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Systemic Sclerosis in Latvia: Patient Characteristics, Peripheral Nervous System Involvement, and Novel Biomarkers

Doctoral Thesis – set of publications – for obtaining the scientific degree "Doctor of Science (*PhD*)"

Sector Group – Medical and Health Sciences

Sector – Clinical Medicine

Sub-Sector – Internal Medicine

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Riga, 2026

Funded by the European Union
NextGenerationEU

2027 National Development Plan

Research project was supported by RSU internal and RSU with LASE external consolidation, project/agreement No 5.2.1.1.i.0/2/24/I/CFLA/005

RSU Riga Stradins University

Research project was supported by RSU PhD grant

Abstract

Systemic sclerosis (SSc) is a rare systemic connective tissue disease (ORPHA code 90291) and among the most severe autoimmune rheumatic disorders, with the mortality rate 3.5 times higher than that of age-matched healthy individuals. Incidence and prevalence vary widely, with lower rates in Northern Europe; no epidemiological data have been reported for Latvia. Peripheral nervous system (PNS) involvement in SSc remains insufficiently characterised, and its pathogenesis is unclear. While ischaemia is the leading hypothesis, additional mechanisms are likely involved. Although serum autoantibodies (Abs) are a hallmark of SSc, no consistent link with PNS damage has been established. Candidate biomarkers – including neurofilament light chain (NfL), growth/differentiation factor 15 (GDF15), glial fibrillary acidic protein (GFAP), and fibroblast growth factor 21 (FGF21) – have shown potential in other conditions but have not been systematically assessed in SSc-related polyneuropathy (PNP). Likewise, metabolome studies in SSc have not addressed PNP.

This study aimed to determine the prevalence of SSc in Latvia, compare it internationally, describe demographic and clinical characteristics with emphasis on PNS involvement, explore pathogenesis, and evaluate potential biomarkers.

Data from both Latvian adult clinical university hospitals, which receive virtually all suspected SSc cases, were analysed. A total of 159 patients meeting ACR/EULAR 2013 criteria between 2016–2021 were identified. Point prevalence was 84.0 (95 % CI 71.9–98.1) per million, highest in the 60–69 age group. The female-to-male ratio was 4.67:1, with females being slightly older at the time of diagnosis (63.12 years versus 59.75 years). Most patients were ANA-positive (82.58 %), with anti-speckled and anti-centromere (ACA) patterns predominating. ACA positivity was more prevalent in females, whereas anti-topoisomerase I (ATA) showed no difference between the sexes.

First non-Raynaud's phenomenon symptoms typically appeared in the fifth decade. Females had earlier onset than males (46.51 ± 13.52 vs 50.5 ± 16.64 years). Despite a small male cohort (n = 18), a trend towards more severe disease was observed, with higher rates of interstitial lung disease (ILD), pulmonary hypertension (PH), and oesophageal dysmotility. Glucocorticoids were used in 68.31 % of patients, most often in diffuse cutaneous SSc (90 %), but also in limited cutaneous SSc (70.59 %) and sine scleroderma (66.67 %).

PNS involvement was systematically assessed. Large fibre neuropathy (LFN) was found in 43 % of 100 patients undergoing nerve conduction studies (NCS). Of 57 patients without NCS changes, quantitative sensory testing (QST) was performed in 38, revealing small fibre neuropathy (SFN) in 29. Neuropathic pain occurred in 40.59 %, more often in LFN (47.62 %) than non-LFN (35.59 %). Neuropathic pain correlated with Total Neuropathy Score (srTNS)

($r = 0.51$, $p < 0.001$), anxiety severity ($r = 0.61$, $p < 0.001$), and lower health-related quality of life (HRQoL) ($r = 0.39$, $p = 0.001$).

Analysing Abs, stratified by PNP presence, ACA (36.08 %), ATA (22.68 %), and anti-Ro52 (22.68 %) were the most frequent Abs. No Abs were significantly associated with PNP, although anti-Ro52 showed a possible protective effect. Anti-myelin-associated glycoprotein and anti-ganglioside Abs were not linked to PNP.

Serum biomarker levels were significantly higher in PNP-positive patients for NfL ($r = 0.62$, $p < 0.001$), GFAP ($r = 0.36$, $p = 0.011$), and GDF15 ($r = 0.65$, $p < 0.001$), while FGF21 showed no significant difference.

Metabolomic profiling revealed reduced aspartic acid, glutamic acid, valine, and citrulline (fold change > 2), and elevated glutamine in SSc patients compared to healthy controls (fold change > 1.5). When comparing SSc with and without PNP, no metabolites met strict discrimination thresholds; using lower cutoffs, PNP-positive patients showed elevated kynurenine and alanine, and reduced aspartic acid, with asparagine reduction shared with PNP-negative patients. Kynurenine and alanine changes were specific to the PNP-positive subgroup.

In conclusion, SSc prevalence in Latvia is lower than in many other regions, consistent with northern European patterns. PNP is highly prevalent, with almost universal PNS involvement. Neuropathic symptoms are linked to poorer HRQoL. No SSc- or inflammatory neuropathy-associated antibodies were associated with PNP. NfL, GFAP, and GDF15 emerge as promising diagnostic biomarkers. Metabolomic profiles suggest that SSc patients with PNP represent a distinct subgroup, with kynurenine and alanine elevations pointing to potential roles for neurotoxicity, mitochondrial dysfunction, and oxidative stress in pathogenesis. The development of PNP in patients with SSc is most likely due to ageing, natural progression and the sequelae of the disease. Future studies are warranted to validate the diagnostic efficacy of these biomarkers and to unravel the complex interplay of factors leading to PNP in patients with SSc. This endeavour should ultimately pave the way for novel therapeutic strategies and a more nuanced understanding of this multifaceted disease.

Keywords: systemic sclerosis, peripheral nervous system, polyneuropathy, autoantibodies, serum biomarkers, metabolome analysis, metabolites.

Anotācija

**Sistēmiskā skleroze Latvijā:
pacientu raksturojums,
perifērās nervu sistēmas
iesaiste un jauni biomarķieri**

Sistēmiskā skleroze (*SSc*) ir reta sistēmiska saistaudu slimība (ORPHA kods 90291) un viena no smagākajām autoimūnām reimatiskām slimībām, ar 3,5 reižu augstākiem mirstības rādītājiem nekā tāda paša vecuma veseliem indivīdiem. Saslimstība un izplatība ievērojami atšķiras starp reģioniem, Ziemeļeiropā slimību novēro retāk, bet Latvijas dati līdz šim nav publicēti. Perifērās nervu sistēmas (PNS) iesaiste *SSc* gadījumā joprojām ir nepietiekami raksturota, un tās patoģenēze nav skaidra. Lai gan galvenā hipotēze ir išēmiska, iespējams, ka ir iesaistīti arī citi mehānismi. Serumā konstatējamās autoantivielas ir *SSc* raksturīga pazīme, tomēr nav pierādīta to saistība ar PNS bojājumu. Potenciālie biomarķieri – neirofilamentu vieglās ķēdes (*NfL*), augšanas/diferenciācijas faktors 15 (*GDF15*), glijas fibrillārais skābais proteīns (*GFAP*) un fibroblastu augšanas faktors 21 (*FGF21*) – tika analizēti citu slimību gadījumos, tomēr ar *SSc* saistītās polineiropātijas (PNP) gadījumā plaši nav pētīti. Tāpat metaboloma pētījumi *SSc* gadījumā līdz šim nav pievērsušies PNP.

Šī pētījuma mērķis bija noteikt *SSc* izplatību Latvijā, salīdzināt to ar starptautiskiem datiem, aprakstīt demogrāfiskās un klīniskās īpatnības ar uzsvaru uz PNS iesaisti, izpētīt patoģenēzi un novērtēt potenciālos biomarķierus.

Analizēti dati no abām Latvijas pieaugušo klīniskajām universitātes slimnīcām, kurās nonāk praktiski visi pacienti ar aizdomām par *SSc*. Identificēti 159 pacienti, kas atbilda ACR/EULAR 2013 kritērijiem laika posmā no 2016. līdz 2021. gadam. *SSc* prevalence 84,0 (95 % CI 71,9–98,1) uz miljonu iedzīvotāju, augstākais rādītājs bija 60–69 gadu vecuma grupā. Sieviešu un vīriešu attiecība bija 4,67:1, sievietes tika diagnosticētas nedaudz vēlāk kā vīrieši (63,12 pret 59,75 gadiem). Lielākā daļa bija *ANA* pozitīvi (82,58 %), ar dominējoši plankumveida un anticentromēru (*ACA*) paterniem; *ACA* biežāk sastopamas sievietēm, bet anti-topoizomerāzes I (*ATA*) antivielu biežums neatšķīrās starp dzimumiem.

Pirmie simptomi, kas nebija saistīti ar Reino fenomenu, parasti parādījās piektajā dzīves desmitgadē. Sievietēm slimība sākās agrāk nekā vīriešiem ($46,51 \pm 13,52$ pret $50,5 \pm 16,64$ gadiem). Neskatoties uz nelielo vīriešu skaitu ($n = 18$), vērojās tendence uz smagāku slimības gaitu ar augstāku intersticiālās plaušu slimības (ILD), plaušu hipertensijas (PH) un barības vada dismotilitātes biežumu. Glikokortikoīdus lietoja 68,31 % pacientu – visbiežāk pie difūzas ādas *SSc* formas (90 %), bet arī limitētas (70,59 %) un *sine scleroderma* (66,67 %) gadījumos.

Tika izvērtēta PNS iesaiste. Lielo šķiedru neiropātija (*LFN*) tika konstatēta 43 % no 100 pacientiem, kuriem veikta neirogrāfija (*NCS*). No 57 pacientiem bez NCS izmaiņām 38 tika veikta kvantitatīvā sensorā testēšana (*QST*), atklājot smalko šķiedru neiropātiju (*SFN*) 29 gadījumos. Neiropātiskas sāpes bija 40,59 % pacientu, biežāk LFN grupā (47,62 %) nekā bez *LFN* (35,59 %). Neiropātisko sāpju intensitāte korelēja ar kopējo neiropātijas punktu skaitu (*srTNS*) ($r = 0,51$, $p < 0,001$), trauksmainību ($r = 0,61$, $p < 0,001$) un sliktāku ar veselību saistītu dzīves kvalitāti (HRQoL) ($r = 0,39$, $p = 0,001$).

Autoantivielu analīzē, stratificējot pēc PNP klātbūtnes, visbiežāk konstatētās antivielas bija ACA (36,08 %), ATA (22,68 %) un anti-Ro52 (22,68 %). Neviena antiviela nebija statistiski nozīmīgi saistīta ar PNP, lai gan anti-Ro52 novēroja aizsargājoša faktora tendenci. Antivielas pret mielīna asociēto glikoproteīnu un gangliozīdiem nebija saistītas ar PNP.

Seruma biomarķieru līmeņi PNP pacientiem bija būtiski augstāki: NfL ($r = 0,62$, $p < 0,001$), GFAP ($r = 0,36$, $p = 0,011$) un GDF15 ($r = 0,65$, $p < 0,001$), savukārt FGF21 atšķirību neuzrādīja.

Metabolomiskajā profilā *SSc* pacientiem, salīdzinot ar veselo kontroli, tika konstatēts samazināts asparagīnskābes, glutamīnskābes, valīna un citrulīna līmenis (> 2 reizes), kā arī paaugstināts glutamīna līmenis (> 1,5 reizes). Salīdzinot SSc pacientus ar un bez PNP, neviens metabolīts nesasniedza diskriminācijas slieksni; lietojot zemākus sliekšņus, PNP pacientiem tika konstatēts paaugstināts kinurenīna un alanīna līmenis un samazināta asparagīnskābe, savukārt asparagīna samazinājums bija kopīgs gan pacietiem ar PNP, gan bez PNP. Kinurenīna un alanīna izmaiņas bija raksturīgas tikai SSc pacietiem ar PNP.

Secinājumā, *SSc* izplatība Latvijā ir zemāka nekā daudzos citos reģionos, kas atbilst Ziemeļeiropas tendencēm. PNP *SSc* pacietu vidū ir ļoti izplatīta, ar gandrīz universālu PNS iesaisti. Neiropātijas simptomi ir saistīti ar zemāku dzīves kvalitāti. Autoantivielu saistība ar PNP attīstību *SSc* pacietiem netika pierādīta. *NfL*, *GFAP* un *GDF15* izceļas kā daudzsološi diagnostiskie biomarķieri. Metaboloma dati liecina, ka *SSc* pacienti ar PNP veido atšķirīgu apakšgrupu, kurā kinurenīna un alanīna līmeņa paaugstinājums norāda uz iespējamu neirotoksicitātes, mitohondriju disfunkcijas un oksidatīvā stresa lomu patoģenēzē. PNP attīstība pacientiem ar *SSc*, visticamāk, saistīta ar novecošanos, slimības dabisko gaitu un tās sekām. Nākotnes pētījumos jāapstiprina šo biomarķieru diagnostiskā efektivitāte un jāizpēta sarežģītā faktoru mijiedarbība, kas veicina PNP attīstību *SSc* pacientiem. Tas nākotnē varētu pavērt ceļu jaunu terapeitisku stratēģiju izstrādei un padziļinātai izpratnei par šo daudzšķautņaino slimību.

Atslēgvārdi: sistēmiska skleroze, perifēra nervu sistēma, polineiropātija, autoantivielas, seruma biomarķieri, metabolome analīze, metabolīti.

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Abbreviations used in the Thesis

Abs	autoantibodies
ACA	anti-centromere antibodies
ACR/EULAR	American College of Rheumatology/European League Against Rheumatism
aHSCT	autologous hematopoietic stem cell transplantation
ANA	antinuclear antibodies
anti-MAG	anti-myelin-associated glycoprotein
ARA	anti-RNA polymerase antibodies
ATA	anti – topoisomerase antibodies
AZA	azathioprine
CENP-A	centromere proteins A
CENP-B	centromere proteins B
CNS	central nervous system
CYC	cyclophosphamide
dcSSc	diffuse cutaneous systemic sclerosis
DM	diabetes mellitus
DN	diabetic neuropathy
DN4	Douleur Neuropathique en 4 questionnaire
EUSTAR	European Scleroderma Trials and Research group
FC	fold changes
FGF21	fibroblast growth factor 21
Fib	fibrillarin
GAD7	Generalised Anxiety Disorder-7 questionnaire
GCs	glucocorticoids
GDF15	growth/differentiation factor 15
GFAP	glial fibrillary acidic protein
HAQ-DI	health assessment questionnaire-disability index
HC	healthy control
HRQoL	health related quality of life
ICAM-1	intercellular adhesion molecule 1
ILD	interstitial lung disease
KL-6	Krebs von den Lungen 6 glycoprotein
lcSSc	limited cutaneous systemic sclerosis
LFN	large fibre neuropathy
MMF	mycophenolate mofetil
mRSS	modified Rodnan skin score

MTX	methotrexate
NCS	nerve conduction studies
NfL	neurofilament light chain
NO	nitric oxide
NOR90	nucleolar organiser region 90
NS	nervous system
PDGFR	platelet-derived growth factor receptor
PH	pulmonary hypertension
PM100	polymyositis/scleroderma 100
PM75	polymyositis/scleroderma 75
PNP	polyneuropathy
PNS	peripheral nervous system
QST	quantitative sensory testing
RP	Raynaud's phenomenon
RP11	RNA polymerase III
RP155	RNA polymerase III
Scl-70	topoisomerase I
SFN	small fibre neuropathy
SP-D	surfactant protein D
srTNS	shortened and revised Total Neuropathy Scoring
SSc	systemic sclerosis

Introduction

Systemic sclerosis (SSc) is a systemic connective tissue disease with an average prevalence of 1 in 6500 adults, listed in the Rare Disease Registry, with ORPHA code 90291 (Orphanet, 2025).

The term 'scleroderma' (translated as thickened, hard skin) has been used since the mid-19th century but the first records date back to 1753, when Carlo Curzio described a 17-year-old girl with marked hardening of the skin all over her body (Rodnan et al., 1962). Since 1980, scleroderma has been defined as a spectrum of diseases that consist of localised scleroderma and SSc (Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee, 1980). Of the two types, localised scleroderma is more frequent with an incidence of 2.7 cases per 100 000, is not usually associated with severe systemic symptoms or Raynaud's phenomenon (RP) and often is self-limited with a good prognosis (Calonje et al., 2020). On the other hand, SSc is considered by many to be one of the most severe autoimmune rheumatic diseases, with the mortality rate 3.5 times higher than that of age-matched healthy individuals (Adigun et al., 2024; Yen et al., 2021). On the other hand, SSc is considered by many to be one of the most severe autoimmune rheumatic diseases (Yen et al., 2021). To verify the truth of this statement, accurate epidemiological data are needed. However, incidence and prevalence vary greatly between different studies, explained mainly by random sampling errors and differences between case definitions and capture methods (Kowal-Bielecka et al., 2013).

Lower prevalence (below 150 cases per million) and incidence (below 10 cases per million per year) are observed in Northern Europe and Japan, whereas higher incidence rates are observed in Southern Europe, North America and Australia (Airò et al., 2020; Furst et al., 2012; Kang et al., 2018). As with other rheumatic diseases, the incidence of SSc varies according to gender. It is observed to be higher in females (female-to-male ratio of 3:1), with a higher gender ratio for younger patients but lower after the age of 50 years (2:1) (Chifflot et al., 2008; Sangha, 2000). The estimated average age of onset is 50 years (Derk et al., 2006). However, after the age of 75 years, the development of the disease is rarely seen (Steen et al., 1997). Gender differences explored in SSc can play an important role in early diagnosis and more accurate prognosis. There is already established higher premature death risk in males with SSc, and more severe expression of the disease, comparing with females with SSc (Hughes et al., 2020). No previous studies on the prevalence of SSc in Latvia have been conducted.

The clinical picture of SSc is highly variable. According to the EUSTAR (European Scleroderma Trials and Research group) database, RP is seen in 96 %, lung damage in 48 %, digital ulcers in 38 %, arthritis with synovitis in 19 %, renal crisis in up to 4 % of SSc patients

(Meier et al., 2012). The involvement of the nervous system (NS) is not isolated in the database but is studied in separate small sample groups. Analysing the results of these studies, the most frequent manifestations of central nervous system (CNS) involvement are headache (23 %) and seizures (13 %), whereas the prevalence of peripheral nervous system (PNS) involvement strongly varies from 17 to 40 %. Different syndromes of PNS involvement have been described, the most frequent being peripheral sensorimotor neuropathy and small fibre neuropathy (SFN) with neuropathic pain (AlMehmadi et al., 2021; Amaral et al., 2013; Averbuch-Heller et al., 1992; Bignotti et al., 2015; Lee et al., 1983). There is a lack of objective data on PNS involvement in SSc, probably due to both small cohorts and variability in study methodologies. No nationwide study of PNS disorders among SSc patients has previously been carried out in the Baltic countries.

Several uncertainties remain in the pathogenesis of SSc. In the 1990s, the main factors in the development of the disease were identified: immune activation, vasculopathy, and overproduction of extracellular matrix with collagen deposition (Denton et al., 1996). The different clinical manifestations of the disease suggest variations in the involvement of these factors. Recent literature describes the pathophysiology of SSc as a chronic progressive process leading to microvascular damage with subsequent autoimmune response and inflammation leading to diffuse tissue fibrosis (Cutolo et al., 2019).

In PNS damage in SSc patients, the pathogenesis remains unclear. One theory of PNS damage is ischaemic, where polyneuropathy (PNP) is associated with RP and its severity. However, when analysing SSc patients with severe RP, the most common clinical manifestation of SSc vasculopathy, as well as pitting scars and ischaemic skin lesions, there was no strong association with PNS damage, suggesting that other mechanisms are involved in the pathogenesis of PNP (Amanzi et al., 2010; Kılıç et al., 2020).

Disease-specific autoantibodies are important to identify different clinical groups of SSc, stratifying patients into more homogeneous subgroups (Cavazzana et al., 2023). Serum autoantibodies directed against multiple intracellular antigens are the serological hallmark of SSc. They are detectable in more than 95 % of patients and are characterised by at least nine SSc specific antibodies directed against nuclear or nucleolar autoantigens (Peoples et al., 2016; Salazar et al., 2015; Tan, 1989). Anti-topoisomerase antibodies (ATA), anti-centromere antibodies (ACA) and anti-RNA polymerase antibodies (ARA), first described in the 1970s–1990s, are classic disease-specific autoantibodies and are included in the 2013 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) SSc classification criteria (Hoogen van den et al., 2013). In general, the presence of SSc specific antibodies may be associated with various clinical manifestations of SSc, such as diffuse

(dcSSc) or limited cutaneous (lcSSc) subtypes, interstitial lung disease (ILD) and pulmonary hypertension (PH) (Cavazzana et al., 2023; Santos, et al., 2023). Unfortunately, there is currently no firm evidence that these antibodies are also associated with PNS damage in SSc. In many systemic connective tissue diseases idea of studying specific antibodies against various nerve structures comes from research done in immune neuropathies like Guillain–Barré syndrome and its subtypes (Souza De et al., 2023; Jin et al., 2021). This approach is still understudied in SSc.

Biomarkers of progression and severity of SSc is yet another understudied issue. Several biomarkers are known to be used to measure and monitor the severity of lung and skin damage (Castro et al., 2010; Utsunomiya et al., 2020). Markers to assess PNS damage and its progression are still not identified. Serum biomarker that has been widely studied in PNS damage due to metabolic or genetic disorders is neurofilament light chain (NfL) (Hayashi et al., 2021; Maalmi et al., 2023). Damaged axons release NfL into the intercellular space to ensure cellular stability (Kahn et al., 2025). Serum NfL concentrations have been found to be elevated in congenital peripheral neuropathies and correlate with the severity of the neuropathy (Sandelius et al., 2018). Serum NfL concentrations have not been studied in SSc patients to date. Growth/differentiation factor 15 (GDF15) is a cytokine belonging to the beta class of transforming growth factors. Its elevated levels are observed in inflammation, myocardial ischaemia and tumours (Wischhusen et al., 2020). Serum GDF15 concentrations in SSc patients were found to be elevated in PH compared to SSc patients without PH (Gamal et al. 2017; Meadows et al., 2021). Elevated levels of the cytokine were also found in SSc patients with ILD and more pronounced skin lesions (Gamal et al. 2017; Wan, et al., 2024). Although there is evidence of increased secretion of GDF 15 from Schwann cells also in PNS damage, there are no known studies in SSc patients with PNS damage (Weng et al., 2022).

Another area of biomarker research in SSc could be metabolome studies. Metabolomics is a field of -omics technology that comprehensively studies metabolites in organisms using high-performance analytical technology (Zhang et al., 2015). Metabolites are known to represent the last downstream of biochemical reaction, and they are widely used in clinical study and drug discovery (Qiu et al., 2023). The clinical application of metabolomics aims to determine the diagnostic biomarkers of disease, pathological mechanisms, and novel drug targets and therapeutic responses. The importance of metabolomics in autoimmune disease has been raised because it can aid in understanding the molecular mechanism behind a specific phenotype of the disease. Metabolome studies have already been carried out in patients with SSc, and several metabolites have been found to be changed compared to healthy controls (HC). Some of these studies have isolated the different manifestations of SSc as ILD or marked

modified Rodnan skin score (mRSS) (Bengtsson et al, 2016; Bögl et al, 2022; Guo et al., 2023; Jud et al, 2023; Morales-González et al., 2023; Ottria, et al, 2020; Smolenska et al., 2020). However, it is noticeable that PNP as a complication of SSc has been ignored in the metabolome studies. Understanding the metabolomic alterations in SSc and its related PNP has the potential to uncover novel biomarkers and therapeutic targets, providing opportunities for improved management and outcomes for patients suffering from this complex disease.

Aim of the Thesis

The aim of this Thesis is to determine the prevalence of SSc in Latvia and to compare it with data from other countries and regions of the world, to summarise demographic and clinical characteristics of SSc patients, with an emphasis on PNS involvement, its pathogenesis and biomarker investigation in patients with SSc.

Objectives of the Thesis

The following objectives are set to reach the aim of the Doctoral Thesis:

1 Select patients diagnosed with SSc who met the ACR/EULAR 2013 classification criteria, by using hospitals database, and determine prevalence of SSc in Latvia.
2 Evaluate clinical characteristics of SSc patients, by examination, that also involves evaluation of mRSS, and previous investigations for ILD, PH, oesophageal dysmotility, while also examining gender-related differences.
3 Determine PNS involvement in SSc patients, by shortened and revised Total Neuropathy Scoring criteria (srTNS), nerve conduction studies (NCS) and quantitative sensory testing (QST), further subclassifying SSc patients in large (LFN) and small fibre neuropathy (SFN) groups, and assessing neuropathic pain by Douleur Neuropathique en 4 (DN4) questionnaire, anxiety symptoms by Generalised Anxiety Disorder -7 (GAD7) questionnaire, and health related quality of life (HRQoL) by Health Assessment Questionnaire-Disability Index (HAQ-DI).
4 Define the autoimmune mechanisms that lead to PNP, by identifying SSc specific antibodies and antibodies that target certain components of the NS, like antibodies against myelin-associated glycoprotein (anti-MAG) and anti-ganglioside antibodies.
5 Define biomarkers that correlate with the detection and progression of PNP in SSc, by identifying NfL, growth/differentiation factor 15 GDF15, glial fibrillary acidic protein (GFAP) and fibroblast growth factor 21 (FGF21).
6 Explore further pathogenesis of PNP in SSc, by metabolite analysis in SSc patients compared to HC and in subgroup analysis distinguished by PNS involvement.

Hypotheses of the Thesis

1 Epidemiology: SSc is a rare disease in Latvia, occurring less frequently than in southern European countries, consistent with a north–south gradient. PNP in SSc is likely underestimated and more common than previously reported, affecting both large and small nerve fibres.

2 Pathogenesis, biomarkers, and metabolic profile: PNP in SSc likely has an autoimmune pathogenesis involving antibodies against peripheral nerve structures. Biomarkers such as NfL, GDF15, GFAP, and FGF21 may help assess its development and severity. Moreover, SSc patients with PNP exhibit distinct metabolite regulation compared with SSc patients without PNP, differing from patterns observed in previous SSc-healthy control comparisons.

Novelty of the Thesis

SSc is a rare disease, which limits studies with large numbers of patients. However, thanks to pooled multi-country or multi-continental registries such as EUSTAR, we can learn about the frequency of different clinical manifestations, type, and relationship to the immunological profile. In this way, the frequency, type and association of ILD with specific Abs in patients with SSc are now much clearer. However, for various reasons, the involvement of the NS in patients with SSc is still underestimated, even considering the above-mentioned registries. This study focused on the development of PNP in patients with SSc, identifying the frequency of this complication, its association with disease duration, type and other disease manifestations, allowing a possible association of PNP with more ischaemic or inflammatory pathway.

The wide range of SSc specific Abs currently available has not been extensively studied in PNP patients. A clearer association of Abs with the development of PNP allows a faster and more accurate identification of at-risk groups, providing them with a more personalised screening in the future.

The treatment of SSc is mainly divided into two groups: against ischaemic damage and against inflammatory process. At present, the pathogenesis of PNP is not clearly known, and there are no guidelines or precise recommendations for the treatment of PNP in SSc. The use of Abs against different components of the NS, which has not previously been performed in SSc patients with PNP, will allow the potential benefits of immunosuppressive therapy in the treatment of PNP to be clarified.

Due to the limited attention paid to PNP in SSc, no biomarkers have been identified that could be used as an indicator for the development and severity of PNP. Biomarkers associated with NS damage have been recognised in studies of other diseases, and if their association with

PNP in SSc patients is demonstrated, these markers could be used in the future to identify SSc patients who require further in-depth investigation or treatment adjustment.

Metabolome research has played an increasingly important role in the study of various diseases in recent years, including SSc. However, the PNP patient group has not been previously isolated. In this case, metabolome analysis would allow to understand whether PNP can be considered as an important distinct cluster in SSc and, by assessing the changes in different metabolites, also the reasons for the development of PNP in SSc.

Study design

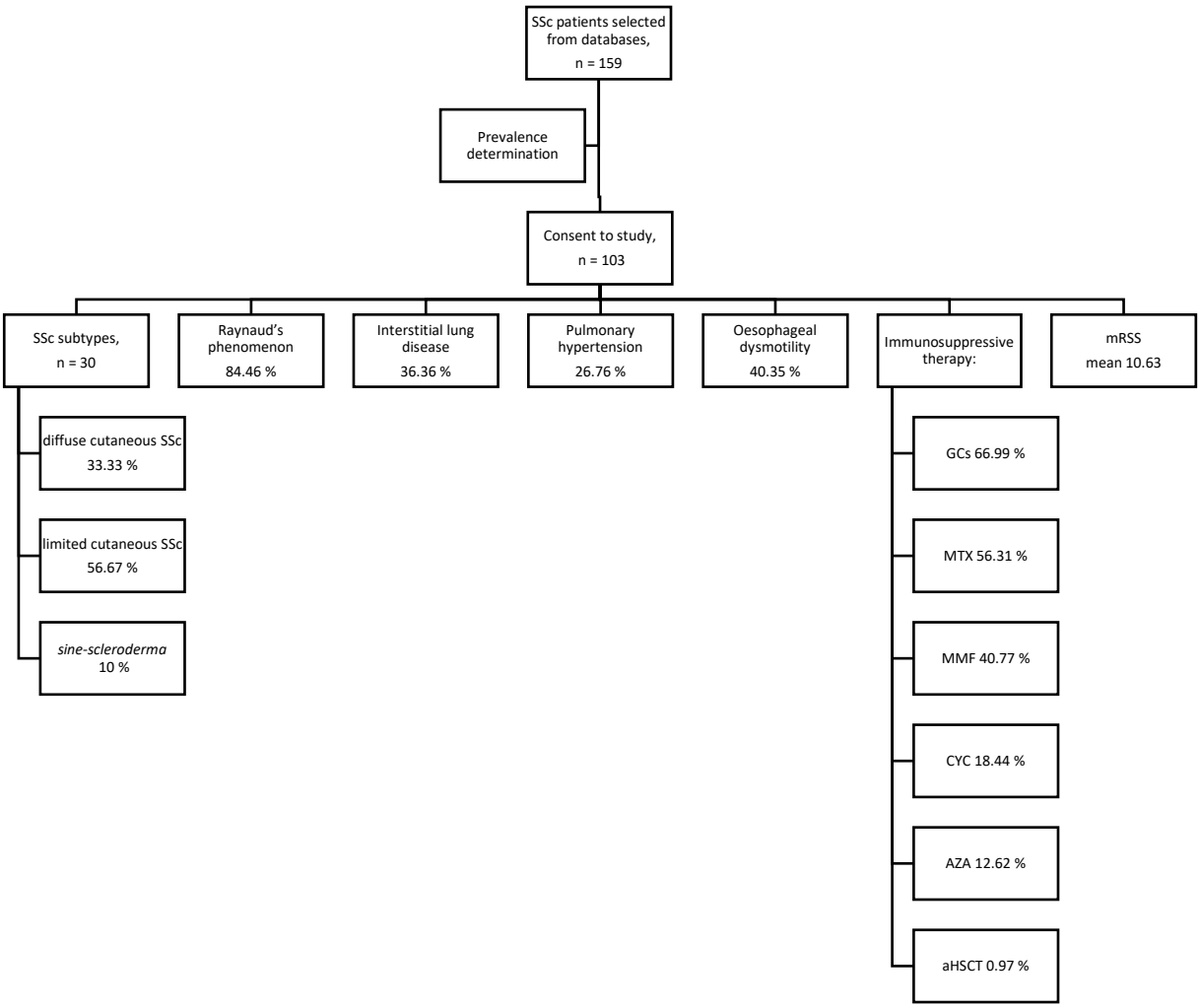

Figure 1.1 **Demographic and clinical data**

SSc systemic sclerosis, GCs glucocorticoids, MTX methotrexate, MMF mycophenolate mofetil, CYC cyclophosphamid, AZA azathioprine, aHSCT autologous hematopoietic stem cell transplantation, mRSS Modified Rodnan Skin Score

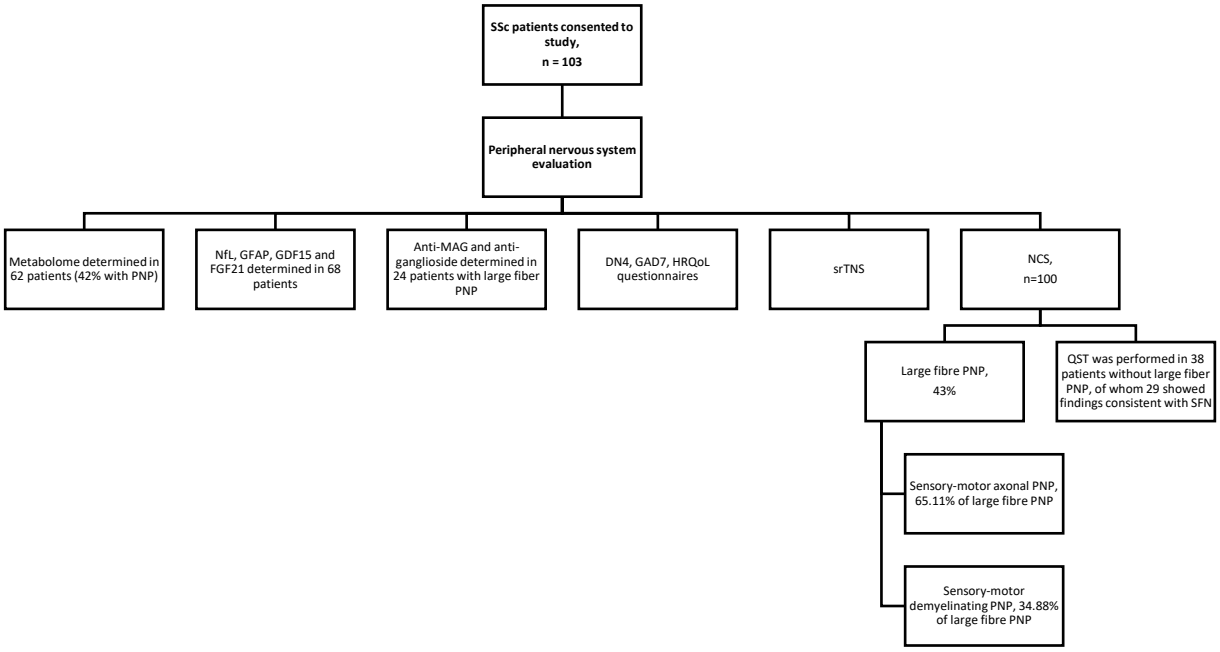

Figure 1.2 **Study of the PNS**

PNP polyneuropathy, NfL neurofilament light chain, GFAP glial fibrillary acidic protein, GDF15 growth/differentiation factor 15, FGF21 fibroblast growth factor 21, anti-MAG anti-myelin-associated glycoprotein, DN4 Douleur Neuropathique en 4 questionnaire, GAD7 Generalised Anxiety Disorder -7 questionnaire, HRQoL health related quality of life, srTNS shortened and revised Total Neuropathy Scoring, NCS nerve conduction studies, QST quantitative sensory testing, SFN small fibre neuropathy

Discussion

To our knowledge, this is one of the few studies on SSc that focuses on the involvement of the PNS, analysing both the prevalence of this complication and its pathogenesis and biomarkers of severity.

Prevalence and gender-specific analysis of a systemic sclerosis cohort in Latvia

The total number of SSc patients in Latvia was unknown, so determining the prevalence of the disease was one of the first tasks, comparing results with other countries in the Northern and Eastern Europe region. The significance of this study lies in the specificity of the country with a small population. In our study for patient selection, we used database from both Latvia's clinical university hospitals for adults with an established team of rheumatologists. Virtually all patients with suspected SSc in Latvia are referred to one of these hospitals, so we were effectively describing the general Latvian population by selecting and evaluating patients from these hospitals. We included patients who were evaluated by a rheumatologist between 2016 and 2021 and had a confirmed diagnosis of SSc. We were able to find 159 SSc patients consulted between 2016 and 2021, and the point prevalence was 84.0 (95 % CI 71.9–98.1) per million. Only a few studies were conducted in Northern or Eastern Europe. One study carried out in Southeast Norway found the point prevalence of SSc to be 99 per million that is compatible with other northern European countries, supporting the notion of a north–south gradient of SSc in Europe, with the lowest prevalence in Northern Europe (Hoffmann-Vold et al., 2012). Opposing results were presented from Sweden, where the prevalence was higher at 235 per million inhabitants (Andréasson et al., 2014). In our study, the point prevalence was lower than the results in review about 50 publications from Europe and North America, with reported prevalence of 70.2–333.9 and 135–443 per million in Europe and North America, respectively (Bergamasco et al., 2019). Although we cannot identify any specific reason for this, the relatively low prevalence is unlikely to be due to study shortages but rather to a possible shortage of rheumatologists in the country and the unavailability of consultations. This would be particularly true for patients with a lcSSc, without severe PH, who do not feel the need to visit their general practitioner. We observed the highest prevalence in the 60–69 age group, that was not similar in other European countries. For example, in Sweden and Italy 70–79 age group had the highest prevalence (Ciaffi et al., 2021; Westerlind et al., 2022). We report a higher mean age in this study for females than males: 63.12 versus 59.75 years. This was not seen in the Norway study, where the difference was minimal (56.7 versus 56.1 years) (Hoffmann-Vold et al., 2012). Also, the mean age of both genders was older than represented in other similar studies: 62.53 ± 12.11 years versus 50.8 ± 12.5 years in Italy and 56.8 ± 12.2 years in Hungary

(Czirják et al., 2008; Foti et al., 2016). A higher female predominance was seen in this study than is reported worldwide, with a female-to-male ratio of 4.67:1 compared to 3:1. However, it was similar to other European reports, where the ratio was estimated to be 3.8–11.5:1, so the study of gender difference should probably be based on regional data rather than on global data linking very different regions together (Bergamasco et al., 2019). The highest gender ratio was observed in the 70–79- year age group (6.75:1), contradicting previous observations of a lower gender ratio after the age of 50 years (2:1) (Sangha, 2000). In younger patients we did find a lower gender ratio (2:1), but this again contradicted the worldwide data (Sangha, 2000). Of course, probabilities must be expressed with caution with the small number of patients. Still, in our study, we probably captured the characteristics of older men avoiding medical help in Latvia.

Next, we evaluated the results of the antibodies previously detected. Of the 159 patients, ANA were available for 155 and ANA pattern of 122. Most of patients were ANA positive (82.58 %) with anti-speckled and anti-centromere patterns present the most. The presence of ANA in patients with SSc is widely observed, with levels as high as 98 % reported (Peoples et al., 2016). Three serum antibodies that are included in the 2013 classification criteria (ACA, ATA and ARA) account for over 70 % of all single antibody specificities detected in previous studies (Hoogen van den et al., 2013; Peoples et al., 2016). Unfortunately, at the time of study, it was not possible to detect ARA, but 84 patients (68.85 %) from the 122 evaluated had either ACA or ATA. In recent data with knowledge of new antibodies associated with SSc, still highest prevalence stands for these two antibodies (Stochmal et al., 2020). Contrary to our results, in the Norway study, there was significant ACA predominance compared to ATA (54.2 % vs. 13.5 %) (Hoffmann-Vold et al., 2012). Previously, many studies reported higher ACA prevalence in Caucasians (McNeilage et al., 1989; Reveille et al., 2001). In contrast, in a study from the USA, evaluating the prevalence of antibodies in a different race, only 17 % of Caucasian patients had positive ACA, with more (19 %) having ARA (Krzyszczak et al., 2011). We found that ACA patients were more likely to be females, whereas the difference was not as significant between ATA positive males and females. In other studies, females were substantially more likely to have ACA, whereas males more likely to have ATA (Peoples et al., 2016). In our study, we present different data from the previous studies. With 100 % Caucasian patients, there was no significant ACA predominance and there was a high prevalence of ATA antibodies. Although ANA positive patients were fewer than in majority of other studies, it could be higher with repeated examination dynamically (Bobeica et al., 2021).

Subsequently, patients were invited to participate in the study; 103 consented, and comprehensive medical and clinical data were obtained, including age at disease onset, SSc

subtype, presence of RP, internal organ involvement, immunosuppressive treatment received, comorbidities, and assessment of the mRSS. Most of patients presented with the first non-RP SSc symptom in the fifth decade of life. Study from Sweden showed similar results (48 ± 4.1 years) (Westerlind et al., 2022). However, disease onset is hard to determine and has not been defined similarly in other studies. The age at which the diagnosis was made is generally analysed and in data from Europe it varies in the range 33.5–59.8 years (Bergamasco et al., 2019). In our view, it is also essential to note patients' observations of their first symptoms, allowing more reliable conclusions of differences between several populations. By contrast, if the focus remains on the time of diagnosis, we may mistakenly assess not the characteristics of the disease but the availability of specialists in different countries. We reported a slight age difference when comparing both genders at disease onset, with females (46.51 ± 13.52) being younger than males (50.5 ± 16.64). Younger female age at onset is not uncommon, and other studies have presented similar findings. In a study from Greece, the age difference was markedly larger but, similarly, the females tended to be younger (Alamanos et al., 2005). In Pittsburg, USA, the results were very similar to ours: 43.8 ± 4.0 years for females; 46.4 ± 13.7 years for males (Peoples et al., 2016). Although the number of males in the study was small (18 patients), we observed a similar trend towards a more severe disease course, with more frequent development of ILD (35.80 % in females and 38.89 % in males) and PH (25.42 % in females and 30.77 % in males), as in other studies (Hughes et al., 2020; Pasarikovski et al., 2016; Volkmann et al., 2022). As the main causes of SSc-related mortality, these data also explain the worse outcomes in males. However, there are no clear data on the difference in the incidence of oesophageal dysmotility between genders. Historically, dysmotility was described as another close symptom to the lcSSc subtype but we observed a higher frequency of dysmotility in males (39.13 % in females and 45.45 % in males), although the lcSSc did not predominate as the most common subtype of disease in them (Arana-Guajardo et al., 2019; Kimmel et al., 2016).

Information on the use of immunosuppressive drugs was also collected, both from medical records and from patients. We found that more than half of patients (68.31 %) received treatment with GCs at any point of the disease. Although this number is exceptionally high, the trend is not exclusive to our study. The German Network for Systemic Scleroderma data showed that 41,3 % of all registered SSc patients were treated with GCs (Hunzelmann et al., 2009). EUSTAR database provided very detailed data on GCs prescribing practices in SSc, with 34 % off patients taking GCs at baseline of the study, but the use of GCs from disease onset was not included. There were no data from Latvia, but interestingly eastern Europe countries tended to prescribe GCs more (Iudici et al., 2023). In the update of the EULAR

recommendations for the treatment of SSc, the experts recognised that GCs, which are used in SSc, are part of the therapeutic strategy in the management of ILD, dcSSc or musculoskeletal involvement (Kowal-Bielecka et al., 2017). However, the evidence regarding their efficacy in SSc is limited (McNeilage et al., 1989). In Latvia, the trend of GCs use was more pronounced in patients with dcSSc (90 %), but it was also used in more than half of patients with lcSSc (70.59 %) and with sine scleroderma (66.67 %). The most difficult to explain the use of GCs was in 57 % of patients who used them without diffuse skin involvement and ILD. Patients enrolled in the study were treated for up to several decades. We think this is also why the number of patients treated with GCs was so high. Previously, higher expectations were placed on GCs in the treatment of SSc. We did not analyse the use of GCs over time, but following further and more recent studies there is a high probability that the use of GCs will decrease in Latvia. It is more likely that, as knowledge of the role of immunosuppressive therapies in SSc develops, data will also show a positive trend towards a reduction in the use of GCs in Latvia.

Prevalence of polyneuropathies among systemic sclerosis patients and impact on health-related quality of life

We further performed a detailed evaluation of PNS involvement in SSc patients. By systematically analysing both LFN from NCS studies and SFN, from QST results in those with NCS results inconsistent with LFN. A total of 100 patients consented to undergo nerve NCS. Standard NCS were conducted using Dantec Keypoint equipment, following the diagnostic protocol for PNP, with assessment of both motor and sensory fibres. Measurements included motor nerve distal latency, amplitude, and conduction velocity, as well as sensory response amplitude and conduction velocity in at least three upper and lower limb nerves (median, ulnar, peroneal, tibial, sural). The examinations were performed at a controlled laboratory temperature (22–24 °C), ensuring a patient skin temperature of ≥ 32 °C to prevent false positive findings. NCS was performed by a certified neurologist specializing in clinical neurophysiology with at least five years of experience in the diagnosis of PNP. LFN was found in 43 % of 100 patients, 15 patients had sensory-motor demyelinating PNP, while 28 had sensory-motor axonal demyelinating PNP.

From 57 patients without LFN, 38 patients consented to undergo QST. Using a Medoc device with a thermode, heat and cold thresholds were measured in patients with SSc to detect small fibre damage that does not appear with the classic NCS method. The thermometer was placed on the dorsal surface of the foot distally (at the base of the II-III toe) and on the dorsal surface of the hand (at the base of the II–III fingers), which correspond to the distal symmetrical zones where PNP symptoms most often manifest, with additional measurements taken on

the anterior–lateral part of the lower leg to assess the function of more proximal fine fibres. The cold detection threshold and warmth detection threshold were determined to assess the condition of C and Aδ fibres, as well as the cold pain threshold and heat pain threshold to assess the sensitivity of the nociceptive system and signs of hyper-/hypoalgesia. The results were obtained using the Method of Limits technique, changing the temperature at a rate of 1 °C/s, with the patient indicating the first sensation (cold, heat, pain). The examinations were performed in a room with a temperature of 22–24 °C, with the patient in a relaxed state; each test was repeated several times, and the final result was calculated as the average value. The data obtained were compared with age- and gender-normalised values to determine sensory function deviations. 29 of 38 patients shoved changes compatible with SFN. If we exclude 19 patients, we could assume the prevalence of PNP in SSc patients as very high, affecting almost 90 % of SSc patients. Even though some subjects had possible secondary causes as risk factors of PNP, we did not find any significant differences between individuals with PNP or without, yet the second group of patients was not big enough to make strong conclusion of PNP to be developed independently of known risk factors. Additionally, we found that neuropathic pain is common amongst SSc patients (in 40.59 %), especially in patients with LFN (47.62 % with LFN, 35.59 % without LFN), and that neuropathic pain has a significant correlation with the srTNS and the severity of anxiety symptoms. Neuropathy-related symptoms, both neuropathic pain and neuropathy severity affected SSc patients' HRQoL. This study revealed a higher prevalence of PNP in SSc than was found in other studies, but the materials and methods used in those studies provide large range of results. A recent systematic review of 113 studies showed a pooled prevalence of neuropathy involvement in 27.37 % of cases, including 26 % (n = 556/2143) with SFN and 10.8 % (n = 231/2143) with LFN (AlMehmadi et al., 2021). However, the titles and abstracts were not selected according to strict criteria regarding evaluated neuropathies, including all works where PNP was reported by symptoms and clinical examination, nerve conduction studies or other detection tools. For LFN some studies performed electrophysiological examinations, while others used imaging techniques, biopsy or other methods (Campello Morer et al., 2003; Devigili et al., 2019; Dyck et al., 1997; Leichenko et al., 1994; Lori et al., 1996; Nitta et al., 1996; Tagliafico et al., 2011). Only a few studies showed similar results to our study. One study on the role of ultrasound imaging in the evaluation of peripheral nerves in SSc showed sensory disturbances revealed by clinical examination in 40 % (n = 10/25) of subjects, but the imaging modalities used revealed abnormalities in 7 of 10 patients (Devigili et al., 2019). However, a PNS examination was performed only on median and ulnar nerves, observing compression neuropathies. We believe that the high prevalence of LFN can be explained by the fact that we were working with

a relatively large study group, and all subjects were evaluated using both clinical symptoms and electrophysiological methods, where motor and sensory components were studied on several nerves of each extremity. Our study suggests that small fibre abnormalities are common in SSc. As mentioned above, in a recent systematic review of PNP in SSc, the prevalence of SFN was more than two times higher than LFN (AlMehmadi et al., 2021). In this study, in 38 patients who did not show abnormalities by NCS, only nine had normal QST results. The high prevalence of SFN may be associated with skin damage due to SSc, but there was not a significant difference between the severity of cutaneous involvement and the presence of SFN. The diagnosis of SFN can be challenging because the diagnostic criteria for SFN are not yet fully established, and the lack of standardised diagnostic criteria for SFN may have implications on our research in terms of the definition of SFN, since our study subjects were defined to have SFN only based on QST results. With the data available from this study, we cannot make strong conclusions regarding small fibre involvement in SSc.

The potential influence of comorbidities and immunosuppressive therapy on the development of PNP in patients with SSc was also assessed. In this cohort, neither comorbidities such as DM nor immunosuppressive agents, including cyclophosphamide, were associated with the occurrence of PNP.

Polyneuropathy in systemic sclerosis: exploring the causes and biomarkers

For further tests, the SSc patients were divided into two groups, patients with PNP and patients without PNP, according to the results of NCS.

As previously mentioned, historically, the classical SS specific Abs, ATA, ACA and ARA, have received the most attention, but currently, novel Abs are assessed in addition to the classical Abs, and their presence in different clinical phenotypes remains a research goal (Cavazzana et al., 2023; Yang et al., 2020). Only a few studies have evaluated the association of these classical Abs with neuropathies in SSc, and the results have varied greatly. In a 1994 study, 35 % of patients with SSc presented neurological symptoms, and 73 % of them had either ARA or ATA, but not ACA (Hietarinta et al, 1994)). On the contrary, in a 2021 systemic review, the authors mentioned that ACA are a risk factor for non-compression neuropathies in patients SSc (AlMehmadi et al., 2021). Similarly, in Brazilian study of 63 patients with SSc, seven were diagnosed with PNP, of whom 6 had ACA and 1 had ARA (Skare et al., 2011). In a Spanish study, ARA, ATA and ACA were present in patients with SSc and PNP, but the authors did not provide the statistical analysis (Iniesta Arandia et al., 2017).

Expanded SSc specific Abs panel have started to play an increasingly important role in research and clinical practice. Although there is wide spectrum of clinical phenotypes in SSc,

information regarding NS involvement is frequently missing (Clark et al, 2022). We could not find published data about expanded SSc specific Abs in patients with SSc and NS damage. The most common SSc specific Abs were anti-Ro52, ACAs and ATA. Only 3 % were positive for ARA, a lower frequency than for Abs that are not included in the SSc classification criteria: anti-Ku, anti-PM100, anti-Th/To and anti-NOR90 Abs. Interestingly, none of our patients was positive for anti-PDGFR, and only one patient was positive for anti-Fib. We did not find significant association between any of the SSc specific Abs and the presence of PNP, although it should be mentioned that anti-Ro52 presence showed protective factor signs for PNP development.

In autoimmune neuropathies, gangliosides are one of the most frequent targets of Abs (He et al., 2015). Gangliosides are nerve fibre glycoproteins that play an important role in both impulse transmission and nerve fibre regeneration. Anti-ganglioside Abs are often detected in the serum of patients with Guillain–Barré syndrome (37–78 % of the cases) (Naik et al, 2017). They have been studied in patients with systemic lupus erythematosus and neuropsychiatric manifestations: the authors detected Abs more frequently in patients with neuropsychiatric manifestations compared with the asymptomatic group (Labrador-Horrillo et al, 2012). There are very few studies on anti-ganglioside Abs in patients with SSc. In 1994, 34 patients with scleroderma, of whom 28 had PNP, were evaluated for the presence of anti-GM1 Abs. The levels were lower in scleroderma patients compared with healthy control, and there was no association with the development PNP (Zeballos et al., 1994). In our study, performed almost 30 years later, we also could not find a significant association between anti-MAG or anti-ganglioside Abs and the development of PNP in patients with SSc. Due to the lack of data on the association between PNP in SSc and NS-specific Abs we initially determined Abs only in a subset of patients with definite PNP, randomly selected. We would most likely not expect a significant change if Abs were detected in all patients with PNP, and even if they were detected at low titres, these data would only show false positives and unnecessarily confound the overall significance of the study.

In this study, no Abs were associated with risk of PNP in patients with SSc. At present, immune-mediated peripheral nerve damage in SSc remains questionable. In the treatment of PNP in patients with SSc, the role of immunosuppressive drugs remains equivocal and, according to our data, there is no reason to expect them to be efficacious. Additional research is necessary to predict PNS damage in patients with SSc so that they can be managed appropriately.

Further we investigated different serum markers as candidate biomarkers for the diagnosis and severity of PNP in SSc. In recent years, successful new candidate serum

biomarkers have been identified for ILD in SSc, including surfactant protein D (SP-D), Krebs von den Lungen 6 glycoprotein (KL-6), CCL18 and intercellular adhesion molecule 1 (ICAM-1) (Elhai et al, 2019; Jee et al, 2023). Unfortunately, researchers have not yet evaluated serum biomarkers for PNS damage in patients with SSc. Thus, we chose to evaluate the most promising biomarkers based on the connection to the PNS. Of these four serum biomarkers, NfL, GFAP, GDF15 and FGF21, three of them showed promise as candidate PNP serum biomarkers in patients with SSc in our study. NfL stand out as novel biomarker for early diabetic neuropathy (DN); there are possible similarities in vascular injury in both DN and PNP in SSc (Maalmi et al, 2023). Our findings showed significantly higher levels of NfL in SSc patients with PNP compared to those without, confirming the already established significant role of NfL as a serum biomarker for neuropathies of different aetiologies (Fundaun et al, 2022). A less-studied biomarker in PNP is GFAP, which has mostly been associated with CNS damage due to its predominant secretion from astrocytes. However, studies have demonstrated the presence of GFAP in the PNS (Fang et al., 2016; Yang et al, 2015). Researchers have reported elevated serum GFAP levels in chronic neuropathies like chronic sensory-motor axonal neuropathy and chronic inflammatory demyelinating PNP (Notturno et al, 2009). Unlike NfL, GFAP has not been widely evaluated in DN, reducing the likelihood of linking this biomarker to neuropathy caused by vascular injury. We did not find any studies of GFAP in SSc, but in our study serum GFAP was significantly elevated in patients with SSc and PNP, compared to SSc patients without PNP. GDF15 and FGF21 have less association with the NS. GDF15 is a cytokine belonging to the transforming growth factor beta superfamily. Elevated GDF15 levels are observed in inflammation, myocardial ischaemia and tumours (Wischhusen et al, 2020). In other studies serum GDF15 levels were elevated in patients with PH in SSc compared with patients with those without PH, as well as in SSc patients with ILD and more pronounced skin lesions (Gamal et al., 2017; Meadows et al, 2011; Wan et al, 2024). There is evidence of increased GDF15 secretion by Schwann cells in nerve injury, and increased GDF15 levels have been found in patients with DN, mainly with more pronounced manifestations of metabolic syndrome (Jennings et al., 2022; Mensching et al, 2012; Weng et al, 2022). We found significantly elevated serum GDF15 levels in the SSc patients with PNP compared with those without PNP. Of note, there have been no other studies that evaluated this serum biomarker in patients with SSc and neuropathies. Only FGF21 showed no significant change between the SSc with PNP and the SSc without PNP groups. This pleiotropic hormone – considered to be a major regulator of energy homeostasis – is mainly synthesised in the liver, pancreas and adipose tissue (Catalán et al., 2018; Cho et al., 2022). Recently, researchers have shown that FGF21 has regenerative capability in the PNS by suppressing oxidative stress, and the FGF21

levels were elevated in patients with DN after aerobic training (Molnár et al., 2022; Lu et al., 2019). While there have been no studies on FGF21 levels in patients with SSc, we found that FGF21 levels did not change significantly in patients with SSc and PNP, indicating that FGF21 has less of a connection to the NS compared with other biomarkers.

By NCS we found that the axonal demyelinating form of PNP was the most common in our patients with SSc. The absence of significant correlations between Abs and PNP has led us to consider alternative pathogenic mechanisms. Comparisons between the patients with and without PNP showed several intriguing differences: the patients with PNP were generally older, with an average age of 67 years compared with 57 years, and it was more prevalent in men (66 % compared with 36 %). These observations indicate that ageing, metabolic factors and ischaemic mechanisms may contribute significantly to the emergence of axon neuropathies, reflecting the patterns observed in cases of idiopathic PNP. In the literature, researchers have noted a higher prevalence of idiopathic PNP in people aged > 60 years. Similar results have been reported in studies focusing on chronic axon idiopathic PNP in people aged > 60 years, with a 3:2 male-to-female ratio (Samuelsson et al., 2020; Zis et al., 2016). As the name suggests, the condition is idiopathic, and metabolic factors are most strongly considered to be involved in the aetiology, but microvasculopathy identified in biopsies shows a different pattern than in DN (Samuelsson et al., 2018; Zis et al., 2016). These coincidences lead us to suspect sequential development of PNP in patients with SSc over time, associated with ageing and a logical progression of the disease with more pronounced vasculopathy and metabolic factor-associated effects. Our regression analysis confirmed this view: it showed that age is a significant predictor of PNP development. Looking into the serum biomarkers we found to be associated with PNP in SSc, NfL and GFAP had already been shown to be associated with axonal injury, strengthening our above hypothesis of the development of PNP in SSc (Gafson et al., 2020; Notturno et al., 2009). On the other hand, GDF15 and FGF21 have mostly been associated with mitochondrial stress and subsequent metabolic changes (Li et al., 2022; Patel et al., 2022). Interestingly, they behaved differently in our study. While the FGF21 levels were slightly higher in patients with SSc and PNP, the difference was not significant. The GDF15 levels were significantly elevated in patients with SSc and PNP, similarly to patients with DN, were metabolic damage plays an important role (Weng et al., 2022). We believe additional studies that detect muscle damage and loss are needed to further investigate the role of mitochondrial damage and metabolic markers in patients with SSc. Our results suggest that the use of serum biomarkers in clinical environments may facilitate early identification of PNS damage in patients with SSc. By dynamically monitoring biomarkers such as the NfL, GFAP and GDF15, it could be possible to detect deterioration of nerve function without further electrophysiological

testing. However, research focusing on hereditary neuropathy has challenged the effectiveness of neurofilament fluctuations as indicators of disease progression, suggesting that these markers may not be suitable for tracking slow-moving diseases due to their lack of specificity and their tendency to reflect general rather than specific nerve damage (Setlere et al., 2023).

Serum metabolomic profiling reveals differences between systemic sclerosis patients with polyneuropathy

The above-mentioned reasons encouraged us to further investigate the pathogenesis of PNP in patients with SSc, which led to the metabolome analysis. The metabolome, a collection of small compound metabolites in an organism, offers insights into the biochemical changes and potential biomarkers associated with diseases like SSc (Zhang et al., 2015). Metabolites can serve as biomarkers for diagnosis, prognosis, and monitoring of disease progression or response to treatment (Qiu et al., 2023). To our knowledge, this is the first metabolome analysis in SSc patients with an emphasis on the presence of PNP. Initially, differences in metabolite regulation were sought between SSc and HC groups (Bengtsson et al, 2016; Bögl et al, 2022; Guo et al., 2023; Jud et al, 2023; Murgia et al., 2018; Morales-González et al., 2023; Ottria, et al, 2020; Smolenska et al., 2020). SSc is a heterogeneous disease with different manifestations and different risks of complications (Nagaraja et al., 2020). Despite this heterogenicity, previous studies have detected several uniform changes in metabolome regulation in SSc patients (Bengtsson et al, 2016; Bögl et al, 2022; Guo et al., 2023; Jud et al, 2023; Murgia et al., 2018; Morales-González et al., 2023; Ottria, et al, 2020; Smolenska et al., 2020). Our study also found several significant differences between SSc patients and HC. We found the concentration of aspartic acid or aspartate to be significantly reduced in SSc patients compared to HC. An important capability of aspartate is to promote macrophage polarisation (Wang et al., 2021). In SSc, at the peak of the late immune response, endothelin-1 induces M2 polarisation, thereby potentiating profibrotic activity (Funes et al., 2018; Soldano et al., 2016). These results suggest that in SSc, tissue damage is not effectively repaired due to the increased and sustained release of cytokines and growth factors from M2 macrophage cells (Christmann et al., 2010). The significant changes in aspartic acid in patients with SSc detected in our study and in previously published studies may indicate changes in macrophage activation, with possibly more pronounced profibrotic activation, as evidenced by correlation with the severity of skin involvement, thereby signalling macrophage dysregulation (Murgia et al., 2018). Another finding in our study was the reduced citrulline concentration in SSc patient samples. Citrulline is an effective substitute for restoring nitric oxide (NO) production in situations of limited arginine availability (Kaore et al., 2013). NO produced by endothelial cells relaxes vascular smooth muscles, resulting in vasodilation and maintaining patency of small

blood vessels and blood flow through microvasculature (Al Jasmi et al., 2020). In SSc, the microvascular bed is the target of an immune–inflammatory injury that leads to dysregulation of vascular tone control and results in progressive disorganisation of the vascular architecture (Matucci Cerinic et al., 2002). Even though the data from our study may differ from previously published data, the elevated concentration of citrulline may still be associated with developing skin fibrosis (Bögl et al, 2022; Smolenska et al., 2020). At the same time, the reduced concentration observed in our study represents an alteration in NO synthesis that could lead to more severe vasculopathy and serve as a marker of vasculopathy in the future. Carnitine was found to be yet another metabolite with reduced concentration in SSc patients. This could be explained by changes in muscle mass in patients with SSc. Not only the skin and subcutaneous tissue are affected, but the normal muscle structure, both the better-known smooth muscle and skeletal muscle, is altered, with an overall loss of muscle mass (Bratoiu et al., 2022; Sari et al., 2021). Further studies could confirm a correlation between muscle mass and carnitine in patients with SSc. Valine concentration was also reduced in SSc patients, and as important metabolite for cellular mitochondrial function and protection against oxidative stress, this could signal mitochondrial dysfunction in SSc (Sharma et al., 2024). The last metabolite with reduced concentration in SSc patients was glutamic acid. It is the most abundant CNS transmitter. Recent data indicate that inflammatory mediators might regulate extracellular glutamic acid concentrations under physiological and pathological conditions (Haroon et al., 2017). Other studies have also found reduced concentrations of glutamic acid in patients with SSc but higher levels in dcSSc (Guo et al., 2023; Murgia et al., 2018). The consensus results of many studies suggest that glutamic acid reduced concentration in SSc patients is not associated with a specific disease complication such as vasculopathy or fibrosis but is a common finding in all SSc patients. It is plausible that these unambiguous changes suggest a role for glutamic acid in the immunoregulation of SSc and that reduced concentration of glutamic acid may be one of the markers of persistent damage due to autoimmunity. Glutamine was the only metabolite with an elevated concentration in SSc patients compared to controls. Interestingly, the uptake of glutamine, but not glutamic acid, is enhanced during T-cell activation (Ardawi et al., 1988). Studying SSc fibroblasts, all of them showed an increase in glutaminase expression, suggesting that altered glutamine metabolism may be a ubiquitous trait in SSc (Henderson et al., 2020). Like our study, reduced concentrations of glutamic acid and elevated concentrations of glutamine have been reported before (Jud et al, 2023; Murgia et al., 2018; Smolenska et al., 2020). It is already speculated that the elevated glutamine concentration can augment collagen synthesis with subsequent fibrosis of the skin and internal organs (Kay et al., 2021; Ung et al., 2022). In the future, with more conclusive data, glutamine could be used as a marker of fibrosis

in patients with SSc, with particular consideration of the role of antifibrotics in each patient. However, evaluating glutamine in conjunction with glutamate as a common marker for both T cell function and profibrotic changes seems more meaningful now. In our study, the potential biomarkers identified by fold changes (FC) analysis were aspartic acid, glutamic acid, glutamine, and carnitine. Aspartate has been found to significantly change in SSc patients compared to HC in other studies as well (Murgia et al., 2018). However, Bengtsson et al. found a concentration of aspartic acid to be significantly elevated in SSc patients compared to HC (Bengtsson et al, 2016). This worrying difference could be explained by the small number of SSc patients enrolled (19 subjects) and the significant difference in prior treatment with immunosuppressive agents between studies, as in the Bengtsson et al. study, patients had not been previously treated with AZA, CYC, MTX or MMF (Bengtsson et al, 2016). We found no similar data on the evidence for glutamic acid, glutamine, and carnitine as a diagnostic biomarker in SSc. We report high predictive scores for glutamine/valine and creatinine/glutamine ratios. We could not find studies with similar data where two metabolite ratios were used to build disease prediction models. Glutamine was the only metabolite with a significantly elevated concentration in patients with SSc compared to HC, and by verifying similar data in other studies, we can be more confident about the ability of these metabolite ratios to perform as biomarkers in SSc (Murgia et al., 2018; Smolenska et al., 2020). Glutamine/valine ratio showed high predictive score in SSc, but this finding is complicated by data from other studies on valine with elevated concentration in patients with SSc, especially in patients with dcSSc and SSc associated ILD (Murgia et al., 2018; Smolenska et al., 2020). SSc patients in our cohort did not have severe skin damage, as evidenced by mRSS in both subgroups, and a low presence of ILD. Interestingly, creatinine/glutamine ratio showed high predictive score in SSc in our study. We have already discussed the compelling data on glutamine, but creatinine showed no significant change in SSc patients in our study or in diligently searched other studies, except for reduced creatinine in SSc associated PH compared to SSc patients without PH (Deidda et al., 2017).

The findings described above were equivalent to previous metabolome studies in patients with SSc. Our study isolated a previously unstudied group of SSc patients with PNP. Differences in some metabolites were observed between SSc patients with and without PNP. In contrast to SSc to HC discrimination, no metabolites had a high FC (> 1.5) or p-value (< 0.1). There were minor changes with FC > 1.2. A possible similarity in the development of PNP in patients with SSc lies in the development of DN. Therefore, we decided to investigate previous metabolome studies in patients with DN, specifically comparing data on metabolites altered in our study in patients with PNP. Kynureine level was elevated in SSc patients with PNP

compared to SSc patients without PNP and HC. The kynurenine pathway, which accounts for the catabolism of approximately 99 % of ingested tryptophan not used for protein synthesis, has links with neurodegenerative diseases, tumor proliferation, inflammation, and depression (Pathak et al., 2024). Possibly due to these findings, the kynurenine pathway is one of the most studied in SSc. ARA positive patients were found to have higher kynurenine levels compared to ATA or ACA positive patients, as well as SSc patients with dcSSc (Campochiaro et al., 2019). Kynurenine levels were higher in PH patients associated with SSc, compared to idiopathic PH or other connective tissue disease-related PH, and may affect the risk of developing PH (Simpson et al., 2023; Wallace et al., 2023). Studies showed that the disturbance of the kynurenine pathway could increase the oxidative compounds, which damage the PNS and CNS through the broken blood-nerve or blood-brain barrier, respectively (Dantzer et al., 2008). Compared to the effects of the kynurenine pathway in various CNS diseases, data on the role of kynurenine in the development of PNS damage are currently very limited. The concentration of kynurenine was found to be elevated in diabetes mellitus (DM) patients with severe PNP and neuropathic pain (Shao et al., 2022; Staats Pires et al., 2020). The possible elevated concentration of kynurenine also in SSc patients with PNP suggests a unifying dysregulation with PH, which would be easier to explain due to a common vasculopathy role of both features that are reinforced by kynurenine elevated concentration in patients with DN (Shao et al., 2022; Staats Pires et al., 2020). Asparagine concentration was also elevated in patients with PNP, compared to SSc patients without PNP, but not to HC. Asparagine is crucial in proliferating cells when cells are starved for nutrients, especially glutamine. Glutamine regulates angiogenesis through multiple mechanisms, and the proliferation of endothelial cells is impaired when exogenous glutamine is unavailable. Instead, endothelial cells rely on asparagine for proliferation, and asparagine can partially rescue these cell defects under low glutamine conditions (Huang et al., 2017; Pavlova et al., 2018). Unlike other metabolites, asparagine has not been described to have marked changes in SSc and various manifestations of the disease. However, a negative correlation with mRSS in SSc patients was found (Jud et al, 2023). In a study with type 2 DM patients, asparagine regulation differentiated between patients with and without DN (Shao et al., 2022). It could be inferred that in SSc patients with PNP, elevated concentration of asparagine signals glutamine deficiency, with changes in endothelial function and regulation of angiogenesis, which could predispose to vasculopathy and ischaemic damage as a cornerstone in the development of PNP. However, the elevated concentration of glutamine observed in patients with SSc in our study strongly differentiates patients with and without PNP, reinforcing the above hypothesis. Another metabolite with elevated concentration in SSc patients with PNP, compared to SSc without PNP

subgroup and HC, was alanine. Changes in the alanine pathway have been shown to play a role in the development of DN. In a study with type 2 DM patients, the serum β-alanine and ratio of β-alanine / L-aspartic acid in DN patients were significantly increased (Shao et al., 2022). When present in high levels, β-alanine is a neurotoxin and damages the brain and nerve tissue (Jong et al., 2010; Schaffer et al., 2018; Shetewy et al., 2016). A possible elevated alanine concentration, like that seen in patients with type 2 DM, indicates neurotoxic functions of alanine, which is a partial scatterer of PNP in SSc or a cause of the progression of the disease. Aspartic acid was the only metabolite with reduced concentration in SSc patients with PNP compared to those without PNP. The role of aspartate in macrophage polarisation, already discussed above, could also indicate polarisation dysregulation in SSc patients with PNP. The role of macrophage polarisation in developing PNP in SSc has not been previously investigated. Still, more data are available on the role of macrophages in developing other autoimmune neuropathies (Yang et al., 2023). PNS resident macrophages are among the least studied subpopulations; however, their differences from other macrophages have been identified (Msheik et al., 2022). Our study identically detects changes in the ratio of alanine/aspartic acid found in DN, further addressing the issue of the underlying mechanics of PNP in patients with SSc (Shao et al., 2022).

Limitations

We are aware of some limitations of the study:

- The sample size of the group: Although we enrolled 103 out of 159 SSc patients who were examined at Latvia's university hospitals in a time frame of five years, we believe that more statistical significance would be found with a larger study group. The small number of SSc patients is explained by the rarity of the disease and the small size of the general population of Latvia. Due to the country specificity, most patients with suspected SSc are referred to the two university hospitals mentioned above, but we cannot exclude a number of patients who were nevertheless not included in this study. Another reason why a proportion of patients may not have been included in our study is the failure to obtain the necessary points to meet the criteria at the time, due to lack of capillaroscopy and impossibility of ARA detection.
- SSc subtypes: We were only able to include skin subtype information for some of the patients. Skin involvement in SSc changes dramatically over time. Usually, mRSS increases rapidly in the first 3–5 years, but then tends to decrease even without specific treatment. We only included data on subtypes from the documentation when

they were mentioned but believe that subtyping patients after several decades of disease would not be correct.

- Small fibre function assessed by QST only: To clarify the involvement of SFN, a skin punch biopsy could be performed to measure epidermal nerve fibre density, since the results of the biopsy can provide more objective diagnostic data for defining SFN.
- HC group included only in metabolome analysis: HC group might have provided more evidence for our findings linking the development of PNP in SSc patients also to natural ageing.
- Comparison of metabolome analysis results between our data in patients with PNP and other publications: We provided additional data on differences in the metabolome in patients with SSc from HC. We compared these data with previously published ones, strengthening the evidence for the role of metabolome alterations in SSc. By performing a study in the PNP group of SSc patients, the data obtained cannot be compared with similar publications, so we chose to investigate the regulation of the altered metabolites found in DN patients, possibly overlooking similarities in the development of PNP in patients with SSc that have not yet been published.

Conclusions

1 SSc is less common in Latvia than in other countries and regions. Due to its location, the data from Latvia are consistent with a north-south gradient in Europe. With its homogeneous racial pattern, Latvia is probably an even more pronounced model for the developing of SSc in northern countries.

2 Most of patients presented with the first non- RP SSc symptom in the fifth decade of life and most common SSc cutaneous type was limited, followed by diffuse, and the least common was sine-scleroderma. Males showed a trend towards a more severe disease course, with more frequent development of ILD and PH.

3 PNP is underestimated in SSc, as we demonstrated an unexpectedly high prevalence of polyneuropathy in Latvian SSc patients, showing that the PNS is affected in almost all patients with SFN to be as common as LFN. The severity of neuropathy symptoms and neuropathic pain were both associated with a higher HAQ-DI, indicating worse HRQoL. SSc patients with PNP (LFN) tended to be older, with longer SSc duration, and male.

4 There was no association between SSc-specific or other inflammatory neuropathy-associated Abs and the development of PNP in patients with SSc. It is likely that the development of PNP in patients with SSc is not solely due to an autoimmune process.

5 Several serum biomarkers – NfL, GFAP and GDF15 – could be used as relevant diagnostic biomarkers for PNP in patients with SSc. Future studies are warranted to validate the diagnostic efficacy of these biomarkers and to unravel the complex interplay of factors leading to PNP in patients with SSc.

6 Metabolomic profiling highlighted alterations consistent with macrophage polarisation changes and mitochondrial dysfunction linked to fibrotic processes and oxidative stress. Notably, elevated kynurenine and alanine levels were distinct to SSc patients with PNP, suggesting a unique metabolic signature for this subgroup. These findings support the hypothesis that direct neurotoxicity and mitochondrial oxidative stress contribute to PNP development in SSc, potentially influenced by aging and disease progression.

Proposals

Practical recommendations for patient care:

- Include PNS screening in the daily care of SSc patients.
- Each patient with SSc should undergo a neuropathy assessment (clinical questionnaire, NCS/QST if available) to enable early identification of PNP and prevent a significant deterioration in quality of life.
- Integrate multidisciplinary care for patients with SSc and neuropathy.
- In addition to rheumatologist supervision, the involvement of a neurologist is necessary, as well as access to rehabilitation and psychological support. This approach reduces the impact of neuropathic pain and anxiety, improving functional ability and quality of life.
- Consider the use of metabolite profiles and NfL, GFAP, GDF15 for a personalised approach and research.
- Including these parameters in patient group stratification and risk assessment could help personalise therapy and improve research design.

Further research on the PNS in patients with SSc is warranted, with particular emphasis on the mechanisms underlying SFN and autonomic nervous system dysfunction.

Publications and reports on topics of Doctoral Thesis

Publications:

1. **Ivanova, K.**, Ribakova, O., Mihailova., A, Možeitoviča, E., Kadiša, A., Zepa, J., Ķēniņa, V., Kurjāne, N., Buliņa, I. 2024. Prevalence and gender - specific analysis of a systemic sclerosis cohort in Latvia // *Orphanet Journal of Rare Diseases*, 19(1):361, 1–9. DOI: 10.1186/s13023-024-03355-y
2. **Ivanova, K.**, Zolovs, M., Blennow, K., Zetterberg, H., Kurjāne, N., Ķēniņa, V. 2024. Polyneuropathy in systemic sclerosis: exploring the causes and biomarkers // *Frontiers in Medicine*, 11:1412706, 1–9. DOI: 10.3389/fmed.2024.1412706
3. **Ivanova, K.**, Žukovs, D., Možeitoviča, E., Rots, D., Kurjāne, N., Ķēniņa, V. 2023. Prevalence of polyneuropathies among systemic sclerosis patients and impact on health-related quality of life // *Polish Journal of Neurology and Neurosurgery (Neurologia i Neurochirurgia Polska),* 57(2), 206–211. DOI: 10.5603/PJNNS.a2023.0018
4. **Ivanova, K.**, Schiemer, T., Vaska, A., Kurjāne, N., Kenina, V., Klavins, K. 2025. Serum Metabolomic Profiling Reveals Differences Between Systemic Sclerosis Patients with Polyneuropathy // *International Journal of Molecular Sciences*, 26(15):7133, 1–15. DOI: 10.3390/ijms26157133

Reports and theses at international congresses and conferences:

1. **Ivanova, K.,** Schiemer, T., Kļaviņš, K., Kurjāne, N., Ķēniņa, V. 2025. Serum metabolomic profiling in systemic sclerosis uncovers potential biomarkers. *RSU Research week 2025: Knowledge for Use in Practice*, Riga, Latvia. (Theses and poster presentation).
2. **Ivanova, K.**, Budreviča, O., Žukovs, D., Možeitoviča, E., Ķēniņa, V., Kurjāne, N. 2022. Polyneuropathy impact on disability in systemic sclerosis patients. *European Alliance of Associations for Rheumatology (EULAR) Annual Meeting,* Copenhagen, Denmark. (Theses and poster presentation).
3. **Ivanova, K.**, Rubins, P., Bulina, I., Zepa, J., Miķēna, S., Andersone, D., Kurjane, N. 2021. Analysis of the hospitalized patients with systemic sclerosis in Pauls Stradiņš Clinical University Hospital Rheumatology department during 2017–2020. *International Scientific Conference on Medicine, LU,* Riga, Latvia. (Theses and oral presentation).
4. Budreviča, O., Možeitoviča, E., Žukovs, D., Ķēniņa, V., Kurjāne, N, **Ivanova, K.** 2022. Predictor Factors of Functional Status in Patients with Systemic Sclerosis. *International Scientific Conference on Medicine, LU,* Riga, Latvia. (Theses and oral presentation).
5. Rubins, P., Miķēna, S., Bulina, I., Zepa, J., Andersone, D., **Ivanova, K**. 2022. Role of Rheumatoid Factor in Patients with Systemic Sclerosis and Extra-Articular Manifestations. *International Scientific Conference on Medicine, LU,* Riga, Latvia. (Theses and oral presentation).
6. **Ivanova, K.,** Rubins, P., Buliņa, I., Zepa, J., Mikena, S., Andersone, D., Kurjāne, N. 2021. Hypocomplementemia and clinical manifestations in patients with systemic sclerosis. *RSU Research week 2021: Knowledge for Use in Practice*, Riga, Latvia. (Theses and poster presentation).
7. Žukovs, D., Možeitoviča, E., Scientific research supervisor: **Ivanova, K.** 2022. Prevalence of large and small fiber neuropathies among systemic sclerosis patients, and their impact on patients' disability and functional status. *RSU International Student Conference*, Riga, Latvia (Theses and oral presentation).
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Acknowledgments

First and foremost, I would like to express my deepest gratitude to my research supervisors, professor Nataļja Kurjāne and associate professor Viktorija Ķēniņa. Their encouragement led me to embark on my PhD journey. They provided invaluable support and guidance in selecting the research topic, recruiting patients, and carrying out analyses and neurological examinations. Their continuous advice and assistance were instrumental in every next step, including enlisting the help of colleagues. Together, we finalised and submitted publications to carefully chosen journals.

I would like to express my deepest gratitude to the heads and senior physicians of the rheumatology centres – Dr. Inita Buliņa, Dr. med. Anda Kadiša, Dr. med. Anna Mihailova, and Dr. med. Jūlija Zepa – whose exemplary professionalism inspired me, who generously shared their expertise regarding patients treated at the centre, and who encouraged those patients to participate in the study.

I am sincerely grateful to all the co-authors for their collaboration, time, support, and advice throughout the creation of the publications.

I also extend my heartfelt thanks to my colleagues – the team of rheumatologists at Pauls Stradiņš Clinical University Hospital – especially to the head, Dr. Inita Buliņa, and the chief physician, dr. med. Jūlija Zepa, for their unwavering support.

I am deeply appreciative of the Rīga Stradiņš University team for their support, including financial assistance in the development of this scientific work, and I am particularly thankful to dr. med. Gailīte for the excellent collaboration.

Finally, I would like to express my profound gratitude to all Latvian rheumatologists, whose lifelong dedication to the care of patients with rheumatic diseases made this research possible.

Annexes

Ivanova *et al. Orphanet Journal of Rare Diseases* (2024) 19:361
https://doi.org/10.1186/s13023-024-03355-y

Orphanet Journal of Rare Diseases

RESEARCH Open Access

Prevalence and gender - specific analysis of a systemic sclerosis cohort in Latvia

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Abstract

Background Systemic sclerosis (SSc) is considered by many to be one of the most severe autoimmune rheumatic diseases with lower prevalence observed in Northern Europe. No previous studies on the prevalence of SSc in Latvia have been conducted and the aim was to study the demographic and clinical data of patients with SSc in northeastern Europe country.

Methods This study was conducted in two main Latvian hospitals for adults and includes patients with SSc who were consulted between 2016 and 2021.

Results During the study period, 159 patients with SSc were consulted. The point prevalence on 1 January 2021 was 84.0 per million. Female to male ratio was 4.67:1, and highest gender ratio was observed in the age group 70–79-year (6.75:1). Antinuclear antibodies were present in 82.58% of patients, without gender difference. Centromere pattern was more frequently observed in females (40.19% vs. 19.04%), in contrast to speckled pattern (50.98% vs. 57.14%). At disease onset females tended to be younger (46.51 ± 13.52) than males (50.5 ± 16.64). Males had more diffuse cutaneous subtype, interstitial lung disease, pulmonary hypertension and esophageal dysmotility. More than half of patients received treatment with glucocorticoids at any point of the disease (68.31%), without gender difference.

Conclusions Systemic sclerosis is less common in Latvia than in other countries and regions. Due to its location, the data from Latvia are consistent with a north-south gradient in Europe. Gender ratio differences persisted in older age groups as well. Antinuclear antibodies presence did not differ between genders, but in female's centromere pattern was much more likely to be present. Males had more severe disease course, but in both genders more than half of patients received treatment with GCs at any point of the disease.

Keywords Systemic sclerosis, Demography, Latvia, Prevalence of systemic sclerosis, Rhaynauds's phenomen, Modified Rodnan skin score, Antinuclear antibodies

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Introduction

The term 'scleroderma' has been used since the mid-19th century but the first records date back to 1753, when Carlo Curzio described a 17-year-old girl with marked hardening of the skin all over her body [1]. Since 1980, scleroderma has been defined as a spectrum of diseases that consist of localized scleroderma and systemic sclerosis (SSc) [2]. Of the two types, localized scleroderma is more frequent with an incidence of 2.7 cases per 100,000, is not usually associated with severe systemic symptoms or Raynaud's phenomenon and often is self-limited with a good prognosis [3].

On the other hand, SSc is considered by many to be one of the most severe autoimmune rheumatic diseases [4]. To verify the truth of this statement, accurate epidemiological data are needed. However, incidence and prevalence vary greatly between different studies, explained mainly by random sampling errors and differences between case definitions and capture methods. Also, SSc is a chronic disease, so its prevalence is influenced by incidence and mortality rates [5].

The average prevalence of SSc worldwide is estimated as 1 in 6500 adults. Lower prevalence (below 150 cases per million) and incidence (below 10 cases per million per year) are observed in Northern Europe and Japan, whereas higher incidence rates are observed in Southern Europe, North America and Australia [6–8].

As with other rheumatic diseases, the incidence of SSc varies according to gender. It is observed to be higher in females (female: male ratio of 3:1) [9], with a higher gender ratio for younger patients (7:1) but lower after the age of 50 years (2:1) [10]. The estimated average age of onset is 50 years. However, after the age of 75 years, the development of the disease is rarely seen [11, 12].

Gender differences explored in systemic connective tissue diseases may play an important role in early diagnosis and more accurate prognosis. There is already established higher premature death risk in males with SSc, and more severe expression of the disease, comparing with females with SSc [13].

No previous studies on the prevalence of SSc in Latvia have been conducted. Latvia is a country in northeastern Europe with a small population, a homogeneous race and two predominant ethnicities. Considering the above-mentioned national population characteristics, by studying the demographic and clinical data of SSc patients we could obtain previously unexplored data that will provide additional information on the characteristics of SSc in northeastern Europe.

Materials and methods

Subjects

This study was conducted in two leading Latvian hospitals, which are the only university hospitals in Latvia for adults.

Patients diagnosed with SSc who met the ACR/EULAR 2013 classification criteria and were consulted by rheumatologists between January 2016 and December 2021 were included [14].

For patient selection we used hospitals databases, where patients with diagnostic codes M34.0–M34.9 were selected according to the 10th revised version of the International Classification of Diseases (ICD-10), which has been used in all Latvian hospitals. Patients with connective tissue diseases other than SSc and patients with localized scleroderma were excluded. The study was approved by the Riga Stradins University medical ethics committee (Institutional Review Board reference no: 22−2/481/2021) and all participants provided written informed consent.

Methods

To assess the presence and pattern of antinuclear antibodies (ANA), previously detected immunological tests were analysed. Analyses were carried out in two laboratories across both clinics. However, ANA were detected using Hep-2 cells in one laboratory at Paul Stradins Clinical University Hospital for all patients in this study.

Patients who agreed to participate in the study were evaluated by one rheumatologist and surveyed and clinically assessed according to the European Scleroderma Trials and Research (EUSTAR)-accepted domains. The domains created by EUSTAR in 2015 include the collection of demographic data, patient complaints, the evaluation of skin conditions according to the modified Rodnan skin score (mRSS) [15]. Interstitial lung disease (ILD) and pulmonary hypertension (PH) were determined after previous investigations including lung computed tomography (CT), transthoracic echocardiography (ECHO), and right heart catheterisation (RHC). Esophageal dysmotility was assessed by patient complaints and previous upper gastrointestinal series.

The age at disease onset was defined as the time of onset of the first non- Raynaud's SSc symptom.

The classification of patients according to subtypes of SSc (diffuse, limited, sine-scleroderma) was not determined during this study, but took into account information provided in previous database.

Statistical analysis

Statistical analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Data normality was assessed using histograms and the Kolmogorov-Smirnov test. For comparison between the groups, the

Kruskal-Wallis H test, Spearman's rank-order correlation and Fisher's exact tests were used; P values of <0.05 were considered to be significant.

Results

Prevalence

Between January 2016 and December 2021, 159 patients with SSc were consulted in Latvia's university hospitals. Of the 159 patients, the majority were females (82%) and only 18% were males. The mean patient age was 62.53 ± 12.11 years, with females slightly older (63.12 ± 11.54 years) than the males (59.75 ± 14.37 years).

On 1 January 2021 the population of Latvia was 1,893,223 and the point prevalence was 84.0 (95% CI=71.9–98.1) per million (Table 1). The prevalence ratio was higher for females −128.7 (95% CI=108.5–152.7) than for males −32.0 (95% CI=22.1–46.2). When adjusted to the European standard population the total prevalence was 62.8 (95% CI=57.8–67.7) and when adjusted to the WHO world standard population it was 49.9 (95% CI=45.9–53.8).

The highest prevalence was found in the 60–69 age group (Fig. 1). In all groups, the rates for females were higher than for males. The difference was statistically significant in all age groups where patients were present.

Antibody characteristics

The presence of ANA was evaluated and found in 82.58% of 155 patients (Table 2). The pattern was checked in 122 patients. Most patients had either speckled pattern (52.45%) or centromere pattern (36.88%). In speckled pattern group of 64 patients, 39 had anti-topoisomerase I. A few patients had homogeneous pattern (6.56%) or nucleolar pattern (5.74%). ANA were found almost equally in females (82.81%) and in males (81.41%), but a difference between genders was observed in ANA patterns. Centromere pattern was more frequently observed pattern in females than in males (40.19% vs. 19.04%), while speckled pattern was the most frequently observed pattern in both genders almost equally with slight male predominance (50.98% vs. 57.14%).

Clinical characteristics.

Of the 159 patients selected, 103 agreed to participate in this study (Fig. 2), of whom 85 were females and 18 were males. All included patients were Caucasians.

Disease duration (from the first non- Raynaud's symptom) ranges from 1 to 41 years. The mean age at disease onset was 47.21 (±14.10) years and the females tended to be younger (46.51±13.52) than the males (50.5±16.64), (Table 3).

The majority (84.46%) of patients had Raynaud's phenomenon. SSc types were only available for 30 patients, most common type was limited (56.67%), followed by diffuse (33.33%), and the least common was sine-scleroderma (10%). Sine-scleroderma was not observed in male patients, but the diffuse type was as common as the limited type.

The mRSS was evaluated in all 103 patients, with a mean score of 10.63, without difference between genders (10.67 in females and 10.36 in males).

99 patients had a CT scan available in the database. Of these, ILD was described in 36 patients (36,36%), slightly

Table 1 Age- and gender-specific prevalence rates (per million) of systemic sclerosis in Latvia, 1 January 2021

	Mean annual population			Number of cases			Prevalence rate (95% CI)			[a]*P* value
Age group (years)	Males	Females	Total	Males	Females	Total	Males	Females	Total	
20–29	98,147	91,079	189,226	1	2	3	10.2 (1.8–57.7)	22.0 (6.0-80.1)	15.9 (5.4–46.6)	0.038
30–39	138,148	130,603	268,751	3	5	8	21.7 (7.4–63.9)	38.3 (16.4–89.6)	29.8 (15.1–58.7)	0.032
40–49	124,588	128,280	252,868	2	5	7	16.1 (4.4–58.5)	39.0 (16.6–91.2)	27.7 (13.4–57.1)	0.002
50–59	122,174	139,441	261,615	5	30	35	40.9 (17.5–95.8)	215.1 (150.7-307.1)	133.8 (96.2–186.0)	<0.001
60–69	103,142	141,844	244,986	11	54	65	106.6 (59.6–191.0)	380.7 (291.8-496.6)	265.3 (208.2-338.1)	<0.001
70–79	57,721	111,032	168,753	4	27	31	69.3 (26.9-178.2)	243.2 (167.1-353.8)	183.7 (129.4-260.7)	<0.001
80–89	25,710	72,230	97,940	2	8	10	77.8 (21.3-283.6)	110.8 (56.1-218.6)	102.1 (55.5–188.0)	0.016
Total	875,225	1,017,998	1,893,223	28	131	159	32.0 (22.1–46.2)	128.7 (108.5-152.7)	84.0 (71.9–98.1)	<0.001
Adjusted to European standard population							26.7 (21.6–31.7)	90.5 (82.6–98.4)	62.8 (57.8–67.7)	<0.001
Adjusted to WHO world standard population							21.8 (17.7–26.0)	71.7 (65.4–77.9)	49.9 (45.9–53.8)	<0.001
	Males	Females	Total	Males	Females	Total	Males	Females	Total	

aP value for statistical differences between the rates for males and females

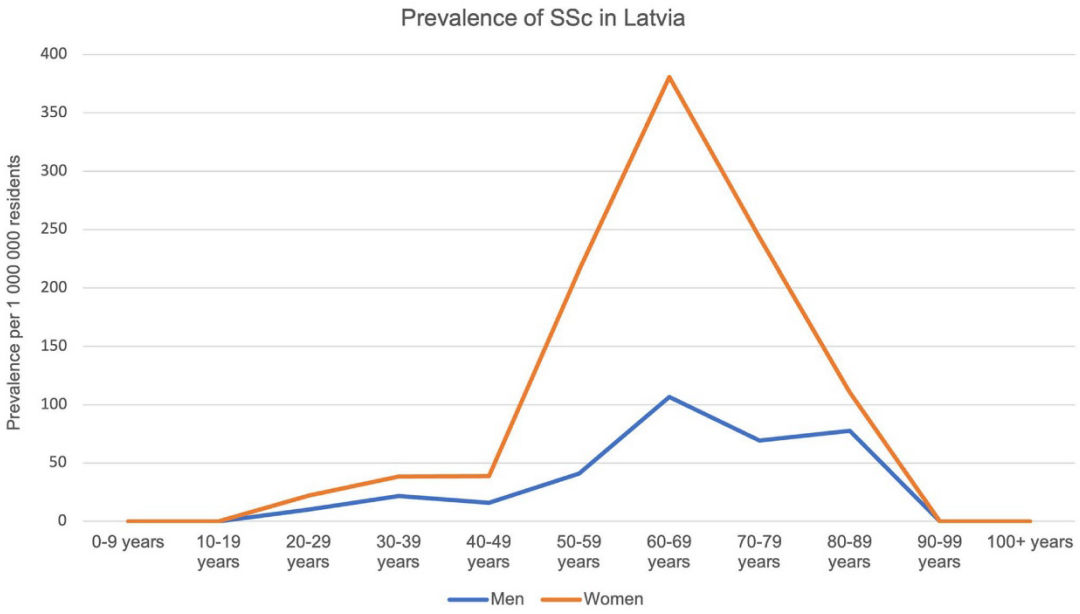

Fig. 1

Table 2 Gender-specific antibody characteristics in patients with systemic sclerosis

		Males	Females	Total
Antinuclear antibodies	ANA positive (n)	22	106	128
	ANA pattern present (n)	21	102	123
	Centromere pattern (n (%))	4 (19.04%)	41 (40.19%)	45 (36.88%)
	Speckled pattern (n (%))	12 (57.14%)	52 (50.98%)	64 (52.03%)
	Anti-topoisomerase I] (n (%)	7 (33.33%)	32 (31.37%)	25 (20.32%)
	Homogeneous pattern (n (%))	2 (9.52%)	6 (5.88%)	8 (6,50%)
	Nucleolar pattern (n (%))	3 (14.28%)	4 (3.92%)	7 (5.69%)

more often in males (35.80% in females and 38.89% in males). ECHO data was available for 71 patients, where PH was suspected and verified by RHC in 19 patients (26.76%), also more often in males (25.42% in females and 30.77% in males). Esophageal dysmotility was evaluated in 57 patients, and present in 23 patients (40.35%), less in females (39.13%) than males (45.45%).

More than half of the patients received treatment with glucocorticoids (GCs) at any point of the disease (66.99%). Methotrexate (MTX) was the next most frequently used immunosuppressant (56.31%), followed by mycophenolate mofetil (MMF) (40.77%). Almost a fifth of patients were treated with cyclophosphamide (CYC) (18.44%). One patient received autologous hematopoietic stem cell transplantation (aHSCT). While treatment with GCs was observed equally between genders, females were more often treated with MTX (60.88% vs. 38.88%) and males with MMF (38.82% vs. 50.00%). Out of 10 patients with diffuse type, 90% received GC therapy, less frequently with limited type (70.59%) and sine-scleroderma (66.67%). Of patients with evaluated SSc type and available lung CT, 8 of 14 (57.14%) received GC without known ILD or diffuse SSc type, (Table 4).

Discussion

The significance of this study lies in the specificity of the country with a small population. There are only two hospitals for adults with an established team of rheumatologists, and we included both. Virtually all patients with suspected SSc in Latvia are referred to one of these hospitals, so we are effectively describing the general Latvian population by selecting and evaluating patients from these hospitals.

There are two main ethnic groups in Latvia – Latvians (62.1%) and Russians (26.9%) – along with other eastern Europeans (ca. 8%), Jews (0.3%) and Romani (0.3%) [16, 17]. The Latvian population mainly consists of Caucasians, as represented in our results with 100% Caucasians. In this study we did not distinguish patients' ethnicity. Still our colleagues in Estonia (where two ethnic groups also predominate, Estonians and Russians) found that the prevalence of SSc, especially CREST syndrome, was

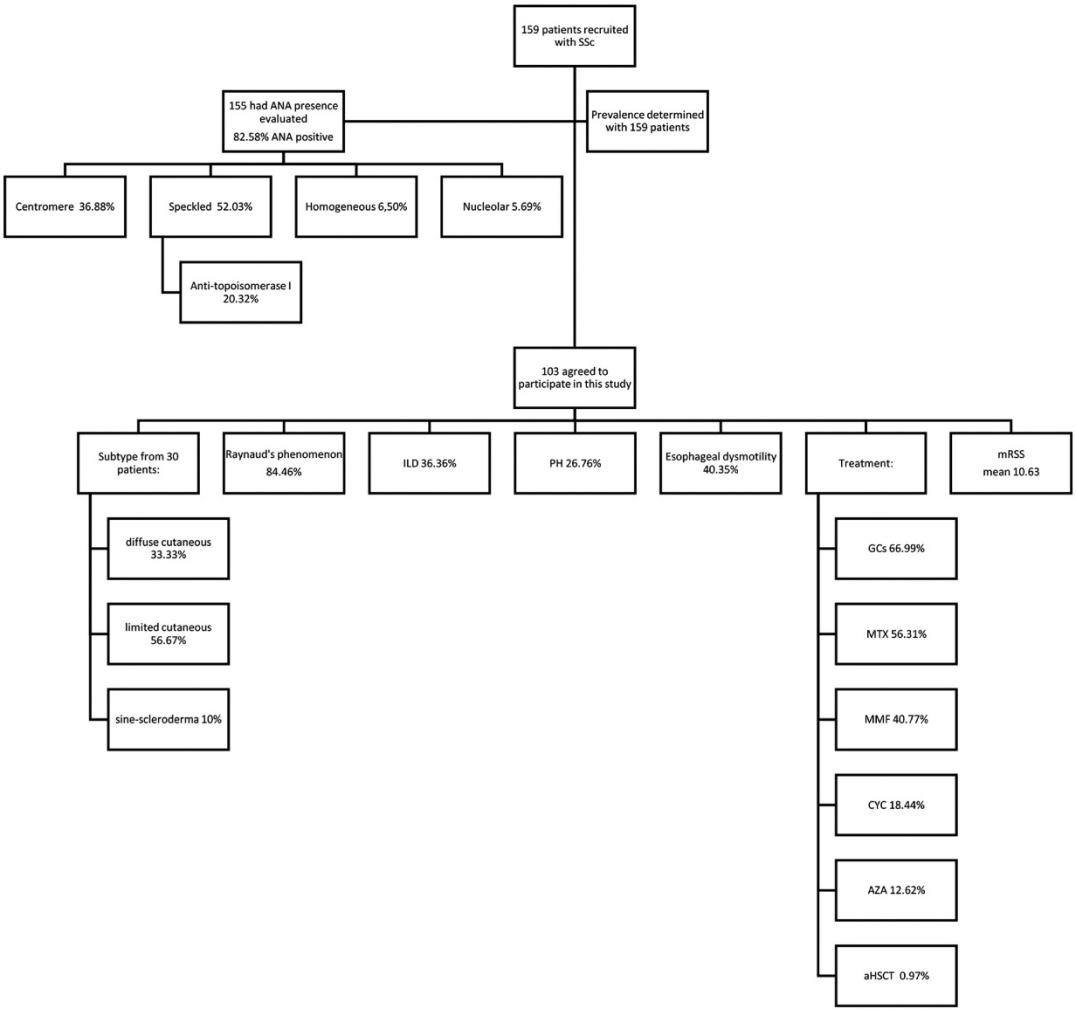

Fig. 2

higher among the Russians [18]. To clarify, the CREST terminology is no longer widely used, and the term used instead is limited cutaneous SSc, as mentioned in our study [19].

Only a few studies were conducted in Northern or Eastern Europe. One study carried out in southeast Norway found the prevalence of SSc to be compatible with other northern European countries, supporting the notion of a north–south gradient of SSc in Europe, with the lowest prevalence in Northern Europe [20]. Opposing results were presented from Sweden, where the prevalence was higher at 235 per million inhabitants [21].

In our study, the point prevalence was 84.0 (95% CI=71.9–98.1) per million, which is lower than the results in review about 50 publications from Europe and North America, with reported prevalence of 70.2–333.9 and 135–443 per million in Europe and North America, respectively [22]. Although we cannot identify any specific reason for this, the relatively low prevalence is unlikely to be due to study shortages but rather to a possible shortage of rheumatologists in the country and the unavailability of consultations. This would be particularly true for patients with a limited subtype of the disease, without severe PH, who do not feel the need to visit their general practitioner.

We observed the highest prevalence in the 60–69 age group, that was not similar in other European countries.

Table 3 Gender-specific clinical characteristics in patients with systemic sclerosis

		Males	Females	Total
Descriptive	Total count, *N*	18	85	103
	Minimum disease Duration, *years*	1	2	1
	Maximum disease Duration, *years*	21	41	41
	Mean (SD) disease duration, *years*	8.95 ± 6.33	15.14 ± 9.87	14.06 ± 9.62
	Minimum age of onset	14	5	5
	Maximum age of onset	80	74	80
	Mean (SD) age of onset	50.5 ± 16.64	46.51 ± 13.51	47.21 ± 14.10
Symptoms	Raynaud's phenomenon, *N* (%)	16 (88.88%)	71 (83.52%)	87 (84.46%)
	modified Rodnan skin score, mean (SD)	10.36 ± 12.95	10.67 ± 8.78	10.63 ± 9.41
SSc types, *N* (from 30 patients)	Sine-scleroderma	0	3 (11.54%)	3 (10%)
	Limited	2 (50%)	15 (57.69%)	17 (56.67%)
	Diffuse	2 (50%)	8 (30.77%)	10 (33.33%)
Interstitial lung disease, *N* (from 99 patients)		7 (38.89%)	29 (35.80%)	36 (36.36%)
Pulmonary hypertension, *N* (from 71 patients)		4 (30.77%)	15 (25.42%)	19 (26.76%)
Esophageal dysmotility, *N* (from 57 patients)		5 (45.45%)	18 (39.13%)	23 (40.35%)
Treatment, *N* (%) (from 101 patients)	Glucocorticoids	12 (66.66%)	57 (67.05%)	69 (66.99%)
	Methotrexate	7 (38.88%)	51 (60.00%)	58 (56.31%)
	Mycophenolate mofetil	9 (50.00%)	33 (38.82%)	42 (40.77%)
	Cyclophosphamide	4 (22.22%)	15 (17.64%)	19 (18.44%)
	Azathioprine	2 (11.11%)	11 (12.94%)	13 (12.62%)
	Autologous hematopoietic stem cell transplantation	0	1 (1.17%)	1 (0.97%)

Table 4 Gender-specific glucocorticoid treatment in different systemic sclerosis types and interstitial lung disease

		Males	Females	Total
Glucocorticoids, *N* (%)	Sine-scleroderma	0	2 (66.67%)	2 (66.67%)
	Limited	1 (50%)	11 (73.33%)	12 (70.59%)
	Diffuse	1 (50%)	8 (100%)	9 (90%)
	Interstitial lung disease	6 (100%)	21 (100%)	27 (100%)
	No interstitial lung disease	6 (54.55%)	36 (69.23%)	40 (63.49%)

For example, in Sweden and Italy 70–79 age group had the highest prevalence [23, 24].

This study did not analyse incidence data for SSc. The main reason for this choice is missing data in hospital databases, and relying only on the medical history from patients can lead to very misleading data.

We report a higher mean age in this study for females than males: 63.12 versus 59.75 years. This was not seen in the Norway study, where the difference was minimal (56.7 versus 56.1 years) [20]. Also, the mean age of both genders was older than represented in other similar studies: 62.53 ± 12.11 years versus 50.8 ± 12.5 years in Italy [25] and 56.8 ± 12.2 years in Hungary [26].

A higher female predominance was seen in this study than is reported worldwide, with a female: male ratio of 4.67:1 compared to 3:1. However, it was similar to other European reports, where the ratio was estimated to be 3.8–11.5:1 [22], so the study of gender difference should probably be based on regional data rather than on global data linking very different regions together.

The highest gender ratio was observed in the 70–79-year age group (6.75:1), contradicting previous observations of a lower gender ratio after the age of 50 years (2:1) [10]. In younger patients we did find a lower gender ratio (2:1), but this again contradicted the worldwide data [10]. Of course, probabilities must be expressed with caution with the small number of patients. Still, in our study, we probably captured the characteristics of older men avoiding medical help in Latvia.

Most of patients evaluated were ANA positive, with anti-speckled and anti-centromere patterns present almost equally. The presence of ANA in patients with SSc is widely observed, with levels as high as 98% reported [27]. Three serum autoantibodies that are included in the 2013 classification criteria (anti-RNA polymerase III, anti-topoisomerase I and anti-centromere) account for over 70% of all single antibody specificities detected in previous studies [14, 27]. Unfortunately, at the time of study, it was not possible to detect anti-RNA polymerase III, but 84 patients (68.85%) from the 122 evaluated had either anti-centromere or anti-topoisomerase I. In recent data with knowledge of new antibodies associated with SSc, still highest prevalence stands for these

two antibodies [28]. Contrary to our results, in the Norway study, there was significant anti-centromere predominance compared to anti-topoisomerase I (54.2% vs. 13.5%) [20]. Previously, many studies reported higher anti-centromere prevalence in Caucasians [29, 30]. In contrast, in a study from the USA, evaluating the prevalence of autoantibodies in a different race, only 17% of Caucasian patients had positive anti-centromere antibodies, with more (19%) having anti-RNA polymerase III [31]. We found that anti-centromere-positive patients were more likely to be females, whereas the difference was not as significant between anti-topoisomerase I positive males and females. In other studies, females were substantially more likely to have anti-centromere antibodies, whereas males more likely to have anti-topoisomerase I [27]. In our study, we present different data from the previous studies. With 100% Caucasian patients, there was no significant anti-centromere antibody predominance and there was a high prevalence of anti-topoisomerase antibodies. Although ANA positive patients were fewer than in majority of other studies, it could be higher with repeated examination dynamically [32].

Most of our patients presented with the first non-Raynaud's SSc symptom in the fifth decade of life. Study from Sweden showed similar results (48±4.1 years) [23]. However, disease onset is hard to determine and has not been defined similarly in other studies. The age at which the diagnosis was made is generally analysed and in data from Europe it varies in the range 33.5–59.8 years [22]. In our view, it is also essential to note patients' observations of their first symptoms, allowing more reliable conclusions of differences between several populations. By contrast, if the focus remains on the time of diagnosis, we may mistakenly assess not the characteristics of the disease but the availability of specialists in different countries.

We report a slight age difference when comparing both genders at disease onset, with females being younger than males. Younger female age at onset is not uncommon, and other studies have presented similar findings. In a study from Greece, the age difference was markedly larger but, similarly, the females tended to be younger [33]. In Pittsburg, USA, the results were very similar to ours: 43.8±14.0 years for females; 46.4±13.7 years for males [27].

Although the number of males in the study was small, we observed a similar trend towards a more severe disease course, with more frequent development of ILD and PH, as in other studies [13, 34, 35]. As the main causes of SSc-related mortality, these data also explain the worse outcomes in males. However, there are no clear data on the difference in the incidence of esophageal dysmotility between genders. Historically, dysmotility was described as another close symptom to the limited subtype but we observed a higher frequency of dysmotility in males, although the limited form did not predominate as the most common subtype of disease in them [36, 37].

We found that more than half of patients (68.31%) received treatment with GCs at any point of the disease. Although this number is exceptionally high, the trend is not exclusive to our study. The German Network for Systemic Scleroderma data showed that 41,3% of all registered SSc patients were treated with GCs [38]. EUSTAR database provided very detailed data on GCs prescribing practices in SSc, with 34% off patients taking GCs at baseline of the study, but the use of GCs from disease onset was not included. There were no data from Latvia, but interestingly eastern Europe countries tended to prescribe GCs more [39]. In the most recent update of the EULAR recommendations for the treatment of SSc, the experts recognized that GCs, which are used in SSc, are part of the therapeutic strategy in the management of ILD, diffuse cutaneous disease or musculoskeletal involvement [40]. However, the evidence regarding their efficacy in SSc is limited [29]. In Latvia, the trend of GCs use was more pronounced in patients with diffuse cutaneous SSc, but it was also used in more than half of patients with limited cutaneous SSc and with sine scleroderma. The most difficult to explain the use of GCs was in 57% of patients who used them without diffuse skin involvement and ILD. Patients enrolled in the study were treated for up to several decades. We think this is also why the number of patients treated with GCs was so high. Previously, higher expectations were placed on GCs in the treatment of SSc. We did not analyse the use of GCs over time, but following further and more recent studies there is a high probability that the use of GCs will decrease in Latvia. It is more likely that, as knowledge of the role of immunosuppressive therapies in SSc develops, data will also show a positive trend towards a reduction in the use of GCs in Latvia.

We are aware of some limitations of the study. Due to the country specificity, most patients with suspected SSc are referred to the two university hospitals mentioned above, but we cannot exclude a number of patients who were nevertheless not included in this study. One of the reasons why a proportion of patients may not have been included in our study is the lack of capillaroscopy data at the time of diagnosis, which could be the reason why the SSc classification criteria were not met. Another shortcoming in meeting SSc classification criteria could be lack of evaluation of anti-RNA polymerase III.

Conclusion

SSc is less common in Latvia than in other countries and regions. Due to its location, the data from Latvia are consistent with a north-south gradient in Europe. With

its homogeneous racial pattern, Latvia is probably an even more pronounced model for the developing of SSc in northern countries. Female to male ratio was 4.67:1, and gender ratio differences persisted in older age groups with highest gender ratio observed in the age group 70–79-year. ANA presence did not differ between genders, but in females centromere pattern was much more likely to be present. Disease developed earlier in females, without significant difference in Raynaud's presence or severity by mRSS. Males had more severe disease course, but in both genders more than half of patients received treatment with GCs at any point of the disease.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13023-024-03355-y.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

We thank Dmitrijs Rots, MD, for his expertise in the analysis of demographic data; and rheumatology colleagues from Latvian hospitals and centers: Sarmite Abolite, Daina Andersone, Janis Arajs, Ineta Balcune, Lelda Dimdina, Renate Diura, Vladimirs Lavrentjevs, Santa Mikena, Pauls Rubins, Evita Sikora, Mihails Tarasovs, Natalija Vellere, Ilze Vinkalna, Tamara Zavgorodnaja, Signe Zelca.

Authors' contributions

KI conducted a survey; performed clinical examination, data collection and data analysis; performed statistical analysis; wrote first draft of the manuscript. OR performed clinical examination, data collection and data analysis; performed statistical analysis. AM conducted a survey. LM performed clinical examination, data collection and data analysis. AK conducted a survey. JZ conducted a survey. VK performed clinical examination, data collection and data analysis. NK performed clinical examination, data collection and data analysis. IB conducted a survey. All authors read and provided critical feedback on manuscript draft, all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work There are no financial or other relations that could lead to a conflict of interest.

Funding

This research received no external funding.

Data availability

The data that support the findings of this study are openly available in Synapse at https://doi.org/10.7303/syn56849053; syn56849053.

Declarations

Ethics approval and consent to participate

The study was approved by the Riga Stradins University medical ethics committee (Institutional Review Board reference no: 22 – 2/481/2021) and all participants provided written informed consent.

Consent for publication

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Competing interests

Not applicable.

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Received: 22 August 2023 / Accepted: 4 September 2024
Published online: 30 September 2024

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Publisher's note

RESEARCH PAPER

Neurologia i Neurochirurgia Polska
Polish Journal of Neurology and Neurosurgery
2023, Volume 57, no. 2, pages: 206–211
DOI: 10.5603/PJNNS.a2023.0018

ISSN: 0028-3843, e-ISSN: 1897-4260

Prevalence of polyneuropathies among systemic sclerosis patients and impact on health-related quality of life

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ABSTRACT

Introduction. Systemic sclerosis (SSc) is a chronic rheumatic disease that affects multiple organ systems, including the peripheral nervous system. However, studies into the involvement of polyneuropathies (PNP) have shown inconsistent results. The aim of this study was to determine the prevalence of small (SFN) and large (LFN) fibre neuropathy among SSc patients and the impact on health-related quality of life (HRQoL).

Material and methods. The study enrolled 67 patients with diagnosed SSc. The severity of neuropathic symptoms was evaluated using shortened and revised total neuropathy scoring criteria. Nerve conduction studies were used for LFN, and quantitative sensory testing was used to evaluate SFN. Neuropathic pain was evaluated using a Douleur Neuropathique en 4 questionnaire, and the severity of anxiety symptoms was assessed using a Generalised Anxiety Disorder-7 scale. The Health Assessment Questionnaire-Disability Index was used to assess HRQoL. Previous data on antinuclear autoantibodies (ANA) test results was obtained. Statistical analysis was performed using SPSS software.

Results. LFN was diagnosed in 47.8% (n = 32/67) and SFN in 40.3% (n = 27/67) of the subjects. ANA positivity was not associated with the presence of LFN/SFN. The severity of neuropathic pain had a significant correlation with anxiety symptoms ($r = 0.61$, $p < 0.001$), the severity of neuropathy symptoms ($r = 0.51$, $p < 0.001$) and HRQoL ($r = 0.45$, $p < 0.001$). The severity of neuropathy symptoms correlated with HRQoL ($r = 0.39$, $p = 0.001$).

Conclusions. We demonstrated that PNP are found in almost all SSc patients. Also, SFN is as common as LFN. Additionally, we found that the severity of neuropathy symptoms and neuropathic pain are both associated with a worse HRQoL.

Key words: systemic sclerosis, large fibre neuropathy, small fibre neuropathy, neuropathic pain, anxiety, health-related quality of life

(Neurol Neurochir Pol 2023; 57 (2): 206–211)

Introduction

Systemic sclerosis (SSc), also known as scleroderma, is a rare chronic rheumatic disease characterised by immune activation, widespread vascular damage, and progressive fibrosis [1, 2]. The hallmark of this disease is thickening and hardening of the skin, but other organ systems are also commonly affected, leading to considerable morbidity and mortality. Many patients complain about one or more symptoms of gastroesophageal reflux disease, but more severe upper and lower gastrointestinal tract involvement can be associated with malnutrition. Restricted joint mobility, arthritis, renal failure, heart and pulmonary complications are the main causes of morbidity and mortality in the course of SSc [2]. Additionally, the peripheral nervous system can also be affected [3]. Neurological involvement includes both

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Received: 24.09.2022 Accepted: 20.01.2023 Early publication date: 14.03.2023

compression (e.g. trigeminal neuropathy, carpal tunnel syndrome, ulnar nerve entrapment) and non-compression (e.g. sensorimotor neuropathy, sensory ataxic neuropathy, multiple mononeuropathies) neuropathies [4].

Neuropathy was previously thought to be a less common SSc finding [5]. However, recent studies have shown that neurological involvement is fairly common. The prevalence of peripheral neuropathy in SSc ranges from 17% [6] to 40% [7], with a pooled prevalence close to 30% [3, 4]. Probably due to the rarity of the disease, the methods used in these studies and the characteristics of the study groups, the results differ and the extent of peripheral nervous system involvement remains unclear. Moreover, there are only a few studies on polyneuropathy that have differentiated small (SFN) from large fibres (LFN). To the best of our knowledge, no nationwide study of peripheral nervous system disorders among SSc patients has previously been carried out in the Baltic countries.

As a chronic systemic disease, SSc affects patients' health-related quality of life (HRQoL), with a number of problems associated with decreased functional status and increased disability [8, 9]. It is unclear whether HRQoL has a direct association with SSc or nervous system involvement, as other factors such as anxiety and neuropathic pain can worsen patients' HRQoL.

The aim of this study was to define the prevalence of SFN and LFN among patients with SSc, based on a population-wide cohort in Latvia, and to identify factors associated with LFN or SFN development. Additionally, we aimed to identify the effects of LFN and SFN, the severity of neuropathic pain, and anxiety symptoms related to HRQoL.

Material and methods

Materials

This study was performed on Latvian patients diagnosed with SSc in accordance with the American College of Rheumatology/European Alliance of Associations for Rheumatology (ACR/EULAR) criteria [10] who were diagnosed or consulted in the period from 1 January 2016 to 30 September 2021 at either of Latvia's adult university hospitals: Riga Eastern Clinical University Hospital and Pauls Stradins Clinical University Hospital. In total, 109 SSc patients were assessed for participation and 67 (54 women and 13 men, age range 23 to 83 years) were enrolled in the study.

Methods

According to the ACR/EULAR criteria [10], patients were assessed for skin thickening on the fingers, fingertip lesions, telangiectasia, abnormal nailfold capillaries, interstitial lung disease (ILD), pulmonary arterial hypertension (PAH), Raynaud phenomenon (RP), and SSc-related autoantibodies. Disease duration was determined based on the occurrence of the first non-Raynaud's phenomenon symptom. The general severity of cutaneous involvement was assessed using the modified Rodnan skin score (mRSS) [11]. Additionally, all subjects were asked regarding specific therapy use (e.g. cyclophosphamides) and common health conditions (e.g. diabetes, thyroid diseases) that are known to be causative for peripheral neuropathy. Previous data on antinuclear autoantibody (ANA) test results was obtained. ANA tests were performed on peripheral blood serum by indirect immunofluorescence using HEp-2 ANA indirect fluorescent antibody (IFA) assays [12].

Enrolled subjects underwent a uniform evaluation of the peripheral nervous system. Firstly, patients were screened using the shortened and revised total neuropathy scoring criteria (srTNS) [13], which consists of three symptom extension components (numbness, tingling, and neuropathic pain) and two objective testing components (tendon reflex and vibration sensibility). Next, the patients were examined using nerve conduction studies (NSC) by a certified neurophysiology expert. Nerve conduction studies were performed on both motor and sensory conduction according to the polyneuropathic examination protocol. Each patient underwent bilateral upper extremities NCS (motor and sensory components of ulnar and median nerves) and bilateral lower extremities NCS (motor component of peroneal and tibial nerves and sensory components of a sural nerve) for nerve conduction latency, amplitude, and velocity. Those subjects who had abnormal NCS results according to the normal values used in Latvian clinical practice [14, 15] in more than one attribute in two separate nerves were diagnosed as having large fibre polyneuropathy. Quantitative sensory testing (QST) was performed in the subjects with normal NSC results in order to evaluate small fibre function for possible abnormalities [16]. Thermal (warm, cold, painful warm/painful cold) sensations were checked. Stimuli were applied to the thenar region of the hands and the dorsal surface of the feet. QST results were compared to normative data, and those subjects who had abnormal values in two separate extremities were diagnosed as having small fibre polyneuropathy.

Additionally, all enrolled subjects completed the Latvian version of the Douleur Neuropathique en 4 (DN4) [17] questionnaire to assess neuropathic pain, the Generalised Anxiety Disorder-7 (GAD-7) [18] scale to assess anxiety symptoms, and the Health Assessment Questionnaire-Disability Index (HAQ-DI) [19] to assess HRQoL. Those patients scoring four or more points on the DN4 questionnaire were defined as having neuropathic pain. More than four points on the GAD-7 questionnaire indicates an increased risk of generalised anxiety. The eight scores of the eight sections of the HAQ-DI were added together and divided by eight to provide the functional disability index.

Statistical analysis

Statistical analysis was performed using SPSS 27.0 software (SPSS Inc., Chicago, IL, USA). Data normality was assessed using histograms and the Kolmogorov-Smirnov test.

Table 1. Clinical characteristics of SSc subjects with and without peripheral neuropathy

Characteristic	No neuropathy (n = 8; 11.9%)	Large fibre neuropathy (n = 32; 47.8%)	Small fibre neuropathy (n = 27; 40.3%)
Median age	62.5 (IQR, 9.25)	66.5 (IQR, 10.50)	57.0 (IQR, 17.50)
Female sex	8 (100%)	24 (75%)	22 (81.5%)
Male sex	0	8 (25%)	5 (18.5%)
Age at scleroderma onset	46.5 ± 15.4	51.0 ± 13.8	40.7 ± 17.4
Median duration of SSc	15.0 (IQR, 13.50)	19.5 (IQR, 17.25)	12.0 (IQR, 13.50)
Scleroderma subtype			
Limited	5 (62.5%)	23 (71.9%)	22 (81.5%)
Diffuse	3 (37.5%)	9 (28.1%)	5 (18.5%)
Median Rodnan score	11.0 (IQR, 14.50)	6.0 (IQR, 10.0)	4.0 (IQR, 10.0)
Antinuclear antibodies	7 (87.5%)	26 (81.3%)	21 (77.8%)
Anti-centromere		*Data not shown*	
Anti-SCL70		*Data not shown*	
Speckled		*Data not shown*	
Nucleolar		*Data not shown*	
Homogenous		*Data not shown*	
Median TNS	2.0 (IQR, 3.50)	7.0 (IQR, 5.25)	0 (IQR, 3.50)
Median DN4 score	3.0 (IQR, 3.50)	4.0 (IQR, 6.0)	3.0 (IQR, 7.5)
Median GAD-7 score	8.0 (IQR, 13.0)	4.5 (IQR, 10.25)	5.0 (IQR, 7.50)
Median HAQDI score	0.81 (IQR, 1.47)	1.63 (IQR, 1.72)	0.63 (IQR, 1.56)
Without risk factors	5 (62.5%)	18 (56.3%)	20 (74.1%)
With risk factors	3 (37.5%)	14 (43.8%)	7 (25.9%)
Treatment with cyclophosphamide	1 (12.5%)	9 (28.1%)	6 (22.2%)
Treatment with chemotherapy	2 (25%)	2 (25%)	0
Diabetes mellitus	0	0	1 (3.7%)
Thyroid diseases	0	3 (9.4%)	2 (3.7%)
Chronic renal diseases	0	4 (12.5%)	3 (3.7%)

TNS — total neuropathy score; DN4 — douleur neuropathique 4; GAD-7 — general anxiety disorder-7; HAQDI — Health Assessment Questionnaire Disability Index

For comparison between groups, the Kruskal-Wallis H test, Spearman's rank-order correlation and Fisher's exact tests were used. P values < 0.05 were considered significant.

Ethical approval

This study was approved by the Ethics Committee of Riga Stradiņš University [Nr. 22-2/481/2021]. All subjects were informed about the rationale and goals of the study, signed an informed consent form, and gave their permission for anonymised publication of their clinical information.

Results

The median age of the study group was 64 years (IQR, 12.0). Out of 67 enrolled patients, 54 (80.6%) were female and 13 (19.4%) were male. The median age at the onset of SSc was 47 (IQR, 19.5) years and the median duration of disease was 16 (IQR, 15.0) years. 52.2% of subjects had the limited subtype of SSc (n = 35/67), while 47.8% had the diffuse type (n = 32/67). A description of the SSc groups divided by the presence of polyneuropathy and its type is set out in Table 1.

Based on the NCS evaluation, LFN was identified in almost half of the SSc individuals (47.8%, n = 32/67). Furthermore, the majority of individuals who did not have LFN showed signs of SFN, evaluated by QST (40.3%, n = 27/67); only 11.9% (n = = 8/67) of the subjects did not fulfil any criterion for SFN or LFN. A comparison of the clinical features, as well as neuropathy risk factors between these three groups, is set out in Table 1.

To identify the aetiology of the peripheral nervous system involvement in SSc, we analysed the prevalence of neuropathy risk factors among the SSc patients. Neuropathy risk factors as

a possible secondary cause were defined in 35.8% of subjects (n = 21/59). These included treatment with cyclophosphamides, chemotherapy, diagnosed diabetes mellitus, thyroid disorders, and chronic renal disease [20–22]. However, the same risk factors were present in 37.5% (n = 3/8) of individuals without neuropathy and there was no difference in risk factor prevalence among the subjects with LFN, with SFN, or without neuropathy ($p > 0.05$). Because we understood that the small number of SSc patients without polyneuropathy affected the statistical power of the given result, we further analysed other factors that could explain the presence of LFN or SFN.

There were no associations between the presence of LFN or SFN and sex ($p = 0.32$), age ($p = 0.63$), disease duration ($p = 0.64$), severity of cutaneous involvement ($p = 0.19$), subtype of SSc ($p = 0.73$), or ANA positivity ($p = 0.91$), nor with any specific subtype of ANA ($p = 0.93$) (ANA subtype data not shown).

LFN patients had higher TNS scores [median TNS = 7.0 (IQR, 5.25)] than SFN patients [median TNS = 0 (IQR, 3.5)] and also higher than subjects without neuropathy [median TNS = 2.0 (IQR, 3.5)], but the difference was not statistically significant ($p = 0.37$).

There were no significant differences between LFN/SFN and the severity of neuropathic pain ($p = 0.46$), anxiety symptoms ($p = 0.75$), or HRQoL ($p = 0.68$). However, the severity of neuropathic pain had a significant correlation with anxiety symptoms ($r = 0.61$, $p < 0.001$), the severity of neuropathy symptoms ($r = 0.51$, $p < 0.001$), and HRQoL ($r = 0.45$, $p < 0.001$). Additionally, the severity of neuropathy symptoms had a moderately strong correlation with HRQoL ($r = 0.39$, $p = 0.001$).

Discussion

In this study, we performed a detailed evaluation of large and small fibre polyneuropathy in a large cohort of SSc patients from Latvia. By systematically analysing both LFN and SFN, we identified that the prevalence of peripheral neuropathy in SSc patients is very high, affecting ~90% of patients. Even though some subjects had possible secondary causes (risk factors) for their neuropathy, we did not find any significant differences between individuals with polyneuropathy and those without, although the second group of patients was not big enough to make a firm conclusion of neuropathy to be developed independently of known risk factors.

Additionally, we found that neuropathic pain is common among SSc patients and that neuropathic pain has a significant correlation with the total neuropathic score and the severity of anxiety symptoms. While the presence of LFN or SFN did not reach statistical significance, neuropathy-related symptoms (both neuropathic pain and severity assessed by the TNS) affected SSc patients' HRQoL.

Our study revealed a higher prevalence of polyneuropathy in SSc than has been found in other studies, but only a few studies have performed as detailed and targeted an evaluation of the peripheral nervous system as we have. Furthermore, the materials and methods used in those studies provide a large range of results. A recent systematic review of 113 studies [4] showed a pooled prevalence of neuropathy involvement in 27.37% of cases, including 26% (n = 556/2,143) with SFN and 10.8% (n = 231/2,143) with LFN when neuropathies were assessed based on small and large fibres.

However, the titles and abstracts were not selected according to strict criteria regarding evaluated neuropathies, including all works where peripheral neuropathy was reported by symptoms and clinical examination, nerve conduction studies or other detection tools. LFN was observed in many studies on isolated or multiple mononeuropathies [23–30], and confirmatory diagnostic tests differed depending on the design of the study. Some studies performed electrophysiological examinations [27, 28, 31], while others used imaging techniques [23, 26, 32], biopsy [26, 30] or other methods. Only a few studies showed similar results to our study. One study on the role of ultrasound imaging in the evaluation of peripheral nerves in SSc [32] showed sensory disturbances revealed by clinical examination in 40% (n = 10/25) of subjects, but the imaging modalities used (ultrasound, computer tomography, magnetic resonance) revealed abnormalities in 7/10 patients. However, a peripheral nervous system examination was performed only on median and ulnar nerves, observing compression neuropathies. We believe that the high prevalence of LFN can be explained by the fact that we were working with a relatively large study group and that all subjects were evaluated using both clinical symptoms and electrophysiological methods, where motor and sensory components were studied on several nerves of each extremity.

Our study suggests that small fibre abnormalities are common in SSc, and that neurological events appear in almost all SSc patients, with the predominant involvement of small fibres, although there are limitations on assessing small fibre function. As mentioned above, in a recent systematic review of peripheral neuropathy in SSc [4], the prevalence of SFN was more than double that of LFN. In our study, SFN was less prevalent than LFN; even so, of those subjects who did not show abnormalities by NCS, only eight had normal QST results. The high prevalence of SFN may be associated with skin changes due to SSc, but there was not a significant difference between the severity of cutaneous involvement and the presence of SFN.

The diagnosis of SFN can be challenging because the diagnostic criteria for SFN are not yet fully established. This lack of standardised diagnostic criteria for SFN may indeed have implications on our research in terms of the definition of SFN, since our study subjects were defined to have SFN solely based on their QST results [33, 34]. We did not detect specific gene mutations for transthyretin familial amyloid polyneuropathy as a rarer underlying cause of SFN and LFN [35, 36]. Neither were autoantibodies in SFN tested, for example antisulfatide and anti-plexin antibodies, which could be specific for small fibre neuropathies and may be a key pointer towards explaining the high frequency of small fibre polyneuropathies in our study [37].

We speculate that the autoimmune nature of polyneuropathy could justify immunomodulatory therapy use such as plasma exchange for those SSc patients who show neuropathic symptoms [38, 39]. Thus more specific examinations of possible autoantibodies should be performed as the next stage of research.

Our study assessed neuropathic pain in SSc patients and showed that LFN and SFN subjects have a tendency towards higher DN4 scores, with no direct association with the severity of neuropathic pain, but a significant association between neuropathic pain and the severity of neuropathy symptoms where both affect SSc patients' HRQoL. Neuropathic pain occurs in many rheumatic diseases and neuropathic pain is thought to be more prevalent in these patients than in the general population [40]. A Danish nationwide cross-sectional registry survey (DANBIO) on pain and pain mechanisms in patients with inflammatory arthritis showed neuropathic pain in 20% of rheumatic arthritis patients, 28% of psoriatic arthritis patients, and 21% of spondylarthritis patients [41]. The prevalence and severity of neuropathic pain in SSc patients is not well-studied and is not yet established. One cross-sectional study on neuropathic pain in SSc patients showed that neuropathic pain was significantly higher in SSc patients compared to control subjects (56.2% *vs.* 13.3%) [42]. In our study, we assessed the severity of neuropathic pain by the DQ4 in all study participants. Only 18 subjects (26.87%) scored zero points on the DQ4. We found neuropathic pain to have an important impact on SSc patients' HRQoL, but it is unclear whether neuropathic pain affects HRQoL independently of, or in relation with, a higher severity of neuropathy symptoms. Moreover, our study supports the concept of neuropathic pain being associated with the severity of anxiety symptoms, showing significance between the DN4 and GAD-7 scores [43].

The main limitation of this study was the size of our study group. Although we enrolled 67 out of 109 SSc patients who were examined at Latvia's university hospitals over the course of 5.75 years, we believe that more statistical significance would be found with a larger study group. The small number of SSc patients is explained by the rarity of the disease and Latvia's small population. Another limitation was the small fibre function being assessed by QST only. To clarify the involvement of SFN, a skin punch biopsy should be performed to measure epidermal nerve fibre density (ENFD), since the results of such a biopsy can provide more objective diagnostic data for defining SFN.

Conclusions

We demonstrated an unexpectedly high prevalence of polyneuropathy in Latvian SSc patients, showing that the peripheral nervous system is affected in almost all patients. Moreover, we found SFN to be as common as LFN. Another important finding in our study is that the severity of neuropathy symptoms and neuropathic pain were both associated with a higher health-related disability index, indicating worse HRQoL. The presence of polyneuropathy was not associated with known risk factors. Therefore it is necessary to seek other reasons for the presence of SFN and LFN in SSc patients, possibly associated with specific antibodies.

Conflicts of interest: *None.*
Funding: *None.*

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frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 02 August 2024
DOI 10.3389/fmed.2024.1412706

OPEN ACCESS

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RECEIVED 05 April 2024
ACCEPTED 25 July 2024
PUBLISHED 02 August 2024

CITATION
Ivanova K, Zolovs M, Blennow K, Zetterberg H, Kurjāne N and Ķēniņa V (2024) Polyneuropathy in systemic sclerosis: exploring the causes and biomarkers. *Front. Med.* 11:1412706. doi: 10.3389/fmed.2024.1412706

Polyneuropathy in systemic sclerosis: exploring the causes and biomarkers

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Introduction: Systemic sclerosis (SSc) is a rare autoimmune disease with multiple organ involvement; however, the contribution of the nervous system (NS) remains relatively understudied. There are no specific data on the role of the autoimmune response and inflammation in the development of peripheral nerve system (PNS) damage in SSc and markers to assess this damage have yet to be identified.

Objectives: The primary objective of this study was to define the autoimmune mechanisms that lead to neuropathy by identifying antibodies (Abs) that target certain component of the NS or are associated with SSc. The secondary objective was to identify markers of NS damage that correlate with the detection and progression of polyneuropathy (PNP).

Methods: This study included patients diagnosed with SSc who met ACR/EULAR 2013 classification criteria at two leading Latvian hospitals between January 2016 and December 2021. Patients underwent a nerve conduction study (NCS). The SSc-associated Abs, Abs against myelin-associated glycoprotein (MAG) and anti-ganglioside Abs (GM1, GM2, GD1a, GD1b and GQ1b) were analysed. Potential serum PNS biomarkers—neurofilament light chain (NfL), glial fibrillary acidic protein (GFAP), fibroblast growth factor 21 (FGF21) and growth/differentiation factor 15 (GDF15)—were measured.

Results: We recruited 103 Caucasian patients diagnosed with SSc. SSc-associated Abs did not differ significantly between patients with and without PNP ($p > 0.05$). Anti-MAG and anti-ganglioside Abs in patients with PNP did not present a significant increase above the reference range. NfL, GFAP and GDF15 were significantly elevated in the presence of PNP ($p < 0.05$), with a moderate to high effect size ($r = 0.36–0.65$). Our regression analysis revealed a strong

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association between the HAQ-DI score, older age, male gender and the risk of developing PNP.

Conclusion: The development of PNP in patients with SSc is most likely due to ageing, natural progression and the sequelae of the disease. Several serum biomarkers—NfL, GFAP and GDF15—could be used as relevant diagnostic biomarkers for PNP in patients with SSc. Future studies are warranted to validate the diagnostic efficacy of these biomarkers and to unravel the complex interplay of factors leading to PNP in patients with SSc.

KEYWORDS

systemic sclerosis, scleroderma, polyneuropathy, nervous system, autoimmune, serum biomarkers

1 Introduction

Systemic sclerosis (SSc) is a rare autoimmune disease with known autoantibodies that help establish a diagnosis and affect the prognosis (1–3). Although multiple organ involvement is widely acknowledged and studied, the contribution of the nervous system (NS) remains relatively understudied (4–6). In most recent classification criteria, NS damage was not included in point assessment, again highlighting its undefined role in SSc (7). Although a few studies have been conducted to establish the prevalence and type of NS involvement in SSc, mostly focusing on peripheral nervous system (PNS), they differed widely in numbers, partly because the authors used different methods of assessing NS damage. Over time, NS involvement in SSc has become more frequent, especially in recent studies, with a range from 17 to 40% (5, 8–11).

While only a few studies have evaluated the prevalence of NS involvement in SSc, there is even less research regarding the true pathogenesis of neuropathy in this rare disease. Most symptoms in patients with SSc can be explained by microvascular damage, the autoimmune response and inflammation, and fibrosis with variable severity (12, 13). The first and to this day the most accepted cause for neuropathy development in SSc is ischaemic damage of the NS (8, 14). Thus, it would be logical to conclude that patients with severe Raynaud's disease, pitting scars and ischaemic skin lesions should develop neuropathy, but the proportion of patients without nerve damage contradicts this view, suggesting that other mechanisms are involved in the pathogenesis of neuropathy in SSc (15, 16).

There are no specific data on the role of the autoimmune response and inflammation in the development of neuropathy in SSc. In many systemic connective tissue diseases, the idea of studying specific antibodies (Abs) against various nerve structures comes from research performed in immune-mediated polyneuropathies (PNP) like Guillain–Barré syndrome (17, 18). This approach is still understudied in SSc and could lead to new insights into neuropathy pathogenesis and a future change in treatment tactics.

Another understudied issue is biomarkers for the progression and severity of SSc. Several biomarkers are used to measure and monitor the severity of lung and skin damage in SSc; however, markers to assess PNS damage and its progression have yet to be identified (19, 20). Neurofilament light chain (NfL) has proved to be useful biomarker for PNP, given that it is related to metabolic and genetic disorders, but it has not been studied in SSc (21, 22). There are other known biomarkers that are mostly or partly secreted from Schwann cells that can associated with PNS damage due to various diseases, including growth/differentiation factor 15 (GDF15) studied in diabetic neuropathies and glial fibrillary acidic protein (GFAP) associated with inflammatory PNP (23, 24).

The primary objective of this study was to define the autoimmune mechanisms that lead to neuropathy by identifying Abs that target certain component of the NS or are associated with SSc. The secondary objective was to identify markers of NS damage that correlate with the detection and progression of PNP.

2 Materials and methods

2.1 Subjects

This study included patients diagnosed with SSc who met the American College of Rheumatology/European Alliance of Associations for Rheumatology (ACR/EULAR) 2013 classification criteria and who received a consultation by rheumatologists at two leading Latvian hospitals between January 2016 and December 2021 (7). Using the hospital databases, patients with diagnostic codes M34.0–M34.9 based on the International Classification of Diseases, 10th Revision (ICD-10) were selected. Patients with connective tissue diseases other than SSc and patients with localised scleroderma were excluded. The age at disease onset was defined as the time of onset of the first non-Raynaud's SSc symptom. The skin condition was evaluated according to the modified Rodnan skin score (mRSS) by a rheumatologist (25).

This study was approved by the Rīga Stradiņš University medical ethics committee (Institutional Review Board reference no 22-2/481/2021). All participants provided written informed consent.

2.2 Methods

The enrolled subjects underwent a uniform evaluation of the PNS. First, the patients underwent a nerve conduction study (NCS) by a certified neurophysiology expert. Motor and sensory conduction were evaluated according to the PNP examination protocol (26). Each patient underwent an NCS of the bilateral upper extremities (the motor and sensory components of the

ulnar and median nerves) and the bilateral lower extremities (the motor component of the peroneal and tibial nerves and the sensory component of the sural nerve) to determine nerve conduction latency, amplitude, and velocity. The patients with abnormal NCS results—considering the normal values used in Latvian clinical practice—in more than one attribute for two separate nerves were diagnosed as having PNP. The patients were divided in two groups according to the NCS results. The first group included patients with PNP, while the second included patients without PNP.

The patients were also evaluated with the Health Assessment Questionnaire Disability Index (HAQ-DI). The use of personal assistance or assistive devices were acknowledged. The scores from each of the eight sections were added together and then divided by eight to obtain the functional disability index. In addition, blood was collected from each patient. After separating the serum, aliquots were stored at −80°C prior to analyses.

The SSc-associated Abs were analysed using a commercial line immunoblot assay (EUROLINE Systemic Sclerosis Profile, Euroimmun). The EUROLINE Systemic Sclerosis (Nucleoli) Profile (IgG) contains 13 recombinant antigens: DNA-topoisomerase I (Scl-70), centromere proteins A and B (CENP-A and CENP-B, respectively), RNA polymerase III (subunits RP11 and RP155), fibrillarin, NOR-90, Th/To, PM-Scl-100, PM-Scl-75, Ku, platelet-derived growth factor receptor (PDGFR) and Ro-52. The detection and interpretation were carried out electronically using the Euroimmun EUROLineScan programme. A signal intensity of 0–5 (negative) and 6–10 (borderline) was considered negative, while a signal intensity of ≥11 was considered positive.

Several nervous system–specific Abs—namely Abs against myelin-associated glycoprotein (MAG) and anti-ganglioside Abs (GM1, GM2, GD1a, GD1b and GQ1b)—were evaluated with GanglioCombi® MAG enzyme-linked immunosorbent assay (ELISA) kits (Bühlmann Laboratories). A signal intensity of 0–29 (negative) and 30–49 (borderline) was considered negative, while a signal intensity of ≥50 was considered positive. These Abs were assessed in patients with PNP first. If the data suggested a significant change in these patients, then the other groups were evaluated.

Two potential serum PNS biomarkers—NfL and GFAP—were measured with a Single molecule array (Simoa) assay (Quanterix, Billerica, MA, United States). Fibroblast growth factor 21 (FGF21) and GDF15 were measured using commercially available ELISAs according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, United States). All measurements were performed in one round of experiments using one batch of reagents by board-certified laboratory technicians who were blinded to the clinical data. The intra-assay coefficients of variation, determined using internal control samples, were below 10%.

2.3 Data analysis

The data distribution was assessed with a normal Q–Q plot and the Shapiro–Wilk test. The Mann–Whitney U test was used to compare SSc-associated Abs, NfL, GFAP, GDP-15 and FGF21 between patients with and without PNP. Additionally, this test was used to compare NfL between the control group and patients with PNP. Differences in SSc-associated Abs between patients with and without PNP were assessed with the chi-square test of homogeneity or Fisher's test.

A binomial logistic regression was conducted to determine factors (age, sex, SSc duration, mRSS and HAQ-DI) related to patients with and without PNP. Forward and backward stepwise regression methods were used to build the model. All possible models and interactions were calculated. The Akaike information criterion (AIC) was used to select the best model. Additionally, receiver operating characteristic (ROC) curve analysis, to determine the area under the curve (AUC), was conducted to evaluate the performance of the regression model as binary classifier. An AUC >0.7 was considered to indicate good performance in distinguishing between patients with and without PNP. The Youden index was used to identify the optimal cut-off point.

3 Results

We initially recruited 103 Caucasian patients diagnosed with SSc (18 men and 85 women). Table 1 summarises the sex-specific clinical and Ab characteristics in these patients.

Among the 103 patients recruited for this study, three declined to undergo an NCS. Following the NCS, the remaining cohort of 100

TABLE 1 Sex-specific clinical and antibody characteristics in patients with systemic sclerosis.

		Men	Women	Total
Descriptive statistic	Number of patients	18	85	103
	Mean (standard deviation) age in years	60.06 (14.92)	61.66 (11.95)	61.38 (12.46)
	Mean (standard deviation) disease duration in years	8.95 (6.33)	15.14 (9.87)	14.06 (9.62)
Symptoms	Raynaud's phenomenon, *n* (%)	16 (88.88%)	71 (83.52%)	87 (84.46%)
	Mean (standard deviation) Modified Rodnan skin score	10.36 (12.95)	10.67 (8.78)	10.63 (9.41)
SSc-associated antibodies	Classical antibodies* *n* (%)	8 (44.44%)	52 (65.82%)	60 (61.86%)
	Scl-70 n (%)	4 (22.22%)	18 (22.78%)	22 (22.68%)
	CENP-A and CENP-B *n* (%)	4 (22.22%)	31 (39.24%)	35 (36.08%)
	RP11 and RP155 n (%)	0	3 (3.80%)	3 (3.09%)
	Novel antibodies** *n* (%)	9 (50%)	35 (44.30%)	44 (45.36%)

*Scl-70 (topoisomerase I), CENP-A and CENP-B (centromere proteins A and B, respectively), RP11 and RP155 (RNA polymerase III). **Fibrillarin, NOR-90, Th/To, PM-Scl-100, PM-Scl-75, Ku, platelet-derived growth factor receptor (PDGFR) and Ro-52.

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TABLE 2 Demographic, clinical and neurophysiological characteristics and comparisons of patients with systemic sclerosis and with or without polyneuropathy (PNP).

Variable	SSc without PNP 57 (57%)	SSc with PNP 43 (43%)	*p*-value
Sex, n (%)			0.0
Male	5 (29.41%)	12 (70.59%)	
Female	52 (62.65%)	31 (37.35%)	
Mean (standard deviation) age in years	57.30 (12.24)	67.07 (10.47)	<0.001
Mean (standard deviation) disease duration in years	12.48 (8.68)	16.26 (10.51)	0.049
Mean (standard deviation) modified Rodnan skin score	8.05 (9.14)	7.36 (9.67)	0.715
Raynaud's phenomenon, n (%)	51 (89.47%)	36 (83.7%)	0.860
Mean (standard deviation) nerve conduction study results			
Nervus peroneus			
Amplitude (mV)	3.32 (1.79)	2.10 (1.28)	<0.001
Velocity (m/s)	45.2 (11.1)	41.7 (3.43)	<0.001
Nervus tibialis			
Amplitude (mV)	8.38 (2.84)	4.90 (2.84)	< 0.001
Velocity (m/s)	46.5(2.58)	40.8 (3.20)	<0.001
Nervus suralis			
Amplitude (mV)	11.7 (6.54)	7.54 (4.73)	0.002
Velocity (m/s)	47.2 (12.2)	41.1 (1.75)	<0.001

patients with SSc was stratified into subgroups based on the presence or absence of PNP. We identified PNP in 43 patients, representing 43% of the cohort. Within this subset, 15 patients had sensory-motor demyelinating PNP, while 28 had sensory-motor axonal demyelinating PNP. Table 2 illustrates the distinctions in demographic, clinical, and neurophysiological characteristics between patients with SSc and with or without PNP.

We assessed SSc-associated Abs in 97 patients; they did not differ significantly between patients with and without PNP ($p > 0.05$). We assessed anti-MAG and anti-ganglioside Abs in 24 patients. All 24 patients had PNP based on the NCS results, but they did not present a significant increase in the Abs above the reference range.

We assessed potential PNS serum biomarkers—NfL, GFAP, GDF15 and FGF21—in 68 patients, 30 with PNP, 38 without PNP. Table 3 summarises the comparison of serum biomarkers concentration between patients with and without PNP. NfL, GFAP and GDF15 were significantly elevated in the presence of PNP ($p < 0.05$), with a moderate to high effect size ($r = 0.36–0.65$). We observed the most pronounced difference for NfL, with significantly lower levels in control subjects (median = 5.2, interquartile range [IQR] 4.3–7.4) compared with those with PNP (median = 15.3, IQR 11.8–25.0; U = 35.0, $p < 0.001$, $r = 0.93$).

The final binomial logistic model was significant ($\chi^2(3) = 30.8$, $p < 0.001$; Table 4). The AUC was 0.81, indicating strong performance in distinguishing between patients with and without PNP (Figure 1).

Our regression analysis revealed a strong association between the HAQ-DI score and the risk of developing PNP. A 1-point increase in the HAQ-DI score was significantly associated with a 95% higher likelihood of PNP (95% confidence interval [CI] 13–236%; $p < 0.001$). Based on the Youden index, individuals with an HAQ-DI score exceeding 0.63 had a greater than 50% probability of developing PNP. Age was also a significant predictor of PNP development. Each additional year of age was associated with a 9% increase in PNP risk (95% CI 4–14%; $p < 0.001$). Using the Youden index, individuals aged ≥63 years had a > 50% chance of developing PNP. Furthermore, we observed a significant sex difference in PNP risk. Women were 86% less likely to develop PNP compared with men (95% CI 46–97%; $p < 0.001$). Finally, we removed SSc duration and the mRSS from the final regression model due to their lack of statistical significance to the model.

TABLE 3 Comparison of biomarker levels in patients with systemic sclerosis (SSc) and with or without polyneuropathy (PNP).

Parameter	SSc without PNP 38 (55.88%)	SSc with PNP 30 (44.11%)	*p*-value	*r*
	Median (interquartile range)	Median (interquartile range)		
Nfl., pg/mL	9.8 (6.0–13.1)	15.3 (11.8–25.0)	<0.001	0.62
GFAP, pg/mL	77.1 (43.9–99.0)	100.5 (67.8–159.8)	0.011	0.36
GDF15, pg/mL	964.5 (705–1,389)	1681.5 (1303–2049)	<0.001	0.65
FGF21, pg/mL	130.7 (65.3–372.5)	148.3 (99.5–287.5)	0.501	NA

FGF21 Fibroblast growth factor 21; GDF15, growth/differentiation factor 15; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; NA, not applicable; r, effect size (Cohen's r).

4 Discussion

To our knowledge, this is one of the few studies on SSc that focuses on the involvement of the PNS, analysing both the prevalence of this complication and its pathogenesis and biomarkers of severity. We found a higher prevalence of PNP in SSc compared to data from other studies, possibly due to detailed and targeted assessment of the

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TABLE 4 Results of final regression model showing the patient's age, sex, and health assessment questionnaire disability index score as predictors of developing polyneuropathy.

					95% confidence interval of the odds ratio	
Predictor	Estimate	Z	*p*-value	Odds ratio	Lower	Upper
Intercept	−4.73	−3.11	0.002	0.01	0.001	0.17
Health assessment questionnaire disability index	0.67	2.39	0.017	1.95	1.13	3.36
Age	0.08	3.43	<0.001	1.09	1.04	1.14
Sex						
Female–Male	−2.00	−2.84	0.005	0.14	0.03	0.54

References: Dependent variable—patients without PNP; sex—male.

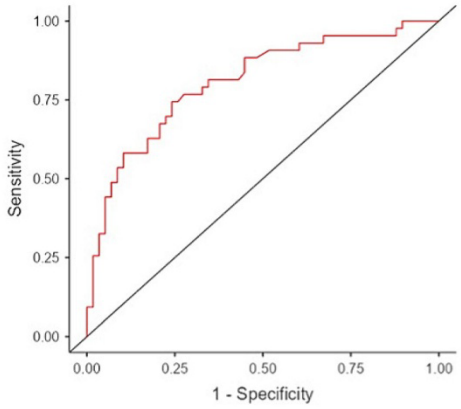

FIGURE 1
Performance of the final binomial logistic model based on receiver operating curve analysis that predicts polyneuropathy in patients with systemic sclerosis.

PNS. Moreover, the materials and methods used in these studies provide a wider range of results. A systematic review of 113 studies found a neuropathy prevalence of 27.37%, including 26% ($n = 556/2143$) with small fibre neuropathy and 10.8% ($n = 231/2143$) with large fibre neuropathy, however, titles and abstracts were not selected according to strict criteria for the neuropathies assessed (8). Confirmatory diagnostic tests for PNP in SSc varied according to study design (27–33). Some studies performed electrophysiological examinations, while others used imaging techniques, biopsies or other methods (4, 27–34). We believe that the high prevalence of PNP in our study can be explained by the fact that we worked with a relatively large study group and that all subjects were assessed using both clinical symptoms and electrophysiological methods, where motor and sensory components were studied on multiple nerves in each limb.

Historically, the classical SSc-specific or SSc-associated Abs—anti-topoisomerase Abs (ATAs), anti-centromere Abs (ACAs) and anti-RNA polymerase Abs (ARAs)—have received the most attention (35). Currently, novel Abs are assessed in addition to the classical Abs, and their presence in different clinical phenotypes remains a research goal (36). Only a few studies have evaluated the association of these classical Abs with neuropathies in SSc, and the results have varied greatly. In a 1994 study, 35% of patients with SSc presented neurological symptoms, and 73% of them had either ARAs or ATAs, but not ACAs (37). On the contrary, in a 2021 systemic review, the authors mentioned that ACAs are a risk factor for non-compression neuropathies in patients SSc (8). Similarly, in Brazilian study of 63 patients with SSc, seven were diagnosed with PNP, of whom 6 had ACAs and 1 had ARAs (38). In a Spanish study, ARAs, ATAs and ACAs were present in patients with SSc and PNP, but the authors did not provide the statistical analysis (39).

Expanded SSc-associated Ab panels have started to play an increasingly important role in research and clinical practice. Although there is wide spectrum of clinical phenotypes in SSc, information regarding NS involvement is frequently missing (40). We could not find published data about expanded SSc-associated Abs in patients with SSc and NS damage. Interestingly, none of our patients was positive for anti-PDGFR Abs, and only one patient was positive for anti-Fib Abs. The most common SSc-associated Abs were anti-Ro52 Abs, ACAs and ATA. Only three patients (3%) were positive for ARAs, a lower frequency than for Abs that are not included in the SSc classification criteria: anti-Ku, anti-PM100, anti-Th/To and anti-NOR90 Abs. We did not find significant association between any of the SSc-associated Abs and the presence of PNP.

In autoimmune neuropathies, gangliosides are one of the most frequent targets of Abs (41). Gangliosides are nerve fibre glycoproteins that play an important role in both impulse transmission and nerve fibre regeneration. Anti-ganglioside Abs are often detected in the serum of patients with Guillain–Barré syndrome (37–78% of the cases) (42). They have been studied in patients with systemic lupus erythematosus and neuropsychiatric manifestations: the authors detected Abs more frequently in patients with neuropsychiatric manifestations compared with the asymptomatic group (43). There are very few studies on anti-ganglioside Abs in patients with SSc. In 1994, 34 patients with scleroderma, of whom 28 had PNP, were evaluated for the presence of anti-GM1 Abs. The levels were lower in these patients compared with healthy individuals, and there was no association with the development PNP (44). In our study, performed almost 30 years later, we also could not find a significant association between anti-MAG or anti-ganglioside Abs and the development of PNP in patients with SSc. Due to the lack of data on the association between PNP in SSc and specific nervous system–specific Abs we initially determined Abs only in a subset of patients with definite PNP, randomly selected. We would most likely not expect a significant change if Abs were detected in all patients with PNP, and even if they were detected at low titres, these data would only show false positives and unnecessarily confound the overall significance of the study.

In this study, no Abs were associated with a more frequent development of PNP in patients with SSc. At present, immune-mediated peripheral nerve damage in SSc remains questionable. In the treatment of PNP in patients with SSc, the role of immunosuppressive drugs remains equivocal and, according to our data, there is no reason to expect them to be efficacious. Additional research is necessary to predict PNS damage in patients with SSc so that they can be managed appropriately.

In recent years, successful new candidate serum biomarkers have been identified for SSc-associated interstitial lung disease (ILD), including surfactant protein D (SP-D), Krebs von den Lungen 6 glycoprotein (KL-6), CCL18 and intercellular adhesion molecule 1 (ICAM-1) (45, 46). For ILD, there has been a focus on searching for biomarkers in SSc that are also related to skin involvement and vascular injury (20, 47). Unfortunately, researchers have not yet evaluated serum biomarkers for PNS damage in patients with SSc. Thus, we chose to evaluate the most promising biomarkers based on the connection to the PNS. Of these four serum biomarkers—NfL, GFAP, GDF15 and FGF21—three of them showed promise as candidate PNP serum biomarkers in patients with SSc.

NfL stand out as novel biomarker for early diabetic sensorimotor PNP; there are possible similarities in vascular injury in both diabetic PNP and PNP in SSc (21). Our findings confirmed the already established significant role of NfL as a serum biomarker for neuropathies of different aetiologies (48).

A less-studied biomarker in PNP is GFAP, which has mostly been associated with central NS damage due to its predominant secretion from astrocytes. However, studies have demonstrated the presence of GFAP in the PNS (49, 50). Researchers have reported elevated serum GFAP levels in chronic neuropathies like chronic sensory-motor axonal neuropathy and chronic inflammatory demyelinating PNP (24). Unlike NfL, GFAP has not been widely evaluated in diabetic neuropathies, reducing the likelihood of linking this biomarker to neuropathy caused by vascular injury. We did not find any studies of GFAP in SSc, but serum GFAP was significantly elevated in patients with SSc and PNP.

GDF15 and FGF21 have less association with the NS. GDF15 is a cytokine belonging to the transforming growth factor beta superfamily. Elevated GDF15 levels are observed in inflammation, myocardial ischaemia and tumours (51). Serum GDF15 levels were elevated in patients with pulmonary hypertension (PH) and SSc compared with patients with SSc but not PH, as well as in patients with SSc, ILD and more pronounced skin lesions (52–54). There is evidence of increased GDF15 secretion by Schwann cells in nerve injury, and increased GDF15 levels have been found in patients with diabetic neuropathy, mainly with more pronounced manifestations of metabolic syndrome (23, 55, 56). We found elevated serum GDF15 levels in the patients with SSc and PNP compared with the patients with SSc but not PNP. Of note, there have been no other studies that evaluated this serum biomarker in patients with SSc and neuropathies.

Only FGF21 showed no significant change between the SSc with PNP and the SSc without PNP groups. This pleiotropic hormone—considered to be a major regulator of energy homeostasis—is mainly synthesised in the liver, pancreas and adipose tissue (57, 58). Recently, researchers have shown that FGF21 has regenerative capability in the PNS by suppressing oxidative stress, and the FGF21 levels were elevated in patients with diabetic neuropathy after aerobic training (59, 60). While there have been no studies on FGF21 levels in patients with SSc, we found that FGF21 levels did not change significantly in patients with SSc and PNP, indicating that FGF21 has less of a connection to the NS compared with other biomarkers. FGF21 expression is significantly increased in the muscles of mice with mitochondrial myopathies, where its levels are directly related to the presence of cytochrome oxidase negative fibres, a marker associated with the severity of the disease. This observation underscores the relevance of FGF21 in muscle pathology, especially under conditions characterised by damaged mitochondrial function (61, 62).

We found that the axonal demyelinating form of PNP was the most common in our patients with SSc. The absence of significant correlations between Abs and PNP has led us to consider alternative pathogenic mechanisms. Comparisons between the patients with and without PNP showed several intriguing differences: the patients with PNP were generally older, with an average age of 67 years compared with 57 years, and it was more prevalent in men (66% compared with 36%). These observations indicate that ageing, metabolic factors and ischaemic mechanisms may contribute significantly to the emergence of axon neuropathies, reflecting the patterns observed in cases of idiopathic PNP. In the literature, researchers have noted a higher prevalence of idiopathic PNP in people aged >60 years. Similar results have been reported in studies focusing on chronic axon idiopathic PNP in people aged >60 years, with a 3:2 male-to-female ratio (63, 64). As the name suggests, the condition is idiopathic, and metabolic factors are most strongly considered to be involved in the aetiology, but microvasculopathy identified in biopsies shows a different pattern than in diabetic neuropathies (64, 65). These coincidences lead us to suspect sequential development of PNP in patients with SSc over time, associated with ageing and a logical progression of the disease with more pronounced vasculopathy and metabolic factor–associated effects. Our regression analysis confirmed this view: it showed that age is a significant predictor of PNP development.

A deeper look into the serum biomarkers we evaluated in patients with SSc revealed three biomarkers associated with PNP. NfL and GFAP had already been shown to be associated with axonal injury, strengthening our above hypothesis of the development of PNP in SSc (24, 66). On the other hand, GDF15 and FGF21 have mostly been associated with mitochondrial stress and subsequent metabolic changes (67, 68). Interestingly, they behaved differently in our study. While the FGF21 levels were slightly higher in patients with SSc and PNP, the difference was not significant. The GDF15 levels were significantly elevated in patients with SSc and PNP, similarly to patients with diabetic neuropathies, were metabolic damage plays an important role (23). We believe additional studies that detect muscle damage and loss are needed to further investigate the role of mitochondrial damage and metabolic markers in patients with SSc.

Our results suggest that the use of serum biomarkers in clinical environments may facilitate early identification of PNS damage in patients with SSc. By dynamically monitoring biomarkers such as the NfL, GFAP and GDF15, it could be possible to detect deterioration of nerve function without further electrophysiological testing. However, research focusing on hereditary neuropathy has challenged the effectiveness of neurofilament fluctuations as indicators of disease progression, suggesting that these markers may not be suitable for tracking slow-moving diseases due to their lack of specificity and their tendency to reflect general rather than specific nerve damage (69).

A strength of this study is the choice of the group of interest: PNP is one of the complications of SSc that seems to have been neglected. To our knowledge, this is the first study that has extensively defined serum tests of different significance in patients with SSc and PNP. Moreover, we analysed both the immune pathogenesis of PNP and the reflection of nervous system damage in serum biomarkers in a univariate manner. However, several limitations must be acknowledged. First, the study did not include a healthy control group, which might have provided more evidence for our findings linking the development of PNP in SSc patients also to natural ageing. Secondly, this study focused on the development of neuropathy as the main complication of SSc, without providing a full description of the patients' other organ involvement such as ILD, PH and others. We included the presence of Raynaud's phenomenon, which partially characterises vasculopathy, and the mRSS, which partially characterises disease severity by skin involvement, but it would also be very useful to include more clinical symptoms. However, the relationship of the different clinical manifestations of the disease to the involvement of the PNS must be demonstrated in future projects.

5 Conclusion

There was no association between SSc-associated or other inflammatory neuropathy-associated Abs and the development of PNP in patients with SSc. The development of PNP in patients with SSc is most likely due to ageing, natural progression and the sequelae of the disease. Several serum biomarkers—NfL, GFAP and GDF15—could be used as relevant diagnostic biomarkers for PNP in patients with SSc. Future studies are warranted to validate the diagnostic efficacy of these biomarkers and to unravel the complex interplay of factors leading to PNP in patients with SSc. This endeavour should ultimately pave the way for novel therapeutic strategies and a more nuanced understanding of this multifaceted disease.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Rīga Stradiņš University medical ethics committee (Institutional Review Board reference no 22-2/481/2021). All participants provided written informed consent. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

KI: Writing – original draft, Writing – review & editing. MZ: Writing – review & editing. KB: Writing – review & editing. HZ: Writing – review & editing. NK: Writing – original draft, Writing – review & editing. VĶ: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. HZ is a Wallenberg Scholar and a Distinguished Professor at the Swedish Research Council supported by grants from the Swedish Research Council (#2023-00356; #2022-01018 and #2019-02397), the European Union's Horizon Europe research and innovation programme under grant agreement No 101053962, Swedish State Support for Clinical Research (#ALFGBG-71320), the Alzheimer Drug Discovery Foundation (ADDF), United States (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C, #ADSF-21-831377-C, and #ADSF-24-1284328-C), the Bluefield Project, Cure Alzheimer's Fund, the Olav Thon Foundation, the Erling-Persson Family Foundation, Familjen Rönströms Stiftelse, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2022-0270), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), the European Union Joint Programme—Neurodegenerative Disease Research (JPND2021-00694), the National Institute for Health and Care Research University College London Hospitals Biomedical Research Centre, and the UK Dementia Research Institute at UCL (UKDRI-1003). KB is supported by the Swedish Research Council (#2017-00915), the Alzheimer Drug Discovery Foundation (ADDF), United States (#RDAPB-201809-2016615), the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden, Sweden (#FO2017-0243), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986), and the European Union Joint Programme—Neurodegenerative Disease Research (JPND2019-466-236). He is supported by the Swedish Research Council (#2017-00915 and #2022-00732), the Swedish Alzheimer Foundation (#AF-930351, #AF-939721, #AF-968270, and #AF-994551), Hjärnfonden, Sweden (#FO2017-0243 and #ALZ2022-0006), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986 and #ALFGBG-965240), the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236), the Alzheimer's Association 2021 Zenith Award (ZEN-21-848495), the Alzheimer's Association 2022-2025 Grant (SG-23-1038904 QC), La Fondation Recherche Alzheimer (FRA), Paris, France, the Kirsten and Freddy Johansen Foundation, Copenhagen, Denmark, and Familjen Rönströms Stiftelse, Stockholm, Sweden.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Serum Metabolomic Profiling Reveals Differences Between Systemic Sclerosis Patients with Polyneuropathy

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Abstract

Metabolome studies have already been carried out in patients with systemic sclerosis (SSc). However, polyneuropathy (PNP) as a complication of SSc has been overlooked in these studies. To the best of our knowledge, this is the first study to examine metabolic changes in SSc patients with PNP. Patients with SSc (n = 62) and a healthy control group (HC) (n = 72) were recruited from two Latvian hospitals. Blood plasma samples were collected and analyzed using an LC-MS-based targeted metabolomics workflow. Our plasma sample cohort consisted of 62 patients with SSc, 42% of whom had PNP. Differences between SSc patients and the HC group with fold changes > 2 were observed for aspartic acid, glutamic acid, valine, and citrulline, all of which were reduced. In contrast to the SSc to HC discrimination, no metabolites had a high fold change; only minor changes were observed using FC > 1.3. We identified elevated concentrations of kynurenine, asparagine, and alanine. Changes in metabolite regulation in patients with SSc, compared to controls, are not identical to those observed in SSc patients with PNP, with elevated concentrations of kynurenine and alanine specific to the SSc subgroup. SSc patients with PNP should probably be considered a distinct population with important metabolomic features.

Keywords: systemic sclerosis; modified polyneuropathy; peripheral nerve system; metabolome; metabolic profiling; modified Rodnan skin score; Raynaud's phenomenon

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Academic Editor: Stergios Boussios

Received: 19 June 2025
Revised: 21 July 2025
Accepted: 22 July 2025
Published: 24 July 2025

Citation: Ivanova, K.; Schiemer, T.; Vaska, A.; Kurjāne, N.; Kenina, V.; Klavins, K. Serum Metabolomic Profiling Reveals Differences Between Systemic Sclerosis Patients with Polyneuropathy. *Int. J. Mol. Sci.* **2025**, 26, 7133. https://doi.org/10.3390/ijms26157133

1. Introduction

Systemic sclerosis (SSc) is a rare autoimmune connective tissue disease characterized by vascular insult, autoimmunity, and tissue fibrosis [1,2]. As a complex disorder, it can affect multiple systems, and its clinical presentation varies widely between individuals [1,2]. Several metabolome studies have already been carried out in patients with SSc, revealing

changes in several metabolites compared to healthy controls (HCs) [3]. Some of these studies have focused on different manifestations of SSc in patients with interstitial lung disease (ILD) or marked modified Rodnan skin score (mRSS) [4,5]. However, it is notable that polyneuropathy (PNP) as a complication of SSc has been ignored in metabolome studies. PNP, a condition involving multiple peripheral nerves that occurs in SSc, contributes to disability and reduced quality of life [6]. Previous studies have documented the involvement of the nervous system (NS) in SSc, although the prevalence has varied widely, ranging from 17% to 40% [7–11]. One possible reason for this variation is the markedly different approaches to PNP detection, ranging from questionnaires to nerve biopsies [12–20]. Therefore, we pre-determined the prevalence of PNP in our study group of SSc patients, which was unexpectedly high [6]. These data further demonstrated the need for metabolome studies in SSc patients with PNP. Understanding the metabolomic alterations in SSc and its related PNP has the potential to uncover novel biomarkers and therapeutic targets, providing opportunities for improved management and outcomes for patients suffering from this complex disease.

Here, to the best of our knowledge, we present the first study of metabolic changes in SSc patients with PNP. Previous studies have included patients with PNP without singling them out in the general SSc population.

The main objective of our study was to determine the metabolome in the SSc patient group compared to HCs and to compare the results with previous metabolome studies in SSc patients. Additionally, our objective was to isolate patients with SSc with diagnosed PNP and to compare the metabolome of this group with patients with SSc without PNP. This approach provided a better understanding of the pathogenesis of PNP in patients with SSc.

2. Results

2.1. Characteristics of the Study Cohort

Our plasma sample cohort consisted of 62 patients with SSc, of which 26 had PNP (42%). Both total SSc patients and the SSc with PNP subgroup were predominantly females (82% and 77%, respectively); however, PNP was much more common among males. In the SSc with PNP subgroup, the mean age was 15 years higher, and the mean SSc disease duration was 8 years longer than the SSc without PNP subgroup. For further information, see Table 1.

Table 1. Description of the subgroups of the SSc cohort without and with PNP.

Variable	SSc Without PNP	SSc with PNP	*p*-Value
Sex, n (%)			0.35
Male	5 (45.5%)	6 (54.5%)	
Female	31 (60.8%)	20 (39.2%)	
Mean age in years (standard deviation)	54.94 (12.533)	69.95 (7.893)	<0.05
Mean disease duration in years (standard deviation)	10.44 (6.596)	18.08 (10.476)	<0.05
Mean modified Rodnan skin score (standard deviation)	7.26 (9.053)	7.65 (8.158)	0.86
Raynaud's phenomenon, %	86	92	0.45

2.2. Metabolites in SSc Patients

We first compared plasma metabolites of all SSc patients with HCs, see Table 2.

Table 2. Description of the SSc cohort group and HC.

Variable		SSc	HC	*p*-Value
Sex, n (%)				0.77
	Male	11 (17.74%)	14 (19.72%)	
	Female	51 (82.26%)	57 (80.28%)	
Mean age in years (standard deviation)		61.19 (13.06)	53.18 (18.65)	<0.05

Based on the PCA analysis (Figure 1a), there was no clear separation between these two groups. However, concentrations of several metabolites changed significantly in the plasma of SSc patients (Figure 1b). The most significant differences with fold changes > 2 were observed for aspartic acid, glutamic acid, valine, and citrulline, all of which were reduced (Figure 1b,c). In particular, the volcano plot showed a general reduction in metabolite concentrations, except glutamine with a fold change >1.5 (A list of all significant metabolites is found in Supplementary Table S1).

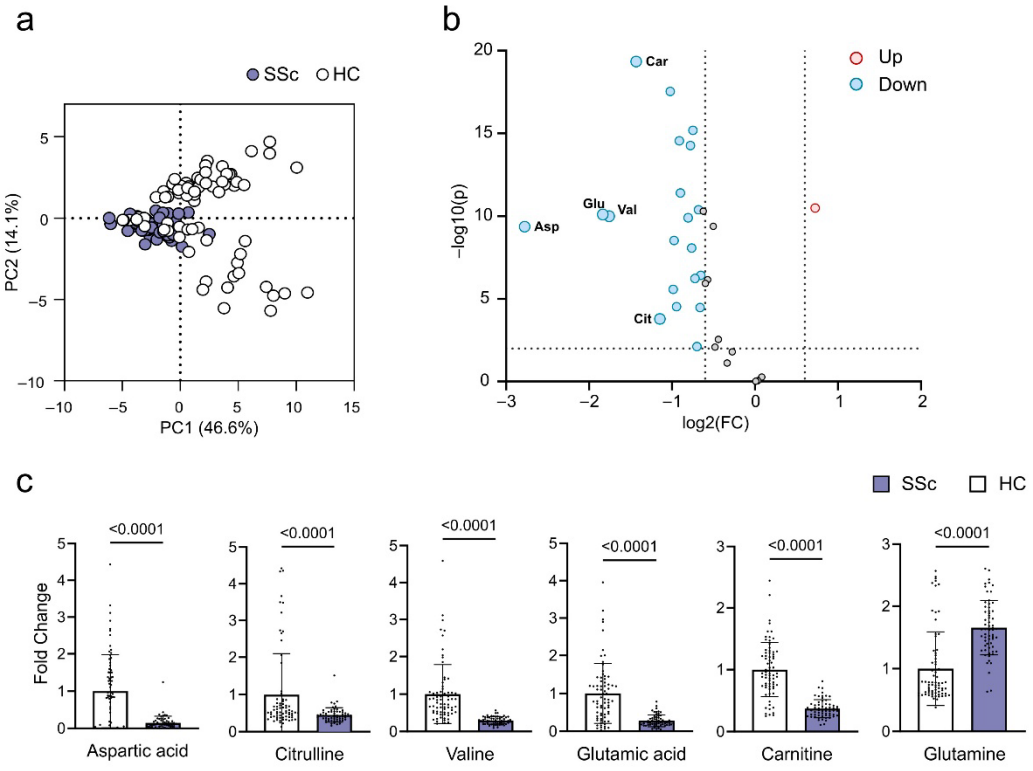

Figure 1. Plasma metabolite changes in systemic sclerosis (SSc) patients compared to healthy controls (HCs). (**a**) PCA plot of SSc patients (purple) and HCs (white), (**b**) Volcano plot of increased (red) and decreased (blue) metabolites using a significance threshold of FC > 1.5 and *p*-value < 0.05; metabolites with FC > 2 are annotated with 3-letter abbreviation. (**c**) Bar plots of metabolites with FC > 2, shown as a fold change relative to the HC average. The bars represent the mean of SSc patients (purple) and HCs (white), individual measurements are overlayed as dots, *p*-values are indicated above each bar. Abbreviations: Asp, aspartic acid; Car, carnitine; Cit, citrulline; Gln, glutamic acid; Val, valine; PC, principal component.

2.3. Disease Prediction Models

Following the hypothesis of using blood plasma metabolites as potential biomarkers for diseases, we tested our dataset on its ability to differentiate SSc from HCs (Supplement Figure S1). We used metabolite and two-metabolite ratios to build disease prediction models. We used both significantly changed metabolites (Figure 2a) and metabolites with high predictive scores (Figure 2b) to build models. Model 1 uses metabolites identified through their fold changes, which resulted in the combination of four metabolites: aspartic acid, glutamic acid, glutamine, and carnitine. Model 2 uses metabolites with high predictive scores, combining ornithine and the metabolite ratios glutamine/valine and creatinine/glutamine (Figure 2d). Both models separated patients from controls with an AUC of 0.954 and 0.993, respectively; model 1 gave a slightly better separation of the two groups (Figure 2a,b). The metabolites used for both models did not have an age correlation in SSc, whereas in HCs glutamic acid showed a positive correlation (Supplement Table S2). Despite this, the removal of glutamic acid from model 1 had no impact on model performance (Supplement Figure S2).

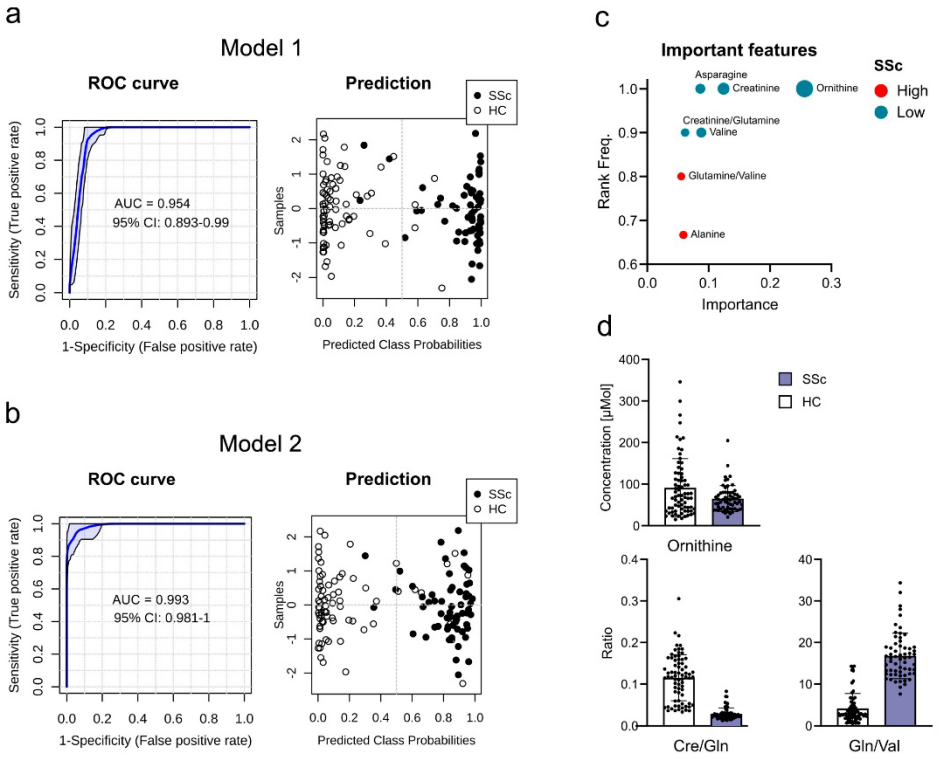

Figure 2. Prediction models distinguishing systemic sclerosis (SSc) patients from healthy controls (HCs). Metabolites were selected based on (**a**) univariant significant changes, or (**b**) high predictive scores. Models were built on linear support vector machine, ROC curves and confidence intervals (CIs) were averaged and calculated from 100 cross-validations, respectively. Class prediction of samples is shown to demonstrate separation power. (**c**) Importance scores of the 7 highest-ranking metabolites for linear support vector machine prediction used for metabolite selection of model 2. (**d**) Plasma metabolite concentrations and their ratios used in model 2. The bars represent mean values for SSc (purple) and HCs (white), individual measurements are overlaid as dots. Abbreviations: Cre, creatinine; Gln, glutamine; Val, valine; ROC, receiver operating characteristic curve; AUC, area under the curve.

2.4. Discrimination of SSc Patients with PNP

We further subdivided the SSc patients based on the diagnosis of PNP. Again, the PCA analysis shows no separation between subgroups (Figure 3a). In contrast to SSc for the discrimination with HCs, no metabolites had a high fold change (>1.5) or *p*-value (<0.05) used for SSc discrimination. There were minor changes using a lower cutoff of FC > 1.3 and *p*-value < 0.1 [21]. When applying these cutoffs, we identified an elevated concentration of the tryptophan metabolite kynurenine and the amino acids asparagine and alanine (Figure 3b). Kynurenine and alanine were specific for the SSc subgroup with PNP, while asparagine was also found to have a reduced concentration when comparing SSc without PNP with HCs (Figure 3c). These findings prompted us to compare significant changes in the total SSc and SSc subgroup with HCs. Most of the metabolite changes were shared between all groups. Arginine and proline changes were only found in SSc with the PNP subgroup, whereas ornithine was only found in SSc without PNP (Figure 3d). Due to the minor changes in the SSc with the PNP subgroup compared to the SSc without the PNP subgroup, we were unable to construct prediction models that could separate these two groups (Supplement Figure S3).

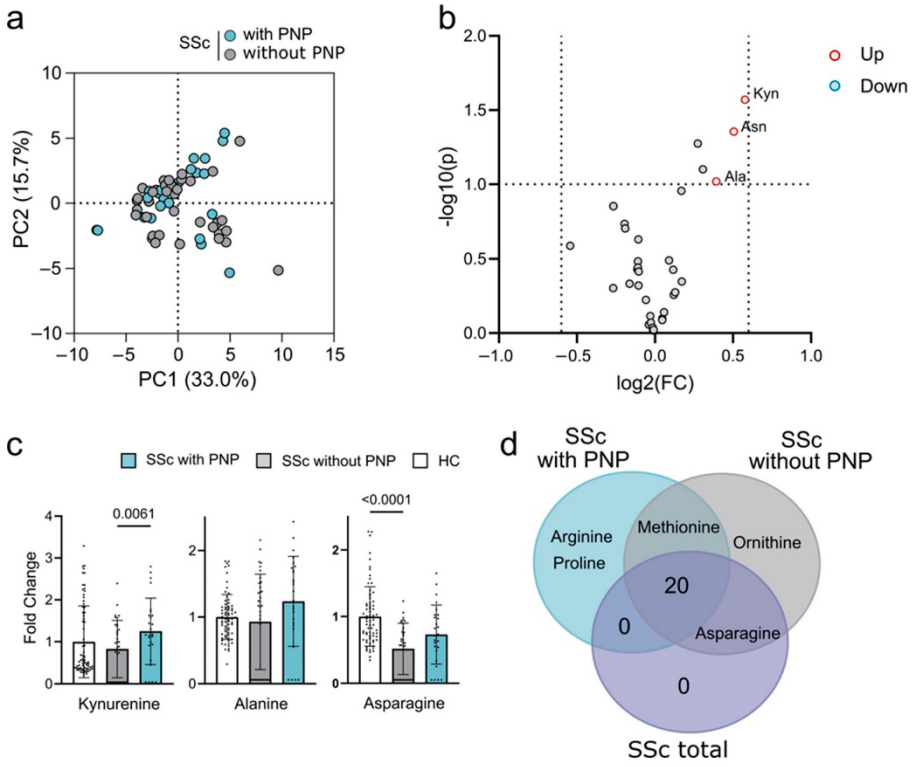

Figure 3. Plasma metabolite changes of SSc patients with and without polyneuropathy (PNP). (**a**) PCA plot of SSc patients with (blue) and without (grey) PNP. (**b**) Volcano plot of increased (red) and decreased (blue) metabolites using a significance threshold of FC > 1.3 and *p*-value < 0.1. Significant metabolites are annotated with a 3-letter abbreviation. (**c**) Bar plots of metabolites with FC > 1.3, shown as a fold change relative to the HC average. The bars represent the mean of SSc patients with (blue) and without (grey) PNP, and HCs (white), individual measurements are overlayed as dots, and *p*-values are indicated above each bar. (**d**) Venn diagram showing the differences from HCs for all SSc patience (purple) and subgroups with (blue) or without (grey) PNP. Abbreviations: Kyn, kynurenine; Asn, asparagine; Ala, alanine.

3. Discussion

The metabolome, a collection of small compound metabolites in an organism, offers insight into the biochemical changes and potential biomarkers associated with diseases such as SSc [22]. Metabolites can serve as biomarkers for diagnosis, prognosis, and monitoring of disease progression or response to treatment [23]. Analyzing metabolic changes can shed light on the underlying mechanisms of SSc and its complications, including PNP. To our knowledge, this is the first metabolome analysis in SSc patients, with an emphasis on the presence of PNP.

Initially, differences in metabolite regulation were sought between the SSc and HC groups. SSc is a heterogeneous disease with different manifestations and risks of complications. Despite this heterogeneity, previous studies have detected several changes in metabolite regulation in SSc patients. Our study also found several significant differences between SSc patients and HCs. Some of these changes were similar, but some data were distinctly different from previously published data, summarized in Table 3.

Table 3. Comparison of the metabolite changes in SSc found in our study compared to the literature. Metabolites without significant change or not mentioned in the paper are indicated with N.A.

Metabolite	Our Study	Murgia et al. 2018 [5]	Jud et al. 2023 [4]	Guo et al. 2023 [21]	Bögl et al. 2022 [24]	Smolenska et al. 2019 [25]	Bengtsson et al. 2016 [26]	Ottria et al. 2020 [27]
Aspartic acid or aspartate	↓	⌄	↑ with higher mRSS	N.A.	N.A.	N.A.	↑	N.A.
Citrulline	↓	N.A.	correlated with other amino acids	N.A.	^	↓ with scleroderma	N.A.	N.A.
Carnitine	↓	N.A.	N.A.	↑ with higher mRSS	N.A.	N.A.	N.A.	↑
Valine	↓	^ in dsSSc	↑ with a higher DETECT score	N.A.	N.A.	↑ in lung involvement	N.A.	N.A.
Glutamic acid	↓	⌄ (↑ in dsSSc)	↑ with higher mRSS	↓	N.A.	↑ in calcinosis and telangiectasia	N.A.	N.A.
Glutamine	↑	^	↓ with higher mRSS	N.A.	N.A.	↑	N.A.	N.A.

We found that the concentration of aspartic acid, or aspartate, was significantly reduced in SSc patients compared to HCs. An important capability of aspartate is to promote macrophage polarization [28]. In SSc, at the peak of the late immune response, endothelin-1 induces polarization of M2, thus potentiating profibrotic activity [29,30]. These results suggest that in SSc, tissue damage is not effectively repaired due to the increased and sustained release of cytokines and growth factors from M2 macrophage cells [31]. In the study by Murgia et al., an analysis of the metabolic profile of SSc patients also showed a reduced concentration of aspartate [5]. The effects of different clinical manifestations of SSc on aspartate levels showed a correlation with mRSS [4]. Significant changes in aspartic acid in patients with SSc detected in our study and in previously published studies may indicate changes in macrophage activation, possibly more pronounced profibrotic activation, as evidenced by correlation with severity of skin involvement, thus signaling macrophage dysregulation.

Another finding in our study was the reduced citrulline concentration in the samples of patients with SSc. Citrulline is an effective substitute for restoring nitric oxide (NO) production in situations of limited arginine availability [32]. NO produced by endothelial cells relaxes vascular smooth muscles, resulting in vasodilation and maintaining the patency of small blood vessels and blood flow through the microvasculature [33]. In SSc, the microvascular bed is the target of an immune-inflammation injury that leads to dysregulation of vascular tone control and results in progressive disorganization of the vascular

architecture. In the Smolenska et al. 2019 study, citrulline showed a trend similar to our study, with a lower concentration in patients with scleroderma [25]. In contrast, citrulline was markedly elevated in patients with SSc in the study by Bögl et al. 2022, especially in the diffuse skin SSc group [24]. Although the data from our study may differ from previously published data, the elevated concentration of citrulline may still be associated with the development of skin fibrosis. At the same time, the reduced concentration observed in our study represents an alteration in NO synthesis that could lead to more severe vasculopathy and serve as a marker of vasculopathy in the future.

Carnitine was found to be yet another metabolite with reduced concentration in SSc patients. In the Ottria et al. 2020 study of 27 individuals with SSc, carnitine was elevated in plasma and monocyte-derived dendritic cells [27]. The reduced concentration of carnitine found in our study could be explained by changes in muscle mass in patients with SSc. Not only is the skin and subcutaneous tissue affected, but the normal muscle structure, including smooth muscle and skeletal muscle, is altered, with a general loss of muscle mass [34,35]. Further studies could confirm a correlation between muscle mass and carnitine in patients with SSc.

Valine concentration was reduced in SSc patients. Valine improves cellular mitochondrial function and protects against oxidative stress [36]. There was no significant difference in the Murgia et al. 2018 [5] study between SSc and healthy controls in valine regulation (5). However, patients with diffuse cutaneous SSc (dcSSC) had higher concentrations than patients with limited cutaneous SSC (lcSSc). SSc patients with lung involvement and subclinical pulmonary arterial hypertension (PAH) were found to have higher concentrations of valine as well [4,25].

The last metabolite with reduced concentration in SSc patients was glutamic acid. It is the most abundant central nerve system (CNS) transmitter. Recent data indicate that inflammatory mediators might regulate extracellular glutamic acid concentrations under physiological and pathological conditions [37]. Other studies have also found reduced concentrations of glutamic acid in patients with SSc but higher levels in dcSSc [5,21]. The consensus results of many studies suggest that reduced glutamic acid concentration in SSc patients is not associated with a specific disease complication such as vasculopathy or fibrosis but is a common finding in all SSc patients. It is plausible that these unambiguous changes suggest a role for glutamic acid in SSc immunoregulation and that a reduced concentration of glutamic acid may be one of the markers of persistent damage due to autoimmunity.

Glutamine was the only metabolite with elevated concentration in SSc patients compared to controls. Interestingly, glutamine uptake, but not glutamic acid, is enhanced during T-cell activation [38]. Studying SSc fibroblasts, all showed an increase in glutaminase expression, suggesting that altered glutamine metabolism may be a ubiquitous trait in SSc [39].

Similarly to our study, reduced glutamic acid concentration and elevated glutamine concentration have been reported before [4,5,25]. However, glutamine was one of the few metabolites to have an elevated concentration in patients with lcSSc, compared to patients with dcSSc (5). It is already speculated that elevated glutamine concentration can increase collagen synthesis with subsequent fibrosis of the skin and internal organs [40,41].

In our study, the potential biomarkers identified by fold changes analysis were aspartic acid, glutamic acid, glutamine, and carnitine.

Aspartate has been found to significantly change in SSc patients compared to HCs in other studies as well. Similarly to our findings, aspartate concentration was significantly reduced in SSc patients in the Murgia et al. 2018 study with an AUC > 0.8 [5]. However, Bengtsson et al. found that the concentration of aspartic acid was significantly elevated in SSc patients compared to HCs [26]. This worrying difference could be explained by the

small number of SSc patients enrolled (19 subjects) and the significant difference in prior treatment with immunosuppressive agents between studies; in the study by Bengtsson et al., patients had not previously been treated with azathioprine, cyclophosphamide, cyclosporine A, methotrexate, or mycophenolate mofetil [26]. We excluded only patients who previously received cyclophosphamide due to neurotoxicity. We found no similar data on evidence for glutamic acid, glutamine, and carnitine as diagnostic biomarkers in SSc.

We report high predictive scores for the glutamine/valine and creatinine/glutamine ratios. An increased glutamine/valine ratio could indicate increased glutaminolysis, possibly to promote proliferation and altered nitrogen metabolism, as more is studied in cancer studies [42]. We could not find studies with similar data in which two metabolite ratios were used to build disease prediction models. Glutamine was the only metabolite with a significantly elevated concentration in patients with SSc compared to HCs, and by verifying similar data in other studies, we can be more confident of the ability of these metabolite ratios to perform as biomarkers in SSc [5,25].

The findings described above were equivalent to previous metabolome studies in patients with SSc. Our study isolated a previously unstudied group of SSc patients with PNP. There is still no consensus on the pathogenesis of PNP development, so metabolome research may reveal new reasons for the development of neuropathies.

Differences in some metabolites were observed between SSc patients with and without PNP. In contrast to SSc versus HC discrimination, no metabolites had a high fold change (>1.5) or a *p*-value (<0.05). There were minor changes with FC > 1.3 and the *p*-value < 0.1.

Due to the lack of published metabolome studies in SSc that specifically isolate patients with PNP, we first examined altered metabolomes in other metabolome studies of SSc. We summarized the findings in Table 4.

Table 4. Comparison of the metabolites differing between systemic sclerosis (SSc) patients with and without polyneuropathy (PNP) in this study, compared to the literature. Metabolites without significant change or not mentioned in the paper are indicated with N.A.

Metabolite	Our Study	Murgia et al. 2018 [5]	Jud et al. 2023 [4]	Guo et al. 2023 [21]	Bögl et al. 2022 [24]	Smolenska et al. 2019 [25]	Bengtsson et al. 2016 [26]	Ottria et al. 2020 [27]
Kynurenine	↑	N.A.	N.A.	N.A.	↑ in dcSSc	N.A.	N.A.	N.A.
Asparagine	↑	N.A.	↓ with higher mRSS	N.A.	N.A.	↓ with scleroderma	N.A.	N.A.
Alanine	↑	↓	↑ in lcSSc	N.A.	reduced concentration	↑ in dcSSc	↓	N.A.

A possible similarity in the development of PNP in patients with SSc lies in the development of diabetic neuropathy (DN). Therefore, we decided to investigate previous metabolome studies in patients with DN, specifically comparing data on altered metabolites in our study in patients with PNP. We summarized the findings in Table 5.

Table 5. Comparison of the metabolites differing between systemic sclerosis (SSc) patients with and without polyneuropathy (PNP) in this study, compared to the metabolite changes in diabetic neuropathy (DN) in the literature. Metabolites without significant change or not mentioned in the paper are indicated with N.A.

Metabolite	Our Study	Staats Pires et al. 2020 [43]	Shao mm et al. 2022 [44]
Kynurenine	↑	↑ in DM Type 1 patients with neuropathic pain compared to diabetic controls	↑ in patients with severe DN compared to patients with mild DN; and without DN
Asparagine	↑	N.A.	↑ in DM Type 2 patients with PNP compared to DM Type 2 patients without DN
Alanine	↑	N.A.	↑ in patients with severe DN compared to patients with mild DN; significantly. ↑ compared to DM Type 2 patients without DN

Kynurenine levels were elevated in SSc patients with PNP compared to those without PNP and HCs. The kynurenine pathway, which accounts for the catabolism of approximately 99% of ingested tryptophan not used for protein synthesis, has links with neurodegenerative diseases, tumor proliferation, inflammation, and depression [45]. Possibly due to these findings, the kynurenine pathway is one of the most studied in SSc. Anti-RNA-polymerase III (ARA) positive patients were found to have higher kynurenine levels compared to anti-topoisomerase I and anti-centromere positive patients, as well as SSc patients with dcSSc [46]. Kynurenine levels were higher in PAH patients associated with SSc compared to idiopathic PAH or other connective tissue disease-related PAH and may affect the risk of developing PAH [47,48]. Studies showed that the disturbance of the kynurenine pathway could increase the oxidative compounds, which damage the peripheral nervous system (PNS) and CNS through the broken blood–nerve or blood–brain barrier, respectively [49]. Compared to the effects of the kynurenine pathway in various CNS diseases, data on the role of kynurenine in the development of PNS damage are currently very limited. The concentration of kynurenine was found to be elevated in diabetes mellitus (DM) patients with severe PNP and neuropathic pain [43,44]. The possible elevated concentration of kynurenine also in SSc patients with PNP suggests a unifying dysregulation with PAH, which would be easier to explain due to a common vasculopathy role of both features that are reinforced by kynurenine's elevated concentration in patients with DN [43,44].

The asparagine concentration was also elevated in patients with PNP compared to SSc patients without PNP, but not to HCs. Asparagine is crucial in proliferating cells when they are starved for nutrients, especially glutamine. Glutamine regulates angiogenesis through multiple mechanisms, and the proliferation of endothelial cells is impaired when exogenous glutamine is unavailable. Instead, endothelial cells rely on asparagine for proliferation, and asparagine can partially rescue these cell defects under low glutamine conditions [50,51]. Unlike other metabolites, asparagine has not been described to have marked changes in SSc and various manifestations of the disease. However, a negative correlation with mRSS was found in SSc patients [4]. In a study with type 2 DM patients, asparagine regulation differentiated between those with and without PNP [44]. It could be inferred that in SSc patients with PNP, an elevated concentration of asparagine signals glutamine deficiency, with changes in endothelial function and regulation of angiogenesis, which could predispose to vasculopathy and ischemic damage as a cornerstone of the development of PNP. However, the elevated glutamine concentration observed in patients with SSc in our study strongly differentiates patients with and without PNP, reinforcing the above hypothesis.

Another metabolite with elevated concentration in SSc patients with PNP, compared to the SSc without the PNP subgroup and HCs, was alanine. Changes in the alanine pathway have been shown to play a role in the development of DN. In a study with type 2 DM patients, the serum β-alanine ratio of β-alanine to L-aspartic acid in DN patients was significantly increased [44]. When present in high levels, β-alanine is a neurotoxin and damages the brain and nerve tissue [52–54]. An elevated concentration of alanine, such as observed in patients with type 2 DM, indicates neurotoxic functions of alanine, which may partially explain PNP in SSc or may be a cause of the progression of the disease.

The study's limitations stem from its strengths. We provided additional data on differences in the metabolome between patients with SSc and HCs. We compared these data with those previously published, thereby strengthening the evidence for the role of metabolome alterations in SSc. However, as the data obtained from the PNP group of SSc patients cannot be compared with similar publications, we chose to investigate the regulation of the altered metabolites found in DN patients. In doing so, we may have

overlooked similarities in the development of PNP in patients with SSc that have not yet been published. We acknowledge that the sample size was relatively small, which can be attributed to the rarity and low prevalence of the disease in the Nordic countries. To draw more definitive conclusions about the role of the metabolome in the development and detection of PNP, a larger patient cohort is required. However, PNP in SSc remains under-recognized, underlining the importance of raising awareness of this complication first. Notably, we observed a surprisingly high prevalence of PNP among SSc patients, with age and sex distributions differing from those of SSc patients without PNP.

4. Materials and Methods

4.1. Subjects

Patients with SSc (n = 62) and HC group (n = 72) were recruited consecutively at the two leading Latvian hospitals, Pauls Stradiņš Clinical University Hospital and Riga East University Hospital. The study was approved by the Rīga Stradiņš University Research Ethics Committee (Institutional Review Board reference no: 22-2/481/2021), and all participants provided their written informed consent. The diagnosis of SSc was made according to the criteria of the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) [55]. The inclusion criteria for the patients were the diagnosis of SSc according to the criteria of ACR/EULAR and an age of 18 years or older. Most off the patients received treatment with immunosuppressive drugs such as azathioprine, cyclophosphamide, methotrexate, mycophenolate mofetil, and glucocorticoids. Patients with previous treatment with cyclophosphamide, chemotherapy due to cancer, diagnosed DM, thyroid disorders, and stage 4-5 chronic renal disease were excluded. HC inclusion criteria were age 18 years or older without acute infections.

4.2. Methods

The enrolled subjects with SSc underwent a uniform PNS evaluation. First, patients underwent a nerve conduction study (NCS) by a certified neurophysiology expert. Motor and sensory conduction were evaluated according to the PNP examination protocol [56]. Each patient underwent an NCS of the bilateral upper extremities (the motor and sensory components of the ulnar and median nerves) and the bilateral lower extremities (the motor component of the peroneal and tibial nerves and the sensory component of the sural nerve) to determine nerve conduction latency, amplitude and velocity. Patients with abnormal results of NCS—considering the normal values used in Latvian clinical practice—in more than one attribute for two separate nerves were diagnosed with PNP. The age at disease onset was defined as the time of onset of the first non-Raynaud's SSc symptom. A rheumatologist evaluated the skin condition according to the mRSS [57].

4.3. Sample Collection and Preparation

Peripheral blood was collected in accordance with the Declaration of Helsinki (1975/83) using an ethylenediamine tetraacetic acid (EDTA) containing BD Vacutainer Blood Collection tube. Plasma separation was performed by centrifuging peripheral blood sample tubes at 4000 rpm, +4 C, for 15 min. Plasma obtained was transferred to −80 °C within 30 min and stored until analysis of the metabolites.

4.4. LC-MS Based Metabolomics

For metabolite extraction, 10 µL of plasma samples were mixed with 10 µL of isotopically labeled internal standard mix and 80 µL of methanol. The samples were vortexed for 15 s and centrifuged at 10,000× g for 10 min. The supernatant was transferred to HPLC vials and used for LC-MS analysis.

Targeted quantitative metabolite analysis was conducted using HILIC-based liquid chromatography and mass spectrometric detection employing an Orbitrap Exploris 120 system. Metabolites were separated on an ACQUITY UPLC BEH Amide 1.7 µm 2.1 × 100 mm analytical column (Waters, Milford, MA, USA). Gradient elution was carried out using 0.15% formic acid and 10 mm ammonium formate in water as mobile phase A and a solution of 0.15% formic acid and 10 mm ammonium formate in 85% acetonitrile as mobile phase B. The initial conditions were set to 100% mobile phase A. After 6 min, the mobile phase A level was reduced to 94.1%. From 6.1 to 10 min, mobile phase A was set to 82.4%, and from 10 to 12 min, mobile phase A was set to 70.6%. The column was then equilibrated for 6 min at initial conditions. The total analysis time was 18 min. The mobile phase flow rate was 0.4 mL/min; the injection volume was 2 µL, and the column temperature was 40 °C. The MS analysis was performed in ESI positive and ESI negative modes, Full Scan mode with a mass range from 50 to 400 m/z. The ESI spray voltage was set to 3.5 kV in positive mode and 2.5 kV in negative mode, the gas heater temperature was set to 400 °C, the capillary temperature was set to 350 °C, the auxiliary gas flow rate was set to 12 arbitrary units, and nebulizing gas flow rate was set to 50 arbitrary units. For quantitative analysis, seven-point calibration curves with internal standardization were used. Tracefinder 5.1 General Quan (Thermo Fisher Scientific, Waltham, MA, USA) software was used for LC-MS data processing and quantification.

4.5. Statistical Analysis

Metabolomics data were analyzed with MetaboAnalyst 6.0 and GraphPad Prism 9.0. Prior to all analysis, metabolites with >50% missing values were removed. For other metabolites, missing values were replaced with 1/5 of the minimal measured value. This imputation technique was chosen based on the assumption that missing values in LC-MS data are typically due to analytes falling under the limit of detection (missing not at random), and such cases seem appropriately handled by determined value replacement [58].

For principal component analysis (PCA) and volcano plots, the data were $\log_{10}$ transformed and pareto scaled. Principal components were selected based on parallel analysis, p-values and fold changes were plotted as $-\log_{10}$ (FC) and $\log_2$ (p), respectively. Metabolite correlation with age was conducted for HCs and SSc patients separately using Pearson's correlation coefficient (r).

For univariate analysis of SSc to HC, a high significance threshold of FC > 1.5 and p-value < 0.05 was chosen. For subgroup analysis, the threshold was lowered to FC > 1.3 and p-value < 0.1. This was done to balance capturing minor changes between the groups with restricting false positives as we expect an average of 10% variation for LC-MS data.

For bar plots, original concentrations were normalized to the average concentration of healthy controls for each metabolite. Metabolites were plotted as mean ± SD, and single measurements were overlayed as dots. Significance between groups was determined with 2-way ANOVA, and corrected with Šidká's multiple comparisons test for two-group comparison, and Tukey's test for three-group comparisons. Adjusted p-values were reported.

For disease prediction models, data were $\log_{10}$ transformed and pareto scaled. Models were built using a linear support vector machine (SVM). For exploratory analysis, 6 different models with fixed feature amounts were created and models were averaged from iterations. For curated models, metabolites were selected based either on univariate significance (volcano plots), or average importance scores for disease classification using SVM. The receiver under operating characteristic (ROC) curves and 95% confidence intervals (CI) were calculated from 100-cross validations, and mean ROC curves were reported. The same data were used for training and class prediction visualization.

5. Conclusions

Here, we present the first known metabolome study of SSc patients with PNP. This subgroup tended to be older, with a longer duration of SSc, and was more often male.

Analyzing the results of our study and comparing them with previous studies in the field of SSc, we conclude that the metabolite alterations identified in our study are similar to those in other studies, showing associations between macrophage polarization changes, fibrotic process stimulation, and mitochondrial dysfunction with oxidative stress-induced damage.

As potential biomarkers for SSc, we found significant changes in aspartic acid, glutamic acid, glutamine, and carnitine, as well as high predictive scores for ornithine, the glutamine/valine ratio, and the creatinine/glutamine ratio.

Changes in metabolite regulation in patients with SSc, as opposed to controls, are not identical to those observed in patients with SSc with PNP, with kynurenine and alanine displaying elevated concentrations specific to the SSc with PNP subgroup. SSc patients with PNP should probably be considered a distinct population with important metabolomic features. Direct neurotoxicity to PNS structures and mitochondrial dysfunction in conjunction with oxidative stress may play a critical role in the development of PNP in SSc patients according to metabolic profiles, possibly a result of aging and sequential progression of SSc. Nevertheless, further studies are required to evaluate the role of these alterations in the pathophysiology of PNP in patients with SSc to uncover novel biomarkers and therapeutic targets in the future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms26157133/s1.

Author Contributions: Author Contributions: Conceptualization, K.I., N.K., V.K. and K.K.; methodology, T.S., A.V. and K.K.; validation, T.S., A.V. and K.K.; formal analysis, K.I., T.S., A.V. and K.K.; investigation, K.I., N.K. and V.K.; resources, K.I., N.K. and V.K.; writing—original draft preparation, K.I. and T.S.; writing—review and editing, K.I., T.S., N.K., V.K. and K.K.; visualization, K.I., T.S. and K.K.; supervision, N.K., V.K. and K.K.; project administration, K.I., N.K. and V.K.; funding acquisition, K.I., N.K. and V.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by RSU internal and RSU with LASE external consolidation, grant number 5.2.1.1.i.0/2/24/I/CFLA/005; and European Union's Horizon 2020 research and innovation programme, grant agreement ID: 857287.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Rīga Stradiņš University Research Ethics Committee (Institutional Review Board reference no: 22-2/481/2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets presented in this study are available upon request.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

SSc	Systemic sclerosis
PNP	Polyneuropathy
HC	Healthy control
ILD	Interstitial lung disease
mRSS	Modified Rodnan skin score

NS	Nervous system
NO	Nitric oxide
dcSSc	diffuse cutaneous Systemic sclerosis
lcSSc	limited cutaneous Systemic sclerosis
PAH	Pulmonary arterial hypertension
CNS	Central nervous system
DN	Diabetic neuropathy
DM	Diabetes mellitus
ARA	Anti-RNA-polymerase III
PNS	Peripheral nervous system
ACR	American College of Rheumatology
EULAR	European League Against Rheumatism
NCS	Nerve conduction study
EDTA	Ethylendiamine tetraacetic acid

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