, mean score of 1.9 points). Only 16 (18.60%)

of our SSc had patent myositis, with moderate impact on daily activities, while significantly more patients had subclinical muscle involvement. Interstitial lung disease was present in 71 (82.55%) patients, while pulmonary arterial hypertension only in few cases (15 individuals, 17.44%). Cardiac arrhythmias were reported in 31 cases (36.04%), being symptomatic in about one third of them. In addition, very frequent gastrointestinal involvement (dysphagia and reflux due to esophageal dysmotility) was reported in 63 cases (73.25%). However, no scleroderma renal crisis or other types of renal involvement were found in the enrolled SSc.

Positive inflammatory syndrome (erythrocyte sedimentation rate, ESR; C reactive protein, CRP) was noticed in up to half of cases, with mild to moderate increase particularly in ESR, while CRP concentrations were abnormal in 30 cases (34.88%).

Specific SSc-autoantibodies were detected as follows: anti-centromere antibody (ACA) positivity in 36 cases (41.86%), all diagnosed with lcSSc subtype, while anti-topoisomerase-1 specificity (anti-topo1 or anti-Scl70 antibodies) in 32 cases (37.20%), all being classified as dcSSc subtype; furthermore, the majority of cases presented with ANA positivity (70 cases, 81.39%) and one third complement consumption (as defined by decreased C3 levels).

SSc-related parameters are summarized in Table 1 and lab assessments in table 2.

Regarding the medication, the majority of patients were on methotrexate (doses ranging between 10 and 20 mg once weekly, given for skin, articular and muscle complains), only four of them on cyclophosphamide (pulse-therapy monthly as they presented with severe symptomatic interstitial lung fibrosis) and five on cyclosporine A (skin, cardiac, erosive arthritis refractory to conventional immunosuppressants); all of them were taking calcium-channel blockers and pentoxifylline for their vascular disease, and those with esophageal signs and symptoms on proton pump inhibitors and prokinetic drugs.

Lipid profile in SSc

A complex assessment of the serum lipid metabolism parameters was performed as per protocol in all SSc patients, supporting the following data: mean total cholesterol level of 182.85 (120-300) mg/dL, mean HDL-cholesterol of 45.23 (35-70) mg/dL, mean LDL-cholesterol of 136 (125-200) mg/dL, while mean serum triglycerides were detected at a serum concentration of 157.2 (115-245) mg/dL (table 3).

Table 1
GENERAL SSC DATA IN STUDIED PATIENTS

Parameters	Values
Demographics	
Age (years)	45.3 (23-65)
Gender (%)	83.87% women
Disease-related	
Disease duration (months)	67 (16-127)
<i>Disease subset</i>	
• dcSSc (%)	44.19
• lcSSc (%)	55.81
Disease activity (MEDSGER)	1.63 (0-2)
Disease severity	4.4 (3-6)
Skin score (modified RODNAN, mRSS)	17 (6-38)
Interstitial lung disease (%)	82.55
Pulmonary hypertension (%)	17.44
Raynaud phenomenon (%)	100
Active digital ulcers (%)	29.07
Digital pitting scars (%)	44.18
Critical digital ischemia (%)	3.48
Myositis (%)	12.90
Arthritis (non-erosive, erosive) (%)	69.76
Cardiac arrhythmias (%)	36.04
Esophageal involvement (%)	73.25%

Table 2
SEROLOGIC AND BIOCHEMICAL ANALYSIS IN SSC

Lab parameters	
Antibodies positivity	
Anti-centromere, ACA (%)	22.58
Anti-topo1 (%)	58.06
ANA (%)	87.09
Inflammatory syndrome	
CRP (mg/dL)	6.2 (1-14)
ESR (mm/hour)	29 (15-64)
Lipid profile	
Cholesterol (mg/dL)	182.85 (120-300)
HDL-cholesterol (mg/dL)	45.23 (35-70)
LDL-cholesterol (mg/dL)	136 (125-200)
Triglycerides (mg/dL)	157.2 (115-245)

Lipid profile	Diffuse SSc	Limited SSc	P
Low HDL-cholesterol (%)	71.05	37.5	<0.05
High LDL-cholesterol (%)	57.89	31.25	<0.05
High triglycerides (%)	69.52	39.58	<0.05
Total cholesterol (%)	71.05	39.58	<0.05

Table 3
LIPID PROFILE IN dcSSc vs. lcSSc

Overall, we reported the aberrant lipid profile in more than half of studied SSc, the so-called *scleroderma-lipid pattern* comprising low HDL-cholesterol and high LDL-cholesterol fractions, with slightly increased in serum triglycerides, without a significant modification in total cholesterol levels.

We also completed an individual subgroup assessment of lipid profile in both disease subsets (dcSSc and lcSSc); we registered consistent differences among dcSSc and lcSSc patients ($p < 0.05$), meaning significant much more lipid abnormalities in diffuse vs. limited disease.

Patients with dcSSc showed lower serum HDL-cholesterol levels (71.05%) and higher LDL-cholesterol (57.89%) concentrations as compared to lcSSc (37.5% for HDL-cholesterol fraction; 31.25% for LDL-cholesterol, respectively) ($p < 0.05$).

Furthermore, significant more cases in the dcSSc subgroup had higher triglycerides (69.52 vs. 39.58%, $p < 0.05$) as well as lower cholesterol levels (71.05% vs. 39.58%, $p < 0.05$) as compared to lcSSc, particularly in patients fulfilling the criteria for CREST syndrome (an acronym for Calcinosis, Raynaud, Esophageal dysmotility, Sclerodactyly, Telangiectasia).

A detailed analysis of fasting lipids revealed the following abnormalities: mean total cholesterol was significantly lower for dcSSc than for lcSSc patients (127.65 mg/dL vs. 190.01 mg/dL, $p < 0.05$); mean HDL-cholesterol concentrations were lower for dcSSc patients than for lcSSc (43.5 mg/dL vs. 61.4 mg/dL, $p < 0.05$); the same trend was detected for the LDL-cholesterol fraction (128.6 mg/dL vs. 167.5 mg/dL) as well as for the triglycerides (112.78 mg/dL vs. 200 mg/dL).

Correlation between lipid metabolism and SSc-related parameters

We further evaluated potential relations between lipid metabolism parameters and different disease related (clinical, serologic) characteristics. We identified significant link between lipid modifications and the extent of skin involvement (high RODNAN skin score positively correlates with lipid anomalies, $p < 0.05$), disease duration (longer history of SSc promotes more dyslipidemic changes, $p < 0.05$), SSc activity (higher EUSTAR score associates with more significant lipid modifications, $p < 0.05$) and SSc severity (higher MEDSGER severity scores, higher lipid abnormalities, $p < 0.05$).

Moreover, patients with seropositivity for either ACA or anti-topo-1 antibodies had significant more dyslipidemic profile than those without serologic abnormalities (ACA negative or anti-topo-1 negative subgroup) ($p < 0.05$).

We assumed that patients with SSc are at risk to develop impaired lipid profile, meaning low HDL-cholesterol and high LDL-cholesterol fractions, high serum triglycerides, compromised total cholesterol levels, potentially involved in cardio-vascular disease and atherosclerosis.

Previously, we have already published data on impaired lipid pattern in different SSc settings and scenarios (patients included in the SASS cohort) [20-24]; however, available data on total cholesterol and its fractions, low-density and

high-density lipoprotein cholesterol, triglycerides and lipoproteins (A, B) in patients diagnosed with SSc, with special emphasis on different disease subsets remains still controversial [21-24].

In addition, it is widely accepted that immune mediated rheumatic conditions such as lupus, rheumatoid arthritis, even spondyloarthropathies, are characterized by abnormal lipid profiles (the pretended *lipid paradox*), both traditional and non-traditional (disease-related) cardio-vascular risk factors accounting for high burden of cardiovascular disease and atherosclerosis [1, 2, 11, 15, 16].

Both inflammation and immune abnormalities are linked with proatherogenic lipoprotein profile, comprising high LDL-cholesterol and triglycerides, together with low HDL-cholesterol and impaired total cholesterol, promoting early accelerated atherosclerosis in autoimmune rheumatic or non-rheumatic pathologies [2, 11, 19-25].

In the current study we reported defective serum lipids in a cohort of consecutive SSc patients, particularly in those classified as diffuse SSc subtype (elevated LDL-cholesterol, decreased HDL-cholesterol, high triglycerides and total cholesterol). Our data partially support data from literature investigating the potential contribution of lipoprotein abnormalities to vascular complications in SSc [12, 16-25].

Certain authors inconsistently reported altered total cholesterol and its fractions (low-density and high-density lipoprotein cholesterol), triglycerides and lipoproteins (LpA and B). Thus, total cholesterol, low density lipoproteins, high density lipoproteins, triglycerides, atherogenic index of plasma were either altered correlating with certain clinical (PAH) and immunological (anti-centromere, anti-topo1 antibodies) parameters [1, 23], or presented no adverse alterations [1, 24].

A closer look to lipid changes in our SSc patients highlighted that abnormal lipids were related to high RODNAN skin score, longer disease duration, anti-topo1 positivity, SSc activity and severity (EUSTAR, MEDSGER severity scale).

Finally, further studies are mandatory in order to clearly define the robust role of lipid abnormalities as traditional cardio-vascular risk factors and potential relations with disease-related parameters in patients with SSc.

Conclusions

Patients with SSc are at risk to develop abnormal lipid profile (with low serum HDL-cholesterol and high LDL-cholesterol levels, high triglycerides and total cholesterol), particularly those with a diffuse cutaneous SSc, those with active and severe disease, and specific autoantibodies. Furthermore, the dyslipidemic profile might represent one of the pathobiological pathways for atherosclerosis in SSc.

References

1. ORESKA S., TOMCIK M., Atherosclerosis and Cardiovascular Risk in Systemic Sclerosis, Systemic Sclerosis, Ph.D. Mislav Radic (Ed.), InTech, DOI: 10.5772/67495. Available from: <https://www.intechopen.com/books/systemic-sclerosis/atherosclerosis-and-cardiovascular-risk-in-systemic-sclerosis>

2. MAGDA S.L., MINCU R.I., MIHAI C.M., CINTEZA M., VINERANU D., Atherosclerosis in Systemic Sclerosis: a Modern Controversy, *A Journal of Clinical Medicine*, 10(3), 2015, p.248-256
3. PAGKOPOULAU E., POUTAKIDOU M., GARYFALLOS A., KITAS G., DIMITROULAS T., Cardiovascular Risk in Systemic Sclerosis: Micro and Macro vascular Involvement., *Indian Journal of Rheumatology*, 2, 2017, p. 211-2017
4. AU K., et al, Atherosclerosis in systemic sclerosis: A systematic review and meta-analysis. *Arthritis & Rheumatism*, 63, 2011, p.2078.
5. FARAG N.A., SEROUGY E.M. METAWEE S.A., AZIZI H.S., Subclinical atherosclerosis and peripheral vascular disease in systemic sclerosis patients: Relation to potential risk factors, *The Egyptian Rheumatologist*, 2015, 37(1):23-28
6. NORDIN A., et al, Ischemic Arterial Events and Atherosclerosis in Patients With Systemic Sclerosis, A Population-Based Case-Control Study, *Arthritis Research and Therapy*, 15, 2013, R 87.
7. SCHIOPU E., AU K.M., MCMAHON, M.A., KAPLAN M.J., DIVEKAR, A., et al, Prevalence of subclinical atherosclerosis is increased in systemic sclerosis and is associated with serum proteins: A cross-sectional, controlled study of carotid ultrasound, *Rheumatology (United Kingdom)*, 53 (4), 2014, p.704-713
8. NUSSINOVITCH U., SHOENFELD Y., Atherosclerosis and macrovascular involvement in systemic sclerosis: Myth or reality. *Autoimmun Rev.*, 2010, doi:10.1016/j.autrev.2010.09.014
9. VETTORI S., MARESCA L., CUOMO G., ABBADESSA S., LEONARDO G., VALENTINI G., Clinical and subclinical atherosclerosis in systemic sclerosis: consequences of previous corticosteroid treatment., *Scand J Rheumatol*, 2010, 39 (6):485-489
10. TSIFETAKI N., GEORGIADIS A.N., ALAMANOS Y., FANIS S., ARGYROPOULOU M.I., DROSOS A.A., Subclinical atherosclerosis in scleroderma patients., *Scand J Rheumatol.*, 39(4), 2010, p. 326.
11. NORDIN A., et al, Patients with systemic sclerosis and anti-centromere antibodies are affected with accelerated atherosclerosis, *Arthritis & Rheum*, 62 (10), 2010.
12. MISSALA I., KASSNER U., STEINHAGEN - THIESSEN E., A Systematic Literature Review of the Association of Lipoprotein(a) and Autoimmune Diseases and Atherosclerosis. Hindawi Publishing Corporation International Journal of Rheumatology, 2012, Article ID 480784, 10 pages
13. HETTEMA M.E., ZHANG D., DE LEEUW K., STIENSTRA Y., SMIT A.J., KALLENBERG C.G., BOOTSMA H., Early atherosclerosis in systemic sclerosis and its relation to disease or traditional risk factors. *Arthritis Res Ther*, 10(2), 2008, R49.
14. ZAKOPOULUS NA et al., Systemic sclerosis is not associated with clinical or ambulatory blood pressure., *Clin Exp Rheumatol* 21, 2003, p.199-204
15. BELIBOU C., ANCUTA C., ANCUTA E., FILOS C., CHIRIEAC R., Carotid intima-media thickness and plaque as surrogate biomarkers of atherosclerosis among consecutive women with systemic lupus erythematosus. *Rom J Morphol Embryol*. 2012; 53(1):29-34.
16. TOMS E.T., PANOULAS V.F., KITAS G.D., Dyslipidaemia in Rheumatological Autoimmune Diseases, *Open Cardiovasc Med J*. 2011, 5: 64-75.
17. NGIAN G., SAHHAR J., PROUDMAN S.M., et al, Prevalence of coronary heart disease and cardiovascular risk factors in a national cross-sectional cohort study of systemic sclerosis, *Annals of the Rheumatic Diseases* 2012;71:1980-1983.
18. PSARRAS A., SOULAIPOULOS S., GARYFALLOS A., KITAS G., DIMITROULAS T., A critical view on cardiovascular risk in systemic sclerosis., *Rheumatol Int.*, 37, 2017, p.85-95
19. DIMITRULAS T., GIANNAKOULAS G., KARVOUNIS H, GARYFALLOS A, SETTAS L, KITAS GD (2014)., Micro- and macro-vascular treatment targets in scleroderma heart disease., *Curr Pharm.*, 20, 2014, p.536-544
20. ZENG Y et al, Macrovascular involvement in systemic sclerosis: evidence of correlation with disease activity., *Clin Exp Rheumatol* 30 (2 Suppl 71), 2012, S76-S80
21. ANCUTA, C., POMIRLEANU, C., IORDACHE, C., FATU, A.M., POPESCU, E., ANCUTA, E., MIHAILOV, C., Periodontal Disease and Lipid Profile in Systemic Sclerosis: an EUSTAR Cohort Experience, *Rev. Chim. (Bucharest)*, 68, no.4, 2017, p.890-893
22. ANCUTA C., BELIBOU C., POMIRLEANU C., MIHAI C., CARREIRA P, ROSALES A.J.L., ALEGRE J.J., RICCIERIV, SALVADOR M.J., CHIRIEAC R., Abnormal lipid profile as a risk factor for atherosclerosis in systemic sclerosis: results from a multicentric EUSTAR cohort. *Journal of Scleroderma and Related Disorders*, 1, 2016, p. 43.
23. LIPPI G et al., Lipoprotein[a] and the lipid profile in patients with systemic sclerosis., *Clin Chim Acta* 364(1-2), 2006, p.345-348
24. BORBA E.F., BORGES C.T.L., BONFA E., Lipoprotein profile in limited systemic sclerosis., *Rheumatol Int*, 25, 2005, p. 379-383
25. GHEORGHE D.N., SURLIN P., HERASCU E., VERE C., et al, Metabolic status of periodontal patients with systemic diseases, *Romanian Journal of Oral Rehabilitation*, 2017, 9 (4):105-119

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