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

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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
AUC	area under the curve
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
LC-MS	liquid chromatography – mass spectrometry
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
ROC	receiver operating characteristic
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). 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 (Abs) are important to identify different clinical groups of SSc, stratifying patients into more homogeneous subgroups (Cavazzana et al., 2023). Serum Abs 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 Abs directed against nuclear or nucleolar autoantigens (Peoples et al., 2016; Salazar et al., 2015; Tan, 1989). Anti-topoisomerase Abs (ATA), anti-centromere Abs (ACA) and anti-RNA polymerase Abs (ARA), first described in the 1970s-1990s, are classic disease-specific Abs and are included in the 2013 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) SSc classification criteria (van den Hoogen et al., 2013). In general, the presence of SSc specific Abs 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 Abs are also associated with PNS damage in SSc. In many systemic connective tissue diseases idea of studying specific Abs against various nerve structures comes from research done in immune neuropathies like Guillain-Barré syndrome and its subtypes (De Souza 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

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 Abs and Abs that target certain components of the NS, like Abs against myelin-associated glycoprotein (anti-MAG) and anti-ganglioside Abs.
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 Abs 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.

1 Materials and methods

1.1 Subjects

This study was conducted in two leading Latvian hospitals, which are the only university hospitals in Latvia for adults, Pauls Stradiņš Clinical University Hospital and Riga East University Hospital, and ORTO Klīnika.

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. For patient selection we used hospital databases, where patients with diagnostic codes M34.0–M34.9 were selected according to the 10th revised version of the International Classification of Diseases, which has been used in all Latvian hospitals. Patients with connective tissue diseases other than SSc and patients with localised 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. Study design described in Figure 1.1 and Figure 1.2.

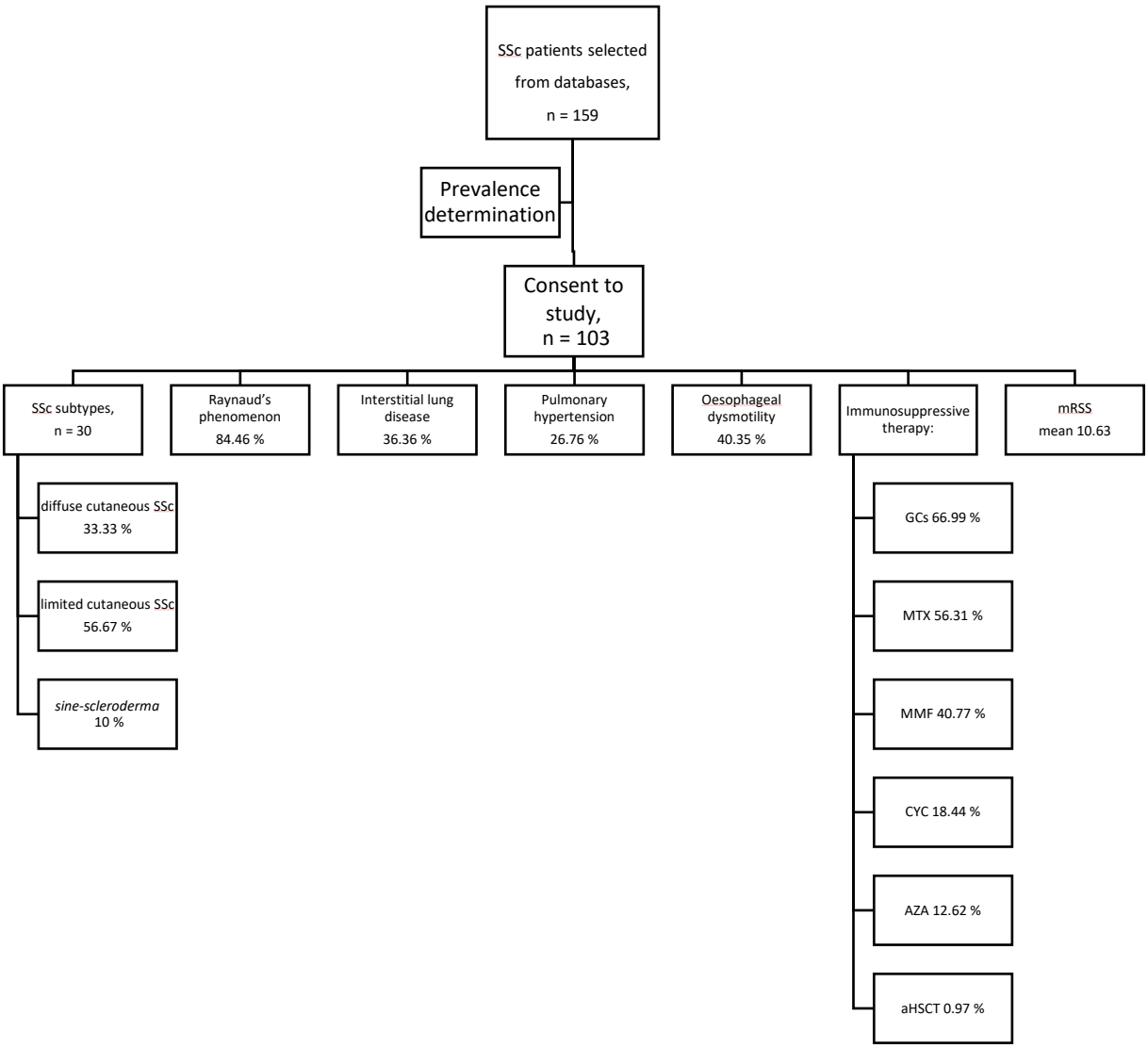

Figure 1.1 **Study design: Demographic and clinical data**

1.2 Presence of antinuclear antibodies (ANA)

To assess the presence and pattern of ANA, previously detected immunological tests were analysed. ANA were detected using Hep-2 cells for all patients in this study.

1.3 Evaluation of clinical characteristics

Patients who agreed to participate in the study were evaluated by one rheumatologist and surveyed and clinically assessed according to the 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 mRSS. ILD and PH were determined after previous investigations including lung computed tomography, transthoracic echocardiography, and right heart catheterisation. Oesophageal 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- RP 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.

Information on previously used immunosuppressive agents and comorbidities was obtained from patients and patient records.

1.4 Determination of PNS involvement

Enrolled subjects underwent a uniform evaluation of the PNS. At first, patients were screened using srTNS, which consists of three symptom extension components (numbness, tingling, neuropathic pain) and two objective testing components (tendon reflex, vibration sensibility). Study design described in Figure 1.1 and Figure 1.2.

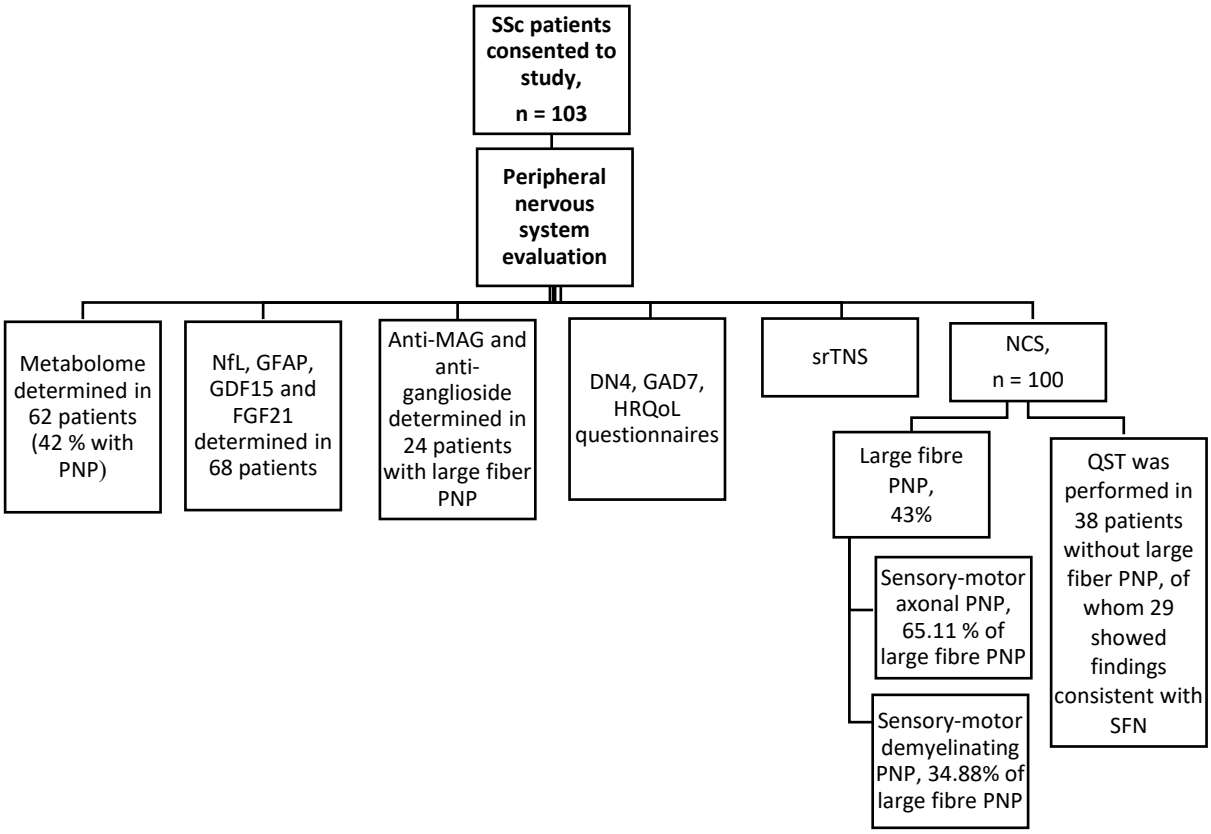

Figure 1.2 **Study design: Study of the PNS**

Next, standard NCS were conducted using Dantec Keypoint equipment, following the diagnostic protocol for PNP, with assessment of both motor and sensory fibres. 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 the nerve conduction latency, amplitude, and velocity. 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. Those subjects who had abnormal NCS results according to the normal values used in Latvian clinical practice in more than one attribute in two separate nerves were concluded to have LFN.

Quantitative sensory testing (QST) was performed in the subjects with normal NSC results in order to evaluate small fibre function for possible abnormalities. 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 thermode 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, and those subjects who had abnormal values in two separate extremities were concluded to may have SFN.

Additionally, all enrolled subjects completed the Latvian version of the DN4 questionnaire to assess neuropathic pain, the GAD-7 scale to assess anxiety symptoms, and the HAQ-DI to assess HRQoL. Those patients who had four or more points on the DN4 questionnaire were defines 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 summed and divided by eight to provide the functional disability index.

1.5 Blood sample collection and analysis

Peripheral blood was collected in accordance with the Declaration of Helsinki (1975/83) using an ethylenediamine tetraacetic acid containing BD Vacutainer Blood Collection tube. Plasma separation was performed by centrifuging peripheral blood sample tubes at 4000 rpm,

+4 C, for 15 minutes. Plasma obtained was transferred to −80°C within 30 minutes and stored prior to analysis.

1.5.1 Detection of SSc specific Abs and Abs that target certain components of the NS

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 anti- 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.

1.5.2 Detection of serum biomarkers

Two potential serum PNS biomarkers – NfL and GFAP – were measured with a Single molecule array (Simoa) assay (Quanterix, Billerica, MA, USA). FGF21 and GDF15 were measured using commercially available ELISAs according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). 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 %.

1.5.3 Metabolite analysis

For metabolite extraction, 10 ul of plasma samples were mixed with 10 uL of isotopically labelled internal standard mix and 80 ul of methanol. The samples were vortexed for 15 seconds and centrifuged at 10,000 x g for 10 min. The supernatant was transferred to high-performance liquid chromatography vials and used for liquid chromatography – mass spectrometry (LC-MS) analysis.

Targeted quantitative metabolite analysis was conducted using hydrophilic interaction 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 x 100 mm analytical column (Waters). 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 minutes, 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 minutes. 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 standardisation were used. Tracefinder 5.1 General Quan (Thermo Fisher Scientific) software was used for LC-MS data processing and quantification.

1.6 Statistical analysis

Data distribution was assessed using histograms, normal Q–Q plots, and the Shapiro–Wilk test. As the data were not normally distributed, non-parametric tests were applied. Comparisons between more than two groups were performed using the Kruskal–Wallis H test, while comparisons between two groups were conducted using the Mann–Whitney U test. Spearman's rank-order correlation was used to assess associations between continuous variables, and Fisher's exact test was applied for categorical variables. A P value < 0.05 was considered statistically significant. A binomial logistic regression analysis was conducted to identify factors associated with PNP. Forward and backward stepwise selection methods were applied to construct the model, and relevant main effects and interactions were considered. The Akaike information criterion was used to select the optimal model. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the discriminatory performance of the final model, and the area under the curve (AUC) was calculated. An AUC ≥ 0.7 was considered to indicate good performance in distinguishing between patients with and without PNP. The Youden index was used to determine the optimal cut-off value.

Metabolomics data were analysed with MetaboAnalyst 6.0 and GraphPad Prism 9.0. For principal component analysis and volcano plots, the data were log10 trans-formed and pareto scaled. Principal components were selected based on parallel analysis, p-values and fold changes (FC) were plotted as -log10 (FC) and log2 (p) respectively. Metabolite correlation with age was done for HC 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. For bar plots, original concentrations were normalised 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 two-group comparison, and turkey's test for three-group comparisons. Adjusted p-values were reported. For disease prediction models, data were log10 transformed and pareto scaled. Models were built using linear support vector machine. 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 support vector machine. The ROC curves and 95 % confidence intervals were calculated from 100-cross validations, and mean ROC curves were re-ported. The same data were used for training and class prediction visualisation.

2 Results

2.1 Prevalence and gender-specific analysis of a SSc cohort in Latvia

2.1.1 Prevalence of SSc in Latvia

Between January 2016 and December 2021, 159 patients with SSc were consulted. Of them, the majority were females (82 %) and only 18 % were males.

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. 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). The highest prevalence was found in the 60–69 age group *(Figure 2.1).* 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.

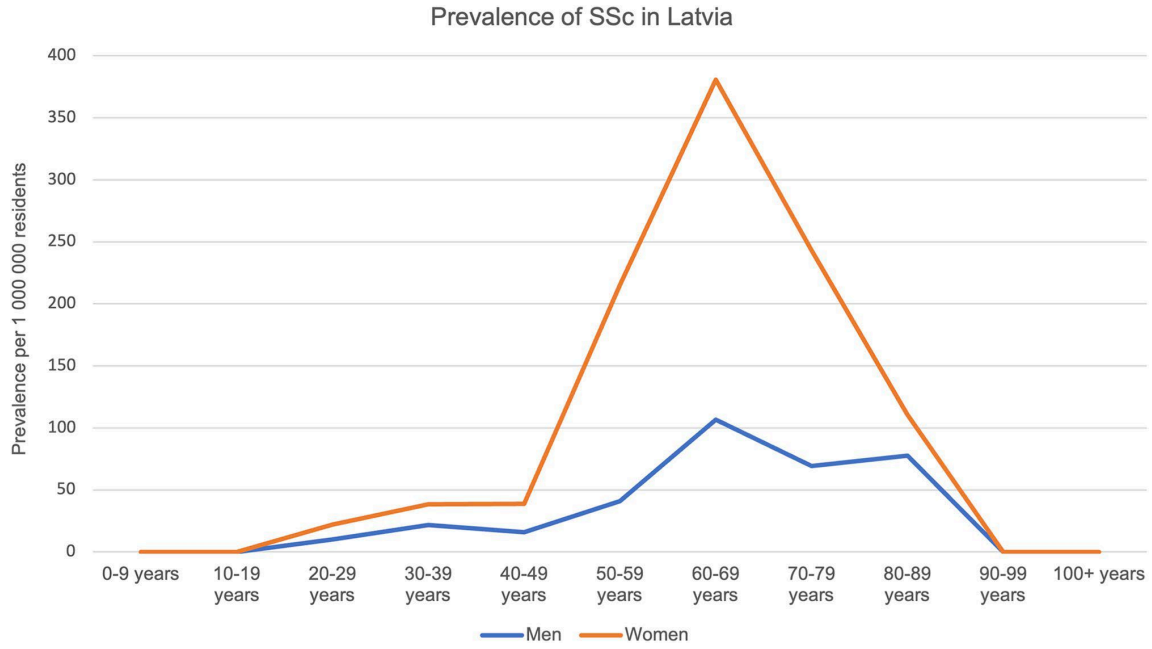

Figure 2.1 **Prevalence of SSc in Latvia**

2.1.2 Clinical and immunological characteristics of SSc patients with gender-related differences

ANA presence and pattern

The presence of ANA was evaluated and found in 82.58 % of 155 patients. The pattern was checked in 123 patients and described in Table 2.1.

Table 2.1

Gender-specific Abs characteristics in patients with SSc

	Males	Females	Total
ANA positive, *n* **ANA pattern present,** *n*	22 21	106 102	128 123
Centromere pattern, *n (%)*	4 (19.04)	41 (40.19)	45 (36.88)
Speckled pattern, *n (%)* **Anti-topoisomerase I,** *n (%)*	12 (57.14) 7 (33.33)	52 (50.98) 32 (31.37)	64 (52.03) 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)

Clinical characteristics

Of the 159 patients selected, 103 agreed to participate in this study, of whom 85 were females and 18 were males. All included patients were Caucasians. Characteristics are described in Table 2.2.

Table 2.2

Gender-specific clinical characteristics in patients with SSc

		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)
	mRSS, 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)
Oesophageal 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%)

2.2 Prevalence of PNP among SSc patients and impact on health-related quality of life

A total of 100 patients consented to undergo nerve NCS. LFN was found in 43 % of 100 patients, 15 patients had sensory-motor demyelinating PNP, while 28 had sensory-motor axonal demyelinating PNP. *Table 2.3* illustrates the distinctions in demographic, clinical, and neurophysiological characteristics between patients with SSc and with or without LFN.

Table 2.3

Demographic, clinical and neurophysiological characteristics and comparisons of patients with systemic sclerosis and with or without LFN

Variable	**SSc without PNP (LFN)** 57 (57 %)	**SSc with PNP (LFN)** 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**

Our regression analysis revealed a strong association between the HAQ-DI score and the risk of developing LFN. A 1-point increase in the HAQ-DI score was significantly associated with a 95 % higher likelihood of LFN *(95 % 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 %; $p < 0.001$).*

From 57 patients without LFN, 38 patients consented to undergo QST. 29 of them shoved changes compatible with SFN.

2.3 PNP in SSc: 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.

2.3.1 SSc specific Abs and Abs that target certain components of the NS

We assessed SSc-associated Abs in 97 patients *(Table 2.4)*; 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

Table 2.4

SSc-associated Abs in patients with and without PNP

SSc specific Abs	SSc without PNP, %	SSc with PNP, %	Total, %	*p-value*
Classical Abs (%)			61.86	–
CENP-A / CENP-B	36.84	37.50	36.08	0.852
Scl-70	24.56	20.00	22.68	0.597
RP-11 / RP-155	1.75	5.00	3.09	0.363
Novel Abs (%)			45.36	–
Ro-52	26.32	17.50	22.68	0.307
PM75 / PM 100	3.51	7.5	6.18	0.381
Ku	5.26	5.00	5.15	0.954
Th / To	1.75	7.5	4.12	0.161
NOR90	7.02	0	4.12	0.087
Fib	0	2.5	1.03	0.230
PDGFR	0	0	0	–

2.3.2 Potential PNS serum biomarkers

We assessed potential PNS serum biomarkers – NfL, GFAP, GDF15 and FGF21 – in 68 patients, 30 with PNP, 38 without PNP *(Table 2.5)*. NfL, GFAP and GDF15 were significantly elevated in the presence of PNP ($p < 0.05$), with a moderate to high effect size ($r = 0.36–0.65$).

Table 2.5

Comparison of biomarker levels in SSc patients with or without polyneuropathy PNP

Parameter	**SSc without PNP** 38 (55.88%)	**SSc with PNP** 30 (44.11%)	*p*	*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 (705–1389)	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

2.4 Serum metabolomic profiling

Our plasma sample cohort consisted of 62 patients with SSc, of which 26 had PNP (42 %).

2.4.1 Metabolites in SSc patients

We first compared plasma metabolites of all SSc patients with HC. Based on the principal component analysis *(Figure 2.3a),* there was no clear separation between these two groups. However, concentrations of several metabolites changed significantly in the plasma of SSc patients *(Figure 2.3b).* The most significant differences with fold changes > 2 were observed for aspartic acid, glutamic acid, valine, and citrulline. All of which were reduced *(Figure 3b, c).* In particular, the volcano plot showed a general reduction in metabolite concentrations, except glutamine with a fold change > 1.5.

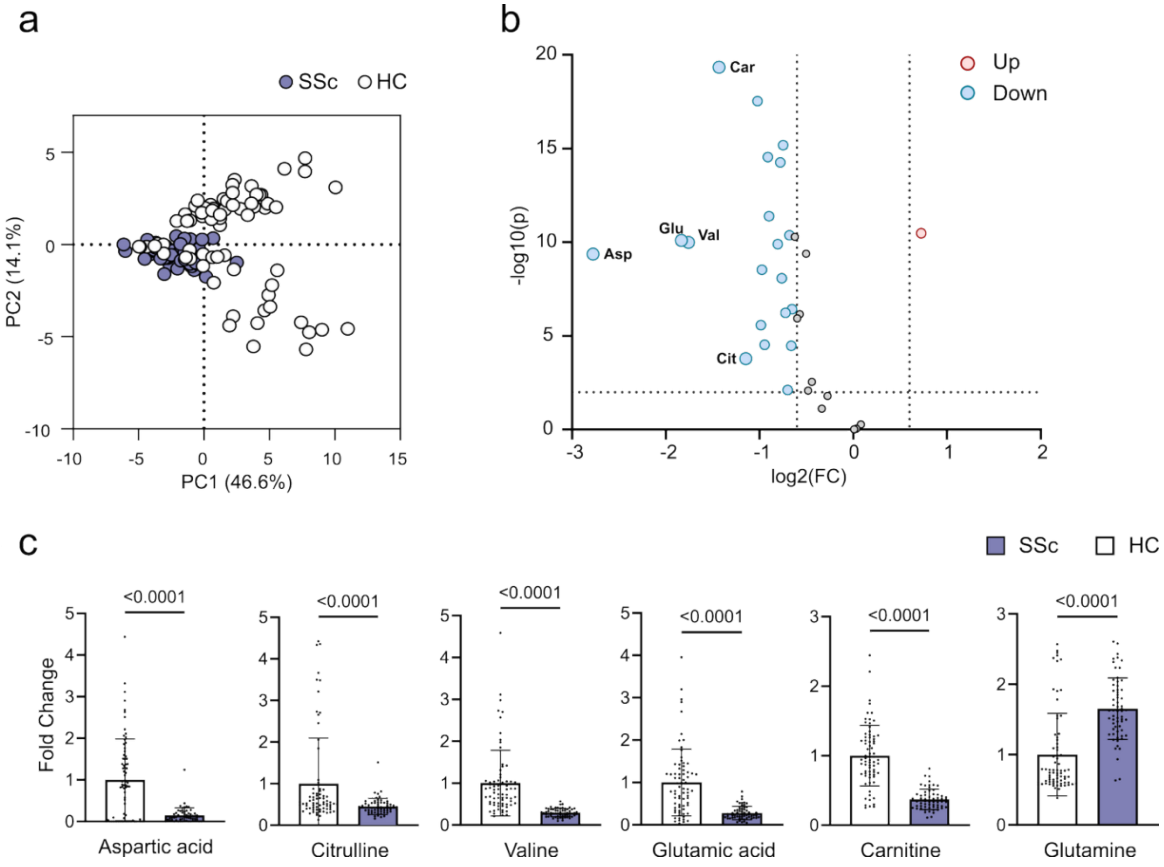

Figure 2.3 **Plasma metabolite changes in SSc patients compared to HC**

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 HC. We used metabolite and two-metabolite ratios to build disease prediction models. We used both significantly changed metabolites *(Figure 2.4a)* and metabolites with high predictive scores *(Figure 2.4b)* 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 2.4d)*. Both models separated patients from controls with an AUC of 0.954 and 0.993, respectively.

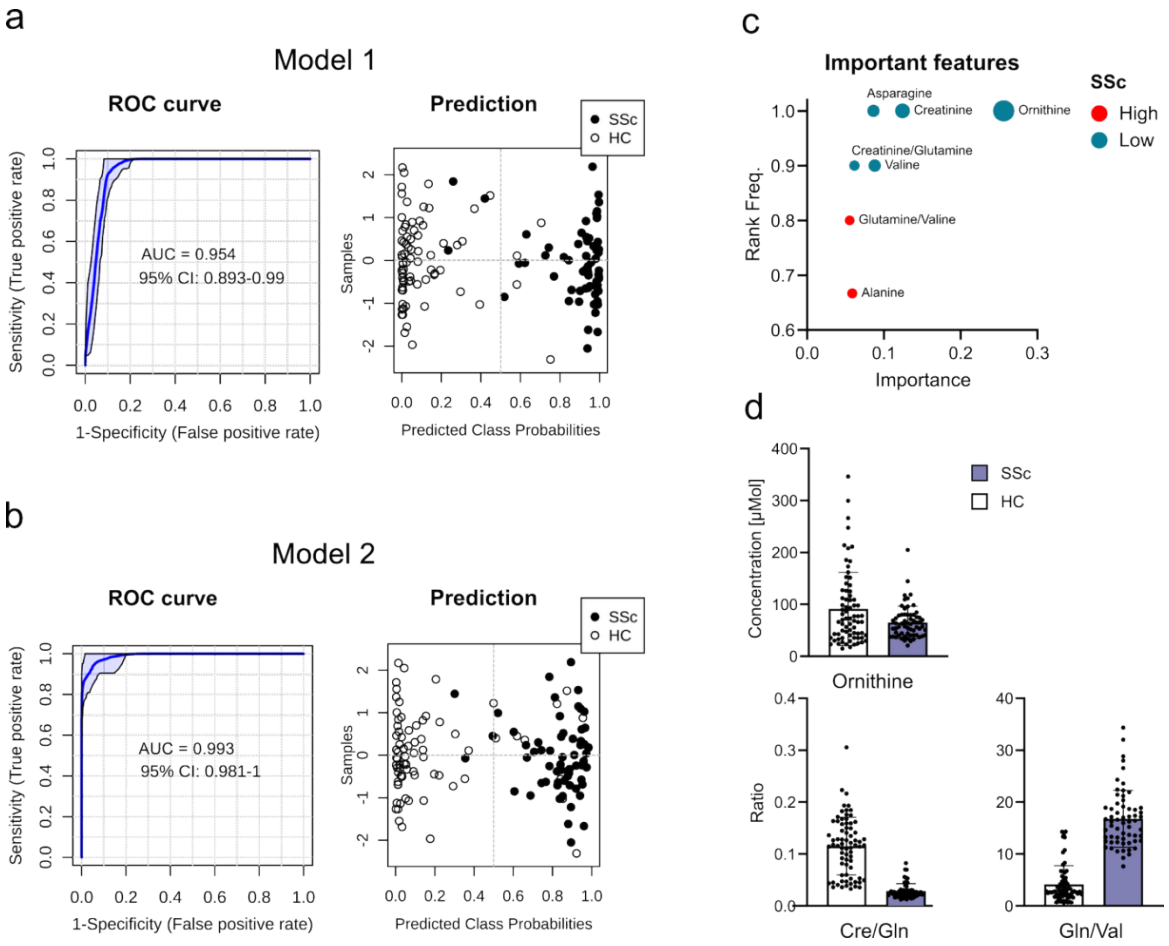

Figure 2.4 **Prediction models distinguishing SSc patients from HC**

2.4.2 Discrimination of SSc patients with PNP

We further subdivided the SSc patients based on the diagnosis of PNP. Again, the principal component analysis shows no separation between subgroups *(Figure 2.5a)*. In contrast to SSc for the discrimination with HC, 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. When applying these cutoffs, we identified an elevated concentration of the tryptophan metabolite kynurenine and the amino acids asparagine and alanine *(Figure 2.5b)*. 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 HC *(Figure 2.5c)*. These findings prompted us to compare significant changes in the total SSc and SSc subgroup with HC. 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 2.5d)*. 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.

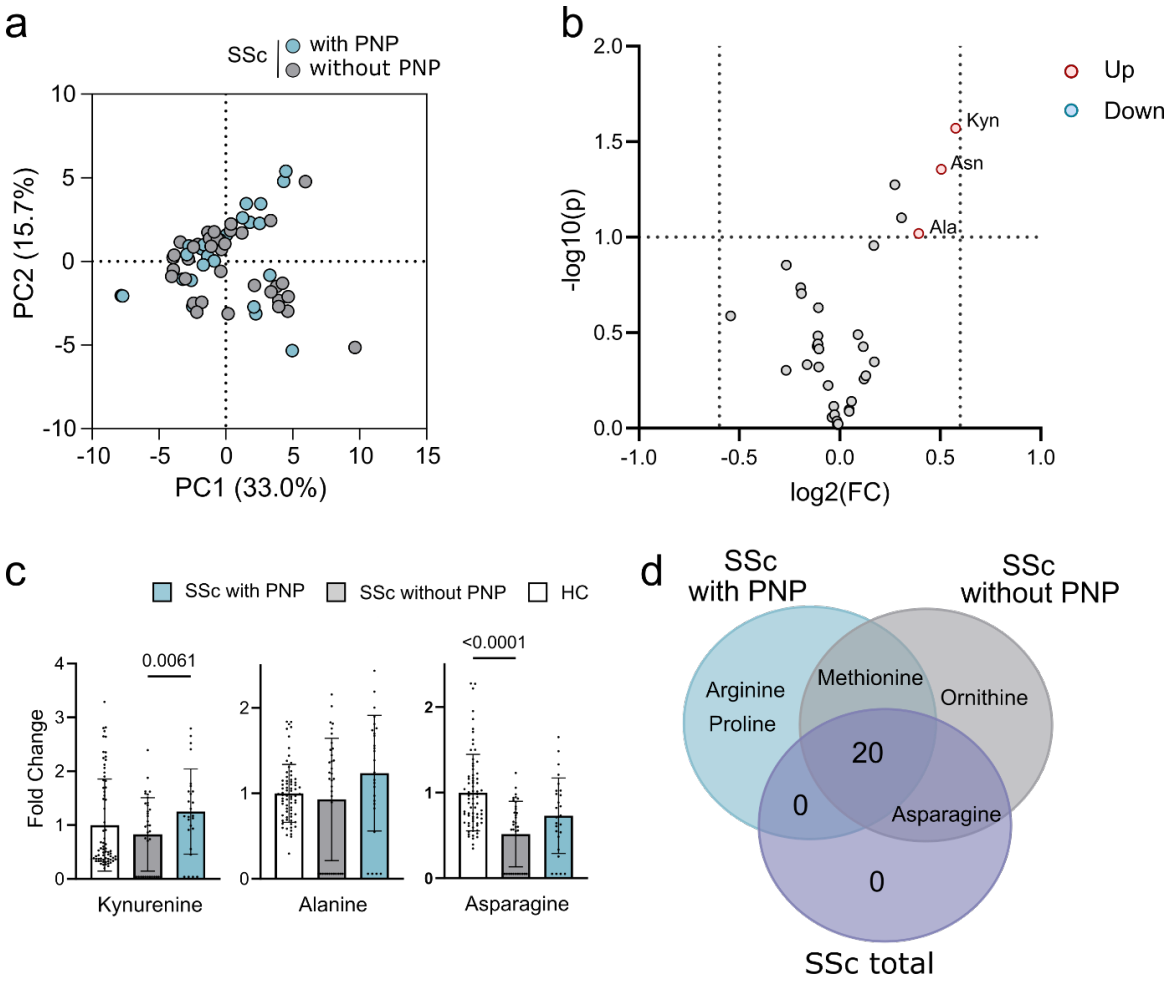

Figure 2.5 **Plasma metabolite changes of SSc patients with and without PNP**

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 SSc 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 Abs 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 Abs that are included in the 2013 classification criteria (ACA, ATA and ARA) account for over 70 % of all single Ab specificities detected in previous studies (van den Hoogen 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 Abs associated with SSc, still highest prevalence stands for these two Abs (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 Abs 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. 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 PNP among SSc patients and impact on HRQoL

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. 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. 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.

PNP in SSc: 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 SSc patients with PNP

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).

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.

List of publications, reports and patents on the topic of the 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).
8. Možeitoviča, E., Scientific research supervisor: **Ivanova, K**. 2024. Potential biomarkers for peripheral nervous system damage in patients diagnosed with systemic sclerosis. *RSU International Student Conference*, Riga, Latvia (Theses and oral presentation).

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