METABOLOMIC PROFILE IN PATIENTS WITH SYSTEMIC SCLEROSIS VERSUS HEALTHY SUBJECTS AND ITS ASSOCIATION WITH DISEASE PHENOTYPES

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ABBREVIATONS

- α-SMA: α-smooth muscle actin
- β2GP1: Anti-β2 glycoprotein-1
- AA: Amino acid
- AA-derived: Amino acid derived
- ACR: American College of Rheumatology
- ACA: Anti-cardiolipin antibody
- AD: autoimmune disease
- ANA: Antinuclear antibody
- CCP3: Cyclic citrullinated peptide third generation
- CENP-B: Centromere B antigen
- dcSSc: Diffuse systemic sclerosis
- dsDNA: Double-stranded DNA
- DU: Digital ulcers
- DMARDs: Disease-modifying antirheumatic drugs
- ECs: Endothelial cells
- ECM: Extracellular matrix
- EG: N-ethylglycine
- EULAR: European League Against Rheumatism
- FC: Fold change
- GC/MS-QTOF: Gas chromatography coupled to quadruple time-of-flight mass spectrometry.
- GER: Gastroesophageal reflux
- GSH: Glutathione
- HC: Healthy controls
- Hcy: Homocysteine
- IL: Interleukin
- ILD: Interstitial lung disease
- IQR: Interquartile range

- lcSSc: Limited systemic sclerosis
- PAH: Pulmonary arterial hypertension
- PCNA: Proliferating cell nuclear antigen
- PF: Puffy fingers
- RP Raynaud's phenomenon
- RF: Rheumatoid factor
- ROS: Reactive oxygen species
- SCR: Scleroderma renal crisis
- SD: Standard deviation
- SSc: Systemic sclerosis
- TG: Thyroglobulin
- TGF-β: Transforming growth factor beta
- TPO: Thyroid peroxidase

ABSTRACT

Background: Systemic sclerosis (SSc) is a chronic autoimmune disease with an unclear etiology. It is characterized by an unpredictable course, high morbidity, and an increased risk of mortality. According to recent studies, identifying altered metabolic pathways may be crucial to comprehend the physiopathology of the disease. Thus, metabolomics might play an important role in a better understanding of these pathogenic mechanisms and a possible tool to identify disease phenotypes.

Objective: To evaluate the differences in the metabolomic profile from amino acid-derived metabolites measured in serum samples of SSc patients compared to healthy subjects and their association with the different disease phenotypes.

Methodology: A case-control study was conducted. The serum concentration of amino acid-derived metabolites from SSc patients (n=38) compared to a control group (n=38) was measured. The metabolite differences in serum samples were analyzed using gas chromatography coupled to quadruple time-of-flight mass spectrometry (GC/MS-QTOF).

Results: The analysis of serum samples of 13 AA-derived metabolites revealed a significant downregulation of N-ethylglycine in SSc patients compared to healthy controls (p= 0,048). Likewise, fold change >1 of L-cysteine, DL-isoleucine, Sarcosine, L-proline, L-leucine, L-valine, Hydroxy-L-proline, Ala-ala, L-alanine, and L-serine showed a downward trend in patients with SSc compared to healthy subjects; however, this change was not statistically significant. Furthermore, our results demonstrated a significant increase in Ala-Ala and L-serine (p= 0,032) metabolites in the diffuse cutaneous SSc subtype, and a significant decrease in DL-isoleucine, L-leucine, and L-valine in SSc-interstitial lung disease patients.

Conclusion: These findings shed light on SSc patients' altered metabolic profiles and pathways, which may offer novel targets for SSc-directed therapies and diagnostics.

Keywords: *Systemic sclerosis; Metabolomics; Metabolic pathways; Amino acids.*

RESUMEN

Introducción: La esclerosis sistémica (ES) es una enfermedad autoinmune crónica con una etiología poco clara. Se caracterizada por un curso impredecible, alta morbilidad y un mayor riesgo de mortalidad. Según estudios recientes, la identificación de rutas metabólicas alteradas puede ser crucial para comprender la fisiopatología de la enfermedad. Por lo tanto, la metabolómica podría desempeñar un papel importante en una mejor comprensión de estos mecanismos patogénicos, así como una posible herramienta para identificar fenotipos de la enfermedad.

Objetivo: Evaluar las diferencias en el perfil metabolómico de los metabolitos derivados de aminoácidos medidos en muestras de suero de pacientes con ES en comparación con sujetos sanos y su asociación con los diferentes fenotipos de la enfermedad.

Metodología: Se realizó un estudio de casos y controles. Se midió la concentración sérica de metabolitos derivados de aminoácidos de pacientes con ES (n=38) en comparación con un grupo de control (n=38). Las diferencias de metabolitos se analizaron mediante cromatografía de gases acoplada a espectrometría de masas con analizador de tiempo de vuelo (GC/MS-QTOF).

Resultados: El análisis de muestras de suero de 13 metabolitos derivados de aminoácidos reveló una regulación negativa significativa de N-etilglicina en pacientes con ES en comparación con controles sanos (p= 0,048). Asimismo, un cambio >1 de L-cisteína, DL-isoleucina, Sarcosina, L-prolina, L-leucina, L-valina, Hidroxi-L-prolina, Ala-ala, L-alanina y L-serina mostró una tendencia a la baja en pacientes con ES en comparación con sujetos sanos; sin embargo este cambio no fue estadísticamente significativo. Además, nuestros resultados demostraron un aumento significativo en los metabolitos Ala-Ala y L-serina (p = 0,032) en la ES difusa y una disminución significativa en DL-isoleucina, L-leucina y L-valina en pacientes con enfermedad pulmonar intersticial.

Conclusión: Estos hallazgos arrojan luz sobre los perfiles y vías metabólicas alteradas de los individuos con ES, que pueden ofrecer dianas terapéuticas novedosas y diagnósticos dirigidos a la enfermedad.

Palabras clave: *Esclerosis sistémica; Metabolómica; Vías metabólicas; Aminoácidos.*

1. PROBLEM STATEMENT

1.1 PROBLEM FORMULATION

Systemic sclerosis (SSc) is a chronic multisystemic autoimmune disease (AD), with an etiology that to date remains poorly understood. This presents a clinical challenge for both physicians and patients affected by it (1). It is characterized by an unpredictable course, high morbidity, and mortality, higher than any other ADs (2). In a 2014 meta-analysis, Rubio-Rivas et al. reported an overall mortality ratio of 2.72, ranging from 1.05 to 5.4 across all studies (3). Recent studies have reported a mortality ratio of 1.39 to 5.1 times greater than that of the general population, with cardiopulmonary compromise impairment being the primary cause of death (4–6) and disease-related mortality at approximately 55% (7).

Worldwide, the prevalence of SSc is approximately 17.6 cases per 100,000 population, and the incidence rate is 1.4 per 100,000 person-years; however, there is significant variability between different geographic populations (8). For instance, prevalence and incidence estimates for North America are approximately 25.9 cases per 100,000 people and 15.1 cases per person-years, respectively (9). In comparison, Japan's prevalence and incidence estimate are approximately 37 cases per 100,000 inhabitants and 6.6 per person-years, respectively (10). In Colombia, in the year 2020, Fernández-Ávila et al., using data from the Integrated Social Protection Information System (SISPRO) corresponding to the years 2012-2016, analyzed the prevalence of the disease. The estimated prevalence was 23.7 cases per 100,000 inhabitants, with Bogotá being the city with the highest number of cases (11).

A striking characteristic of the disease is its variability from patient to patient, observing heterogeneity in clinical manifestations, serological profiles, and the rate of disease progression (12). Although skin fibrosis is the disease's hallmark, cardiopulmonary, renal, and gastrointestinal complications determine the clinical outcome (13). Concerning the extension of the disease in the skin, two subtypes are distinguished: diffuse systemic sclerosis (dcSSc) and limited systemic sclerosis (lcSSc). Each subtype is characterized by different clinical manifestations, involvement of different organs, and disease progression, with dcSSc having a

rapid evolution with extensive skin changes and early onset of comorbidities, increasing the mortality rate (14).

The National Registry of Patients with Orphan Diseases in Colombia, according to the list of diseases issued in Resolution 5265 of 2018 of the Ministry of Health and Social Protection, reports 52,753 confirmed cases in the country. Of these, the first ten diseases concentrate 32% of the total registered cases, finding dcSSc in eighth place with 919 cases, representing 1.7% (15).

Due to its high mortality, the difficult management of the disease, and the diagnostic challenge that it entails, the search for new techniques that allow the identification of SSc in the early phases to quickly initiate management has been encouraged, as well as techniques that allow the characterization of the clinical presentations of the disease to administer individualized therapies.

From metabolomics, an analytical technique that measures and compares metabolites in biological samples (16), this study aims to evaluate the metabolomic profile from amino acid (AA)-derived metabolites in patients diagnosed with SSc in comparison to healthy subjects and determine if deregulations can be associated with the disease presentation and the different clinical presentations according to disease phenotypes.

1.2 JUSTIFICATION

Understanding the possible pathophysiological mechanisms that trigger SSc and determining the etiology of the disease will enable the development of management schemes capable of modifying its course, positively impacting the prognosis of the disease and decreasing mortality rates.

Omics sciences aim to characterize and quantify groups of biological molecules throughout the organism according to their structure, function, and dynamics. These sciences focus on specific approaches such as early prevention and disease-modifying therapies (17). Investigating in omics sciences, specifically in metabolomics, seeks to increase knowledge about the

pathophysiology of the disease and the early characterization of patients. This approach can elucidate possible alterations in the metabolic pathways responsible for the mechanisms underlying the development of the disease, and the different clinical presentations according to disease phenotypes.

Metabolomics offers new insights into the altered metabolic state in patients with SSc, reflecting the disease phenotype. Therefore, metabolite alterations may provide unique phenotypic information and reflect individual responses (18). This technique offers new insights into the metabolic state in patients with SSc in order to apply this knowledge for early detection, diagnosis, disease typing, and treatment control, as well as may be promising for the discovery of new biomarkers preventing the progression of the disease to irreversible advanced stages (19).

Consequently, this research aims to compare the metabolomic profile of individuals with SSc with healthy individuals and ascertain if deregulations are linked to the disease presentation and the various clinical manifestations based on phenotypes.

1.3 RESEARCH QUESTION

What are the differences in the metabolomic profile measured in serum samples and its association with the disease phenotype in patients diagnosed with systemic sclerosis versus healthy subjects?

1.4 PICO STRATEGY

Population	Patients diagnosed with Systemic sclerosis by ACR/EULAR 2013 criteria
Intervention	Metabolomic profile in patients with Systemic sclerosis
Compare	Metabolomic profile in patients with healthy subjects
Outcome	Disease development and phenotypes

2. THEORETICAL FRAMEWORK

2.1 SYSTEMIC SCLEROSIS

Systemic sclerosis is a complex and chronic multisystemic disease of the connective tissue of unknown etiology but of autoimmune basis, characterized by a pathogenic triad consisting of vascular damage, alteration of the immune system, and progressive fibrosis of the skin and internal organs (Fig. 1) (12,14). The organs and systems that are most frequently compromised are the skin, lungs, heart, kidneys, digestive tract, and the osteoarticular system (20). A characteristic of the disease is patient-to-patient variability and heterogeneity in clinical manifestations, serological profiles, and the rate of disease progression (13).

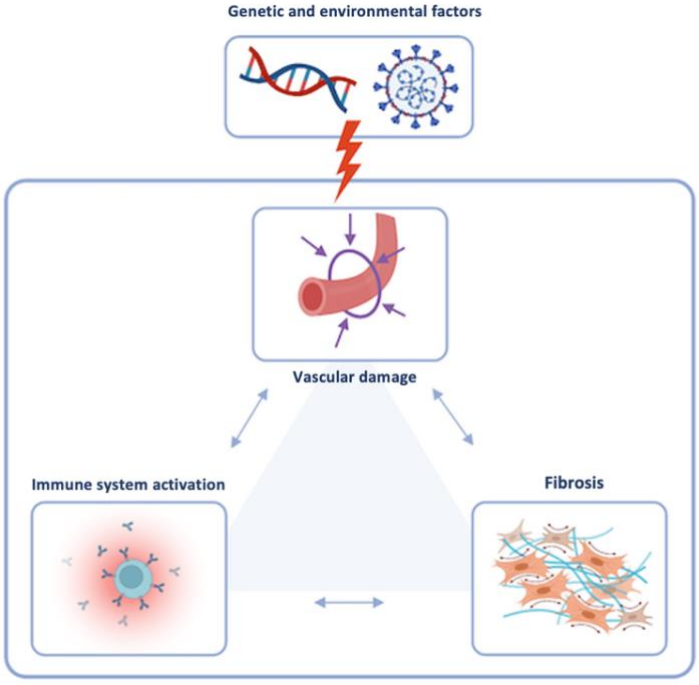

Figure 1. Pathogenic triad of systemic sclerosis. The onset of SSc is caused by both genetic and environmental factors. SSc is distinguished by vascular changes, inflammation and autoimmunity, as well as excessive fibrosis of multiple organs. *Made with BioRender*.

2.1.1 EPIDEMIOLOGY

Globally, the prevalence of SSc is estimated to be 17.6 cases per 100,000 people, and an incidence rate is 1.4 per 100,000 person-years (8,21). Both epidemiological measures have

significant variability between geographic populations; for example, in Europe, the prevalence ranges from 7.2 to 33.9 per 100,000 people, and the incidence is approximately 0.6 to 2.3 per 100,000 person-years (22), whereas, in North America, the prevalence and incidence estimates are approximately 25.9 cases per 100,000 inhabitants and 15.1 per person-years respectively (9). . The prevalence and incidence rates in Japan are approximately 37 cases per 100,000 people and 6.6 per person-years, respectively (10). On the contrary, in Korea, the prevalence is 7.7 cases per 100,000 people (6). The ratio of affectation by gender, female: male, ranges from 3:8 to 15, and the age group with the highest frequency of affectation is between 45 and 64 years (23–25).

Fernández-Ávila et al., in 2020, using data from the Integrated Social Protection Information System (SISPRO) corresponding to the years 2012-2016, analyzed the prevalence of the disease in Colombia. The estimated prevalence was 23.7 cases per 100,000 people, with Bogotá being the city with the highest number of cases. It is more common in the female sex in the age group of 65-69 years (77%) and has a female: male ratio of 3-7:1 (11). According to the list of orphan diseases released by the Ministry of Health and Social Protection in Resolution 5265 of 2018, the National Registry of Patients with Orphan Diseases in Colombia reports 52,753 confirmed cases. Of these, the first ten diseases comprise 32% of the total registered cases, with diffuse cutaneous systemic ranking eighth with 919 cases, accounting for 1.7% (15).

Although the prevalence of SSc is relatively low, the disease burden is substantial. Recent studies have reported a mortality ratio that is 1.39 to 5.1 times higher than the general population, with cardiopulmonary compromise being the leading cause of death (4–6), with disease-related mortality of approximately 55% (7), being the most important causes of disease related mortality interstitial lung disease (ILD), pulmonary arterial hypertension (PAH), scleroderma renal crisis (SCR), and cardiac involvement (26).

2.1.2 PATHOPHYSIOLOGY

The pathophysiology of SSc is complex and remains poorly understood (13). This disease is characterized by a pathogenic triad consisting of endothelial dysfunction, abnormalities of the innate and adaptive immune system with autoantibody production and cell-mediated autoimmunity, and fibroblast dysfunction with excessive collagen deposition, leading to progressive fibrosis of the skin and internal organs (27,28). Environmental factors that may contribute to the development of the disease have been identified, such as previous occupational exposure to compounds such as silica, solvents, pesticides, and epoxy resins (29). Infectious agents such as Parvovirus B19, cytomegalovirus, Epstein-Barr virus, and retroviruses may disrupt T and B cell tolerance by molecular mimicry and simultaneous activation of innate responses in the presence of pathogen-associated molecular patterns, tailoring the immune system to enhances responses. Therefore, these viruses have been proposed as initiating triggers of SSc (30). On the other hand, genome-wide association analyses have identified HLA and non-HLA genes with polymorphisms associated with SSc (31). The analysis of the commitment of these genes has revealed the importance of cytokines in the pathophysiology of SSc, reinforcing the link between genes and the immune response (32).

Endothelial damage is one of the earliest events in the natural history of SSc, along with Raynaud's phenomenon (RP) and digital ulcers (DU). Endothelial dysfunction promotes vasoconstriction by inducing the secretion of endogenous vasoconstrictive agents and reducing the secretion of vasodilators such as nitric oxide, leading to an alteration of tissue oxygenation (27). Additionally, abnormalities in the innate and adaptive immune systems contribute to the development of SSc. The Th2 subtype, which makes up the majority of skin-infiltrating T cells, is associated with an increase in related cytokines such as Interleukin (IL)-4, IL-13, and IL5, which have been linked to fibrosis. Activation of fibrotic mechanisms leads to a remodeling of the extracellular matrix (ECM) through collagen production (27,33). On the one hand, pro-inflammatory cytokines such as IL-1, TNF, and IL-6 released by stressed cells can activate macrophages, triggering the production and activation of transforming growth factor (TGF-β) (34). Fibroblasts isolated from skin lesions of patients with SSc have a phenotype similar to activated myofibroblast. This fibroblast is characterized by the expression of α-

smooth muscle actin (α-SMA) and excessive production of collagen and other ECM macromolecules due to different stimuli, mainly from the TGF-β pathway. These characteristics lead to increased collagen production by SSc fibroblasts (35,36).

2.1.3 CLINICAL MANIFESTATIONS

SSc is mainly characterized by developing skin manifestations; however, it is also associated with manifestations and complications in internal organs, mainly in the gastrointestinal tract, lungs, heart, and kidneys (Fig. 2).

2.1.3.1 CUTANEOUS MANIFESTATIONS

Skin fibrosis, or scleroderma, refers to the thickening and hardening of the skin, this being the hallmark of SSc (37). Scleroderma is characterized by extensive fibrosis in the dermis along with large ECM deposits, mainly type I collagen (38). Dermal induration in patients with SSc generally occurs in three phases: early edematous phase, in which the swollen fingers have a "sausage finger" appearance, followed by an induration phase in which the skin acquires a firm consistency. , developing a shiny appearance, and finally, an atrophic phase where the thinning of the skin over the joints begins; however, patients may be left with permanent joint contractures, called sclerodactyly (39,40). This induration is also commonly associated with hyperpigmentation or hypopigmentation, especially the "salt and pepper" appearance characterized by depigmentation with perifollicular pigment retention in the indurated areas (41), as well as the presence of vascular dilations, called telangiectasias, which appear most frequently on the face and hands (39).

On the other hand, RP affects 95% of patients with SSc in the initial stages of the disease (42). RP is usually bilateral, primarily affecting the fingers and toes, and is characterized by episodic vasospasm of digital arteries resulting in pain, paresthesia, and discoloration of the fingers (43). This phenomenon occurs in 3 different phases: an ischemic phase characterized by the fingertips' pallor, a blue phase during which the fingers become painfully cyanotic, and a red phase due to revascularization (44). Other skin signs that may occur are nail fold alterations (80%), UD (40%), and skin calcifications (25%) (37).

2.1.3.2 GASTROINTESTINAL MANIFESTATIONS

The gastrointestinal tract is the second most affected system in people with SSc. Up to 90% of patients manifest gastrointestinal symptoms, affecting any part of the tract, from the oral cavity to the rectum, with the esophagus and anorectum being the most affected (45). These alterations manifest mainly as dysmotility, related to fibrosis and myopathy, that lead to abnormalities in the compliance and contractility of the digestive tract wall (46).

The clinical presentation is variable, ranging from dysphagia, heartburn, and reflux to abdominal distension, nausea, vomiting, diarrhea, constipation, and fecal incontinence. However, severe complications can occur, such as intestinal atrophy and excessive growth of bacteria that lead to poor absorption of nutrients and severe malnutrition (47).

2.1.3.3 PULMONARY MANIFESTATIONS

The primary causes of death in patients with SSc are pulmonary complications such as ILD, followed by PAH (7). ILD has a prevalence of 16 to 47% in patients with SSc and commonly presents with dyspnea, cough, and a nonspecific interstitial pneumonia pattern on computed tomography, with a minority of cases meeting the criteria for usual interstitial pneumonia (48). On the other hand, PAH occurs with a prevalence of 5-15% (49,50) and is characterized by presenting nonspecific clinical symptoms that include dyspnea, fatigue, weakness, chest pain, dizziness or syncope, and cough (51). This pathology presents with arterial remodeling and an increase in pulmonary vascular resistance secondary to abnormal vascular proliferation that produces right heart failure, which can eventually lead to death (52).

2.1.3.4 CARDIAC MANIFESTATIONS

In patients with SSc, there is a broad clinical spectrum of symptomatic and asymptomatic cardiac involvement that can manifest with myocardial damage, fibrosis of the conduction system, pericardial system, and, less frequently, valvular disease (53). There are contradictions regarding the development of atherosclerotic disease in patients with SSc since the prevalence of atherosclerosis of the large coronary arteries is similar to that of the general population, unlike other ADs where atherosclerotic involvement is more significant (54).

2.1.3.5 RENAL MANIFESTATIONS

Renal involvement occurs mainly in the form of SCR, which consists of the appearance of acute arterial hypertension and progressive renal failure. Likewise, patients with SSc may present with abnormal kidney function, proteinuria, chronic renal vascular disease, kidney injury due to nephrotoxic drugs, calcium oxalate nephropathy, glomerulonephritis, and vasculitis associated with anti-neutrophil cytoplasmic antibodies (55).

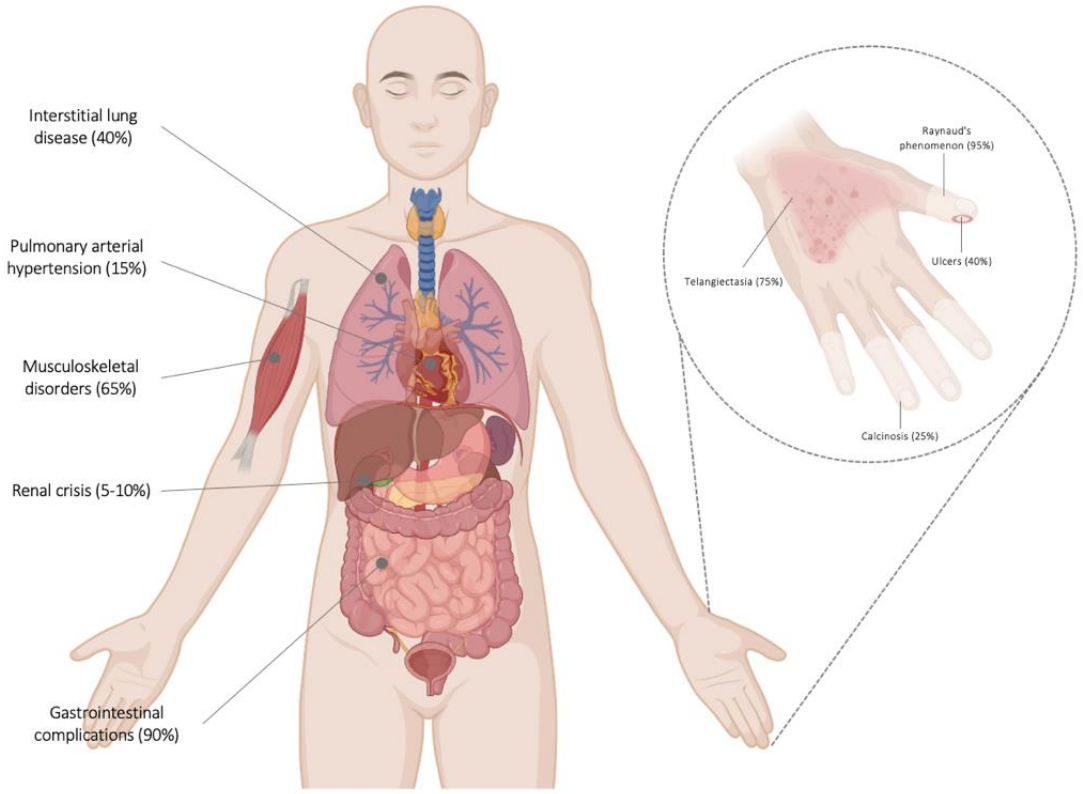

Figure 2. Main clinical complications associated with systemic sclerosis. The frequency of complications is indicated in parentheses. Adapted from: *Allanore, Y., Simms, R., Distler, O. et al. Systemic sclerosis. Nat Rev Dis Primers 1, 15002 (2015). https://doi.org/10.1038/nrdp.2015.2. Made with BioRender.*

2.1.4 CLINICAL SUBTYPES

Depending on the degree of skin involvement, SSc can be classified into two main clinical subtypes: lcSSc and dcSSc. Although systemic manifestations can accompany both subtypes,

each has distinct clinical manifestations, and the severity of involvement of the internal organs and the disease progression is highly variable (Fig. 3A-3B) (39,56).

The most frequent subtype presentation, lcSSc (25), generally has a gradual onset with the early development of RP, sometimes occurring years before skin thickening becomes apparent (20). In this subtype, skin fibrosis is limited to a specific area such as the face and distal limbs, with minor systemic involvement (42). Along with RP, abnormalities in the capillaries of the nail folds can be observed, as well as positive anticentromere antibodies, and organ involvement with a predominance of PAH (57). CREST syndrome acronym refers to the clinical features that patients with lcSSc can present: Calcinosis, RP, esophageal involvement, sclerodactyly, and telangiectasias (58); however, since not all patients manifest these five characteristics, some clinicians agree that this term should be dropped, and these patients should be classified in the lcSSc subtype (59).

In contrast, in the dcSSc subtype, RP coexists with skin fibrosis extended proximally to knees, elbows, and the trunk, typically in a symmetrical pattern (42), with a more aggressive progression characterized by severe internal organ manifestations, mainly in the gastrointestinal tract, lungs, heart, and kidneys with the early development of complications, increasing the mortality rate (60). This subtype is generally associated with early disease and a less favorable prognosis (61).

On the other hand, a small percentage of SSc patients can be classified under another clinical subtype: sclerosis sine scleroderma (62). This subtype is characterized by the absence of classical skin thickening and the presence of a positive antinuclear antibody (ANA), RP or nailfold capillary and internal organ involvement of ILD, PAH, renal dysfunction, or esophageal dysmotility (63,64).

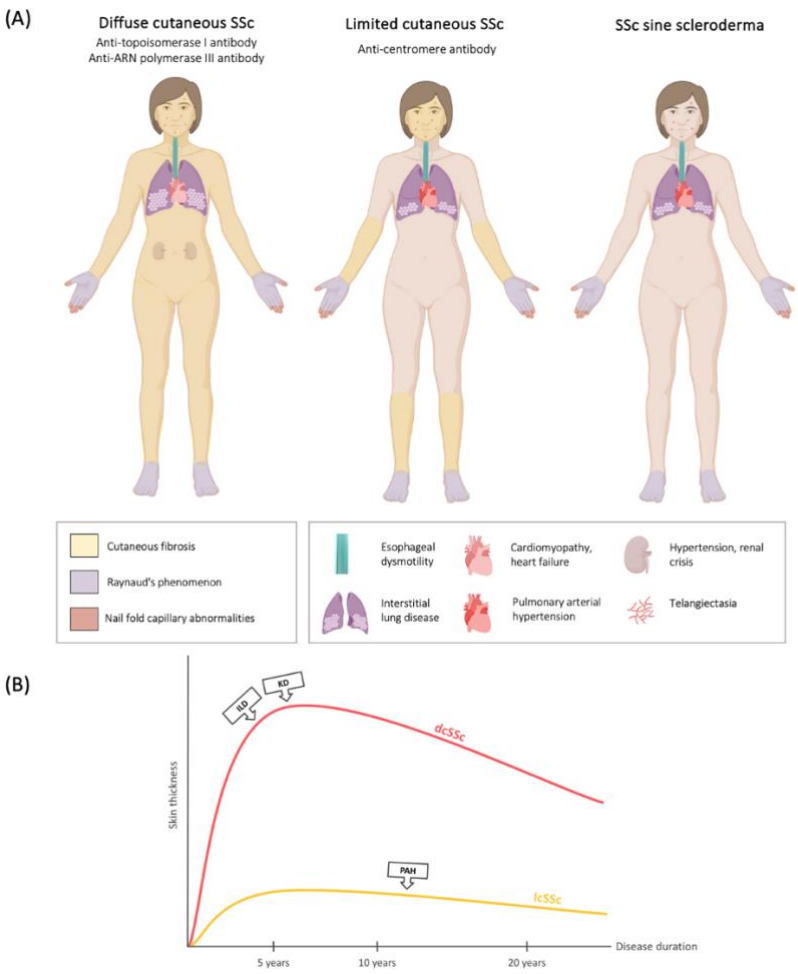

Figure 3. (A) Clinical classification of systemic sclerosis and disease progression. (B) SSc progression according to clinical subtypes. Typically, dcSSc subtype is associated with early internal organ involvement, within 5 years of disease onset, and a worse prognosis, whereas lcSSc subtype develops internal organ involvement later in the disease and have a better prognosis. *Adapted from: Bolognia J, Schaffer J, Duncan K, Ko C. Systemic Sclerosis and Sclerodermoid Disorders. In: Dermatology Essentials. 2nd ed. Elsevier Inc; 2022. p. 326–37. Made with BioRender.*

2.1.5 CLASSIFICATION CRITERIA

The first preliminary classification criteria for the disease were proposed in 1980 by the American College of Rheumatology (ACR), with a single major criterion being proximal scleroderma and minor criteria being sclerodactyly, digital pitting scars of fingertips or loss of substance of the distal finger pad, and bibasilar pulmonary fibrosis. A definite case of SSc was

achieved with one major criterion or two or more minor criteria (65). In 1988, LeRoy and collaborators attempted to improve the limitations of the previous preliminary criteria proposed by the ACR by introducing in the classification the definitions of dcSSc and lcSSc based on the extent of fibrosis on the skin, where morphological characteristics by capillaroscopy and association of antibodies to each of the subtypes appeared for the first time, in the case of dcSSc the anti-topoisomerase antibodies and for lcSSc the anticentromere antibodies (66); however, in 2001, LeRoy and Medsger proposed the classification criteria that actually would allow the classification of SSc into lcSSc and dcSSc, introducing the definition of limited systemic sclerosis or sine scleroderma (Fig. 4) (67).

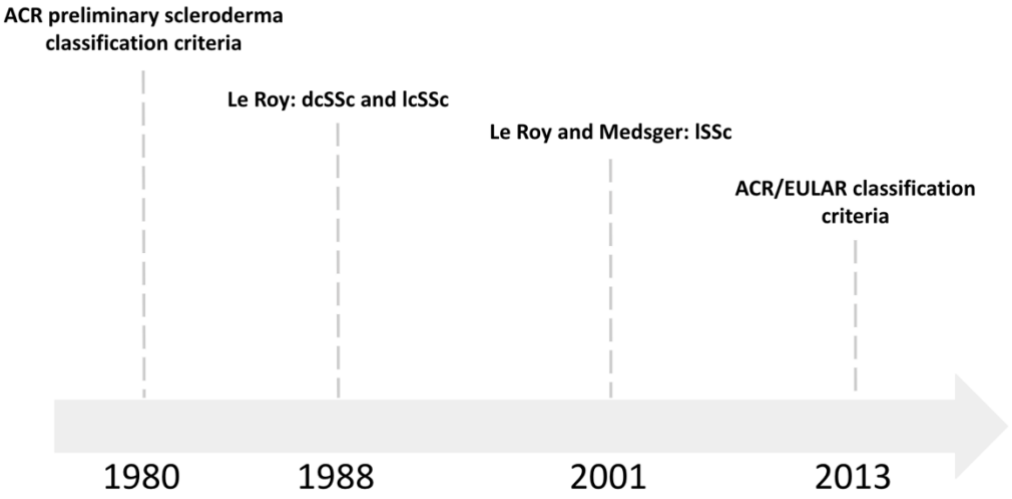

Figure 4. Timeline of the different classification criteria for systemic sclerosis.

In 2013, the ACR and the European League Against Rheumatism (EULAR) published new classification criteria that guarantee that the population of patients studied is clearly defined and homogeneous. In this scheme, the presence of the single major criterion (increased skin thickness on the fingers of both hands, reaching proximal to the metacarpophalangeal joints leads to a definitive diagnosis of SSc. If the patient does not meet this requirement, but the sum of items gives a result of ≥ 9 points, the patient is classified as having a definitive diagnosis of SSc (Table 1). These 2013 criteria demonstrate higher sensitivity (91%) and specificity (92%) than previously used criteria (68).

Table 1. ACR/EULAR 2013 Systemic sclerosis classification criteria.

Item	Sub-item	Weight or score*
Skin thickening of the fingers of both hands extending proximal to the metacarpophalangeal joints (*sufficient criterion*)	NA	9
Skin thickening of the fingers *(only count the highest score)*	Puffy fingers	2
	Sclerodactyly of the fingers (distal to the metacarpophalangeal joints but proximal to the interphalangeal joints)	4
Fingertip lesions *(only count the highest score)*	Digital tip ulcers	2
	Fingertip pitting scars	3
Telangiectasia	NA	2
Abnormal nailfold capillaries	NA	2
Lung involvement	Pulmonary arterial hypertension and/or interstitial lung disease	2
Raynaud phenomenon	NA	3
SSc-related autoantibodies	Any of the centromere, topoisomerase I, and RNA polymerase III specific antibodies	3
These criteria apply to any patient considered for inclusion in a systemic sclerosis study. The criteria do not apply to patients with skin thickening sparing the fingers or to patients with scleroderma-like disorder that better explains their manifestations. *A Summary score $\geq$ 9 is sufficient to fulfill the criteria.		

Table adapted and reproduced from Van Den Hoogen F, 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.

2.1.6 TREATMENT

The management of SSc represents a challenge due to its variability and heterogeneity of symptoms. The treatment of the disease is guided according to the clinical manifestations and the main complications according to the affected organ or system, with a preference for therapies focused on more than one affected organ system (Table 2) (69,70).

Table 2. Current pharmacological treatments for the management of systemic sclerosis.

Therapeutic agent	Benefit
Diffuse systemic sclerosis	
Mycophenolate Cyclosporin Hematopoietic stem cell transplant	Improves modified Rodnan score.
Raynaud's phenomenon	
Calcium channel blockers Fluoxetine Sildenafil, Tadalafil	Reduction in the frequency and severity of attacks of RP
Iloprost	Reduction in the frequency and severity of attacks of RP and DU.
Bosentan, Ambrisentan	Reduction in the development of new DU.
Interstitial lung disease	
Mycophenolate Cyclosporin	Improvement in FVC, radiographic fibrosis, and dyspnea.
Tocilizumab	Stabilization of CVF in early dcSSc with and without ILD.
Nintedanib	Reduction in the annual rate of decline in CVF.
Pulmonary hypertension	
Riociguat Sildenafil, Tadalafil	Improvement in exercise capacity, hemodynamics, as well as functional class.
Bosentan	Improvement in 6-minute walk distance; delay in progression to clinical worsening.
Ambrisentan + Tadalafil	Reduced risk of clinical failure.
Selexipag	Reduction in hospitalizations and disease progression.
Prostanoids	Improvement in 6-minute walk distance and time to clinical worsening.
Gastrointestinal disease	
Proton-pump inhibitor	Improvement in GER symptoms; decreased risk of upper gastrointestinal ulcers.
H2 blockers	Improvement in GER symptoms.
Prokinetic agents	Improvement in symptoms related to gastrointestinal tract dysmotility.
Renal disease	
Angiotensin-converting enzyme inhibitors	Improvement in morbidity and mortality due to SCR.
Heart disease	
Calcium channel blockers	Prevention and treatment of left ventricular systolic dysfunction.
Angiotensin-converting enzyme inhibitors Calcium channel blockers	Improvement in myocardial perfusion.

Table adapted and reproduced from *Bukiri H, Volkmann ER. Current advances in the treatment of systemic sclerosis. Curr Opin Pharmacol. 2022 Jun;64:102211*

2.2 METABOLOMICS

Metabolomics is an omics science that focuses on the study of the metabolome, defined as the complete set of metabolites and small molecules, such as metabolic intermediates, hormones, secondary metabolites, and other signaling molecules found within cells, fluids, and tissues, identifying them based on their structure, function, and dynamics in the organism (71,72). Metabolites are small molecules with a low molecular weight (less than 1.5 kilodaltons (kDa) in a biological system, both exogenous and endogenous (73).

Metabolites are the final product of the entire biological cascade of our body. They are the primary products of different metabolic pathways, so their concentrations are influenced by genetic predisposition and exposure to environmental stimuli (74), which is why interactions between gene expression, proteins, and the environment are directly reflected in the metabolome (75), making metabolomics a more complex omics science. The metabolome reflects the phenotype; therefore, metabolite alterations may provide unique phenotypic information and reflect individual responses (76–78). Among the emerging omics sciences, metabolomics is the youngest of all and, along with the others, plays a vital role in the future of personalized medicine (79). It offers a valuable approach for the identification of disease-related metabolites in fluids or tissues as well as the classification and characterization of disease or treatment-associated molecular patterns generated from metabolites (72).

2.3 SYSTEMIC SCLEROSIS-ASSOCIATED METABOLITES

Recent studies suggest a link between disease-causing disturbances of the immune system and the mechanisms that regulate cellular metabolism (80,81). The leading deregulated metabolic families detected that could play an essential role in systemic sclerosis are AA-derived metabolites, fatty acyls, glycerophospholipids, and sphingolipids (82).

For example, Smolenska et al. (83) compared plasma samples from 42 patients diagnosed with SSc and 27 healthy controls (HC). They performed direct metabolomics analyses looking for 36 AA-related metabolites. Analysis revealed increased concentrations of nitric oxide synthase,

an inhibitor of asymmetric dimethylarginine, in patients with SSc. Regarding the extent of skin involvement, patients with dcSSc revealed higher concentrations of beta-alanine, sarcosine, and L-arginine compared with patients diagnosed with lcSSc.

Szamosi et al. (84) and Motegi et al. (85) evaluate through direct metabolomics analyses levels of AA-related metabolites, specifically homocysteine (Hcy), finding no difference in Hcy levels between SSc and patients with HC; however, SSc patients with vascular or thromboembolic events had significantly higher Hcy concentrations than those without these manifestations (84). Furthermore, increased Hcy levels were associated with ILD associated with SSc (85).

In a study conducted in 2021, Bögl et al. (86) compared plasma samples from 52 patients diagnosed with SSc and 48 HC. The results identified a deregulation of metabolites derived from AA such as kynurenine, tryptophan, dimethylarginine, citrulline, proline, ornithine and phenylacetylglutamine, which were associated with four altered metabolic mechanisms: inflammation, vascular damage, fibrosis and intestinal disorders, specifically dysbiosis, characteristic of the pathophysiology of SSc.

Furthermore, Fernández-Ochoa et al. (87) found deregulations in metabolic families such as acylcarnitine, acylglycines, and metabolites derived from AA, specifically proline, histidine, and glutamine, leading to alterations in beta-oxidation of fatty acids and AA pathways in patients with scleroderma.

On the other hand, Geroldinger-Simic et al. (Ref), in 2020, documented significant changes in the levels of phospholipids, such as plasmalogens and sphingomyelins, in the plasma of SSc patients compared to HC. The results also demonstrated a significant association between the alteration of phospholipids, specifically plasmalogens of phosphatidylcholine and phosphatidyl-ethanolamine, and the presentation of different clinical manifestations (88).

These results indicate disturbances in fatty acid beta-oxidation, lipid metabolism, and AA pathways in patients with scleroderma that are associated with inflammation, vascular endothelial dysfunction, and fibrosis, relevant to the pathophysiology of SSc.

3. HYPOTHESIS

Ho: There are no differences in the metabolomic profile derived from amino acids measured in serum between patients diagnosed with Systemic Sclerosis and healthy subjects.

Ha: There are differences in the metabolomic profile derived from amino acids measured in serum between patients diagnosed with Systemic Sclerosis and healthy subjects.

4. OBJECTIVES

4.1 GENERAL OBJECTIVE

Evaluate the differences in the metabolomic profile from AA-derived metabolites measured in serum in a cohort of patients with SSc compared to healthy subjects.

4.2 SPECIFIC OBJECTIVE

1. Describe the sociodemographic and clinical characteristics of the study sample.
2. Determine the most common clinical subtype in this population according to the different clinical manifestations and to which metabolomic profile it is associated.
3. Identify the differential metabolites between diffuse and limited cutaneous SSc.
4. Analyze the association between alterations in the metabolomic profile from AA-derived metabolites measured in serum in patients with SSc and the different disease phenotypes.

5. METHODOLOGY

5.1 METHODOLOGICAL APPROACH

A quantitative approach study was carried out. Based on the collected data, the association between the outcome variables, given by the presence or absence of SSc, and the exposure variables, given by the primary altered metabolites and metabolic pathways in these patients, was described. In order to establish accurately the study population's patterns, the previously established hypothesis was tested, relying on numerical measurement, counting, and statistical analysis.

5.2 STUDY TYPE AND DESIGN

A analytical observational case-control study was conducted. Alterations in AA-derived metabolites were evident in the presence of the disease and persisted throughout the analyzed studies. Differences in the levels AA-derived metabolites evaluated between patients diagnosed with SSc (cases) and healthy subjects (controls) were measured.

5.3 POPULATION

The target population consisted of two groups: cases, defined as any subject of both sexes over the age of 18 diagnosed with SSc according to the ACR/EULAR 2013 classification criteria (68) evaluated at the Center for Autoimmune Diseases Research (CREA). The cases included individuals from different socioeconomic strata, as well as the level of education and occupation. Controls, defined as any subject of both sexes over 18 years of age, of the same age and gender as the cases, who does not currently have ADs, immunodeficiencies, acute infections, a history of malignancy, or is currently receiving anti-inflammatory, antimalarial, antirheumatic, immunosuppressive, or biological therapy. This group of control individuals is part of a parallel study on ADs conducted at the CREA center.

5.4 SAMPLE DESIGN

Regarding the selection, a non-probabilistic convenience sampling was conducted. After reading the informed consent form, all patients assessed in the participating institution with a

confirmed diagnosis of SSc were included in the study (n= 38). As for the control group, thirty-eight age and sex matched healthy volunteers were included. In order to determine whether patients met the inclusion criteria, the medical records of those who attended sessions held by the institution were reviewed as part of the selection process.

5.4.1 Inclusion Criteria for Cases

- Patients over 18 years of age of both sexes with a diagnosis of SSc according to ACR/EULAR 2013 criteria (68).
- Residents of Colombia.
- Patients with a complete medical records and available blood samples.
- Those who voluntarily agreed to participate in the study and signed the informed consent form.

5.4.2 Exclusion Criteria for Cases

- Patients with overt polyautoimmunity.
- Patients with inflammatory diseases.
- Patients with a history of infection within the last two months.
- Patients with a diagnosis of juvenile-onset systemic sclerosis.
- Patients with a diagnosis of localized scleroderma or sclerodermiform syndromes.
- Patients with a history of cancer.

5.4.3 Inclusion Criteria for Controls

- Healthy subjects over 18 years of age of both sexes.
- Residents of Colombia.
- Subjects with a complete medical history and available blood samples.
- Those who voluntarily agreed to participate in the study and signed the informed consent form.

5.4.4 Exclusion Criteria for Controls

- Subjects with a history of ADs.

- Subjects with a history of inflammatory diseases.
- Subjects with a history of immunodeficiencies.
- Subjects with acute infections at the time of sample collection or a history of infection within the last two months.
- Subjects with a history of malignancy.
- Subjects currently under anti-inflammatory, antirheumatic, antimalarial, immunosuppressive, or biological treatment.

5.5 VARIABLE DESCRIPTION

5.5.1 Diagram of variables

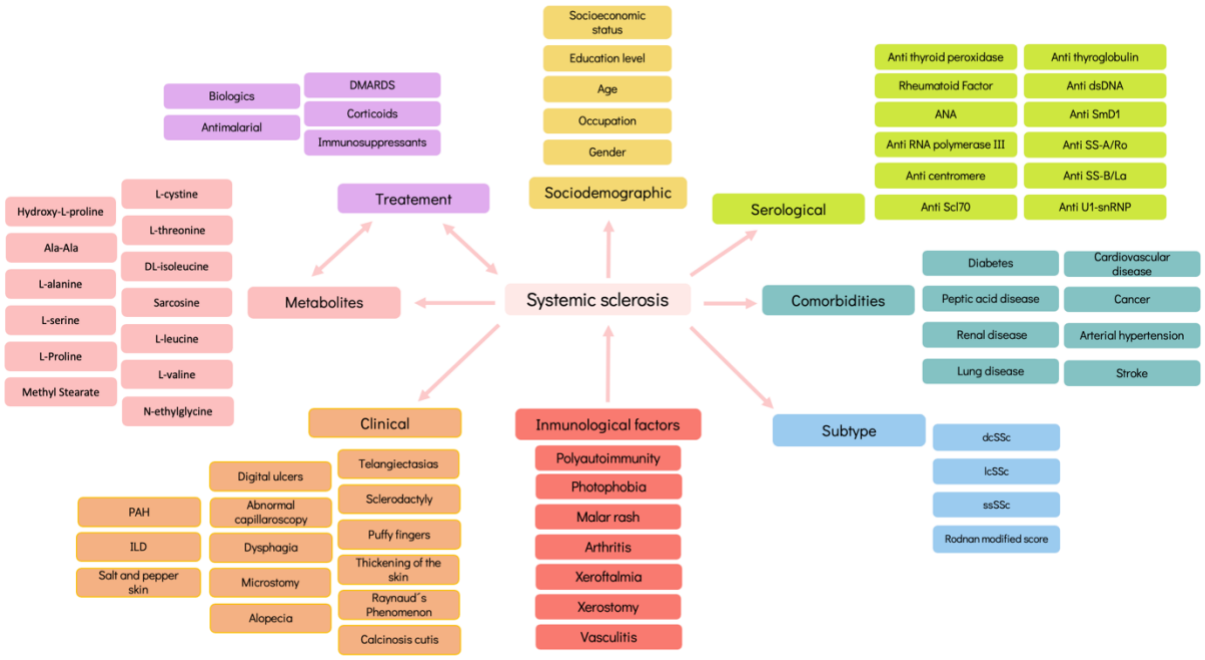

Figure 5. Diagram of variables. The variables considered for the study were divided into different categories: sociodemographic, serological profile, comorbidities, SSc subtype, immunological factors, clinical, metabolomic profile and treatment.

5.5.2 Table of variables

Further information is available in the annexed section. (See Appendix 1- Table 3)

5.6 INFORMATION GATHERING TECHNIQUES

With prior authorization from the Universidad del Rosario ethics committee, the information was collected from the medical records retrieved from the "CREA System" database, which holds data on patients who attend inclusion sessions at the institution (CREA). From there, cases and controls were selected based on the predetermined criteria.

5.6.1 SOURCE OF INFORMATION

In this study, secondary sources of information were used. Data on sociodemographic, clinical, serological, and laboratory information were collected through predefined institution-backed forms and electronic medical records.

5.6.2 INFORMATION COLLECTION INSTRUMENT

Sociodemographic information such as age, gender, occupation, and socioeconomic status was obtained through medical forms. These forms also inquired about clinical, surgical, pharmacological history, familiar history and comorbidities. On the other hand, habits such as consumption of coffee and tobacco were asked (Appendix 2). Furthermore, distinctive signs and symptoms, complications, and previous diagnostic tests were acquired through medical forms (Appendix 3). Finally, to rule out polyautoimmunity, one last form was used to ascertain any signs or symptoms common to other ADs (Appendix 4). A data collection format was developed in Excel, including all variables of interest. Additionally, for measurements of laboratory variables, targeted metabolomics techniques were employed for the quantitative measurement of selected metabolites derived from AA using an Agilent Technologies 7890B gas chromatograph coupled to an Agilent Technologies GC/Q time-of-flight mass selective detector TOF 7250, equipped with a split/splitless injection port (250 °C, split ratio 30) and an Agilent Technologies 7693A autosampler (GC/MS-QTOF). Likewise, an indirect ELISA kit and IMTEC ANA-LIA Maxx were used to measure serum autoantibodies.

5.6.3 OBTAINING INFORMATION PROCESS

Data collection was carried out by healthcare personnel (research assistant physicians and laboratory assistants) from CREA through self-administered medical form evaluations under the guidance of an expert physician with support from previous medical records between 2017 and 2019. Blood was collected from each individual by experienced health personnel using the venipuncture process. After coagulation occurred within 30 minutes, the blood sample in a dry tube with SST was centrifuged at 3500 rpm for 10 minutes. Following centrifugation, the serum was divided into aliquots and stored at –80°C for further analysis.

Sera samples were assessed for the detection of anti-thyroid peroxidase (TPO) antibodies, IgG anti-thyroglobulin (Tg) antibodies, IgM rheumatoid factor, IgG anti-cyclic citrullinated peptide third generation (CCP3) antibodies, IgM and IgG anti-cardiolipin antibodies (ACAs), IgM and IgG anti-β2 glycoprotein-1 (β2GP1) antibodies, , and were all quantified by enzyme-linked-immunosorbent assay (ELISA). All the assay kits were from Inova Diagnostics, Inc (San Diego, CA, USA). The remaining 17 autoantibodies were evaluated using immunoblotting (double-stranded DNA (dsDNA), nucleosomes, histones, SmD1, proliferating cell nuclear antigen (PCNA), P0, SS-A/Ro60, SS-A/Ro52, SS-B/La, centromere B antigen (CENP-B), Scl70, U1-snRNP, AMA M2, Jo-1, PM-Scl, Mi-2, Ku) using IMTEC ANA-LIA Maxx from Human Diagnostics (Magdeburg, Germany). For serum determinations, the results were as follows: anti-TPO antibodies (positive > 100 Units OMS), anti-Tg antibodies (positive > 0.6 Units OMS), IgM Rheumatoid factor (positive > 6 Units), IgG CCP3 antibodies (positive > 20 Units), IgM and IgG ACAs (positive > 20 GPL), IgM and IgG β2GP1 (positive > 20 SGU), dsDNA (positive > 301 UI/ml), SS-A/Ro (positive > 20 Units), SS-B/La (positive > 20 Units). As for ANA-LIA, these results were considered positive when the assay results were above a threshold value. Lastly, antinuclear antibodies (ANAs) were evaluated by using an ELISA assay. Positive results were considered from dilution 1/80.

On the other hand, for the measurement of metabolites, extraction was carried out by taking 50 µL of each sample and adding 150 µL of cold methanol, followed by vortexing for 3 minutes. Subsequently, the samples were cooled to -20 °C for 20 minutes and centrifuged at 13,000

rpm at 4 °C for 10 minutes. Of the previously prepared extracts, 50 µL were dried in a SpeedVac for 2 hours at 35 °C. Next, 10 µL of O-methoxyamine in pyridine (15 mg/mL) was added, vortexed for 5 min, and incubated in the dark at room temperature for 16 hours. The silylation process was carried out by adding 10 µL of N,O-bis(trimethylsilyl)fluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS), vortexing for 5 min and incubation at 70°C for 1 hour.

Furthermore, the samples were allowed to cool to room temperature for 30 minutes, and 100 µL of methyl stearate in heptane was added as an internal standard (5 mg/L) and vortexed for 5 minutes. The temperature of the transfer line to the detector, the source filament, and the quadrupole were maintained at 280, 230, and 150 °C, respectively. The electron ionization (EI) source was operated at 70 eV. An Agilent Technologies J&W HP-5MS column (30 m, 0.25 mm, 0.25 µm) was used; the carrier gas was helium at a constant flow of 0.7 mL/min. The oven temperature was programmed from 60°C (1 min) @10°C/min to 325°C (10 min). The temperature of the transfer line to the detector, the source filament, and the quadrupole were maintained at 280, 230, and 150 °C, respectively. Detection by mass spectrometry was carried out between 50 to 600 m/z at a speed of 5 spectra/s. Finally, the obtained information was recorded in an Excel program format that included all variables and was subsequently exported to the "CREA System" database.

5.7 BIAS AND BIAS CONTROL

ERROR CONTROL

- Sampling error:
  - Biological samples were arranged randomly within the sequence.
  - Measurement of metabolites and autoantibodies to all included patients, independent of previous results.
- Measurement errors:
  - Measurement by trained personnel.
  - Standardized technique (ELISA - ANA LIA - GC/MS-QTOF).
  - Quality control by the manufacturer (INOVA Diagnostics - IMTEC - Agilent)

BIAS CONTROL:

- Selection biases: As it is a non-probabilistic convenience sampling, not all subjects have the same possibility of participating in the study. This bias cannot be controlled and will be discussed as a limitation.
- Memory biases: Questioning of the patients was guided
- Information biases: Errors when tabulating data extracted from medical records and medical forms. Double information entry was carried out, and the data source was reviewed when discrepancies arose. Additionally, missing data will be recorded in the database as missing. The collection instruments were applied to the entire study population.
- Confounding biases: The following were identified as possible confounding biases: sex, since the disease occurs more frequently in women, and age, since it is more common at older ages in the general population. Confusion may occur in the outcome due to the transversal nature of the symptoms in ADs. Patients with overt polyautoimmunity were excluded from the study**.**

5.8 PROCESSING TECHNIQUES AND DATA ANALYSIS

In the univariate analysis, quantitative continuous variables were expressed as mean and standard deviation (SD) and median and interquartile range (IQR), while categorical variables were analyzed by frequencies. Where appropriate, a Mann–Whitney or Paired t-test was employed to assess associations between outcomes of interest and other variables. The significance threshold of 0.05 and a Fold change (FC) threshold equal to 1 were established for the study. FC was calculated for each metabolite by comparing the means of the two groups in order to estimate the variation between them. Statistical analyses were done using the Jamovi 2.3.22 program and MetaboAnalyst server (https://www.metaboanalyst.ca/). Graphics were done using *GraphPad* Prism 10 software. The analysis of the data acquired by GC-MS included the deconvolution phase and identification of the metabolites. This procedure was carried out using the Agilent MassHunter Unknowns Analysis program. Next, the retention times were aligned using the Agilent Mass Profiler Professional program, and the results

obtained were exported to the Agilent MassHunter Quantitative program to carry out data integration. AAs were identified using the Fiehn and NIST libraries in the MassHunter Personal Compound Database and Library Manager Software B.08.00 program. As a final step in the analysis, AA annotated in the GC-MS processing were filtered based on presence and reproducibility. Only those present in at least 80% of the samples were retained, ensuring a coefficient of variation in the Quality Controls of less than 20%. Finally, to evaluate the quality of the data and ensure the stability of the analytical system, the clustering of the data of the quality control samples was verified by an unsupervised principal component analysis (PCA) using the SIMCA-P program (Appendix 6- Supplementary Figure 1).

5.9 DISCLOSURE OF RESULTS

First, this protocol will be shared in the institutional repository of the Universidad del Rosario. The results of this study are expected to be published in an indexed journal. We are committed to socializing them with the community from which the data was extracted.

6. ETHICAL CONSIDERATIONS

Under Resolution No. 8430 of 1993 of the Ministry of Health, which establishes the scientific, technical, and administrative standards for health research in Colombia, this study adjusts the definition of risk-free research, given that the source of information is secondary. No interventions or modifications were made to the population. After signing the informed consent, sample collection and the start of the survey were requested. The informed consent form is shown in Appendix 5. This thesis is framed within the project ¨Biomarkers in Autoimmunity - BIOMA¨, which is intended to study and evaluate biomarkers based on their biological plausibility as essential tools for diagnosing and prognosis ADs. This project and its consent were approved by the research ethics committee of the Universidad del Rosario, under Resolution 008430 of 1993 and 002378 of 2008.

The CREA staff of the Universidad del Rosario contacted patients. All data and information collected were stored in a safe electronic database and will be handled in compliance with the 1581 statutory law of 2012. The identity of the patients will not be disclosed. The use and conservation of the information will be governed by Resolution 839 of 2017, which states the clinical history management in Colombia.

Likewise, the development of this study is based on the fundamental principles of ethics: beneficence, non-maleficence, justice, and autonomy, focused on maximizing resources.

Similarly, the authors state no conflicts of interest in conceptualizing, developing, and presenting results.

7. RESULTS

7.1 SOCIODEMOGRAPHIC AND CLINICAL CHARACTERISTICS

The sociodemographic and clinical characteristics of the enrolled patients are summarized in Tables 4 and 5. To decrease potential confounding factors, we matched participating individuals (n = 38 per group, n = 76 total) by age and sex. The mean age was 54.8±10.8 years, with most patients being women (n= 37, 97.3%). Thirty-three patients were classified as lcSSc (86.8%), while only five were classified as dcSSc (13.2%). As for comorbidities, gastroesophageal reflux disease, high blood pressure, and dyslipidemia predominated in both study groups.

Table 4. Sociodemographic characteristics and clinical history.

Characteristics	SSc (*n*=38)	HC (*n*=38)
Gender (Female/male)	37/1	37/1
Age, *mean* (SD)	54.8 (± 10.8)	54.8 (± 10.9)
Marital status, *n* (%)		
Single	6 (15,8%)	10 (26,3%)
Married	15 (39,5%)	13 (34,2%)
Widowed	3 (7,9%)	2 (5,3%)
Divorced	8 (21,1%)	5 (13,2%)
Cohabitation	6 (15,8%)	8 (21%)
Socioeconomical stratum, *n* (%)		
Low (1 and 2)	14 (36,8%)	14 (36,8%)
Medium (3)	15 (39,5%)	16 (42,1)
High (4,5 and6)	9 (23,7%)	8 (21,1%)
Comorbidities, *n* (%)		
Diabetes mellitus 2	2 (5,3%)	2 (5,3%)
Dyslipidemia	11 (28,9%)	6 (15,8%)
Renal disease	5 (13,2%)	3 (7.9%)
Anemia	7 (18,4%)	2 (5,3%)
Depression	8 (21,1%)	1 (2,6%)
Gastroesophageal reflux disease	22 (57,9%)	6 (15,8%)
Cancer	5 (13,2%)	2 (5.3%)
Arterial hypertension	12 (31,6%)	9 (23,7%)
Thrombosis	8 (21.1%)	2 (5,3%)
Cardiovascular disease	3 (7,9%)	1 (2,6%)

Abbreviations: SSc, Systemic sclerosis; HC, Healthy controls; SD, Standard deviation.

Table 5. Serological and clinical characteristics.

Characteristics	SSc (*n*=38)
Clinical subtypes, *n* (%)	
dcSSc/lcSSc	5 (13,2%) / 33(86,8%)
Serological data, *n* (%)	
Anti-Centromere	22 (57,9%)
Anti-Scl70	2 (5,3%)
Anti-RNA polymerase III	0 (0,0%)
ANA	29 (76,3%)
Clinical characteristics, *n* (%)	
Raynaud phenomenon	37 (97,4%)
Telangiectasia	33 (86,8%)
Sclerodactyly	30 (78,9%)
Fingertip pitting	15 (39,5%)
Dysphagia	15 (39,5%)
GER	14 (36,8%)
Hyperpigmentation	13 (34,2%)
Alopecia	13 (34,2%)
Microstomy	12 (31,6%)
Puffy fingers	11 (28,9%)
Abnormal capillaroscopy	11 (28,9%)
Hypopigmentation	11 (28,9%)
PAH	10 (26,3%)
ILD	9 (23,7%)
Calcinosis cutis	9 (23,7%)
Cough	7 (18,4%)
Dyspnea	6 (15,8%)
Fingertip ulcers	5 (13,2%)
Nail dystrophy	5 (13,2%)
Renal crisis	5 (13,2%)
Treatment, *n* (%)	
Prednisone	11 (28,9%)
Chloroquine	6 (15,8%)
Hydroxychloroquine	1 (2,6%)
Cyclophosphamide	2 (5,3%)
Mycophenolate mofetil	1 (2,6%)
Azathioprine	1 (2,6%)
Methotrexate	13 (34,2%)

Abbreviations: SSc, Systemic sclerosis; dcSSc, Diffuse cutaneous systemic sclerosis; lcSSc, Limited cutaneous systemic sclerosis; PAH, Pulmonary arterial disease; ILD, Interstitial lung disease; GER, Gastroesophageal reflux; ANA, antinuclear antibody.

Regarding serological features, 57.9% of SSc patients had positive autoantibodies indicative of the disease. Twenty-two patients (57.9%) were positive for the anticentromere antibodies, whereas two patients (2,3%) showed positivity towards anti-Scl70-antibodies. Anti-RNA polymerase III antibodies were not detected in any individual. Twenty-nine patients (76.3%) were positive for ANAs, with the centromere pattern (n=21; 55.3%) and the speckled pattern (n=7; 18.4%), these two being the most prevalent patterns.

As for clinical characteristics of the disease, the most common manifestation was RP, found in almost every SSc patient at the moment of evaluation (n=37; 97,4%), followed by telangiectasias (86,8%), sclerodactyly (78,9%), and fingertip pitting (39,5%). Likewise, gastrointestinal involvement was the most prevalent complication of SSc, predominating gastroesophageal reflux (GER) with fourteen affected SSc patients 39,5%), and dysphagia 39,5%), followed by alterations of the respiratory tract due to cough (18,4%) and dyspnea (15,8%). Regarding cardiopulmonary complications, there were no differences in the occurrence of PAH (26,3%) and ILD (23,7%).

Finally, regarding treatment, twenty-five of the thirty-eight patients with SSc received treatment at the time of evaluation (65,8%). Of these, 13 patients were receiving management with DMARDs, specifically methotrexate, followed by 11 patients who, at the time of evaluation, were receiving management with corticosteroids, specifically prednisone; eight were receiving immunosuppressants, including mycophenolate mofetil and cyclophosphamide, and finally seven patients were receiving antimalarials.

7.2 IMMUNOLOGICAL CHARACTERISTICS

Immunological data is described in Table 6. After analyzing these autoantibodies, we could infer that the Rheumatoid factor, SS-A/Ro, and U1-snRNP are the most prevalent autoantibodies in this population. The remaining evaluated autoantibodies were not observed in these subjects (data not shown). Furthermore, an interesting percentage of familial autoimmunity, given by a first-degree family history of ADs, was discovered, along with the existence of additional AD (latent polyautoimmunity).

Table 6. Immunological data.

Characteristics	SSc (*n*=38)
Familial autoimmunity, n (%)	12 (31,6%)
Latent polyautoimmunity, n (%)	17 (44,7%)
Antibodies, n (%)	
Rheumatoid factor	21 (55,3%)
SS-A/Ro	14 (36,8%)
U1-snRNP	10 (26,3%)
B2GP1 IgM	6 (15,8%)
ACA IgM	6 (15,8%)
SS-B/La	6 (15,8%)
TPOAbs	5 (13,2%)
TgAbs	4 (10,5%)
dsDNA	3 (7,9%)
ACA IgG	2 (5,3%)
SmD1	2 (5,3%)
CCP3	1 (2,6%)
B2GP1 IgG	1 (2,6%)
Jo-1	1 (2,6%)

Abbreviations: B2GP1, beta-2 glycoprotein 1 antibody; ACA, Anticardiolipin antibody; TPOAbs, Thyroid peroxidase antibody; TgAbs, thyroglobulin antibody; dsDNA, double stranded DNA antibody; SmD1, Smith antibody; CCP3, cyclic citrullinated peptide antibody.

7.3 BIVARIATE ANALYSIS

Deregulated amino-acid derived metabolites in SSc patients vs HC.

An initial analysis of serum samples was carried out between patients with SSc and HC. A total of 13 AA-derived metabolites were evaluated. The data showed that only N-ethylglycine (EG) was found to be significantly down-regulated in patients with SSc compared to the healthy subjects (p= 0,048); however, the FC of L-cysteine, DL-isoleucine, Sarcosine, L-proline, L-leucine, L-valine, Hydroxy-L-proline, Ala-ala, L-alanine and L-serine showed a downward trend in patients with SSc compared to healthy subjects, without statistical significance (Fig. 6) (Appendix 6 - Supplementary Table 1).

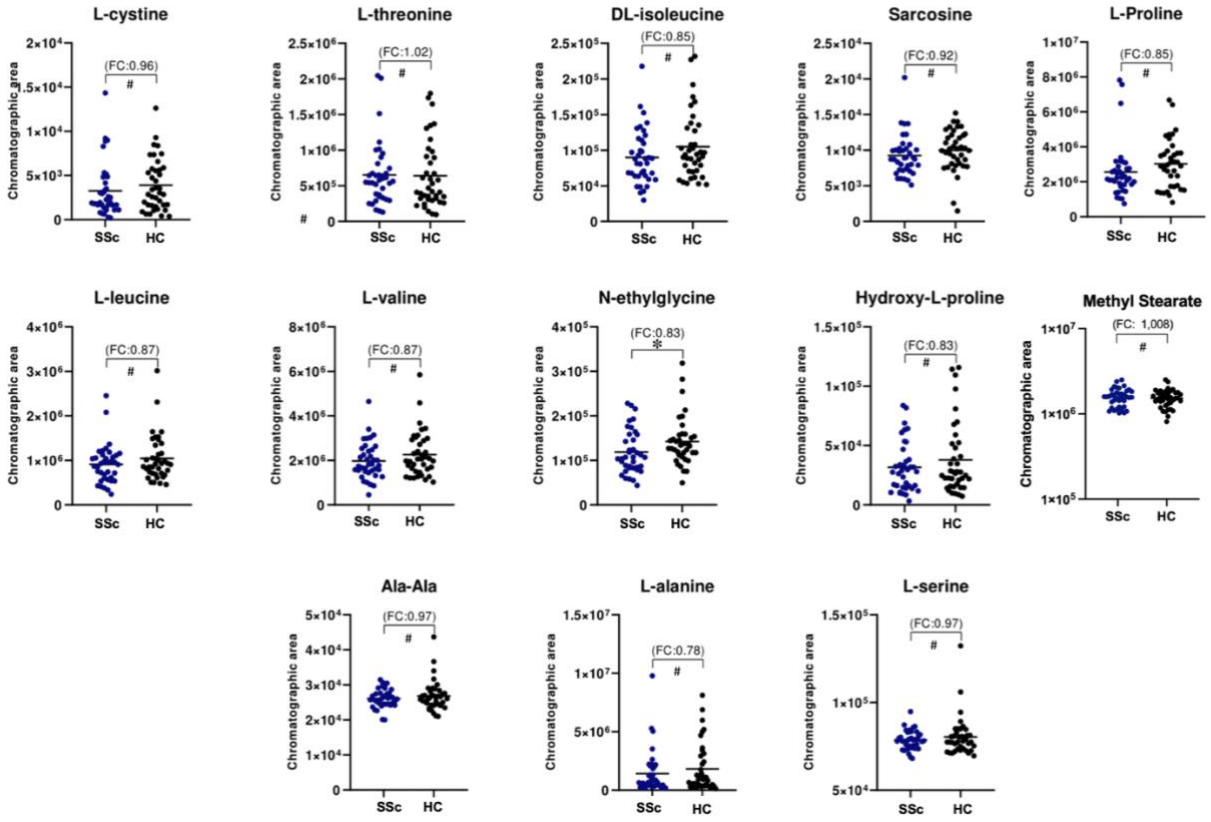

Figure 6. Scatter-plot of the evaluated metabolites between SSc and HC samples from the GC-MS analysis. SSc, Systemic sclerosis; HC, Healthy controls; FC, Fold Change; *= $p < 0.05$, # = not significant in univariate analysis.

Altered Metabolism in lcSSc and dcSSc subtypes.

In a further approach, an analysis was performed between serum samples of patients with lcSSc (n=33) and patients with dcSSc (n=5) subtypes to identify possible differential metabolites (Appendix 6 - Supplementary Table 2 and Figure 2). Of the 13 evaluated metabolites, data showed a statistically significant increase in Ala-Ala (p= 0,041) and L-serine (p= 0,032) metabolites in the dcSSc group as compared with the lcSSc patients (Fig. 7).

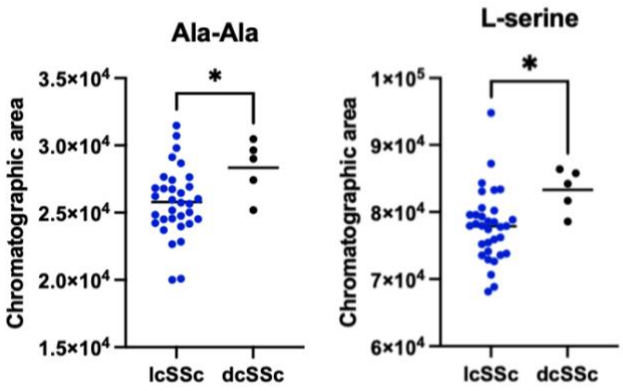

Figure 7. Scatter-plot of significant differential metabolites between dcSSc and lcSSc samples from the GC-MS analysis. dcSSc, Diffuse cutaneous Systemic sclerosis; lcSSc, Limited cutaneous Systemic sclerosis; *= $p < 0.05$.

Deregulated metabolites associated with clinical manifestations, serological profile, and pulmonary complications.

To further examine whether alterations in the concentrations of AA-related compounds could be related to the disease's different clinical manifestations and complications, we analyzed the serum samples within the SSc patient group to determine possible associations. Regarding pulmonary complications, data showed that DL-isoleucine (p= 0,021), L-leucine (p= 0,035), and L-valine (p= 0,032), three branched-chain amino acids (BCAAs) were significantly decreased in SSc-ILD patients in comparison to non-ILD patients (Fig. 8A). In contrast, findings showed that SSc-PAH patients had significantly higher levels of L-leucine than non-PAH patients (p= 0,044) (Fig. 8B). In SSc patients with puffy fingers (PF), significantly increased serum levels of L-serine (p= 0,04) were observed, as compared to SSc patients without PF (Fig. 8C). No other clinical associations were observed. Increased Ala-Ala levels were significantly associated with positive ANA (p= 0,029) and U1-snRNP (p=0,04) antibodies. Similarly, positive TPO antibodies were significantly associated with increased levels of valine (p=0,041) and DL-isoleucine (p=0,042) compared to TPO-negative patients (Fig. 9).

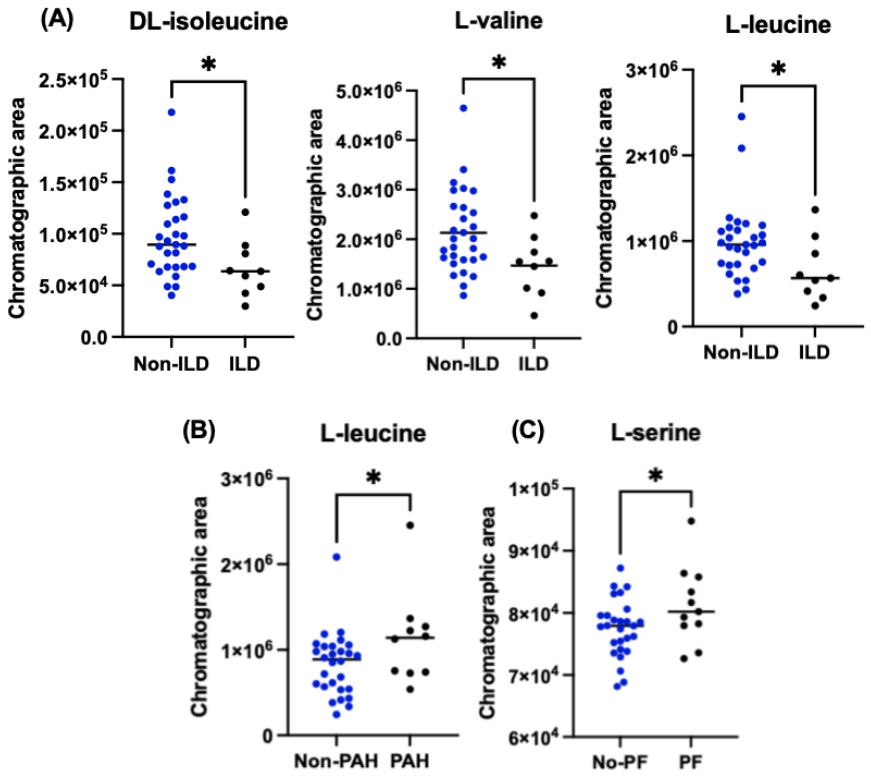

Figure 8. Association of amino-acid derived metabolites from serum with pulmonary complications (A-B) and clinical symptoms (C). ILD, Interstitial lung disease (n=9); PAH, Pulmonary arterial disease (n=10); PF, Puffy fingers (n=11);*= $p < 0.05$.

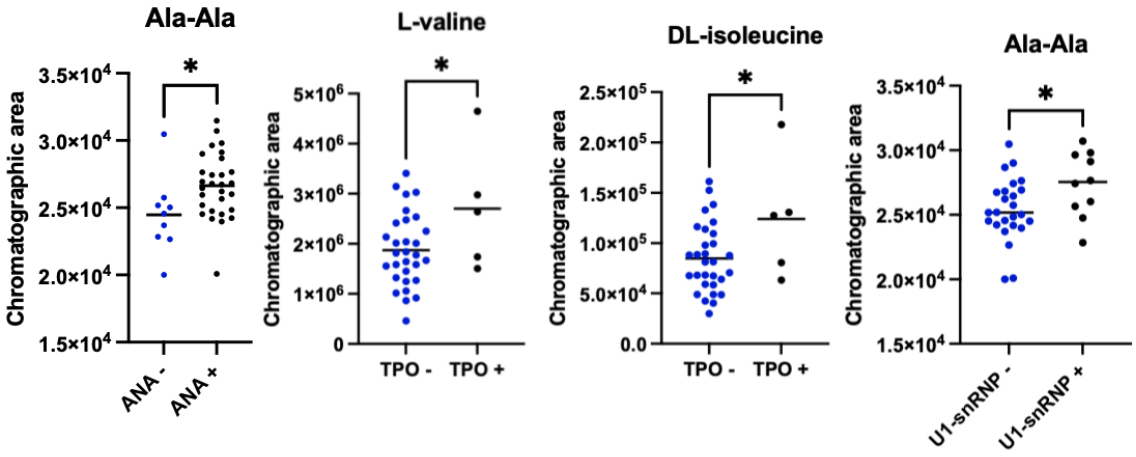

Figure 9. Association of amino-acid derived metabolites from serum with serological profile. TPO, anti-thyroperoxidase (n=5); ANA, antinuclear antibody (n=29);*= $p < 0.05$.

8. DISCUSSION

SSc is distinguished by a wide range of clinical presentations, making it difficult to understand its clinical and physiopathology features (89). A more profound comprehension of the disease pathophysiology is essential to tackle the factors contributing to the disease development and progression and to discover efficient therapies to increase the long-term survival of SSc patients.

According to recent research, there may be a connection between disease-causing immune system disruptions and the processes that govern cellular metabolism (80,81). Therefore, metabolomic characterization represents a promising approach that could applied for diagnosis, disease typing, and individual treatment of SSc (90). AA-derived metabolites, fatty acyls, glycerophospholipids, and sphingolipids are the most commonly found altered metabolic families that could be essential in SS development and progression (82). Altered AA metabolism is a common finding in SSc patient samples, and it may be associated with protein synthesis and catabolic processes for energy production (91). Thus, this study aimed to evaluate the differences in the metabolomic profile derived from AA in patients with SSc using the GC/MS-QTOF technique.

Our results showed one discriminant metabolite, EG, which was significantly lower in SSc patients than HC subjects. EG is a well-known metabolite of the anesthetic medication lidocaine; however, it is also an endogenously produced metabolite. It has a chemical structure similar to glycine, with an additional ethyl group attached to the amino group (92). Previous research has shown that, in addition to analgesic effects, lidocaine therapy has anti-inflammatory potential through a yet unknown mechanism (93). Furthermore, EG has been demonstrated to operate as an artificial substrate for the glycine transporter GlyT1, lowering GlyT1-dependent glycine absorption (94). Glycinergic neuron activity has been shown to affect itch and pain perception in inflammatory pain conditions (95). GlyT1 regulates the extracellular concentration of glycine in a synergistic manner. Therefore, EG binding to this transporter has been shown to cause a significant increase in glycine concentration, thus ameliorating the symptoms (96). Werdehausen et al. (97) experimented with *in vivo* extracellular recordings

from the lumbar spinal cord of rats, demonstrating the inhibitory effects of EG on inflammation-induced neuronal hyperexcitability. These results suggest that EG and not lidocaine alone is responsible for the anti-inflammatory effects. Proposing the exact mechanism of EG concentration in SSc pathophysiology and its association with inflammation is challenging, but it warrants further investigation.

Although EG was the only discriminant metabolite with statistical significance, the FC of the remaining metabolites showed a downward trend in SSc patients compared to HC. L-Leucine, for example, is transported via a transporter composed of SLC7A5 and SLC3A2 subunits that are up-regulated upon TCR engagement during T cell activation (98). Reports showed that SLC7A5 deficient mouse show abnormal responses to antigen stimulation, clonal expansion, or effector differentiation, resulting in decreased T cell activation and effector function (99). Furthermore, leucine is also required for Treg cell function as it boosts mTORC1 activity in Treg cells via the small G proteins RagA/B and Rheb1/2, which drives its suppressive action by inducing the expression of inducible T cell costimulator and CTLA4 (100), suggesting that decreased levels of this metabolite could generate immune dysfunction. Furthermore, L-serine is an essential compound in the synthesis of several sulfur-containing substances, including glutathione (GSH) (101). By neutralizing reactive oxygen species (ROS) and signaling molecules that play an important role in inflammatory diseases, GSH is the primary antioxidant in all tissues (102). Low levels of GSH have been associated with different chronic pro-inflammatory conditions, cardiovascular, kidney, and liver diseases, as well as ADs, highlighting its importance as an antioxidant (103).

Contrary to recent studies that showed proline up-regulation, our results showed a downward trend of L-proline and its derivate, Hydroxy-L-proline. Proline is a critical component in the synthesis of collagen and the ECM (104). It constitutes approximately 10% of total AA in collagen and is increased in TGFβ stimulated fibroblasts, boosting collagen formation and accounting for fibrosis (105,106); however, proline deficiency also acts as an essential AA at times of increased body stress and might be a predictor of poor prognosis in individuals with SSc (107).

The amino acid metabolism in dcSSc differed from that in lcSSc. In dcSSc patients, Ala-Ala, a dipeptide consisting of two L-alanine units joined by a peptide, was significantly increased compared to the lcSSc subtype. L-alanine is required for T cells to transition from quiescence to activation (108). For their initial activation, naive T cells rely on extracellular alanine pools. Alanine deficiency impairs cell growth, proliferation, and effector functions and skews activation-induced metabolic reprogramming (109). As a result, elevated Ala-Ala levels may indicate an exacerbated immune response in the dcSSc subtype compared to the lcSSc subtype. Similarly, L-serine levels were significantly increased in patients with dcSSc compared to lcSSc. Serine has been shown to behave as an immunometabolite, controlling adaptive immunity directly by modulating the proliferative capacity of T cells, playing a vital role in effector T cell responses (110).

Cardiopulmonary complications account for the majority of deaths in SSc patients (111). In this context, screening for ILD and PAH in SSc patients has become crucial. Our study showed that SSc patients with PAH demonstrated significantly increased serum levels of L-leucine, a BCAAs metabolite, in comparison to non-PAH patients. According to recent epidemiological studies, increased levels of BCAAs may predict worse outcomes in a number of cardiovascular diseases, including PAH (112). Zhenyukh et al. (113) performed *in vitro* and *ex vivo* studies in human endothelial cells (ECs) and aorta from male C57BL/6J mice, respectively, finding that increased BCAAs level generate inflammation and oxidative stress in ECs by triggering ROS production from mitochondria and NADPH oxidases, nuclear factor kappa B κB activation and subsequent up-regulation of adhesion molecules ICAM-1 and E-selectin, which facilitate inflammatory cells adhesion and endothelial dysfunction which could contribute to increased cardiovascular risk.

Differing to what has been reported in the literature, where elevated levels of BCAAs in patients with ILD are related to enhanced pro-inflammatory phenotype (114), in our study, SSc-ILD patients demonstrated lower levels of BCAAs than non-ILD patients. However, the anti-fibrotic effects of BCAAs are shown in liver fibrosis models. Lee et al. (115) conducted *in vitro* experiments with human stellate hepatic cell lines, exposing them to TGF-β1 and BCAAs. Expression of α-SMA decreased after BCAA administration, concluding that BCAA treatment

ameliorates hepatic fibrosis by directly influencing the active state of hepatic stellate cells via suppression of the TGF -β signaling pathway. Therefore, low levels of BCCAs could be expected in SSc-ILD patients.

Metabolomics, a rapidly evolving field in biomedical research, has promise for personalized or predictive medicine research and may help find new biomarkers (116,117). Nevertheless, this study has some limitations. The limitations of our study are related to a limited number of patients, a single small cohort, and a single time-point examination. Furthermore, some patients had drug intake, and it is feasible that the therapy may affect metabolomics. Nevertheless, there is currently limited data that shows how metabolite changes after drug intake in patients with SSc. Validation in external longitudinal multicenter cohorts will be required to evaluate the future utility in clinical practice. More subgroup studies on patients with additional system involvement and collecting relevant differential metabolites can be carried out with a larger sample size. Further research is needed to understand the correlation and interaction mechanisms between the metabolic pathways and differential metabolites screened in this study, the relevant biochemical indicators and risk factors, and the dynamic changes of metabolic pathways and metabolites. Still, we expect metabolomics to provide more accurate and validated biomarkers for detecting SSc.

9. CONCLUSIONS

In conclusion, our metabolomic approach allowed the identification of amino acid-derived metabolites that discriminate between SSc and HC patients. This approach may be helpful to better understand and clarify the pathophysiology of SSc, classify patients into different subtypes, and predict the different clinical manifestations of the disease. It may represent a preliminary step for future, more extensive studies. While several variables may still influence the metabolic profile, the findings point to the possibility of a disease-specific metabolic fingerprint; however, further research is required to evaluate the role of these alterations in the pathophysiology of the disease and evaluate whether they have potential as targets for treatment or as indicators for prognosis and response to treatment in addition to diagnosis.

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11. APPENDIX

Appendix 1

Table 3. Table of variables

VARIABLE	NAME	DEFINITION	TYPE	CLASSFICATION	SCALE OF MEASUREMENT	SPECIFIC OBJECTIVE
ID	Identification	Identification of the patient	Numerical ratio	Independent	ID number	Objective 1
SEX	Gender	Distinguish between male and female	Categorical nominal	Independent	0 = Women 1 = Men	Objective 1
AGE	Age	Patient age in years	Numerical ratio	Independent	(Years) N/A: Not available	Objective 1
MARIT_STA	Marital status	Current marital status	Categorical nominal	Independent	1 = Single 2 = Married 3 = Widowed 4 = Divorced 5 = Cohabitation N/A: Not available	Objective 1
OCUP	Occupation	Current occupation	Categorical nominal	Independent	1 = Manual exclusive 2 = Intellectual exclusive 3 = Mixed 4 = Housewife 5 = Unemployed 6 = Retired 7 = Student N/A: Not available	Objective 1
EDU_LEV	Education level	Highest degree of education an individual has completed	Categorical ordinal	Independent	1 = Primary school 2 = Secondary school 3 = Technical level 4 = Professional 5 = Postgraduate degree N/A: Not available	Objective 1
SES	Socioeconomic status	Socioeconomic stratum to which the patient belongs	Categorical ordinal	Independent	1 = 1 and 2 2 = 3 and 4 3 = 5 and 6 N/A: Not available	Objective 1
DM2	Diabetes Mellitus 2	History of diabetes Mellitus 2	Categorical nominal	Independent	0 = No 1 = Yes N/A: Not available	Objective 1
DYS	Dyslipidemia	History of dyslipidemia	Categorical nominal	Independent	0 = No 1 = Yes N/A: Not available	Objective 1
RENAL_DIS	Renal disease	History of renal disease	Categorical nominal	Independent	0 = No 1 = Yes N/A: Not available	Objective 1

ANEM	Anemia	History of anemia	Categorical nominal	Independent	0 = No 1 = Yes N/A: Not available	Objective 1
OSTEO	Osteoporosis	History of osteoporosis	Categorical nominal	Independent	0 = No 1 = Yes N/A: Not available	Objective 1
DEPRES	Depression	History of depression	Categorical nominal	Independent	0 = No 1 = Yes N/A: Not available	Objective 1
EPILEP	Epilepsy	History of epilepsy	Categorical nominal	Independent	0 = No 1 = Yes N/A: Not available	Objective 1
ACID_DIS	Peptic acid disease	History of peptic acid disease	Categorical nominal	Independent	0 = No 1 = Yes N/A: Not available	Objective 1
CANC	Cancer	History of cancer	Categorical nominal	Independent	0 = No 1 = Yes N/A: Not available	Objective 1
COPD	Chronic Obstructive Pulmonary Disease	History of Chronic Obstructive Pulmonary Disease	Categorical nominal	Independent	0 = No 1 = Yes N/A: Not available	Objective 1
AHT	Arterial hypertension	History of arterial hypertension	Categorical nominal	Independent	0 = No 1 = Yes N/A: Not available	Objective 1
STROK	Stroke	History of stroke	Categorical nominal	Independent	0 = No 1 = Yes N/A: Not available	Objective 1
TRHOMB	Thrombosis	History of thrombosis	Categorical nominal	Independent	0 = No 1 = Yes N/A: Not available	Objective 1
CVD	Cardiovascular disease	History of cardiovascular disease	Categorical nominal	Independent	0 = No 1 = Yes N/A: Not available	Objective 1
FAM_EIA	Familial autoimmune disease	Familial autoimmune disease	Categorical nominal	Independent	0 = No 1 = Yes N/A: Not available	Objective 1
POLYA	Polyautoimmunity	Presence of more than 1 autoimmune disease	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	-
AGE_DX	Age of diagnosis	Age at disease diagnosis	Numerical ratio	Independent	(Years) N/A: Not available	Objective 2
AGE_ONSET	Age onset	At what age did the symptoms start?	Numerical ratio	Independent	(Years) N/A: Not available	Objective 2
ONSET_WAY	Onset of the disease	Initial symptoms and time of onset	Categorical nominal	Independent	Description of the onset	Objective 2

SKIN_THICK_MCP	Thickening of the skin with extension to the MCP region	Presence of skin thickening of the fingers of both hands extending proximal to the MCP joints (sufficient criterion)	Categorical nominal	Dependent	0 = No 1 = Yes (9 points) N/A: Not available	Objective 3 and 4
SKIN_THICK_FINGER	Thickening of the skin of the fingers	Presence of skin thickening of the fingers	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 3 and 4
PUFFY_FINGER	Puffy fingers	Presence of swollen digits extending beyond the normal confines of the joint	Categorical nominal	Dependent	0 = No 1 = Yes (2 points) N/A: Not available	Objective 3 and 4
SLERODAC	Sclerodactyly	Presence of thickening and hardening of the skin of the fingers distal to the MCP joint, but proximal to the proximal joint	Categorical nominal	Dependent	0 = No 1 = Yes (4 points) N/A: Not available	Objective 3 and 4
FINGERTIP_LESION	Fingertip injury	Presence of fingertip lesions	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 3 and 4
FINGERTIP_PITTING	Fingertip pitting scars	Presence or absence of pinhole-sized digital concave depressions with hyperkeratosis as a result of ischemia.	Categorical nominal	Dependent	0 = No 1 = Yes (2 points) N/A: Not available	Objective 3 and 4
FINGERTIP_ULCER	Digital ulcers	Presence or absence of ulcers on the skin of the fingertips	Categorical nominal	Dependent	0 = No 1 = Yes (3 points) N/A: Not available	Objective 3 and 4
TELANGIEC	Telangiectasias	Presence of macular dilated superficial blood vessels	Categorical nominal	Dependent	0 = No 1 = Yes (2 points) N/A: Not available	Objective 3 and 4
CAPILAROSCOPY	Abnormal capillaroscopy	Presence of enlarged capillaries and/or capillary loss with or without pericapillary hemorrhages at the nailfold	Categorical nominal	Dependent	0 = No 1 = Yes (2 points) N/A: Not available	Objective 3 and 4
PULMONARY_FIND	Pulmonary abnormalities	Presence of pulmonary abnormalities	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 3 and 4
PAH	Pulmonary arterial hypertension	Presence of mean pulmonary artery pressure increase ≥ 25 mmHg on right heart catheterization	Categorical nominal	Dependent	0 = No 1 = Yes (2 points) N/A: Not available	Objective 3 and 4

ILD	Interstitial lung disease	Presence of pulmonary fibrosis seen on high-resolution computed. tomography or chest radiography, or occurrence of “Velcro” crackles on auscultation, not due to another cause	Categorical nominal	Dependent	0 = No 1 = Yes (2 points) N/A: Not available	Objective 3 and 4
RAYNAUD	Raynaud phenomenon	Presence of cold skin and changes in skin coloration of fingers exposed to cold or stress	Categorical nominal	Dependent	0 = No 1 = Yes (3 points) N/A: Not available	Objective 3 and 4
SSc_AB	Systemic sclerosis related autoantibody	Presence of systemic sclerosis-related autoantibodies	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 3 and 4
CENP	Anti-centromere	Presence of anti-centromere antibodies or centromere pattern seen on ANA in blood by serological analysis	Categorical nominal	Dependent	0 = No 1 = Yes (3 points) N/A: Not available	Objective 3 and 4
Scl70	Anti-Scl70	Presence of anti SCL70 antibodies in blood by serological analysis	Categorical nominal	Dependent	0 = No 1 = Yes (3 points) N/A: Not available	Objective 3 and 4
RNA	Anti-RNA polymerase III	Presence of anti-RNA polymerase III antibodies in blood by serological analysis	Categorical nominal	Dependent	0 = No 1 = Yes (3 points) N/A: Not available	Objective 3 and 4
CRITERIA_POINTS	Criteria points	ACR/EULAR 2013 criteria total score	Numerical ratio	Dependent	Number N/A: Not available	Objective 3 and 4
CRITERIA_FULL	Criteria fulfillment	Whether or not it meets inclusion criteria (Criteria points ≥ 9)	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 3 and 4
ANA	Antinuclear antibodies	Presence of antinuclear antibodies in blood by serological analysis	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 3 and 4
ANA_DIL	ANA dilution	Antinuclear antibodies dilution	Numerical ratio	Dependent	Dilution N/A: Not available	Objective 3 and 4
ANA_PATT	ANA pattern	Antinuclear antibodies patterns	Categorical nominal	Dependent	Pattern type	Objective 3 and 4
DIF_SSc	Diffuse cutaneous systemic sclerosis	Presence of skin lesions affecting the distal and proximal parts of the	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 2

		extremities, trunk, and face				
LIM_SSc	Limited cutaneous systemic sclerosis	Presence of skin lesions limited to the hands, forearms, feet, and face	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 2
CALCINOSIS	Calcinosis cutis	Presence of calcium deposits in the skin	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
MORPHEA	Morphea	Presence of isolated patches of hardened and thick skin	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
MICROSTO	Microtomy	Presence of reduction in the size of the oral aperture	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
HYPO_PIG	Hypopigmentation	Presence of an area of skin lighter than the baseline skin color	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
HYPER_PIG	Hyperpigmentation	Presence of an area of skin darker than the baseline skin color	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
ALOPE	Alopecia	Presence of partial or complete absence of hair from areas of the body where it normally grows	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
NAIL_DYSTRO	Nail dystrophy	Presence of nail changes (distortion and discoloration of normal nail-plate structure)	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
DISCOID_LES	Discoid lupus	Presence of erythematous, scaly papules and plaques preferentially occurring on sun-exposed skin areas	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
RENAL_CRISIS	Renal crisis	Presence of abrupt onset of severe hypertension accompanied by rapidly progressive renal failure	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
RENAL_INSU	Renal insufficiency	Presence of kidney impairment	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
PROTE	Proteinuria	Presence of more than 150 milligrams of	Categorical nominal	Dependent	0 = No 1 = Yes	Objective 4

		protein in urine per day			N/A: Not available	
HEMAT	Hematuria	Presence of blood cells in urine	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
GER	Gastroesophageal reflux	Presence of gastroesophageal reflux	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
DYSPHA	Dysphagia	Presence of difficulty swallowing	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
VOLVU	Volvulus	Presence of an obstruction caused by twisting of the stomach or intestine.	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
COUGH	Cough	Presence of cough	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
DISNEA	Dyspnea	Presence of difficult or labored breathing	Categorical nominal	Dependent	0 = No 1 = Yes N/A: Not available	Objective 4
NYHA	NYHA functional class	New York Heart Association Functional Classification	Categorical ordinal	Dependent	0 = Class I 1 = Class II 2 = Class III 3 =Class IV N/A: Not available	Objective 4
RODNAN_TOTAL	Rodnan total points	Rodnan total points	Numerical ratio	Dependent	Number N/A: Not available	Objective 4
PHOTOFO	Photophobia	Presence of abnormal intolerance to visual perception of light.	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
MALAR_RASH	Malar rash	Presence of erythematous flat or raised rash across the bridge of the nose and cheeks	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
ARTHRI	Arthritis	Presence of joint inflammation	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
ARTHRAL	Arthralgia	Presence of joint pain	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
XEROF	Xerophthalmia	Presence of dry eye	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
XEROS	Xerostomia	Presence of dry mouth	Categorical nominal	Confounding	0 = No 1 = Yes	Objective 4

					N/A: Not available	
INTER_FEVER	Intermittent fever	Presence of intermittent fever	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
ORAL_ULCER	Oral ulcers	Presence of oral ulcers	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
PERIODONT	Periodontal disease	Presence of periodontal disease	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
SKIN_ULCER	Skin ulcers	Presence of skin ulcers	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
ANEM	Anemia	History of anemia	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
PLEURAL_EFFU	Pleural effusion	Presence of buildup of fluid between the layers of tissue that line the lungs and chest cavity.	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
PULM_EMBO	Pulmonary embolism	Presence of blood clot in the blood vessels to the lungs.	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
PERICARD	Pericarditis	Presence of swelling and irritation of the pericardium	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
MIOCARD	Myocarditis	Presence of swelling and irritation of the myocardium	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
SEIZURE	Seizures	History of seizures	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
PSYCOS	Psychosis	History of psychosis	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
VASCUL	Vasculitis	History of vasculitis	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
CNS_INVOL	Central nervous system involvement	Presence of alterations in the central nervous system	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
PNS_INVOL	Peripheral nervous system involvement	Presence of alterations in the peripheral nervous system	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4

MYAL	Myalgias	Presence of muscle pain	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
URTICARIA	Urticaria	Presence of raised, itchy rash that appears on the skin	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
GASTRITIS	Gastritis	History of gastritis	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
W_LOSS	Weight loss	Unintentional weight loss	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
INFERTI	infertility	History of infertility	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
PERI_EDE	Periorbital edema	Presence of swelling around the eyes	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
VASC_TROMB	Vascular thrombosis	Presence of blood clots in blood vessels	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
EPIESCLE	Episcleritis	Presence of acute unilateral or bilateral inflammation of the episclera	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
UVEIT	Uveitis	Presence of inflammation of the uvea	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
SKIN_NOD	Skin nodules	Presence of skin nodules	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
LUNG_NOD	Lung nodules	Presence of lung nodules	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
GOITER	Goiter	Presence of enlarged thyroid gland	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
MENS-IRREG	Menstrual irregularity	Presence of menstrual irregularity	Categorical nominal	Confounding	0 = No 1 = Yes N/A: Not available	Objective 4
TREATMENT	Treatment	Actual treatment	Categorical nominal	Interaction	0 = No 1 = Yes N/A: Not available	Objective 1
CORTICO	Corticoids	Corticosteroid treatment	Categorical nominal	Interaction	0 = No 1 = Yes N/A: Not available	Objective 1

CORTICO_TYPE	Corticoid type	Type of corticosteroid treatment	Categorical nominal	Interaction	1 = Hydrocortisone 2 = Prednisone 3 = Methylprednisolone 4 = Deflazacort 5 = Dexamethasone 6 = Betamethasone N/A: Not available	Objective 1
ANTIMALA	Antimalarial	Antimalarial treatment	Categorical nominal	Interaction	0 = No 1 = Yes N/A: Not available	Objective 1
ANTIMALA_TYPE	Antimalarial type	Type of antimalarial treatment	Categorical nominal	Interaction	1 = Chloroquine 2 = Hydroxychloroquine N/A: Not available	Objective 1
IMMUNOSUP	Immunosuppressants	Immunosuppressants treatment	Categorical nominal	Interaction	0 = No 1 = Yes N/A: Not available	Objective 1
IMMUNOSUP_TYPE	Immunosuppressants type	Type of immunosuppressants treatment	Categorical nominal	Interaction	1 = Cyclophosphamide 2 = Mycophenolate mofetil 3 = Azathioprine 4 = Fingolimod 5 = Tacrolimus 6 = Copaxone 7 = Interferon N/A: Not available	Objective 1
DMARDS	Disease-modifying antirheumatic drugs	DMARDS treatment	Categorical nominal	Interaction	0 = No 1 = Yes N/A: Not available	Objective 1
DMARDS_TYPE	DMARDS type	Type of DMARDS treatment	Categorical nominal	Interaction	1 = Methotrexate 2 = Sulfasalazine 3 = Cyclosporine 4 = Leflunomide 5 = Gold salts 6 = D-penicillamine N/A: Not available	Objective 1
Anti_CD20	Anti-CD20	Anti-CD20 treatment	Categorical nominal	Interaction	0 = No 1 = Yes N/A: Not available	Objective 1
Anti_CD20_TYPE	Anti-CD20 type	Type of Anti-CD20 treatment	Categorical nominal	Interaction	1 = Rituximab 2 = Ocrelizumab N/A: Not available	Objective 1
Anti_TNF	Anti-TNF	Anti-Tumor Necrosis Factor treatment	Categorical nominal	Interaction	0 = No 1 = Yes N/A: Not available	Objective 1
Anti_TNF_TYPE	Anti-TNF type	Type of Anti-Tumor Necrosis Factor treatment	Categorical nominal	Interaction	1 = Infliximab 2 = Etanercept 3 = Adalimumab	Objective 1

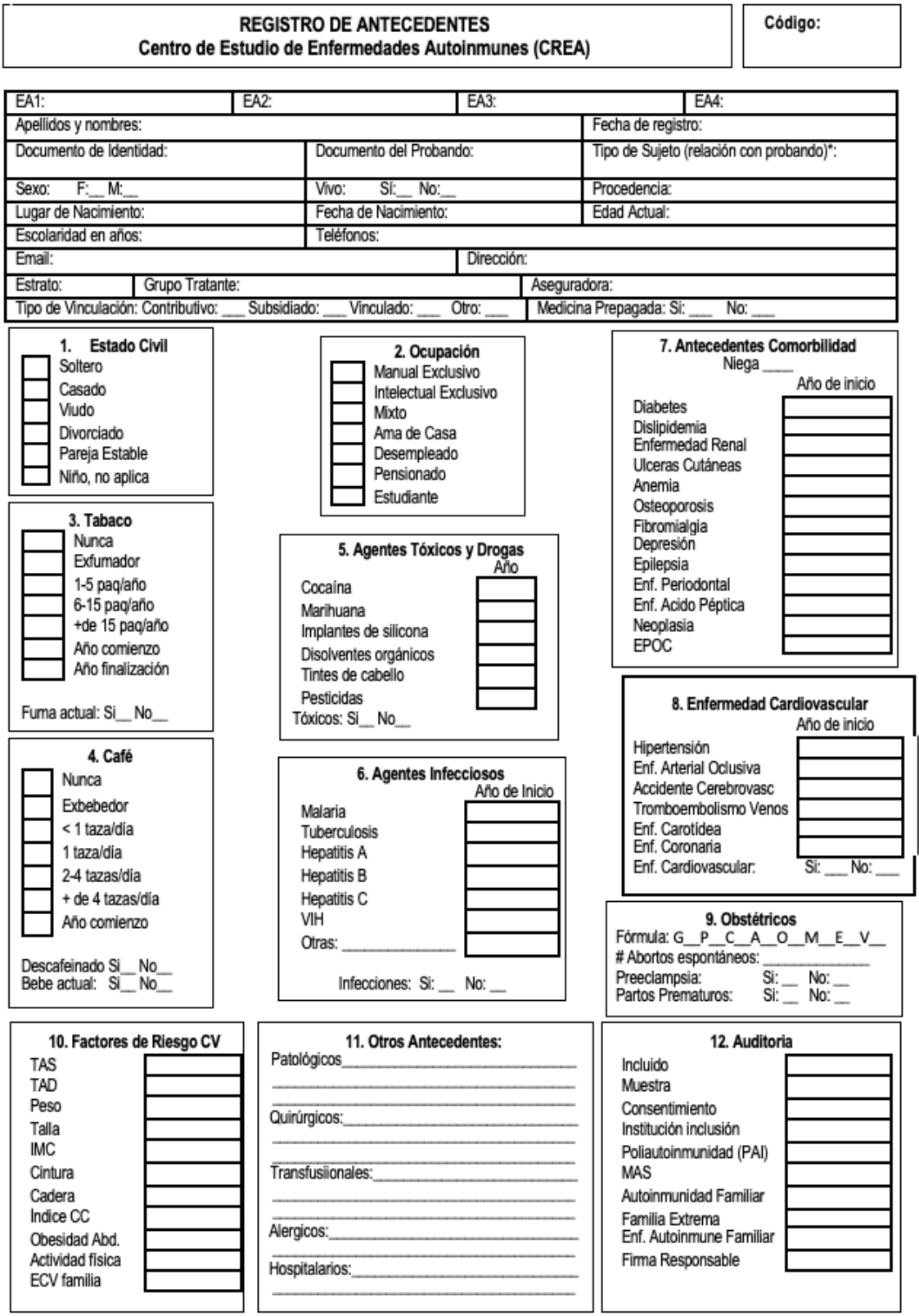
					4 = Certolizumab 5 = Golimumab N/A: Not available	
BIOLOGICS	Biologics	Biologics treatment	Categorical nominal	Interaction	0 = No 1 = Yes N/A: Not available	Objective 1
BIOLOGICS_TYPE	Biologics type	Type of Biologics treatment	Categorical nominal	Interaction	1 = Tocilizumab 2 = Anakinra 3 = Abatacept 4 = Belimumab 5 = Eculizumab 6 = Natalizumab 7 = Denosumab 8 = Ustekinumab N/A: Not available	Objective 1
OTHER	Other treatment	Other treatment	Categorical nominal	Interaction	0 = No 1 = Yes N/A: Not available	Objective 1
OTHER_TYPE	Other type	Type of treatment	Categorical nominal	Interaction	1 = Mitoxantrone 2 = Isopto Carpine 3 = Pilocarpine 4 = Pyridostigmine N/A: Not available	Objective 1
HB	Hemoglobin	Hemoglobin value	Numerical ratio	Dependent	Value N/A: Not available	Objective 4
MCV	Mean corpuscular volume	Mean corpuscular volume value	Numerical ratio	Dependent	Value N/A: Not available	Objective 4
LEUKO	Leukocytes	Leukocytes value	Numerical ratio	Dependent	Value N/A: Not available	Objective 4
LYNFO	Lymphocytes	Lymphocytes value	Numerical ratio	Dependent	Value N/A: Not available	Objective 4
PLATE	Platelet count	Platelets value	Numerical ratio	Dependent	Value N/A: Not available	Objective 4
RETICU	Reticulocytes	Reticulocytes value	Numerical ratio	Dependent	Value (%) N/A: Not available	Objective 4
CRP	C Reactive protein	C Reactive protein value	Numerical ratio	Dependent	Value N/A: Not available	Objective 4
ESR	Erythrocyte sedimentation rate	Erythrocyte sedimentation rate value	Numerical ratio	Dependent	Value N/A: Not available	Objective 4
CPK	Creatinine phosphokinase	Creatinine phosphokinase value	Numerical ratio	Dependent	Value N/A: Not available	Objective 4
CREAT	Creatinine	Creatinine value	Numerical ratio	Dependent	Value N/A: Not available	Objective 4
PROTE_VALUE	Proteinuria	Proteinuria value	Numerical ratio	Dependent	Value N/A: Not available	Objective 4

HEMAT_VALUE	Hematuria	Hematuria value	Numerical ratio	Dependent	Value N/A: Not available	Objective 4
SAMPLE_ID	Sample ID	Sample identification of the patient	Numerical ratio	Dependent	ID	Objective 4
FR_RESULT	FR result	Positivity or negativity of Rheumatoid Factor	Categorical nominal	Dependent	0 = Negative (≤6 Units) 1 = Positive (>6 Units) N/A: Not available	Objective 4
CCP3_RESULT	CCP3 result	Positivity or negativity of CCP3 autoantibody	Categorical nominal	Dependent	0 = Negative (<20 Units) 1 = Weak positive (20-39 Units) 2 = Moderate positive: (40-59 Units) 3 = Strong positive (≥60 Units) N/A: Not available	Objective 4
TPO_RESULT	TPO result	Positivity or negativity of thyroperoxidase autoantibody	Categorical nominal	Dependent	0 = Negative (0 - 100 OMS Units) 1 = Positive (>100 OMS Units) N/A: Not available	Objective 4
THYR_RESULT	Antithyroglobulin result	Positivity or negativity of Antithyroglobulin autoantibody	Categorical nominal	Dependent	0 = Negative (<0,6 OMS Units) 1 = Weak positive (0,6 - 1 OMS Units) 2 = Moderate to strong positive: (>1 OMS Units) N/A: Not available	Objective 4
B2GPIM_RESULT	B2GPI IgM result	Positivity or negativity of B2GPI IgM autoantibody	Categorical nominal	Dependent	0 = Negative (0 - 20 SMU) 1 = Positive (>20 SMU) N/A: Not available	Objective 4
B2GPIG_RESULT	B2GPI IgG result	Positivity or negativity of B2GPI IgG autoantibody	Categorical nominal	Dependent	0 = Negative (0 - 20 SMU) 1 = Positive (>20 SMU) N/A: Not available	Objective 4
ACAM_RESULT	ACA IgM result	Positivity or negativity of ACA IgM autoantibody	Categorical nominal	Dependent	0 = Negative (<12,5 MPL) 1 = Indeterminate (12,5 - 20 MPL) 2 = Weak to moderate positive (>20 - 80 MPL) 3 = Strong positive (>80 MPL) N/A: Not available	Objective 4
ACAG_RESULT	ACA IgG result	Positivity or negativity of ACA IgG autoantibody	Categorical nominal	Dependent	0 = Negative (<15 GPL) 1 = Indeterminate (15 - 20 GPL	Objective 4

					2 = Weak to moderate positive (>20 - 80 GPL) 3 = Strong positive (>80 GPL) N/A: Not available	
dsDNA_RESULT	dsDNA result	Positivity or negativity of dsDNA autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
NUCLEO_RESULT	Nucleosomes result	Positivity or negativity of nucleosome autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
HISTON_RESULT	Histones result	Positivity or negativity of histones autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
SmD1_RESULT	SmD1 result	Positivity or negativity of SmD1 autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
PCNA_RESULT	PCNA result	Positivity or negativity of PCNA autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
P0_RESULT	P0 result	Positivity or negativity of P0 autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
SSARo60_RESUL	SS-A/Ro60 result	Positivity or negativity of SS-A/Ro60 autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
SSARo52_RESULT	SS-A/Ro52 result	Positivity or negativity of SS-A/Ro52 autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
SSBLa_RESULT	SS-B/La result	Positivity or negativity of SS-B/La autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
CENP_RESULT	CENP B result	Positivity or negativity of CENP B autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
Scl70_RESULT	Scl70 result	Positivity or negativity of Scl70 autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
U1snRNP_RESULT	U1-snRNP result	Positivity or negativity of U1-snRNP autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
M2_RESULT	AMA M2 result	Positivity or negativity of AMA M2 autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
Jo1 _RESULT	Jo-1 result	Positivity or negativity of Jo-1 autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4

PMScl_RESULT	PM/Scl result	Positivity or negativity of PM/Scl autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
Mi2_RESULT	Mi-2 result	Positivity or negativity of Mi-2 autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
Ku_RESULT	Ku result	Positivity or negativity of Ku autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
DFS70_RESULT	DFS70 result	Positivity or negativity of DFS70 autoantibody	Categorical nominal	Dependent	0 = Negative 1 = Positive N/A: Not available	Objective 4
L-CYSTINE	L-cystine result	L-cystine value	Numerical ratio	Independent	Value N/A: Not available	Objective 3 and 4
L-THREONINE	L-threonine result	L-threonine value	Numerical ratio	Independent	Value N/A: Not available	Objective 3 and 4
DL-ISOLEUCINE	DL-isoleucine result	DL-isoleucine value	Numerical ratio	Independent	Value N/A: Not available	Objective 3 and 4
SARCOSINE	Sarcosine result	Sarcosine value	Numerical ratio	Independent	Value N/A: Not available	Objective 3 and 4
L-LEUCINE	L-leucine result	L-leucine value	Numerical ratio	Independent	Value N/A: Not available	Objective 3 and 4
L-VALINE	L-valine result	L-valine value	Numerical ratio	Independent	Value N/A: Not available	Objective 3 and 4
N-ETHYLGLYCINE	N-ethylglycine result	N-ethylglycine value	Numerical ratio	Independent	Value N/A: Not available	Objective 3 and 4
HYDROXY-L-PROLINE	Hydroxy-L-proline result	Hydroxy-L-proline value	Numerical ratio	Independent	Value N/A: Not available	Objective 3 and 4
ALA-ALA	Ala-Ala result	Ala-Ala value	Numerical ratio	Independent	Value N/A: Not available	Objective 3 and 4
L-ALANINE	L-alanine result	L-alanine value	Numerical ratio	Independent	Value N/A: Not available	Objective 3 and 4
L-SERINE	L-serine result	L-serine value	Numerical ratio	Independent	Value N/A: Not available	Objective 3 and 4
L-PROLINE	L-Proline result	L-Proline value	Numerical ratio	Independent	Value N/A: Not available	Objective 3 and 4
METHYL STEARATE	Methyl Stearate result	Methyl Stearate value	Numerical ratio	Independent	Value N/A: Not available	Objective 3 and 4

Appendix 2. Clinical and laboratory history form



REGISTRO DE ANTECEDENTES
Centro de Estudio de Enfermedades Autoinmunes (CREA)

Código:

EA1:	EA2:	EA3:	EA4:
Apellidos y nombres:			Fecha de registro:
Documento de Identidad:	Documento del Probando:		Tipo de Sujeto (relación con probando)*:
Sexo: F:__ M:__	Vivo: Si:__ No:__		Procedencia:
Lugar de Nacimiento:	Fecha de Nacimiento:		Edad Actual:
Escolaridad en años:	Teléfonos:		
Email:		Dirección:	
Estrato:	Grupo Tratante:	Aseguradora:	
Tipo de Vinculación: Contributivo: ___ Subsidiado: ___ Vinculado: ___ Otro: ___		Medicina Prepagada: Si: ___ No: ___	

1. Estado Civil
- Soltero
- Casado
- Viudo
- Divorciado
- Pareja Estable
- Niño, no aplica

2. Ocupación
- Manual Exclusivo
- Intelectual Exclusivo
- Mixto
- Ama de Casa
- Desempleado
- Pensionado
- Estudiante

3. Tabaco
- Nunca
- Exfumador
- 1-5 paq/año
- 6-15 paq/año
- +de 15 paq/año
- Año comienzo
- Año finalización

Fuma actual: Si__ No__

4. Café
- Nunca
- Exbebedor
- < 1 taza/día
- 1 taza/día
- 2-4 tazas/día
- + de 4 tazas/día
- Año comienzo

Descafeinado Si__ No__
Bebe actual: Si__ No__

5. Agentes Tóxicos y Drogas

	Año
Cocaína	
Marihuana	
Implantes de silicona	
Disolventes orgánicos	
Tintes de cabello	
Pesticidas	

Tóxicos: Si__ No__

6. Agentes Infecciosos

	Año de Inicio
Malaria	
Tuberculosis	
Hepatitis A	
Hepatitis B	
Hepatitis C	
VIH	
Otras: _____	

Infecciones: Si: __ No: __

7. Antecedentes Comorbilidad
Niega ____

	Año de inicio
Diabetes	
Dislipidemia	
Enfermedad Renal	
Ulceras Cutáneas	
Anemia	
Osteoporosis	
Fibromialgia	
Depresión	
Epilepsia	
Enf. Periodontal	
Enf. Acido Péptica	
Neoplasia	
EPOC	

8. Enfermedad Cardiovascular

	Año de inicio
Hipertensión	
Enf. Arterial Oclusiva	
Accidente Cerebrovasc	
Tromboembolismo Venos	
Enf. Carotídea	
Enf. Coronaria	

Enf. Cardiovascular: Si: ___ No: ___

9. Obstétricos
Fórmula: G__P__C__A__O__M__E__V__
Abortos espontáneos: _____
Preeclampsia: Si: __ No: __
Partos Prematuros: Si: __ No: __

10. Factores de Riesgo CV

TAS	
TAD	
Peso	
Talla	
IMC	
Cintura	
Cadera	
Indice CC	
Obesidad Abd.	
Actividad física	
ECV familia	

11. Otros Antecedentes:
Patológicos_____
_____
_____
Quirúrgicos:_____
_____
_____
Transfusionales:_____
_____
_____
Alergicos:_____
_____
Hospitalarios:_____
_____

12. Auditoria

Incluido	
Muestra	
Consentimiento	
Institución inclusión	
Poliautoinmunidad (PAI)	
MAS	
Autoinmunidad Familiar	
Familia Extrema	
Enf. Autoinmune Familiar	
Firma Responsable	

Formulario para Registro de Medicamentos
Centro de Estudio de Enfermedades Autoinmunes (CREA)

Grupo Farmacológico	Medicamento	Tto Actual	Tto Previo	Dosis Media	Comentarios
Inmunoglobulinas					
Corticoides	Hidrocortisona				
	Prednisolona				
	Metilprednisolona				
	Deflazacort				
	Dexametasona				
	Betametasona				
Antimaláricos	Cloroquina				
	Hidroxicloroquina				
DMARDs	Metrotexate				
	Sulfasalazina				
	Ciclosporina				
	Leflunomida				
	Sales de Oro				
	Dpenicilamina				
Inmunosupresores	Ciclofosfamida				
	Micofenolato Mofetil				
	Azatioprina				
	Fingolimod				
	Tacrolimus				
	Copaxone				
	Interferon				
Anti-CD20	Rituximab				
Anti-TNF	Infliximab				
	Etanercept				
	Adalimumab				
	Certolizumab				
	Golimumab				
Biológicos	Tocilizumab				
	Anakinra				
	Abatacept				
	Belimumab				
	Eculizumab				
	Natalizumab				
	Denomumab				
	Ustekinumab				
Otros	Mitoxantrone				
	Isoptocarpina				
	Pilocarpina				
	Piridostigmina				

Observaciones Medicamentos

Formulario para Registro de Laboratorios
Centro de Estudio de Enfermedades Autoinmunes (CREA)

Apellidos y Nombres:	Identificación:

Fecha	Laboratorio	Resultado	+/-
	Hb		
	Leucocitos1		
	Linfocitos Ab1		
	Plaquetas1		
	Leucocitos2		
	Linfocitos Abs2		
	Plaquetas2		
	VCM		
	PCR (mg/L/mg/dL)		
	VSG		
	VDRL		
	FTA-ABS		
	CPK		
	Coombs		
	Reticulocitos		
	PTT		
	Triglicéridos		
	Colesterol Total		
	HDL		
	LDL		
	Glicemia		
	Creatinina		
	Dep. Creatinina		
	Proteinuria1		
	Proteinuria2		
	Hematuria1		
	Hematuria2		
	Cilindruria1		
	Cilindruria2		
	Leucocituria2		
	Acs Hep C		
	Ags Hep B		
	VIH		
	Aldolasa		
	T3		
	T4		
	TSH		

Fecha	Anticuerpos	+/-	Dilución	Patrón
	ANA			

Fecha	Anticuerpos	+/-	Dilución	Título
	p-ANCA / MPO			
	c-ANCA / PR3			
	AntiMúsculo Liso			
	AntiMitocondriales			
	DNA			
	Anti-Centrómero			
	Anti-RNA polimerasa 3			

Fecha	Anticuerpos	+/-	Título
	Cardiolipina IgG1		
	Cardiolipina IgG2		
	Cardiolipina IgM1		
	Cardiolipina IgM2		
	B2GPI IgG		
	B2GPI IgM		
	B2GPI IgM		
	Ro		
	La		
	Sm		
	RNP		
	Factor Reumatoide		
	CCP		
	Ac Lúpico		
	C3		
	C4		
	ScL-70		
	IgA		
	IgG		
	IgM		
	Electroforesis Proteínas		Monoclonal
			Policlonal
	Crioglobulinas		
	AntiKu		
	Anti-Jo1		
	SRP		
	Mi2		
	Anti-GBM		
	AntiTPO		
	AntiTg		
	AntiTSHR		

Observaciones Laboratorio

Appendix 3. Specific form for patients with systemic sclerosis

Cumple criterios: Si__ No__ Cuantos ___	**Compromiso:** Limitado__ Difuso. __ CREST __	**Fecha de Registro:** Pág.1/4

FORMULARIO PARA PACIENTES CON ESCLERODERMIA
CENTRO DE ESTUDIO DE ENFERMEDADES AUTOINMUNES (CREA)

Nombres y Apellidos:	
Doc. Identidad:	**Edad de inicio de los síntomas:**
Forma de inicio:	**Edad al diagnóstico:**

ITEM	SUB-ITEM	PUNTAJE	
Engrosamiento de la piel de los dedos, que se extienda mínimo hasta región metacarpofalangica (criterio diagnostico)	_	9	
Engrosamiento de la piel de los dedos	"Puffy fingers"	2	
	Esclerodactilia de los dedos (distal a la articulación metacarpofalangica pero proximal a la articulación interfalangica proximal)	4	
Lesiones en la punta de los dedos	Ulceras en la punta de los dedos	2	
	Cicatriz en la punta de los dedos (pitting scars)	3	
Telangiectasias	_	2	
Anormalidad en la capilaroscopia	_	2	
Hipertensión pulmonar y/o enfermedad intersticial pulmonar	Hipertensión pulmonar *	2	
	Enfermedad intersticial pulmonar *	2	
Fenómeno de Raynaud	_	3	
Autoanticuerpos relacionados con SSC (anticentromero, anti-topoisomerasa I (anti-Scl-70), anti-RNA polimerasa III)	ANA Patrón centromérico (Anti centrómero)	3	
	Anti-topoisomerasa I (anti-Scl-70)	3	
	Anti-RNA polimerasa III	3	
	PUNTAJE TOTAL	28	

Nota: para el diagnóstico debe tener mínimo 9 puntos, y si cumple más de un criterio de la categoría, solo se suma el de mayor puntaje

* Si está presente alguno de los 2 compromisos pulmonares diríjase al formulario de **"Hallazgos Radiológicos en Enfermedad Pulmonar de Pacientes con Esclerodermia".**

1.Manifestaciones en piel

Calcinosis	
Morfea	
Microstomía	
Hipopigmentación	
Hiperpigmentación	
Alopecia	
Distrofia ungueal	
Lupus discoide	

2. Enfermedad renal

Crisis renal	
Insuficiencia renal	
Proteinuria	
Hematuria	

3. Gastrointestinal

Reflujo gastroesofágico	
Disfagia	
Vólvulo	

Pruebas de función esofágica

Vías digestivas altas: Si__No___

Endoscopia VDA: Si___ No____

pHmetría: Sí___No___

4. Pulmonar

	Año de inicio
Tos	
Disnea	
Clase funcional NYHA	

4.1 Pruebas de función pulmonar

Espirometría: Si_____ No_____

Normal____ Restr.___ Obstr____

CVF_____% VEF1_____%

VEF1/CVF_____%

DLCO: Si___ No____ %____

Normal _____ Anormal._____

5. Ecocardiograma: Si___ No__

Normal _____ Anormal._____

HTP: Si___ No___

PSAP: _____ mm Hg

FEVI: ____ %

EKG: Si ___ No ___

Arritmia: Si ___No ___Tipo:_____

6. Biopsia

Piel ___ Pulmón ___ Renal___

Lectura: _____

_____

7. Rodnan skin score

Cara___

Mano D___

Dedos D___

MS proximal D___

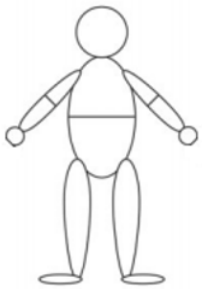
MS distal D___

Abdomen___

MI proximal D___

MI distal D___

Pie D___



Mano I___

Dedos I___

MS proximal I___

MS distal I___

Tórax___

MI proximal I___

MI distal I___

Pie I___

Puntaje total: _____

Observaciones:

Appendix 4. Transversal variables form of autoimmune diseases

FORMULARIO VARIABLES TRANSVERSALES DE LAS ENFERMEDADES AUTOINMUNES

Nombres y Apellidos			
Fecha diligenciamiento			
Documento de Identidad		**Código Muestra**	
Dx1		**Dx2**	
Dx3		**Dx4**	

Incluido proyecto PolyA		**SI**		**NO**					
Cali		**Funinderma**		**Artmédica**		**Otro**		¿Cuál?	

*Tenga en cuenta que algunas de las variables pueden haber sido diligenciadas en los formatos específicos de cada patología, sin embargo, debe llenar acá también cada uno de los campos. No deje ninguna de las variables sin respuesta.

Variable	Presente	Ausente	Sin inf	Observaciones
Fotofobia				
Rash malar				
Raynaud				
Artritis				
Artralgias				
Xeroftalmia				
Xerostomía				
Fiebre intermitente				
Insuficiencia renal				
Úlceras bucales				
Enf. periodontal				
Úlceras en piel				
Anemia				
Telangiectasias				
Derrame pleural				
TEP				
Pericarditis				
Miocarditis				
Convulsiones				
Depresión				
Psicosis				
Vasculitis				
Compromiso SNC				
Compromiso SN perif				
Mialgias				

Variable	Presente	Ausente	Sin inf.	Observaciones
Calcinosis				
Urticaria				
Alopecia				
Disfagia				
Gastritis				
Pérdida de peso				
Infertilidad				
Edema periorbitario				
Esclerodactilia				
Abortos espontáneos				
Preclampsia				
Pretérmino				
Trombosis vascular				
Epiescleritis				
Uveítis				
Nodulosis cutánea				
Nodulosis pulmonar				
Bocio				
Alteración menstrual				
Morfea				
Distrofia ungueal				
Microstomía				
OBSERVACIONES				

Laboratorio	Fecha	Resultado	+/-	Observaciones (Especificar si el paciente traía el resultado o se tomó la muestra el día de inclusión)
PCR				
VSG				
Fibrinógeno				
Parcial de orina				
Aplica solo si el paciente tiene LES:				
Hb				
Leucocitos				
Linfocitos				
Plaquetas				

¿El paciente es clasificado como Naive? Si ___ No __ **¿El paciente es clasificado como sin TX**? Si ___ No __

Fecha de la toma de muestra: _____ Hora: _____

Diligenciado por _____

Appendix 5. Inform consent

CONSENTIMIENTO INFORMADO PARA LA TOMA DE MUESTRAS BIOLÓGICAS Y LA PARTICIPACIÓN EN UN TRABAJO DE INVESTIGACIÓN EN ENFERMEDADES AUTOINMUNES

Centro de Estudio de Enfermedades Autoinmunes CREA - Universidad del Rosario

Es muy importante que usted lea y entienda los siguientes puntos sobre la realización de este estudio:

1. La participación en este estudio es totalmente voluntaria.
2. La naturaleza de esta investigación, sus propósito, sus limitaciones, sus riesgos, sus inconvenientes, incomodidades y cualquier información pertinente al resultado de este, le serán explicados por el grupo de atención clínica.
3. Si tiene algún interrogante sobre el estudio por favor no dude en manifestarlo a alguno de los investigadores, que con mucho gusto, le contestará sus preguntas.
4. **CONFIDENCIALIDAD**: Los registros médicos de cada individuo permanecerán archivados en el **Centro de Estudio de Enfermedades Autoinmunes (CREA)**, perteneciente a la Escuela de Medicina y Ciencias de la Salud de la **Universidad del Rosario**. Las historias médicas, los resultados de exámenes y la información que usted nos ha dado, son de carácter absolutamente confidencial, de manera que, solamente usted y el grupo de atención clínica tendrá acceso a estos datos. Por ningún motivo se divulgará esta información sin su consentimiento. La finalidad y uso de los datos personales por usted suministrados, serán para fines científico de investigación y de contacto con el paciente.
5. En aplicación del artículo 15 de la constitución política de la ley 1581 de 2012, la Universidad del Rosario informa a todos los participantes en este estudio, que sus datos personales se tratarán en concordancia con la política interna de protección de datos personales, puesta a su disposición a través del siguiente link http://repository.urosario.edu.co/bitstream/handle/10336/4503/PoliticaTratamientoProteccionDatosPersonales.pdf. Igualmente se dispuso del siguiente correo para la recepción de solicitudes habeas.data@urosario.edu.co.

EXPLICACIÓN DEL ESTUDIO

OBJETIVO:

El objetivo de este trabajo es identificar qué genes (códigos o huellas dactilares de las células) y cuales mecanismos se presentan más frecuentemente en pacientes con enfermedades autoinmunes, sus familiares sanos o que padezcan también, de alguna enfermedad autoinmune. Estos genes (ADN y ARN) están presentes en todas las células de su organismo, incluidas las de la sangre y la saliva. De estos tipos de muestra, extraeremos los genes, las células y otras sustancias circulantes (moléculas que viajan por la sangre o que están en la saliva) con el fin de analizarlas en un laboratorio respaldado por el CREA perteneciente a la Universidad del Rosario.

PROCEDIMIENTO:

Si Usted decide tomar parte de este estudio, llenaremos un registro con sus datos y la información relevante de su condición de salud. Adicionalmente, le tomaremos una muestra de 20 mililitros de sangre que es necesaria para analizar en el laboratorio y será obtenida de la vena de su brazo. Esta es la manera usual como se obtiene sangre para el análisis. Es posible que sienta un poco de dolor cuando la aguja entre en su brazo. En una de cada 10 personas queda una pequeña cantidad de sangre debajo de la piel, lo cual causará un moretón.

Hay un pequeño riesgo (1 de cada 100) de que la vena se coagule por un tiempo corto. El riesgo de infección o pérdida de mucha sangre es muy bajo (menos de 1 de cada 100). En algunos casos especiales (que nosotros individualizaremos) tomaremos una muestra adicional de saliva, la cual se recolecta en un recipiente especial (Oragene) y cuyo procedimiento no implica ninguna molestia para usted.

Las moléculas que extraeremos de la sangre o de la saliva (ADN, ARN, suero o células) serán almacenadas anónimamente en un biobanco bajo custodia del CREA hasta que se realicen los siguientes estudios con fines de investigación:

1. **Extracción de células de la sangre**. A partir de la muestra de sangre se realizará la separación de las células mononucleadas no granulocíticas (PBMCs). Una vez aisladas, estas células serán almacenadas en nitrógeno líquido indefinidamente hasta usarlas en su totalidad en los estudios de investigación. Una vez éstas sean cultivadas, se almacenarán en las incubadoras del cuarto de cultivo por un tiempo máximo de 7 días. Este material será utilizado para la extracción de ADN y para las pruebas funcionales como se describe a continuación.

2. **Extracción de ADN.** El ADN podrá ser obtenido a partir de la muestra de sangre, de la muestra de saliva así como de células de la sangre almacenadas. El ADN será utilizado para los análisis genéticos y epigenéticos como se describe a continuación.

3. **Extracción de ARN.** El ARN será extraído a partir de la muestra de sangre. El ARN será utilizado para la realización de análisis genéticos como se describe a continuación.

4. **Análisis genéticos:** Con el ADN y el ARN previamente extraídos se realizarán unas pruebas con el que se podrán determinar alteraciones en algunos genes (llamadas científicamente polimorfismos) relacionados con las enfermedades autoinmunes. Para ello se utilizarán pruebas conocidas como microarreglos, PCR en tiempo real y captura de exones.

5. **Análisis epigenéticos**: Con el ADN previamente extraído se evaluarán los cambios que van más allá de los genes (conocidos científicamente como cambios epigenéticos). Para ello se utilizaran pruebas conocidas como Metiloma y Ensayo ChIP-Seq.

6. **Análisis funcionales** A las células de la sangre previamente extraídas se les determinará la presencia de proteínas que podrían estar involucradas en el desarrollo de enfermedades autoinmunes.

7. **Pruebas serológicas.** A partir de la muestra de sangre se realizará la separación del suero de los otros componentes sanguíneos con el fin de determinar la presencia de moléculas que defienden al organismo (comúnmente llamados anticuerpos).

RESULTADOS:
Todo lo que aprenderemos de usted durante la investigación será confidencial. Si publicamos los resultados del estudio en una revista o libro científico, no lo identificaremos a usted de ninguna manera.

Si usted lo desea le ofrecemos la posibilidad de conocer los resultados globales de las familias que participen en este estudio mediante una reunión con usted y sus familiares. El impacto de estos resultados en el manejo o prevención de enfermedades como la que usted o su familiar padecen, no será inmediato ni modificará directamente su situación.

BENEFICIOS:
No le garantizamos que su participación en el estudio lo beneficie a usted. No recibirá ninguna compensación por participar en este estudio. Usted no tendrá costos adicionales por su participación. Su decisión para tomar parte en este estudio es voluntaria. Usted tiene libertad de decidir si no quiere participar en él en cualquier momento. Si decide no participar, o parar en cualquier momento, esto no afectará su cuidado médico actual ni futuro, como habitualmente se ha venido realizando.

Para la finalidad del estudio es necesario conservar sus muestras en un biobanco que estará compuesto por ADN, ARN, suero y células. Estas muestras serán almacenadas indefinidamente hasta su uso, bajo la custodia del CREA. Cada muestra será registrada en forma anónima y sólo se utilizará con fines de investigación en inmunogenética, la cual se realizará para identificar, evaluar y determinar posibles alteraciones a nivel inmunológico y genético que puedan conllevar al desarrollo de las enfermedades autoinmunes en la población colombiana. Por lo tanto, es importante que usted tenga en cuenta que ni usted ni su familia se beneficiarán directamente de estos estudios pero que indirectamente usted, su familia y otros individuos afectados podrían beneficiarse.

Si tiene preguntas ahora, tiene la libertad de hacerlas. Si tiene preguntas adicionales más tarde sobre la investigación que se hará en sus muestras o necesita cualquier información adicional, usted puede comunicarse directamente con:

Juan Manuel Anaya Cabrera –Adriana Rojas Villarraga, Carrera 24 N 63 C 69 Tercer Piso. Teléfono: 3499650-3474570 ext. 591, Cel 3208656253. Centro de Estudio de Enfermedades Autoinmunes -CREA, perteneciente a la Escuela de Medicina y Ciencias de la Salud de la Universidad del Rosario

Dr. Ramón Fayad Naffah. Presidente del Comité de Ética en Investigación, la Escuela de Medicina y Ciencias de la Salud de la Universidad del Rosario. Teléfono: 3474570 ext. 380 - 249

AUTORIZACIÓN PARA LA TOMA DE MUESTRAS Y REALIZACIÓN DEL ESTUDIO
MECANISMOS COMUNES DE LAS ENFERMEDADES AUTOINMUNES

Fecha: _____

Yo, _____ identificado con el documento de identificación No: _____ de _____, acepto voluntariamente que se tome una muestra de _____, con el fin de analizar las siguientes moléculas: ADN, ARN, suero y células de la sangre con fines de investigación. Así mismo declaro que se me ha explicado la ausencia de riesgos mayores y el manejo que se le dará a las muestras.

Autorización para Almacenamiento de Muestras. (Marque Con Una X)

____ Deseo que la muestra que me fue extraida sea DESECHADA una vez completado el estudio.

____ Autorizo conservar de manera anónima la muestra que me fue extraída con la posibilidad de emplearla junto con el resultado del estudio, para que adelante nuevas pruebas, si fuese necesario, sin necesidad de tomar una nueva muestra, en las situaciones señaladas a continuación:

- En estudios de investigación en inmunogenética específicos para la(s) entidad(es), objeto de esta toma de muestra, siempre y cuando se conserve en anonimato mis datos de identificación.
  Sí: __ No: __
- En estudios de investigación en inmunogenética de entidades distintas a la(s) entidad(es) objeto de esta toma de muestra, siempre y cuando se conserve en anonimato mis datos de identificación.
  Sí: __ No: __
- En estudios de investigación en inmunogenética colaborativos con otras instituciones nacionales y/o internacionales, siempre y cuando exista acuerdo interinstitucional previo y se conserve en anonimato mis datos de identificación.
  Sí: __ No: __

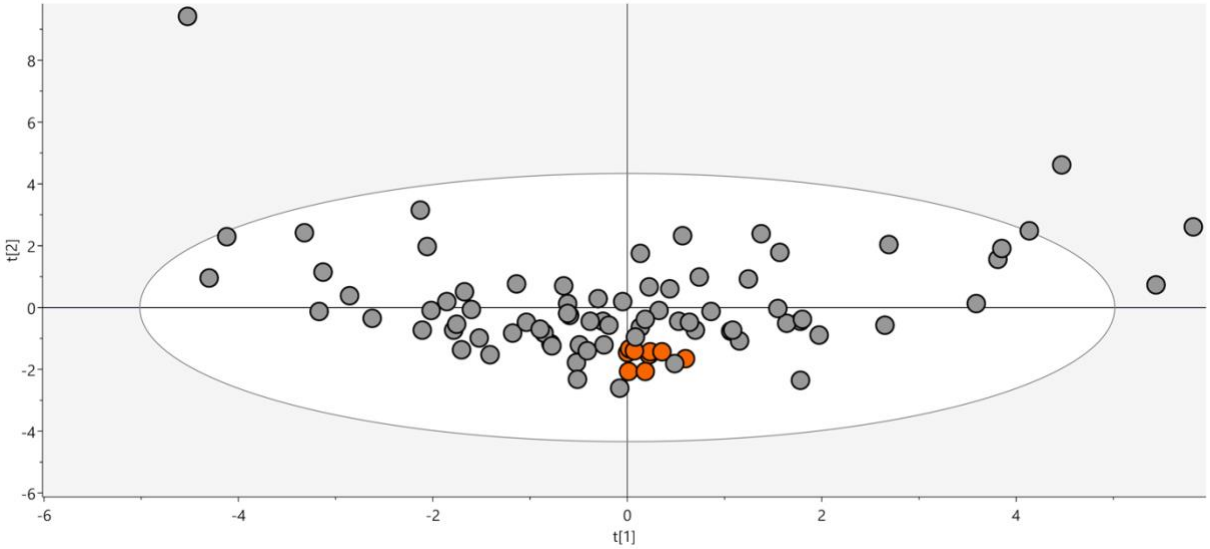
Firma: _____
Nombre: _____
Documento de Identidad: _____

Testigo 1: _____
Firma: _____
Doc. Ident: _____
Tel: _____
Relación con paciente: _____

Testigo 2: _____
Firma: _____
Doc. Ident: _____
Tel: _____
Relación con paciente: _____

Firma del Investigador: _____
Registro Médico: _____
Fecha: _____

Appendix 6.

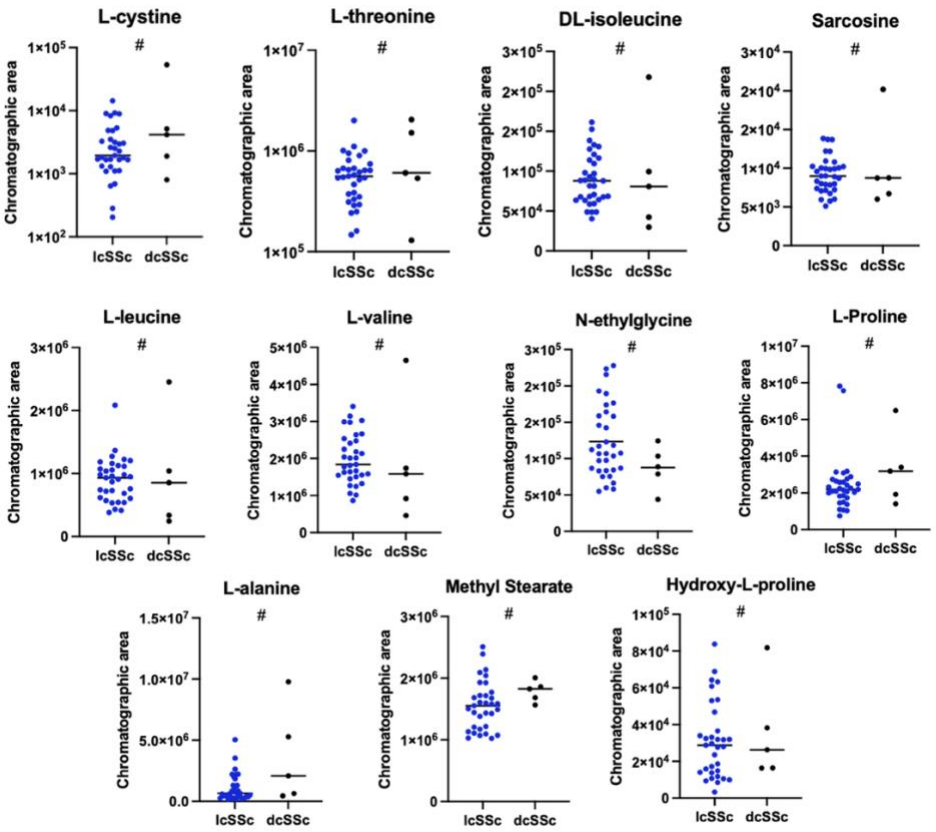


Supplementary Figure 1. Principal component analysis (PCA).

Supplementary Table 1. Results of deregulated metabolites in serum of SSc compared to HC.

Metabolite	**%Change**	**FC**	***p*-value**
L-cystine	-3,3397	0,966603	0,455
L-threonine	2,097926	1,020979	0,625
DL-isoleucine	-14,1925	0,858075	0,255
Sarcosine	-7,2994	0,927006	0,286
L-leucine	-12,7239	0,872761	0,349
L-valine	-12,856	0,87144	0,396
N-ethylglycine	-16,5891	0,834109	***0,048***
Hydroxy-L-proline	-16,5143	0,834857	0,429
Ala-Ala	-2,5	0,975	0,988
L-alanine	-21,8326	0,781674	0,863
L-serine	-2,25333	0,977467	0,841
L-Proline	-14,5438	0,854562	0,282
Methyl Stearate	0,87842	1,008784	0,576

Abbreviations: FC, Fold change.



Supplementary Figure 2. Scatter-plot of the evaluated metabolites between lcSSc and dcSSc samples from the GC-MS analysis.; # = not significant in univariate analysis.

Supplementary Table 2. Results of deregulated metabolites in serum of lcSSc compared to dcSSc.

Metabolite	***t*-stat**	***p-value***
L-cystine	-0.963	0,390
L-threonine	-1.01	0.585
DL-isoleucine	-0.139	0,896
Sarcosine	-0.365	0,733
L-leucine	-0.211	0,843
L-valine	0.163	0,878
N-ethylglycine	1.54	0,132
Hydroxy-L-proline	72	0,675
Ala-Ala	-2.12	***0,041***
L-alanine	-1.45	0.220
L-serine	-2.23	***0,032***
L-Proline	57	0,290
Methyl Stearate	-1.29	0.204