Systemic sclerosis: can we identify patients at risk?

Leeuwen, N.M. van

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SYSTEMIC SCLEROSIS:

CAN WE IDENTIFY PATIENTS AT RISK?

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Door

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General introduction and outline

SYSTEMIC SCLEROSIS

A complex and heterogeneous auto-immune disease

Systemic Sclerosis (SSc) is a relatively rare disease. The prevalence in the Netherlands has been estimated at 8.9 per 100.000 inhabitants using the American College of Rheumatology (ACR) preliminary criteria and LeRoy's criteria [1]. The diagnosis of SSc is generally suggested by the presence of Raynaud's Phenomenon (RP) consecutively followed by skin thickening associated with the presence of additional organ manifestations, microvascular abnormalities and SSc specific autoantibodies [2]. RP is present in 95% of the patients, it precedes other symptoms by years, and is classically viewed as reversible vasospasm due to functional changes in digital arteries of the hands and feet [3]. After the skin, gastro-intestinal (GI) complaints are most prevalent in SSc [4]. More life threatening organ complications include interstitial lung disease (ILD), pulmonary arterial hypertension (PAH), and cardiac involvement [5, 6]. Compared to the general population, but also to patients with other rheumatic diseases or chronic conditions, health-related quality of life (HRQoL) is significantly more affected in SSc patients. Optimal management of SSc is challenging due to severity, heterogeneity and complexity of the disease. SSc can be progressive and many of the disease features can aggravate over time. Therefore annual screening of SSc patients for disease severity and/or progression is advocated using standardized programs with a multidisciplinary approach [7]. Here, patients are seen on a yearly basis by a multidisciplinary team including a rheumatologist, pulmonologist, specialized rheumatology nurse, physical therapist, and if needed, a dietician, occupational therapist, cardiologist, gastroenterologist or dermatologist. Even with this approach it remains difficult to accurately identify the trajectory of the disease in the individual patient. The first five years after diagnosis are most critical in determining the individual patient's prognosis [8]. With the introduction of new diagnostic guidelines in 2013, the diagnosis is more frequently set in an early phase [9]. The ACR/European League against Rheumatism (EULAR) 2013 classification criteria for SSc reflect the need to create an earlier treatment window, based on better understanding of the pathogenesis. These criteria recognize the importance of nailfold capillaroscopy (NC) to identify the degree of vasculopathy in early diagnosis, and the role of specific SSc autoantibodies in clinical manifestations and disease prognosis. However, even with the use of the ACR/EULAR 2013 criteria patients with very early or mild SSc can be missed. To detect patients with very early disease, the EULAR scleroderma Trials and Research group (EUSTAR) developed the Very Early Diagnosis of Systemic Sclerosis (VEDOSS) criteria [10, 11]. Unfortunately, earlier diagnosis has not led to improvements in determining the prognosis in the individual patient due to lack of accurate prognostic markers [12] and tailormade assessment and treatment of the individual SSc patient remains challenging. In addition, with the shift towards early recognition it is important and helpful to know how early diagnosis affects patients, as illness perceptions directly influence illness behaviour [13, 14].

The ACR/EULAR criteria for the classification of SSc*

Item	Sub-item(s)	Weight/score #
Skin thickening of the fingers of both hands extending proximal to the metacarpophalangeal joints	-	9
Skin thickening of the fingers (only count the higher score)	Puffy fingers Sclerodactyly	2 4
Fingertip lesions (only count the higher score)	Digital tip ulcers Fingertip pitting scars	2 3
Telangiectasia	-	2
Abnormal nailfold capillaries	-	2
Pulmonary arterial hypertension or interstitial lung disease	-	2
Raynaud Phenomenon	-	3
Scleroderma related autoantibodies	Anti-centromere Anti-topoisomerase anti-RNA polymerase III	3

Table 1. *these criteria are applicable to any patients considered for inclusion in a systemic sclerosis study. The criteria are not applicable to patient with skin thickening sparing the fingers or to patients who have a scleroderma-like disorder that better explains their manifestation. # The total score is determined by adding the maximum weight in each category. Patients with a total score > 9 are classified as having definite systemic sclerosis. Source: ARD

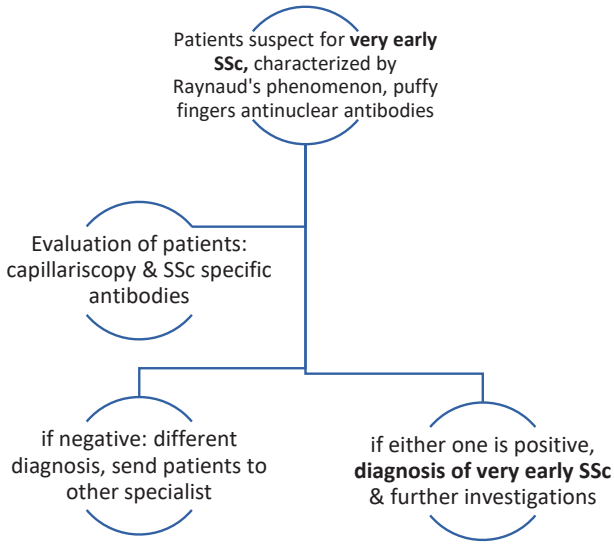

Figure 1. Very early SSc (VEDOSS) criteria.

Pathophysiology

SSc is characterized by a dysregulation in the innate and adaptive immune response, by vascular damage and excessive collagen deposition in the extracellular matrix [15]. The clinical and pathologic manifestations of SSc are the result of this triad: (1) innate and adaptive immune system abnormalities leading to production of autoantibodies and cell-mediated autoimmunity, (2) microvascular endothelial cell/small vessel fibroproliferative vasculopathy, and (3) fibroblast dysfunction generating excessive accumulation of collagen and other matrix components in all organs. From early autopsy studies we learned that multiple organ systems are involved in SSc pathogenesis as the following abnormalities were frequently found; interstitial lung fibrosis (74%), arteriolar thickening (29%), myocardial fibrosis (81%), pericardial lesions (53%), muscle atrophy and fibrosis of the esophagus (74%), lesions of reflux esophagitis (40%), muscle atrophy of the small intestine, dilatation and/or fibrosis of the duodenal loop or other segments of the small intestine (48%) and/or renal lesions (58%) [16]. Histologic examination of early SSc skin reveals changes consistent with damaged endothelial cells, including endothelial cells undergoing apoptosis, that precede the development of fibrosis by months to years. In addition, skin biopsies from SSc patients with a longer disease duration show a significant increase in myofibroblasts. In the end, as the vessels of the vascular system lose their elasticity, the vessel media and adventitia become fibrotic and more prone to vessel occlusion; which results in end organ damage. There is a certain interplay between these three characteristics (immunity, vascular system and fibrosis). However, it has not yet been established which of these processes is of primary importance, or how they are related during the development and progression of the disease [17]. There are reasons to presume that vasculopathy and dysregulated immunity precedes fibrosis in SSc. It remains unclear whether the activation of immune pathways ultimately drives the disease pathogenesis or rather represents a defective attempt to limit or even reverse excessive extracellular matrix deposition and progressive vasculopathy [18].

SSc specific autoantibodies

Current research is indicating a prominent role of both the innate and adaptive immune response in SSc pathophysiology. In addition, the SSc specific autoantibodies produced by B cells have important diagnostic and prognostic values in SSc [17, 19]. B cell receptors and T cell receptors are the proteins on which adaptive immunity is based. Effector B cells, called plasma cells, secrete soluble forms of B cell receptors, namely Immunoglobulins or antibodies, the main weapon of the adaptive immune response. These antibodies are used by the immune system to identify and neutralize foreign objects such as pathogenic bacteria and viruses. The antibody recognizes a unique part of the pathogen, the antigen. An autoantibody is mistakenly targeting and reacting with non-foreign proteins commonly expressed human tissue. In SSc, disease specific

autoantibodies recognize self-proteins that are commonly expressed in the nucleus of human cell, so-called anti-nuclear autoantibodies (ANA). ANA are detected in more than 95% of the SSc patients. Anti-centromere antibody (ACA), anti-topoisomerase antibody (ATA) and anti-RNA polymerase III autoantibodies (ARA) are the most specific and most prevalent in SSc. These SSc specific autoantibodies bear clinical significance as biomarkers to help with early diagnosis and prediction of the disease course, and they might also be helpful to understand pathophysiological processes of the disease [20]. Presence of ACA in SSc patients is associated with limited cutaneous SSc (lcSSc), calcinosis and GI involvement [20, 21]. Presence of ACA generally carries a better prognosis than many other SSc associated autoantibodies with respect to survival. ATA is associated with diffuse cutaneous SSc (dcSSc), ILD and their presence indicates an unfavorable prognosis [22, 23], ARA is associated with rapid progressive skin involvement and renal crisis. Evidence suggests an active role for SSc specific autoantibodies in the pathogenesis process beyond associations with the disease [18, 24-27]. In addition to their role as diagnostic biomarkers, each of the different autoantibodies correlates with typical clinical phenotype which suggests that the immune response involved in these specific autoantibody production may play a role in disease pathophysiology. However, the exact role of the SSc specific autoantibodies remains unclear [28, 29].

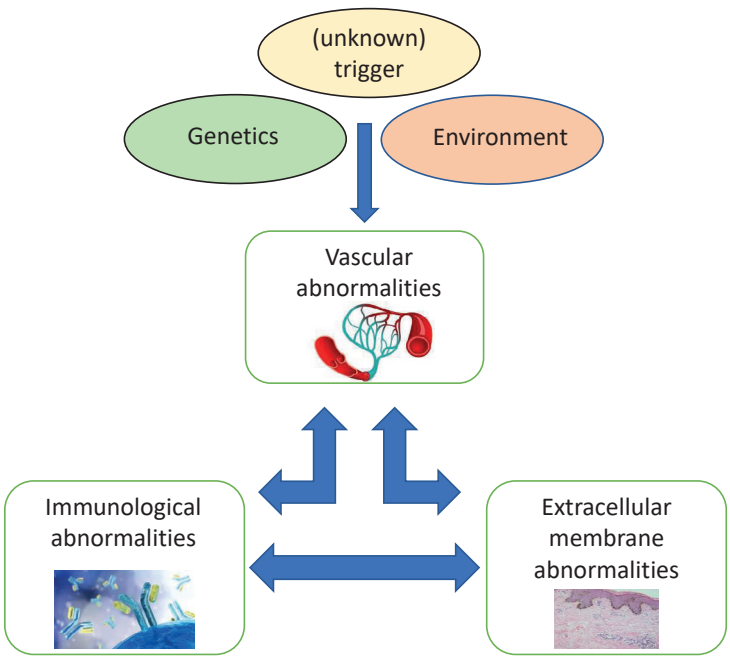

Figure 2. Schematic overview of SSc pathogenesis. Source: Annu. Rev. Pathol. Mech.Dis.2011.6:509-537

Microangiopathy

Clinically, obliterative microangiopathy leads to RP symptoms, and these symptoms precede other SSc symptoms by many years. RP with microvascular damage appears to be one of the best predictors of development of SSc [30]. Microvascular damage is characterized by alterations of the capillaries with a decrease in capillary density, enlarged capillaries, hemorrhages, disorganization of the vascular array and an abnormal morphology. Some suggest that vascular injury is the primary mechanism driving pathogenesis in patients with SSc [31-33]. A spectrum of changes can be seen in the vascular system that ranges from endothelial cell activation with enhancement of inflammatory properties, and apoptosis with capillary destruction and devascularization of the tissues leading to defective angiogenesis and vasculogenesis. There are several hypotheses on how vascular alterations may lead to fibrosis. It has been reported that vascular endothelial cells (EC's) undergo endothelial-to-mesenchymal transition (endoMT), in which EC's acquire extracellular matrix producing myofibroblasts features [34]. Myofibroblasts play an important role in the fibrosis pathogenesis, and myofibroblast may arise from different sources including perivascular pericytes.

The structural alterations in the capillaries of patients with SSc are well recognized and studied by nailfold capillaroscopy (NC), a non-invasive and safe technique that determines the degree of microangiopathy by using standardized magnification to visualize the capillaries in the nailfold [35]. In SSc, specific patterns of capillary changes and the degree of these changes have been defined [36]. These specific changes have been incorporated both in the ACR/EULAR 2013 classification criteria for SSc, as well as in the criteria for very early diagnosis of SSc. More severe microangiopathy is associated with more severe disease and with disease progression in SSc patients, underlining the importance of microangiopathy in SSc pathophysiology [35, 37-44]. Next to disease specific autoantibodies, NC therefore can be seen as an important biomarker to diagnose SSc and to predict complications in SSc.

Fibrotic changes

One of the hallmarks in the pathogenesis of SSc is fibrosis of the skin and internal organs. Excessive extracellular matrix (ECM) deposition results in altered architecture of organs and tissues which in the end leads to dysfunction. In healthy persons fibrosis is seen as a process aiming at repair; in SSc the triggers for uncontrolled fibrosis are still poorly understood. Profibrotic mediators released from infiltrating leukocytes, activated endothelial cells, and degranulated platelets may be the cause of fibroblast activation and collagen release during the early stages of fibrosis. Endogenous activation of fibroblasts due to epigenetic modifications or biochemical or physical factors may play a role in disease progression later in the disease course [45, 46].

Survival and treatment

The overall survival of SSc has improved over the last couple of years. This is assumed to be at least partly caused by earlier initiation of adequate treatment based on regular screening and greater awareness for organ involvement [47]. Survival rates at 1,5 and 10 years of disease are 94.2%, 80.0% and 65.7% respectively, with cardiopulmonary involvement as number one cause of death in SSc [6]. Treatment options in SSc consist of supportive medication on the one hand and therapies that aim to reduce inflammation, fibrosis and vasculopathy on the other hand [48]. Patients are treated based on their clinical manifestations and related severity. Treatment of fibrotic complications consists mainly of immunosuppressive drugs combined with symptomatic treatment. Vascular complications including PAH, digital ulcers are treated with vasoactive drugs including phosphodiesterase inhibitors and endothelin receptor antagonists; renal crisis is treated with ACE-inhibitors. Methotrexate is often the first choice for skin involvement, Mycophenolate Mofetil (MMF) or Cyclophosphamide should be considered for the treatment of SSc-related ILD, in particular in patients with progressive ILD [48]. In SSc patients with rapid progressive disease and high risk of early mortality, including skin and long involvement, hematopoietic stem cell transplantation is the best treatment option [49]. The last years evidence has been provided that anti-fibrotic treatment by direct inhibition of fibroblast activation (nintedanib) might be benefical in SSc. Treatment with nintedanib was shown to decrease the deterioration of pulmonary function in patients with SSc-ILD [50]. Unfortunately several clinical trials evaluating efficacy of different immunosuppressives failed to meet the primary endpoint [51-53]. Several explanations might account for this failure. Besides true inefficacy of a specific drug, also the lack of reliable outcome measures and clinical heterogeneity of the disease with lack of adequate biomarkers to identify the right target population might account for this. In conclusion, currently, in SSc patients it remains difficult to decide when, who and with what to treat. Multiple international trials are still gathering data on new possible treatments for SSc and new trials start on a yearly basis.

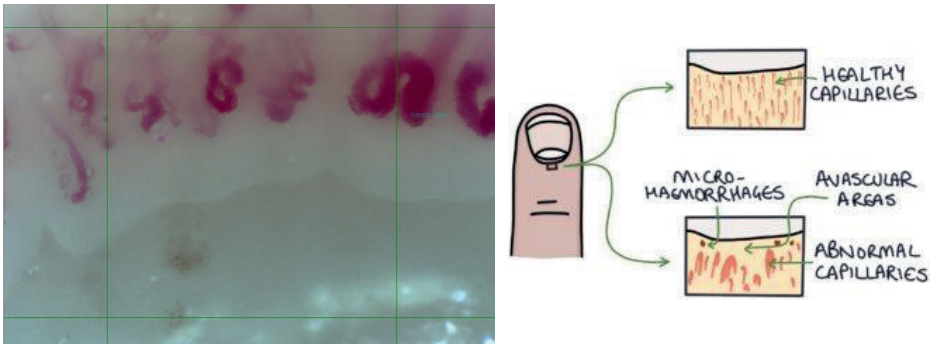

Figure 3. Image of nailfold capillaroscopy with a 1mm grid (Inspectis Pro videocap LUMC and https://zerotofinals.com/). We see an abnormal pattern (active SSc pattern, with capillary loss and dilatations).

AIMS AND OUTLINE OF THIS THESIS

The aim of this thesis is to identify biomarkers for risk stratification in SSc. Accurate biomarkers ensure that the correct patients are managed with the appropriate follow-up strategy and treatments at a time when this will have meaningful impact on the disease course.

Part I Impact of Systemic Sclerosis on patients
In the first part of this thesis we evaluate the impact of SSc and the impact of disease specific characteristics on SSc-related quality of life. We realized that there is little to no information if and how a diagnosis of SSc can impact patients' life in both mild and severe disease, and with the switch towards earlier diagnosis this is critical information. Therefore, in **chapter 1** we evaluated the illness and risk perceptions in recently diagnosed SSc patients in a small explorative study. In **chapter 2** we evaluated which disease specific characteristics have the largest impact on quality of life and on the evolution of quality of life over time in SSc patients.

Part II Disease progression in Systemic Sclerosis
Part II describes disease progression in SSc from different angles. First, in **chapter 3** we describe in detail the prevalence of disease progression in the Leiden cohort, for all organ domains. Second, we focus on gastro-intestinal involvement and it's progression over time by combining data of two large prospective cohorts in **chapter 4**, and finally in **chapter 5** we evaluate whether disease progression can be predicted at an individual level by applying machine learning.

Part III Role of microangiopathy and specific autoantibodies in Systemic Sclerosis
In the last part of this thesis, we investigated the role of two well-known biomarkers more extensively. In **chapter 6** we assess the associations between SSc specific autoantibodies and the degree of microangiopathy, and we also evaluated the association between sex and the degree of microangiopathy in the existing literature. In **chapter 7** we focus on a very specific rare autoantibody associated with SSc, anti-U3RNP autoantibody (anti-fibrillarin), we assess the prevalence of cardiopulmonary involvement in these patients, the degree of microangiopathy as shown by NC, and the association between NC and cardiopulmonary involvement. In **chapter 8** we evaluate whether ACA and ATA specific isotype expression and organ involvement associates with the degree of microangiopathy in SSc. As last, in **chapter 9** we investigate whether we can use the ACA isotype response as a biomarker to predict which ACA positive SSc patients progress over time.

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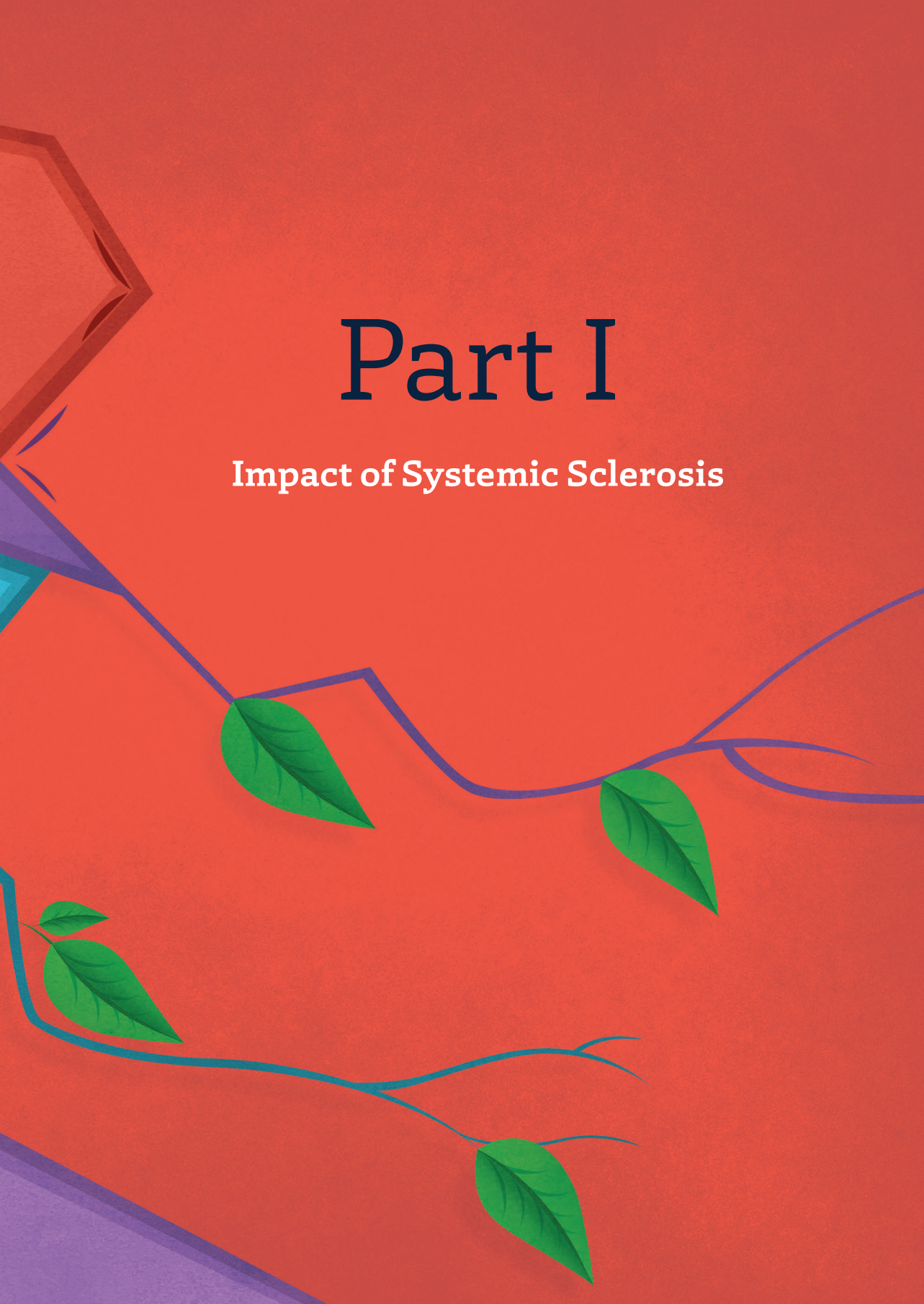
Part I

Impact of Systemic Sclerosis



Chapter 1

Illness perceptions, risk perceptions and worries in patients with early systemic sclerosis: a focus group study

Nina M. van Leeuwen, Maaike Boonstra, Tom W.J. Huizinga,
Ad A. Kaptein, Jeska K. de Vries-Bouwstra

Published Muscoloskeletal Care

Objectives: This study explores illness perceptions, risk perceptions, and degree of worry in patients with recently diagnosed Systemic Sclerosis (SSc). Specifically, it aims to answer whether and how early diagnosis in a stage that disease is relatively mild can impact patients' life, and if and how disease severity associates with illness perceptions and risk perception.

Methods: Patients with a diagnosis of SSc <2 years were invited to participate in a focus group discussion for in-depth exploration of illness perceptions, risk perceptions, and worry. In addition, illness perceptions, risk perceptions and worries were evaluated with the use of questionnaires. In order to explore how patients perceive SSc, we asked them to draw their disease. Physician global assessment of disease severity was used to measure disease severity. Associations between disease severity, illness/risk perceptions, drawings, and elements of the focus group were assessed.

Results: We observed three dimensions of illness perception as most relevant for patients: personal control, concern, and consequences. Patients with SSc experienced many symptoms and felt low personal control. Concerns about the future were often mentioned, and a majority of patients scored high on the worry questionnaire. None of the patients were pre-occupied with prognosis or death. All drawings illustrate the impact of SSc on daily life and psychological well-being. Illness perceptions were highly variable between patients and did not associate with disease severity.

Conclusion: This study illustrates that a diagnosis of early SSc has significant impact on patients' life,

INTRODUCTION

Systemic Sclerosis (SSc) is a chronic and incurable connective tissue disease with a heterogeneous presentation and disease course (1). Skin fibrosis is characteristic, but interstitial lung disease (ILD), peripheral vasculopathy, and gastro-intestinal involvement are also common. More severe disease complications such as myocardial disease and pulmonary arterial hypertension (PAH), though less frequent, are associated with increased mortality and require monitoring (2). The first 5 years of the disease are most critical in determining the individual patient's prognosis (3). With the improvement of diagnostic guidelines, the diagnosis is more frequently made in an early phase (4). However, earlier diagnosis has not led to improvements in determining the prognosis in the individual patient due to lack of accurate prognostic markers and the fact that early diagnosis lengthens the time-window in which prognosis is unclear (5). It is important and helpful to know how this affects patients, as illness perceptions directly influence illness behaviour (6,7). The patient's perception of risk for severe complications of SSc, however, has hardly been studied.

Several studies indicate that among persons with chronic illnesses individual's subjective beliefs about their condition are strongly associated with outcomes such as pain, physical health status, and mental health status (8,9,10,12). Beliefs regarding the patient's condition are referred to as illness perceptions. These comprise the patient's own ideas about the disease, its cause, how the disease evolves over time, what the consequences will be, and how the disease should be treated, and emotional responses to the illness and its consequences. Previous evaluation of illness perceptions in longstanding SSc showed that patients generally held strong views about the chronic nature and negative consequences of the disease. The unpredictable disease course and being at risk for organ involvement were found as important areas of illness perceptions (13-15). Interestingly, illness perceptions contributed more to physical and mental health in SSc than disease variables commonly used to describe disease severity (16,17).

Risk perceptions comprise the result of individual patient characteristics, including for example coping strategies, in combination with illness perceptions. These risk perceptions have a major impact on level of worry (18), illness behaviour (e.g., adherence with medication, seeking help from health care providers, refraining from work, and sexual activities, etc.), and commitment to medical care, which in turn affects the outcome of the illness and its medical management (19,20). However, in depth interviews on these issues in early SSc are lacking. The traditional method to elucidate patients' illness and risk perceptions includes questionnaires and focus group interviews. Although of value, these methods might influence patients' answers due to social desirability. A personal

drawing of the disease can illustrate the psychological and social impact of the disease of the individual patient and can reveal issues that remain unspoken during focus group discussions because of patient embarrassment, stigma, and shame (21). Indeed, a recent review revealed that drawings supplement and potentially outperform traditional data collection approaches (21).

In the current study we explore illness perceptions, risk perceptions and degree of worry in patients with recently diagnosed SSc, when prognosis is still uncertain. We performed an explorative, in-depth study combining quantitative measures such as questionnaires, qualitative measures such as a focus-group, and individual drawings in a selected group of SSc patients.

METHODS

Participants

Patients from the Combined Care Pathway Systemic Sclerosis (CCISS) were eligible for inclusion (22). This is an observational cohort of SSc patients, with annual follow-up at the rheumatology department at the Leiden University Medical Centre. Following written informed consent, patient data is collected systematically, including results of physical examination and extensive screening for organ involvement. For the current study, we selected patients aged 18-60 years that had received the diagnosis of SSc according to the ACR/EULAR 2013 criteria (American College of Rheumatology/ European League against Rheumatism) between 1-2 years prior to the start of this study (23). This time frame was chosen to allow recently diagnosed SSc patients an appropriate amount of time to develop personal illness/risk perceptions, while excluding patients with well-established disease (>2 years) that might have already developed severe disease-related morbidity and in whom it was not possible to asses future risk perceptions. For that same reason, we excluded patients with severe organ involvement requiring stem cell transplantation and/ or end-stage organ involvement. In addition, patients had to have completed a second evaluation in the care program and started with any kind of medication (prescribed by the rheumatologist) because of SSc. Patients with a psychiatric medical history were excluded.

Brief Illness Perception Questionnaire (BIPQ)

Illness perceptions were assessed using the BIPQ (24). The BIPQ consists of nine questions: 1. perceived consequences; 2. timeline (acute-chronic); 3. amount of perceived personal control; 4. treatment control; 5. identity (symptoms); 6. concern about the disease; 7. coherence of the illness; 8. emotional representation; and 9. causal perception. Item 6 and 8 overlap, with assessment of concern about the illness and assessment of the emotional aspects and mood of patients. Item 9 allows the patient to give three factors that in his/ her opinion have caused the disease. Each item is rated on a 10-point scale, where higher scores in question 1, 2, 5, 6 and 8 represent stronger negative endorsement with the illness perception. Higher scores in question 3, 4 and 7 represent positive endorsement with that perception.

Risk perceptions and worry

Perceived risks of disease complications, intensive treatment, and death were assessed using the adapted questionnaire from Cameron and Diefenbach, 2001 (19), consisting of three questions each with two subquestions:

1.1) How likely do you think it is that, at some point in your life, you will get a disease complication that will influence your way of life?, 1.2) How vulnerable do you think you are to develop a disease complication that will influence your way of life, at some point in your life?; 2.1) How likely do you think you are to get a disease complication that requires intensive treatment such as chemotherapy (cyclophosphamide) or stem cell transplantation, at some point in your life?, 2.2) How vulnerable do you think you are to develop a disease complication that requires intensive treatment such as chemotherapy (cyclophosphamide) or stem cell transplantation, at some point in your life?; 3.1) How likely do you think it is that, at some point in your life, you will get a disease complication that will result in death?, 3.2) How vulnerable do you think you are to develop a disease complication that will result in death, at some point in your life?.

Each item is rated on a 7-point Likert scale ranging from 1 (not at all) to 7 (almost certain or extremely). To calculate scores for risk perception, ratings of subscores were added (range 2-14) for each pair of questions.

Perceived worry was assessed with the following questions, also adapted from Cameron and Diefenbach, 2001: "1. To what extent are you worried about the disease worsening?" and "2. To what extent are you concerned about the disease worsening?". These items were also rated on a 7-point Likert scale ranging from 1 (not at all) to 7 (almost certain or extremely). Addition of the two questions generated a total worry score (range 2-14).

Focus Group and Drawings

A focus group discussion was held in an informal setting in a meeting room of the LUMC (outside the outpatient clinic) with coffee, tea, and biscuits and lasted 2 hours. The discussion was chaired by a health psychologist experienced in group discussions (AAK), one researcher (NvL), and one rheumatologist (JdVB) observed the meeting. Audio of the discussion was recorded and transcribed verbatim. Focus group discussions are valuable because discussions between patients indicate not only what patients think, but also how they think and why they think that way (25). A focus group generates rich narrative data that provides in-depth insights into patient perspectives on living with SSc. The optimal size for a focus group is between four and twelve participants; we included nine participants (four cancellations). This sample size created a large enough group to facilitate discussion without inhibiting balanced participation. Having a homogeneous group facilitates a narrative of shared experiences, fosters group comfort and cohesion, and improves the quality of group interaction (26,27). The study was designed in accordance with suggestions from the patient board of the Department of Rheumatology of the LUMC. Patients with different rheumatic disease, including two patients with SSc, take part in this board and are involved in research

as performed by members of the department. The rheumatologist involved in SSc (JdVR) proposed to investigate the impact of prognosis and the value of biomarkers form a patients' perspective in SSc during one of board meetings. The a priori themes evolved out of discussions with the rheumatologist specialized in SSc and a medical psychologist, and included prognosis, mortality and information on the disease. Two SSc patients participating in the Combined Care in Systemic Sclerosis cohort of the LUMC were involved in the development of the focus group discussion and evaluated the questionnaires. Before the interview, all patients were asked to complete the questionnaires and make a drawing representing their SSc. No further instructions were given regarding the drawing, and patients were not asked to draw a specific organ or whatsoever. Patients were asked to provide a brief written explanation of their drawing to make its content more readily identifiable (21). The dimensions of the BIPQ, the drawings, and a priori formulated themes were used as a guideline during the focus group discussion (Table 1). Patients were invited to discuss further issues that had not been brought up but that they felt to be important too.

Topic guide focus group	
Brief Illness perception questionnaire dimensions	- Consequences: How much does your illness affect your life? - Timeline: How long do you think your illness continues? - Personal control: How much control do you feel you have over your illness? - Treatment control: How much do you think your treatment can help your illness? - Identity: How much do you experience symptoms from your illness? - Concern: How concerned are you about the disease? - Coherence: How well do you feel you understand your disease? - Emotional: How much does your illness affect you emotionally? - What are the mosty important factors that you believe caused your illness?
A priori formulated themes	- Prognosis - Mortality - Provision of information
Drawings	- What did you draw? - Can you explain what the drawing mean to you?

Table 1. Topic guide focus group.

Disease severity

Disease severity was evaluated using the physician global assessment tool, measured with a 0-100 mm visual analogue scale (VAS; scale 0-100 (28,29)). The VAS is a score to evaluate SSc organ system symptoms including Raynaud's phenomenon, gastrointestinal

tract, cardiac and lung involvement, pain, and overall disease severity (28,29). All scores were given by the same physician (JdVB), as this physician was the treating rheumatologist for all included patients. A higher score indicates a more severe disease.

Analysis

Due to the small sample size, statistical testing and formal correlation analyses were not possible. Instead, summary scores and within-patient relationships between dimensions were analysed. Illness perceptions were assessed with the BIPQ during the focus group and in the drawings. Risk perceptions and worry were assessed in the questionnaires and during the focus group. Mean scores on the BIPQ, risk perception, and worry are presented. The relationship between BIPQ, risk perception, worry questionnaires, and the drawings were evaluated. Per patient, we explored the association between illness/risk perceptions as measured by the questionnaire and disease severity as measured by the physical global assessment tool for disease severity (Visual Analogue Scale Score). Individual stories of patients in the focus group transcript were analysed using interpretative phenomenological analysis (IPA) by two researchers (AAK and NvL) independently, and coded according to the dimensions of the BIPQ (30). The dimensions used for coding were: perceived consequences, timeline (acute-chronic), amount of perceived personal control, treatment control, identity (symptoms), concern about the disease, coherence of the illness, and emotional representation. These dimensions were also used to code the drawings. Differences in coding between the two researchers were discussed with the third researcher (JdVB) until consensus was achieved. Characteristics of patients were analysed using SPSS software.

RESULTS

Characteristics of participants

Of the 23 approached persons with recently diagnosed SSc that were approached, nine agreed to participate in the focus group discussion. Unfortunately, four had to cancel the focus group due to illness (two), a car accident (one) and anxiety related to the meeting (one) on the day the discussion was scheduled. Of these four, two did complete the questionnaire and made a drawing. The clinical characteristics of the seven patients with complete or partial data are summarized in Table 2.

Brief Illness Perception Questionnaire

Figure 2 illustrates the diversity in BIPQ scores for each illness perception per patient. The mean patients' BIPQ scores for each illness perception is shown in Table 3 (and Figure 1). The mean BIPQ score was high for timeline (mean ± SD, 9.6 ± 0.4), which indicates that the participants perceive SSc as a condition that will last forever. The participants perceived SSc as reasonably controllable with treatment (mean ± SD, 6.9 ± 2.3). As shown in Figure 2, the level of personal control varied considerably between patients (mean ± SD, 3.9 ± 3.4, range 0-10). The majority of patients feel little personal control over SSc and experiences quite a lot of concern (mean ± SD, 5.7 ± 1.5). The patient with the highest score on perceived consequences (''SSc affects my life severely'') scored highest on identity (''many severe symptoms'') and on treatment control (''treatment is extremely helpful''). Two patients with the lowest score for personal control (''absolutely no control over the disease'') both scored high on the dimension concern (''extremely concerned'') and low on the dimension treatment control (''treatment is not helpful'').

Self-reported causal perception

Most frequently mentioned causal factors for SSc were stress (n= 4) and genes (n= 3). Bad luck, menopause, and heavy physical work were also causal factors mentioned by the patients.

Worry and risk perceptions

Worries on symptom deterioration were present in all patients, with a mean ± SD score of 7.5 ± 2.7 on a scale of 2-14 (Figure 2). The majority of the patients (n= 6) felt themselves to be at risk for disease complications (mean ± SD, 7.1 ± 2.7 on a scale of 2-14), which is also shown on the BIPQ dimension timeline and concern. The mean score ± SD for perceived risk of patients on receiving intensive treatment somewhere in the future was 6.1 ± 2.7 (n= 5 scored above the midpoint) and the score for perceived risk of dying due to a SSc related complications was 4.9 ± 2.2 (n= 3 scored above the midpoint).

Baseline characteristics

	Sex	Age	Time since onset Raynaud	Time since onset non-Raynaud	Disease subset	Pitting scars	Digital ulcers
P1	F	43	1.5	1	L	no	no
P2	M	50	0.5	0.5	L	yes	no
P3	M	53	5	1.5	D	yes	yes
P4	F	41	5	4	L	no	no
P5	F	52	33	0.5	L	no	no
*P6	F	59	22	1	L	yes	yes
*P7	F	45	6	2	L	no	no

Table 2. Disease duration is given in years, mRSS= modified Rodnan Skin Score, ILD= interstitial lung disease, PAH= pulmonary arterial hypertension, SSc= systemic sclerosis, M= male, F= female, L= limited cutaneous skin involvement,

Scores of BIPQ dimensions

Dimensions	**Mean all 7 patients**	**Standard deviation**	**number of participants above midpoint >5 (total n=7)**
Consequences	5.3	2.5	3
Timeline	9.6	0.4	7
Personal control	3.9	3.4	3
Treatment control	6.9	2.3	5
Identity	4.9	2.0	3
Concern	5.7	1.5	4
Coherence	5.1	2.7	4
Emotional	4.9	2.1	3

Table 3. BIPQ, Brief Illness Perception Questionnaire. Means ± SD (standard deviations) of BIPQ dimensions, and number of participants scoring above midpoint, range 0-10.

mRSS	ILD	PAH, cardiac involvement, renal crisis	SSc specific autoantibody	VAS score	Immunotherapy	Drawing
5	no	no	yes	24	Hydroxychloroquine	E#
10	no	no	yes	24	No	F#
14	no	no	no	58	Methotrexate	C
5	yes	no	yes	31	Methotrexate	B
0	no	no	yes	24	No	D
5	yes	no	yes	20	No	G#
0	no	no	yes	22	No	A

D= diffuse cutaneous skin involvement. *Did not participate in the focus group due to sickness, but did fill in the questionnaires and made a drawing. # Figure S5 supplementary file

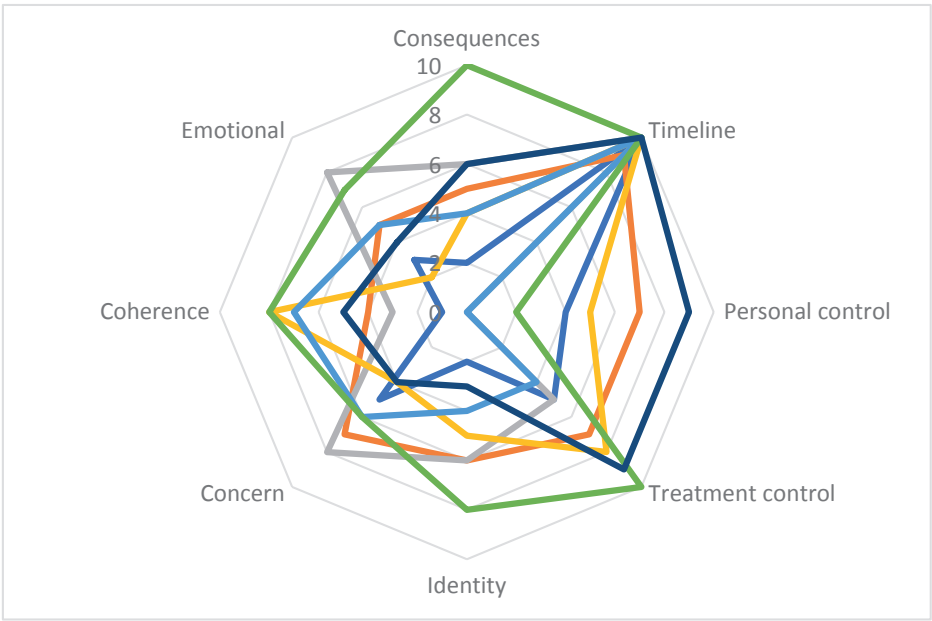


Figure 1. Brief Illness Perception Questionnaire dimensions. Every colour represents one patient, range 0-10.

Figure 2. Score on worry and risk questionnaire range 2-14. Every colour represents one patients.

Focus group

Identity, consequences, personal control, and concern were the illness perception dimensions mentioned most frequently. Living with SSc appears to be a dynamic process where symptoms, physical health, and mental health can change daily. This process includes regaining control over personal life. Patients mentioned the following regarding personal control:

- *A certain mindset is what you need, making specific things less important. (P3)*
- *I changed my lifestyle to minimize the symptoms I experienced. (P4)*
- *I thought I would be the one whose disease would disappear. Admitting to having a chronic disease did take a long time. (P3)*
- *All my thoughts and concerns, I keep them behind closed doors and act like they do not exist. (P5)*

Some patients experienced a mismatch between their mental capacity and physical health. The majority of patients changed their lifestyle to benefit their health. Particularly, patients had to change from a full-time to a part-time job, change to a less physically demanding job, give up or change their sport routine, sleep more hours, or make decisions about participating in activities which they took part in without issues before getting ill. The consequences of the disease were expressed in different ways:

- *My husband and children live in high gear around me, and I am already glad if I can make it to first gear. (P5)*
- *Every time I wear the gloves for the Raynaud Phenomenon, I feel obliged to explain this to everyone. (P1)*
- *I would love to have an extra battery, or a docking station which loads my energy levels during the night. (P5)*

At start of the symptoms, the majority of patients had their symptoms dismissed or these were misdiagnosed. When a diagnosis was finally made, this brought great relief. The dimension identity came forward during the focus group in the following quotes:

- *Finally hearing the diagnosis fit like a puzzle piece. (P4)*
- *It [the disease] does not show on the outside. People often tell me that I look good without knowing what happens on the inside. (P2)*
- *I am more tired than before, which is hard to accept. (P3)*

A lot of concerns were raised about the future and the disease progression, which also caused mood swings and concerns.

- *I fear how the disease will evolve. (P4)*
- *The disease brings a lot of insecurities, and you do not know what tomorrow will bring. (P2)*
- *Which level of the disease course do you step in? The disease course can vary from mild to severe, where on this scale am I?. (P4)*

Some patients described that after diagnosis, they searched for information about SSc, but that the pictures of patients and/or statistics on reduced life expectancy upset them. Most patients were displeased by reactions from their social environment. Especially patients without visible features of SSc were frustrated by family members who told them "they were looking good" or "were doing fine". Family members' and physicians' lack of knowledge about SSc makes patients feel as though an explanation of the disease and symptoms is continuously necessary. Despite feeling unsupported by their personal environment, none of the participating patients were interested in meeting other patients in support groups.

Drawings

In Figure 3, four drawings are depicted (see supplementary for additional drawings). Descriptions by participants of their drawings provide insight into how they are affected by SSc. All drawings were made in black and white. No one drew about treatments, or hospital visits. The drawings include symptoms, restrictions, and how these aspects affect patients emotionally. In the drawings, several dimensions of illness perception can be recognized. The most recognized dimensions are personal control (3 times) and identity (3 times). In most drawings more than one illness perception can be found. The portrayed hands and shoulders (drawing A in Figure 3, E and G in the supplementary file) demonstrate which symptoms individuals associate with SSc, i.e. , the illness *identity*. Three drawings also included portrayal of the participants' *concerns* regarding possible complications of SSc (e.g., Figure 3B), especially not knowing what to expect. Interestingly, no aspect of the drawings was coded to the *timeline* item, which explores patients' perceptions of the expected duration of SSc. Some aspects went beyond existing illness perceptions. For example, some drawings showed aspects of an individual's *social environment* such as family (drawing F, supplementary file). Other aspects include activities that were restricted due to the disease (Figure 3A-D). Finally, drawings in Figure 3C and 3D use metaphors. The scale in Figure 3D stands for finding balance in life and the life metaphor in drawing C (" *with each step the road becomes clear"*) illustrates how this patient deals with the disease.

Disease severity and its association with illness perceptions, risk perceptions and worry

The mean ± SD score for disease severity was 29.0 ± 13.2 (range in the study population 20-58mm). The patient with the highest score on the VAS (58mm, P3) had the lowest score on the BIPQ domain concern (score of 4) and drew drawing C, which is mostly about personal control. The patient with the lowest score on the VAS (20mm, P6) scored high on the BIPQ domain personal and treatment control (score of 9) and low on identity (score of 3), and drew the calcinosis in drawing G (supplementary file) which concerns the domain identity (symptoms). The patient (P5) with the highest score on the domains concern (score 7) and perceived consequences (score 10) and the lowest score on personal control (score 2) had a VAS score of 24mm and drew the scale in drawing D.

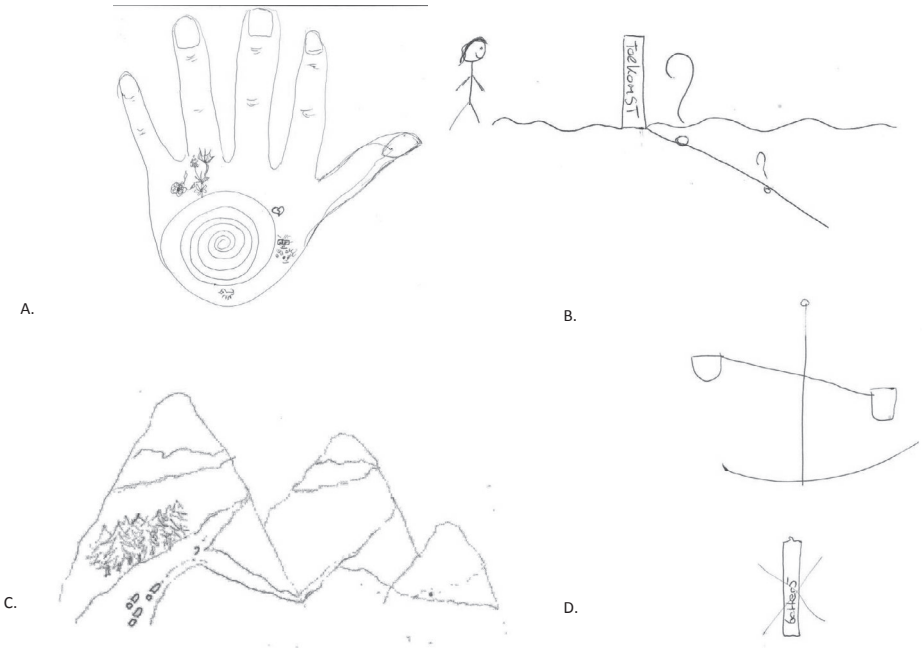


Figure 3 . Examples of drawings showing illness representations/perceptions with explanations by the patients.

A. ''My hands do the work, my hands make artwork. However, at this moment my hands are in pain and my fingers are getting thicker. It started with a little discomfort but it is getting worse. I have two kids, divorces, private business, a lot of insecurities, but I am strong''.

B. Toekomst= future. ''Most difficult part is not knowing what the future will bring. Will it get worse or will it stabilize''?

C. ''In every step the road will follow. The disease is like a mountain trail, will the trail fo to a new top or to the next valley''.

D. ''This is a scale, drawn because very day I have to make sure to be in balance and I cannot do too much in one day. The disease brings a lot of restrictions and I can be very tired which frustrates me, my batter is empty very fast''.

DISCUSSION

In this study, we explored illness perceptions, risk perceptions, and degree of worry in a few representative, recently diagnosed SSc patients who had not yet developed severe complications and still had an uncertain prognosis. Our study shows that being diagnosed with SSc can have a major impact on daily life, even in an early, relatively mild disease phase, and that patients describe a broad range of illness perceptions.

The BIPQ showed that these patients believed SSc could be reasonably controlled with treatment, and that patients with a low score on personal control were hampered more by concern. The worry and risk questionnaire indicated that the majority of patients thought they were at risk for disease complications, even in this early stage of the disease. Although patients expressed loss of personal control in the BIPQ, they also described different ways of adjusting their lifestyle to regain personal control during the focus group discussions. In addition to the defined illness perceptions in the BIPQ, the drawings revealed relevant perceptions including social environment and restrictions of specific activities. This demonstrates the additive value of the drawings, as previously described (31-33).

Illness perceptions do not seem to reflect disease severity, as patients with the highest scores on identity and perceived consequences were not the patients with the most severe disease according to physician global assessment. As illness perceptions influence illness behaviour (seeking medical help, medication adherence e.g.), it is important for physicians to be aware of this decoupling of patient perception of disease from objectifiable disease activity. For example, a patient who is short of breath might think this is just a sign of needing to rest because they perceive their current disease to be stable and mild. As such, they will not seek medical care, while in reality, this patient might be at risk for ILD progression.

To our knowledge, the BIPQ has not been used in SSc before, but some studies used the more traditional Revised Illness Questionnaire (34) to evaluate illness perceptions in SSc and found that that illness perceptions were a significant contributor to physical and mental health in SSc (16,17). They also found that unpredictable disease course and being at risk for developing disease complications were important areas of illness perceptions in these patients (13,14).The BIPQ has been used in patients with other rheumatic conditions including clinically suspect arthralgia (CSA), rheumatoid arthritis (RA), and psoriatic arthritis (PsA). In CSA patients, identity, consequences, personal control, and concern were identified as relevant, similar to what we found in SSc patients,. However, the CSA patients more often drew the timeline dimension compared to the SSc group. This might reflect the fact that CSA patients are at risk of developing a disease, while SSc

patients already realise the chronicity of their disease. In contrast to the SSc patients in our study, none of the CSA patients identified with being a patient (35). As SSc has the highest mortality rate among rheumatic diseases, one might expect SSc patients to score more negatively on multiple dimensions. However, although SSc patients showed more concern and lower personal control compared to patients with RA and PsA (24), they were comparable for the other dimensions. One explanation for this could be that we only included patients with recently diagnosed SSc without active severe complications to evaluate how patients deal with the diagnosis of a chronic disease with possible future disease complications. This might explain why SSc patients score relatively low on identity (symptoms) and consequences. The fact that SSc patients score higher on concern than patients with RA or PsA indicates that they are aware of the possible future complications. Questionnaires exploring worry and risk have not been performed before in SSc, precluding direct comparison with other studies in SSc.

Milette et al. (36) performed a study in SSc regarding patients' perspectives on coping and disease managements. Challenges discussed in that study referred to situations that hindered the possibility of coping well, including issues such as accessing information, and dealing with negative emotions. We identify part of these issues in this study as well: after a diagnosis was made patients, had negative experiences caused by internet based information, but on the other hand felt little understanding in their personal environment. Khanna et al (37) showed that both internet-based self-management websites and educational patient-focussed books are improving self-efficacy in SSc patients.

Limitations of this study could be that the participants who were able to physically attend and participate in these focus groups represent a subgroup of SSc patients who are potentially healthier than other SSc patients. Furthermore, given that the patients included in this study were both willing and able to attend in focus groups, this sample may also be over represent individuals with SSc who are comfortable in participating in groups. We acknowledge that the sample size of this cross-sectional study design is too small to provide evidence of causality. However, we aimed to explore illness perceptions and risk perceptions in early SSc and show subjective associations among the variables.

The strength of this study was the combined quantitative and qualitative analysis of illness perceptions, risk perceptions, and worry in recently diagnosed SSc patients, resulting in an unbiased approach, which has not been done before.

As shown in this study, a recent diagnosis of SSc can have a major impact on daily life and psychological well-being even in patients with mild disease. The concerns expressed by the patients advocate for patient information and education on an individual level

and in accordance with individual illness perceptions. Physicians should be aware that these illness perceptions can influence health outcomes and are not always in line with objectifiable disease measures. A multidisciplinary approach of patient-centred care that encompasses strategies to promote self-esteem, self-efficacy, and open communication may help to improve SSc related health and quality of life.

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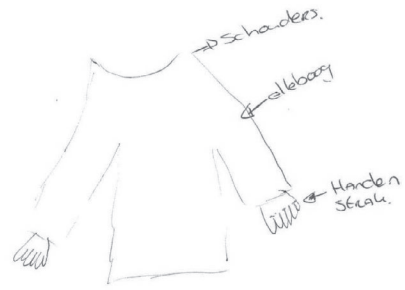
SUPPLEMENTARY FILE



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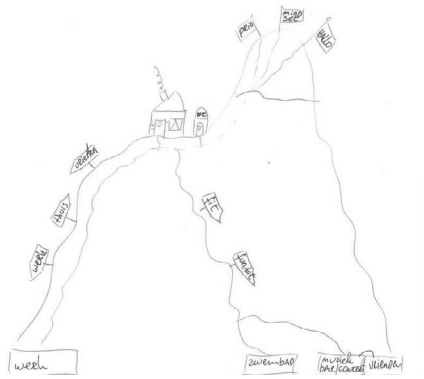

F.

G.

Figure S4. Examples of drawings showing illness representations/perceptions with explanations by the patients.

E. Schouder= shoulder, elleboog= elbow, handen strak= hand tight. '' My shoulder, elbow and hands are tight''.

F. '' Learning where you are in life, who you are and where you want to go. Reflection on yourself, this is something I learned due to SSc. I learned to embrace my life, mindset is very important. My aim is to do the right things and keep busy with things that are important for me and make me happy. I drew my house, work, swimming pool and family/friends but als an 'out house' to go and flush my concerns''.

G. '' Calcinosis in my fingers and a digital ulcer is how the disease started''.

Chapter 2

Health-related quality of life in patients with systemic sclerosis: evolution over time and main determinants

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Published Rheumatology

Objectives

In systemic sclerosis (SSc) patients, disease specific determinants that influence health-related quality of life (HRQoL) over time have not been described. We aim to, in patients with SSc 1) evaluate if and how HRQoL changes over time, and 2) assess how different SSc domains and functional impairments contribute to changes in HRQoL over time.

Methods

All SSc patients from the Leiden SSc cohort were included; patients with disease duration <24 months were classified as incident cases. HRQoL was assessed prospectively on an annual basis using the EQ-5D and the SF36. To assess baseline associations between clinical characteristics and HRQoL, linear regressions were performed. To identify possible associations between SSc characteristics and HRQoL change over time linear mixed-models were performed in both incident and prevalent cases.

Results

In total, 492 SSc patients were included (n=202 incident cases), with a median follow-up duration of 3.4 years. At baseline, presence of organ involvement was independently associated with a worse SF36 physical component score and lower EQ-5D score. Over time, gastro-intestinal symptoms, Raynaud and digital ulcers were independently associated with deterioration of HRQoL in both incident and prevalent cases. In prevalent cases, pulmonary arterial hypertension (PAH) was associated with a decrease in HRQoL over time. Worse functioning as measured by six-minute-walking distance, mouth-opening, finger-to-palm distance and grip-strength contributed significantly to deterioration of HRQoL over time.

Conclusion

In SSc, key clinical burdens that contribute to worsening of HRQoL over time include digital ulcers, Raynaud and gastro-intestinal involvement. In addition, PAH is a significant burden in prevalent disease.

INTRODUCTION

Systemic sclerosis (SSc) is a complex connective tissue disease characterized by deregulation of the immune system, vasculopathy and excessive collagen deposition leading to fibrosis of the skin and internal organs (1). SSc is a heterogeneous disease, in which multiple manifestations are associated with considerable morbidity and mortality (2). Two major clinical subtypes, namely limited cutaneous (lcSSc) and diffuse cutaneous SSc (dcSSc), can be recognized according to the extent of skin involvement (3). Given its severe and systemic character, health-related quality of life (HRQoL) is significantly affected in SSc patients both compared to the general population, and to patients with other rheumatic diseases or chronic conditions (4-6).

HRQoL is a patient reported outcome that includes domains related to physical, mental, emotional and social functioning. It focuses on the impact health status has on quality of life. Several tools are available to evaluate HRQoL in SSc patients. Some are specific for distinct organ systems or manifestations, while others are generic and can be applied to SSc and to a broad spectrum of rheumatic and non-rheumatic diseases. Among the generic indices, the Short Form-36 (SF36) and the EuroQol Five-Dimensional descriptive system (EQ-5D) are widely used given their reliability and construct validity (7, 8). The SF36 is a multidimensional questionnaire evaluating both physical and mental functioning. The EQ-5D is simple, quickly completed, and provides a multidimensional description of HRQoL. However, because the EQ-5D contains only a few questions, it could be considered simplistic and not capable of fully assessing individuals' HRQoL. These patient-reported outcomes are frequently included as secondary endpoints in randomized trials, highlighting the importance of addressing HRQoL indices when the efficacy of novel therapies is investigated (9).

Previous studies have evaluated SSc-related HRQoL cross-sectionally (9-12). Pain, dyspnea, digital ulcers (DU), Raynaud's phenomenon (RP) and gastrointestinal (GI) manifestations have been shown to have a negative influence on HRQoL (9-12). Most of the available evidence originates from studies with cross-sectional designs and focusing on one clinical characteristic. Other studies evaluated data from randomized controlled trials with a relatively short reassessment period (12-14). Due to the chronic nature of SSc, it is of additional importance to assess which disease manifestations have largest impact on disease related HRQoL longitudinally (4). This is of additional importance for design of therapeutic trials where manifestations with highest clinical burden should be taken into account. Therefore, using both the SF36 and EQ-5D, we evaluated the main determinants of HRQoL in a monocentric unselected cohort of SSc patients with prospective and longitudinal data available. First, we evaluated which factors are associated with HRQoL at first evaluation. Second, and as main purpose of our study, we evaluated if and how HRQoL changes over time and how different SSc manifestations impact on HRQoL over time.

METHODS

Study design and patients

For the current study all SSc patients followed at the Leiden University Medical Center (LUMC) from the ongoing, prospective, observational SSc cohort were included (time period 2009-2019). Patients with disease duration < 24 months were classified as incident cases. Patients had to fulfill the criteria of the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2013 for SSc (15) and had to have a clinical diagnosis of SSc. All patients undergo annual evaluation in the LUMC and clinical, laboratory, and imaging variables are systematically recorded in the research database; the Combined Care in Systemic Sclerosis (CCISS, approved by the local Ethics Committee P09.003/SH/sh in Leiden) registry (16). Questionnaires are collected on an annual basis. The cohort study is designed in accordance with the ethical principles of the Declaration of Helsinki. All patients gave written informed consent.

Health-related quality of life assessment

The Dutch version of the SF36 was used. Eight areas are covered in this questionnaire including: physical function, physical role, bodily pain, general health, vitality, social function, emotional role and mental health. The score ranges from 0 (poor health status) to 100 (good health status). Evidently, scores can be summarized in two global scores: the physical component score (PCS) and the mental component score (MCS) (17).

The EQ-5D is a generic tool consisting of five questions on mobility, self-care, usual activities, pain/discomfort and anxiety/depression, with three potential answers (1= no problem, 2= moderate problem, 3= severe problem) for each item. A sum utility score is calculated using nation-specific algorithms (18). The Dutch tariff (19) was applied in the present study. Results vary from -0.59 to 1. Negative scores indicate a patient's perception of a health status worse than death, while a score of 1 means perfect health. The second part of the questionnaire consists of a single visual analogue scale (VAS) through which patients are asked to rate their health of the day from 0 to 100. Higher values represent better health (20).

Patient characteristics and independent variables

For organ involvement, the following definitions were applied: DU were recorded as present when there was clear visible tissue breakdown. Both ischemic and mechanical (results of microtrauma and increased skin tension) ulcers were included in this definition. Interstitial lung disease (ILD) was defined based on the combination

of forced vital capacity (FVC) <70% and evidence for ILD on high-resolution computed tomography (HRCT). An experienced radiologist evaluated the HRCT for ground glass opacifications, reticulations and honeycombing. Pulmonary arterial hypertension (PAH) was defined as a mean pulmonary arterial pressure (mPAP) ≥ 25 mmHg at rest as assessed by right heart catheterization (RHC); including presence of pre-capillary PH, defined by a pulmonary capillary wedge pressure (PCWP) ≤15 mmHg and a PVR >3 Wood units (WU) on RHC. All patients with suspicion for PAH were referred for RHC. To evaluate myocardial involvement, we used a modified Medsger score. The Medsger scale mainly relies on the left ventricular ejection fraction (LVEF) for determination of myocardial involvement (21). However, the use of this parameter alone could lead to an underestimation of presence of myocardial involvement. Therefore, we used a combined value where patients had to have at least two of the following: arrhythmias (> 2% ventricular or supraventricular arrhythmia, atrial fibrillation), conduction problems, decreased LVEF < 54%, diastolic or systolic dysfunction, pericarditis or pericardial effusion. Myositis was defined based on a combination of creatine kinase (CK) measurements, proximal muscle weakness, if available, histology. Presence of gastro-intestinal (GI) involvement was defined based on the composite of severe GI symptoms according to the University of California Los Angeles GI tract (UCLA-GIT) questionnaire and/or the presence of gastric antral vascular ectasia (GAVE), and/or fecal incontinence, and/or weight loss and/or >10% or parenteral nutrition (22, 23). As the Dutch version of the UCLA-GIT was translated and validated in 2012 we do have some missing questionnaires in patients entering the cohort before 2012; baseline n= 143 missing (of 492), n= 74 missing (of 387) at 1 year follow-up, n= 26 missing (of 298) at 2 year follow-up.

In addition to specific organ dysfunction, the impact of functional assessments on HRQoL was evaluated. For this, handgrip strength measured in kilograms by a handheld dynamometer (24), finger range of motion measured by the standard finger-to-palm (FTP) method (25) (full fist closure was recorded as zero), mouth-opening measured by the maximal interincisal distance (26), and the six-minute walking distance were evaluated in relation to HRQoL (27).

As sensitivity analyses, we evaluated the association between the Health Assessment Questionnaire (HAQ), and the SSc specific version Scleroderma HAQ (SHAQ) and HRQoL measured by the EQ-5D and SF36. The HAQ comprises twenty items divided into eight domains, with a final composite score ranging from 0 (no disability) to 3 (maximal disability). The sHAQ evaluates five additional domains (scored on a 0-100 VAS) assessing disability induced by SSc specific symptoms, including DU, Raynaud's phenomenon, lung complaints, gastro-intestinal symptoms and disease severity (28).

The SHAQ, SF36, and EQ-5D are frequently used to evaluate HRQoL in SSc. The SHAQ has been extensively validated in SSc (7, 8), and is designed to measure functional ability or disability in SSc. It is quickly completed by the patients; however, by definition, it does not investigate the psychological aspect of HRQoL (7). The SF36 and EQ-5D include both physical and mental aspects of HRQoL.

Statistical analyses

Descriptive statistics were used to summarize baseline demographic and clinical characteristics of the included patients. Continuous variables are presented as mean (SD) or median (IQR) and categorical variables are presented as counts and percentages. The association between HRQoL based on the EQ-5D or SF36 (dependent variable) and the independent variables (organ involvement and functional performance) were expressed as the beta (b) and the standard error (SE), or as a P value (considered significant when less than 0.05). The UCLA GIT questionnaire was collected on an annual basis since 2012, for the missing numbers we used next observation carried backward. Univariable and multivariable linear regression models were constructed for both incident and prevalent cases, and the following confounders were fixed in the multivariable model: age, smoking, socio-economic status (defined as International Standard Classification of Education criteria), cardiopulmonary comorbidities and disease duration. Additional relevant variables based in univariable analyses were also included. Global curves for our outcomes over time were evaluated for both the incident and the prevalent cases. Linear mixed-effect models were used to assess changes in HRQoL score (MCS, PCS and EQ-5D) over the observation time, to control for repeated measurements, and to identify SSc characteristics associating with change in HRQoL during follow-up. The mixed models were separately performed in the incident and prevalent cases to adjust for the different disease durations. Time and risk factors were fixed effects in the analyses. All models included random intercept and slope to account for the longitudinal aspect of the data, and a compound symmetry correlation matrix was used. We selected the most fitting variance-covariance structure with the aid of the Akaike's score. The continuous predictors were mean centered to help interpreting the coefficients. The beta coefficient for each of individual independent variables of interest can be used to compare the strength of the effect of each variable on the dependent variable. For every 1-unit change in the predictor variable (independent), the outcome variable (dependent) will change by the beta coefficient value. Based on the number of tests performed, we corrected for multiple testing using the Bonferroni method. Statistical analyses were performed on SPSS version 26.

RESULTS

Patient group

In total, 492 patients with SSc were included. Mean age was 55 years, 79% of the patients were female, and 24% had dcSSc. At baseline, median duration since first non-Raynaud symptom was 3 years. Of the 492 included patients, 202 patients could be included in the incident cohort (disease duration since non-Raynaud < 24 months). The baseline characteristics are shown in Table 1.

Baseline associations with health-related quality of life (cross-sectional)

The SF36 mean scores of the total group were 62.6 (SD 22) on the MCS and 47.7 (SD 20) on the PCS, and the overall mean score on the EQ-5D was 0.66 (SD 0.26). Patients with diffuse cutaneous SSc (dcSSc), lower education level, shorter disease duration and cardiopulmonary comorbidities had worse quality of life as measured by SF36 and EQ-5D (supplementary table S1).

Of the evaluated organ systems in the incident cases, mRSS, Raynaud and GI symptoms were identified as independent determinants of HRQoL at baseline (table 2), organ involvement as a composite variable also independently associated with the PCS of the SF36 (multivariable β -11.1, $p < 0.001$) and with the EQ-5D (multivariable β -0.12, $p < 0.001$; table 2). In the prevalent cases (supplementary table S2) only severe GI and Raynaud symptoms were found as independent determinant of HRQoL. Secondly, we evaluated associations between functional assessments and HRQoL at baseline in which positive associations between the six-minute walk test and the PCS of SF36 (multivariable β 0.02, $p = 0.001$), MCS (multivariable β 0.02 $p = 0.003$), and the EQ-5D (multivariable β 0.13, $p = 0.001$) were identified in the incident cases (table 2), these associations were not found in the prevalent cases (supplementary table S2).

HRQoL changes over time

Evaluating the MCS, PCS and EQ-5D mean scores over time (follow-up period: 8 years) we found that the MCS (β -1.32, $p < 0.001$) and PCS (β -1.30, $p < 0.001$) worsened over time with respectively -1.32 and -1.30 points every year on a scale from 0-100, while the EQ-5D (β 0.01, $p < 0.001$) improved over time, although the extent of change was minimal (supplementary file table S3). The global curves of outcomes reflecting HRQoL (MCS, PCS and EQ-5D) over time for the incident and prevalent are shown in figure 1. Interestingly, incident cases showed worse HRQoL during the first two years of follow-up, but after two till three years of follow-up the curves of the incident and prevalent cases were quite similar for the SF36 and the EQ-5D.

	Total cohort N=492 (100%)	Incident N=202 (41%)	Prevalent N=290 (59%)
Female, n(%)	390 (79)	153 (76)	237 (82)
Age, mean (SD)	55 (14)	53 (14)	57 (14)
High education, n(%)	101 (21)	43 (21)	58 (20)
Current smoker, n(%)	79 (16)	30 (15)	49 (17)
Disease duration since NR, median (IQR)	3.2 (0.8-10.3)	0.7 (0.3-1.2)	8 (5-15)
Follow-up duration, median (IQR)	3.4 (2.0-6.2)	3 (1-5)	4 (2-7)
Disease characteristics			
Diffuse cutaneous subset, n(%)	118 (24)	51 (25)	67 (23)
Anti-centromere positive, n(%)	194 (39)	81 (40)	113 (39)
Anti-topoisomerase positive, n(%)	116 (24)	55 (27)	61 (21)
Digital Ulcers, n(%)	62 (13)	17 (8)	45 (16)
Modified Rodnan Skin score, median (IQR)	4 (0-6)	3 (0-7)	4 (2-6)
Organ involvement			
Interstitial lung disease, n(%)	183 (37)	62 (31)	121 (42)
FVC % of pred, mean (SD)	97 (23)	97 (25)	98 (21)
DLCO % of pred, mean (SD)	64 (24)	65 (28)	63 (22)
Pulmonary arterial hypertension, n(%)	26 (5)	8 (4)	18 (6)
LVEF < 54%, n(%)	31 (6)	11 (5)	20 (7)
Renal crisis, n(%)	14 (3)	6 (3)	8 (3)
Severe GI involvement, n(%)	82 (16)	35 (17)	47 (16)
Myositis, n(%)	8 (2)	8 (4)	0 (0)
Functional impairment			
Six minute walk test (m), mean (SD)	395 (259)	416 (260)	377 (265)
Mouth opening (mm), mean (SD)	31 (37)	26 (45)	33 (35)
Grip strength (kg), mean (SD)	13 (36)	11 (39)	16 (31)
Finger-to-palm (cm), mean (SD)	9.7 (22)	9 (23)	12 (20)
Medication at baseline			
Mycophenolate mofetil, n(%)	19 (4)	7 (4)	12 (4)
Methotrexate, n(%)	68 (14)	27 (13)	41 (14)
Cyclophosphamide, n(%)	11 (2)	9 (5)	2 (1)
Azathioprine, n(%)	14 (3)	6 (3)	8 (3)
Hydroxychloroquine, n(%)	22 (5)	9 (5)	13 (5)

Table 1. Baseline characteristics of the included patients. FVC= forced vital capacity, DLCO= diffusing capacity for carbon monoxide, pred= predicted, LVEF= left ventricular ejection fraction, GI= gastro-intestinal, n= number, SD= standard deviation, IQR= interquartile range

Clinical characteristics and worsening of quality of life over time (longitudinal)
To identify SSc patients at risk for worsening of HRQoL, we assessed factors associating with HRQoL change over an 8-year follow-up period in both the incident and prevalent cohort including 1977 measurements for each of the questionnaires (in total, n= 775 inception cohort, n= 1202 prevalent cohort). In the multivariable linear mixed-effect models Raynaud, GI symptoms and presence of DU were identified as independent risk factors for worsening of HRQoL over time in both cohorts (Table 3). In the incident cohort, also mRSS skin score and cardiac involvement were identified as risk factors for worsening of HRQoL over time. PAH was found to be an important risk factor for worsening of HRQoL in the prevalent cohort (PCS and EQ-5D). In the incident cases Raynaud symptoms were independently associated with a change in physical (PCS) and general health status (EQ-5D). The mRSS score was independently associated with a worsening PCS and EQ-5D score. GI symptoms were independently associated with worsening of the PCS and the EQ-5D. Presence of DU was independently associated with worsening of the PCS.

To evaluate which functional impairments affect worsening of HRQoL in SSc, we evaluated the six-minute walk test, mouth opening, finger-to-palm and grip strength in multivariable linear mixed effect models (Table 4). All functional outcomes were independently associated with worsening HRQoL over time as measured by components of the SF36 in both the incident and the prevalent cases. Only the fingertip to palm distance showed a significant association with worsening of HRQoL as measured by EQ-5D in both cohorts. The largest difference between the incident and prevalent cases was observed in the results for the six-minute walk test, which had a larger impact on HRQoL in the incident patients.

As a sensitivity check to confirm that both SF36 and EQ-5D measurements capture global disability in SSc, we evaluated associations between the SHAQ and the SF36 and EQ-5D. All scores, both cross-sectionally and over time, showed significant and strong association with SHAQ (table 5). Difference between the incident and prevalent cases were predominantly seen in the VAS digestive, which only showed a significant association with the SF36 in the prevalent cases (table 5).

	Univariable linear regression					
	MCS		PCS		EQ-5D	
Predictors	B (SE)	P	B (SE)	P	B (SE)	P
mRSS > 15	-8.04 (4.4)	0.07	**-5.6 (3.8)**	**0.001**	**-0.006 (0.002)**	**0.01**
Digital Ulcer	0.13 (0.16)	0.44	-0.01 (0.15)	0.91	-0.001 (0.002)	0.72
ILD	-1.01 (3.5)	0.77	-4.89 (3.1)	0.12	0.004 (0.04)	0.92
PAH	2.38 (8.3)	0.77	-10.54 (7.4)	0.16	-0.028 (0.1)	0.77
Severe GI	**-12.19 (4.2)**	**0.004**	-8.99 (3.7)	0.02	**-0.13 (0.05)**	**0.01**
Myositis	-3.31 (8.2)	0.69	-10.86 (7.4)	0.14	-0.14 (0.1)	0.14
Myocardial	0.063 (3.7)	0.98	-1.06 (3.3)	0.75	0.004 (0.04)	0.93
Renal crisis	-8.37 (9.4)	0.38	-14.29 (8.4)	0.09	0.40 (0.11)	0.72
Six minute walk test	0.01 (0.006)	0.02	**0.023 (0.005)**	**<0.001**	**0.005 (0.0)**	**<0.002**
Mouth opening	0.027 (0.04)	0.46	0.012 (0.03)	0.72	-0.06 (0.0)	0.41
Fingertip to palm	-0.21 (0.07)	0.77	- 0.13 (0.06)	0.04	**-0.003 (0.001)**	**<0.001**
Grip strength	0.035 (0.04)	0.41	0.012 (0.04)	0.75	-0.001 (0.0)	0.26
Raynauds VAS	**-58.14 (19.19)**	**0.003**	**-46.95 (17.26)**	**0.007**	-0.46 (0.23)	0.04
Organ involvement	-6.75 (3.28)	0.04	**-11.30 (2.8)**	**<0.001**	**-0,11 (0.04)**	**0.003**

Table 2. Associations at baseline between clinical and functional assessments and HRQoL in incident SSc cases (n= 202) . MCS= mental component score, PCS= physical component score, VAS= visual analogue scale, SE= standard error, mRSS= modified Rodnan Skin Score, ILD= interstitial lung disease, PAH= pulmonary arterial hypertension,

	Incident cohort				
	MCS Adjusted Estimate (95% CI)	P	PCS Adjusted Estimate (95% CI)	P	EQ-5D Adjusted Estimate (95% CI)
mRSS> 15	-2.7 (-7.3-1.8)	0.2	**-6.2 (-10.2- -2.2)**	**0.002**	**-0.06 (-0.1- -0.005)**
ILD	4.9 (0.3-9.5)	0.04	0.8 (-3.3-4.9)	0.7	0.02 (-0.04-0.06)
PAH	2.4 (-7.0-11.7)	0.6	1.4 (-6.9-9.7)	0.7	0.01 (-0.07-0.10)
GI	-3.07 (-6.5-0.4)	0.08	**-2.3 (-5.4- - 0.7)**	**0.002**	**-0.05 (-0.09- -0.009)**
Myositis	-1.4 (-8.6-5.9)	0.7	-6.2 (-12.6 - 0.1)	0.05	- 0.07 (-0.2-0.01)
Cardiac	**-22.2 (-34.7- -9.7)**	**0.001**	-11.8 (-23.2- - 0.3)	0.04	-0.2 (-0.2- -0.007)
Renal crisis	1.2 (-10.5-12.9)	0.8	0.6 (-9.9-11.2)	0.9	0.1 (-0.02-0.2)
Digital Ulcers	-0.1 (-4.9-4.7)	0.9	**-5.0 (-9.2- -0.8)**	**0.002**	- 0.06 (-0.12- -0.006)
VAS Raynaud	-0.04 (-0.1-0.02)	0.2	**- 0.09 (-0.2- -0.04)**	**<0.001**	**-0.002 (-0.003- - 0.001)**

Table 3. Linear mixed model performed in inception and prevalent cohort. mRSS, GI, myocardial, DU and Raynaud symptoms are associated with HRQoL changes over time in incident cohort. PAH, GI, DU and Raynaud symptoms are associated with HRQoL changes over time in prevalent cohort. Linear mixed model to evaluate association between organ involvement and HRQoL over

Multivariable linear regression

MCS		PCS		EQ-5D	
B (SE)	P	B (SE)	P	B (SE)	P
-8.62 (4.6)	0.06	**-16.23 (3.9)**	**<0.001**	**-0.13 (0.05)**	**0.01**
0.11 (0.17)	0.50	-0.004 (0.15)	0.98	-0.001 (0.002)	0.70
-1.68 (3.6)	0.64	-4.92 (3.2)	0.13	-0.005 (0.04)	0.90
-	-	-	-	-	-
-11.86 (4.3)	**0.007**	-8.69 (3.9)	0.03	-0.12 (0.05)	0.02
-	-	-	-	-	-
0.7 (4.2)	0.87	1.95 (3.8)	0.61	0.003 (0.05)	0.95
-	-	-	-	-	-
0.02 (0.006)	**0.003**	**0.02 (0.006)**	**<0.001**	**0.13(0.002)**	**<0.001**
0.03 (0.04)	0.39	0.02 (0.03)	0.65	0.00 (0.00)	0.53
-0.02 (0.07)	0.78	-0.13 (0.06)	0.04	**-0.003 (0.001)**	**0.001**
0.04 (0.04)	0.37	0.014 (0.04)	0.72	0.0001 (0.00)	0.36
-58.68 (19.5)	**0.003**	**-49.28 (17.4)**	**0.005**	-0.46 (0.23)	0.04
-6.88 (3.3)	0.04	**-11.13 (3.9)**	**<0.001**	**-0.12 (0.04)**	**0.002**

GI= gastro-intestinal. Multivariable linear regression adjusted for age, socio-economic status, comorbidities and smoking. Bold indicates significant associations after Bonferroni correction. We were underpowered to evaluate pulmonary arterial hypertension, myositis and renal crisis in this multivariable analysis.

	Prevalent cohort					
	MCS Adjusted		PCS Adjusted		EQ-5D Adjusted	
P	Estimate (95% CI)	P	Estimate (95% CI)	P	Estimate (95% CI)	P
0.003	1.6 (-3.0-6.2)	0.5	-1.06 (-4.9-2.7)	0.6	-0.06 (-0.1--0.009)	0.02
0.6	2.3 (-1.4-6.03)	0.2	1.2 (-2.1-4.6)	0.5	-0.01 (-0.05-0.03)	0.5
0.8	-6.4 (-13.6-0.79)	0.08	**-9.6 (-16.0- - 3.1)**	**0.004**	**-0.1 (-0.2- - 0.07)**	**<0.001**
0.002	-2.6 (-5.2-0.10)	0.06	-2.07 (-4.2- -0.09)	0.03	**- 0.05 (-0.07 - - 0.02)**	**0.003**
0.09	1.5 (-16.2-19.2)	0.9	2.6 (-11.8-17.0)	0.7	- 0.009 (-0.2-0.2)	0.9
0.04	1.3 (-11.1-13.7)	0.8	0.6 (-10.5-11.6)	0.9	- 0.09 (-0.2-0.04)	0.2
0.09	1.3 (-11.8-14.4)	0.8	-5.6 (-17.9-6.8)	0.4	0.05 (-0.07-0.2)	0.4
0.03	-2.8 (-5.5- -0.02)	0.04	**-3.0 (-5.3- - 0.8)**	**0.009**	-0.02 (-0.05-0.01)	0.2
<0.001	**-0.1 (-0.2- - 0.09)**	**<0.001**	**-0.1 (-0.2- -0.09)**	**<0.001**	**- 0.002 (-0.002--0.001)**	**<0.001**

time adjusted for age, socio-economic status, comorbidities and smoking. In this table the main effect are shown. P value cut-off after Bonferroni p <0.006. Bold indicates significant associations after Bonferroni correction. mRSS= modified Rodnan Skin Score, ILD= interstitial lung disease, PAH= pulmonary arterial hypertension, GI=gastro-intestinal, VAS= visual analogue scale.

	Incident cohort					
	MCS Adjusted Estimate(95% CI)	P	PCS Adjusted Estimate(95% CI)	P	EQ-5D Adjusted Estimate(95% CI)	P
6MWT	**0.01 (0.007-0.02)**	**<0.001**	**0.02 (0.010-0.02)**	**<0.001**	0.0001 (0.0004-0.002)	0.002
Mouth open	**0.05 (0.02-0.07)**	**<0.001**	**0.02 (0.004-0.04)**	**0.02**	0.00006 (-0.0003-0.0002)	0.64
FTP	**-0.07 (0.04-0.10)**	**<0.001**	**0.03 (0.002-0.06)**	**0.03**	**-0.0005 (-0.0008- - 0.0001)**	**0.009**
Grip strength	**0.06 (0.04-0.09)**	**<0.001**	**0.04 (0.02-0.06)**	**0.001**	-0.0001 (-0.0004-0.0002)	0.46

Table 4. linear mixed model; function impairment and HRQoL performed in incident and prevalent cohort. Adjusted for age, socio-economic status, comorbidities and smoking. In this table both the main effect as the time coefficients are shown.

	Incident cohort					
	MCS Adjusted Estimate(95% CI)	P	PCS Adjusted Estimate(95% CI)	P	EQ5D Adjusted Estimate(95% CI)	P
DIG VAS	-0.06 (-0.1-00.002)	0.05	-0.05 (-0.1-0.003)	0.06	**-0.001 (-0.002- -0.0004)**	**0.002**
DU VAS	**-0.09 (-0.2- -0.01)**	**0.02**	**-0.1 (-0.2- -0.05)**	**0.001**	**-0.002 (-0.002 - -0.0006)**	**0.001**
Pulm VAS	**-0.1 (-0.2- -0.02)**	**0.006**	**-0.1 (-0.2-0.07)**	**<0.001**	**- 0.001 (-0.002- -0.0001)**	**0.02**
Severity VAS	**-0.09 (-0.2- -0.03)**	**0.002**	**-0.2 (-0.2- -0.1)**	**<0.001**	**-0.002 (-0.003- -0.001)**	**<0.001**
HAQ-DI	**-9.6 (-11.8- -7.3)**	**<0.001**	**-12.3 (-13.2- -10.5)**	**<0.001**	**-0.005 (-0.01-0.00)**	**0.05**

Table 5. Linear mixed model HAQ and SF-36 and EQ5D performed in incident and prevalent cohort. Adjusted for age, socio-economic status, comorbidities and smoking. In this table both the main effect as the time coefficients are shown.

Prevalent cohort

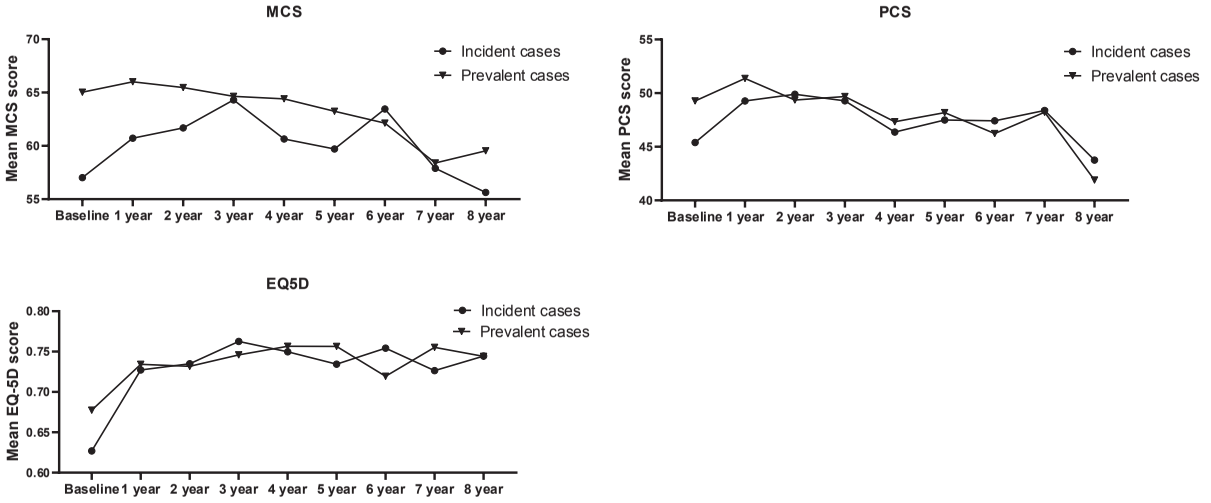
MCS Adjusted Estimate(95% CI)	P	PCS Adjusted Estimate(95% CI)	P	EQ-5D Adjusted Estimate(95% CI)	P
0.003 (-0.002-0.008)	0.21	**0.005 (0.001-0.009)**	**0.01**	0.0002 (-0.0007-0.0003)	0.5
0.03 (0.01-0.05)	**<0.001**	**0.02 (0.002-0.03)**	**0.02**	0.0001 (-0.0002-0.0002)	0.9
-0.08 (0.05-0.11)	**<0.001**	**-0.06 (0.03-0.08)**	**<0.001**	**-0.0003 (-0.0006- -0.0001)**	**0.04**
0.04 (0.02-0.06)	**<0.001**	**0.02 (0.005-0.04)**	**0.01**	0.00002 (-0.0002-0.0002)	0.9

P value cut-off after Bonferroni p <0.013. Bold indicates significant associations after Bonferroni correction. 6MWT= six-minute walk test, Mouth open= mouth opening, FTP= fingertip-to-palm.

Prevalent cohort

MCS Adjusted Estimate(95% CI)	P	PCS Adjusted Estimate(95% CI)	P	EQ5D Adjusted Estimate(95% CI)	P
-0.1 (-0.2- -0.09)	**<0.001**	**- 0.09 (-0.1- -0.05)**	**<0.001**	**-0.001 (-0.002- -0.001)**	**<0.001**
-0.1 (-0.2- -0.08)	**<0.001**	**-0.1 (-0.2- -0.07)**	**<0.001**	**-0.001 (-0.002- -0.0008)**	**<0.001**
-0.1 (-0.2- -0.07)	**<0.001**	**-0.1 (-0.2-0.09)**	**<0.001**	**-0.001 (-0.002- - 0.0008)**	**<0.001**
-0.1 (-0.2- -0.1)	**<0.001**	**-0.2 (-0.2-- 0.7)**	**<0.001**	**-0.003 (-0.002- -0.002)**	**<0.001**
-1.7 (-2.6- -0.8)	**<0.001**	**-3.04 (-3.9 - -2.1)**	**<0.001**	**-0.006 (-0.008- -0.002)**	**<0.001**

DIG= digestivus, DU= digital ulcera, Pulm= pulmonary, Severity= disease severity, VAS= visual analogue scale, HAQ DI= health assessment questionnaire disability index, bold indicates p value < 0.05.



	Baseline	1 year FU	2 year FU	3 year FU	4 year FU	5 year FU	6 year FU	7 year FU	8 year FU
Incident cases, n	202	167	124	94	73	51	33	31	18
Prevalent cases, n	290	220	174	155	112	102	82	67	48

Figure 1. Mean scores over time of the EQ-5D and both component scores of the SF35 (MCS= mental component score, PCS= physical component score) in the incident and prevalent cases.

DISCUSSION

In this study we aimed to explore which disease-specific characteristics are associated with change of HRQoL in patients with SSc over time. Over time, HRQoL in SSc slightly worsened, and key clinical burdens determining worsening of HRQoL included DU, Raynaud and GI involvement. In addition, functional impairment as reflected by worse walking distance, mouth opening, and hand function, independently impact on worsening of HRQoL. Some differences were found for the incident and prevalent cases, where skin score and six-minute walk test had a larger influence on HRQoL in incident cases and PAH had a larger influence on HRQoL in prevalent cases.

Literature on longitudinal variations of HRQoL in SSc is scarce. Most published studies have been conducted on cross-sectional data. Our results on cross-sectional associations at baseline are largely in line with previous findings. Indeed, the extent of skin involvement has frequently been identified as one of the factors affecting HRQoL in SSc. Compared with lcSSc, patients with dcSSc had poorer HRQoL scores (4, 10, 29) and the degree of skin involvement measured by mRSS had a negative impact on HRQoL (5). GI manifestations have also been studied. Both Franck-Larsson et al. (30) and Omair et al. (31) showed that lower GI symptoms, and especially fecal incontinence, contributed negatively to HRQoL in patients with SSc, while Franzt et al. (9) revealed how GI complications were significantly associated with perception of disease severity. Our results are also in accordance with previous studies showing that Raynaud Phenomenon is one of the most common symptoms influencing HRQoL in SSc (32). Although hands are frequently involved in SSc and dedicated programs have been developed to improve hand function (25), the impact of hand disability on HRQoL has not been extensively explored. In our study, we show that functional disability of the hand, measured by finger-to-palm distance and grip strength, significantly contributed to worsening of HRQoL over time.

It is important to note that severe GI involvement, and also Raynaud's phenomenon significantly impact SSc-related HRQoL, both cross-sectionally and over time, while other disease manifestations including ILD did not seem to affect the patient's perception of HRQoL. As elegantly hypothesized by Frantz et al.(9), caring physicians naturally focus their attention on life-threatening manifestations. Consequently, physicians might assume that these life-threatening manifestations will also considerably impact HRQoL from the patients' perspective. Our results indicated that daily life of SSc patients is significantly affected by relatively less severe, but troublesome and difficult to control symptoms, including Raynaud's and GI complaints.

Strikingly, in the multivariable analyses, presence of ILD did not impact HRQoL over time which is opposite from what one might have expected. We can only speculate about the explanation for this observation. For example, it is known that not all SSc-ILD patients actually experience symptoms attributable to ILD. However, by using a combined definition for presence of ILD, based on interstitial lung abnormalities on HRCT together with FVC % of predicted (<70), we aimed to identify patients with more severe, and clinical relevant ILD. Interestingly, the pulmonary VAS (of the HAQ) and the six-minute walk test did show an association with change in SF36 and EQ-5D, indicating that indeed patients who actually experience dyspnoea do experience worse quality of life. This observation might indicate that the clinically applied definitions for SSc-ILD are not completely in line with symptoms as experienced by the patients. Another explanation might be that SSc-ILD patients are treated more aggressively and earlier in the disease course which might come to benefit of other disease manifestations as well, and consequently is beneficial for HRQoL (33). Finally, patients with SSc-ILD might reflect a study population with more severe disease. In this subgroup, SSc-HRQol might be more severely affected from the start and, while over time, these patients learn to cope with their situation. Consequently, presence of ILD does not result in more deterioration of SSc-HRQoL over time.

There are some limitations to our study. The first limitation is intrinsic to the use of patient reported outcomes, which are self-administered questionnaires that largely dependent on patients' perceptions and not always reflect disease activity or severity of specific manifestations in an objective way. However, the used questionnaires are all validated in SSc (7, 28, 34, 35). To confirm construct validity a sensitivity analysis using SHAQ was performed which showed highly significant associations with SF36 and EQ-5D both cross-sectionally and over time. Second, consensus about the definition of specific SSc related organ involvement is not unanimously among experts, as for example for cardiac complications. The definitions applied to define organ involvement have been largely based on the Medsger scale with adjustments where deemed necessary. Our work has major strengths too: most importantly the longitudinal design. Few studies investigated determinants of HRQoL evolution over time in SSc patients. We were able to include patients with up to 8 years of follow-up. Secondly, most published articles investigate the effect of a specific disease manifestation, the effect of medication or a selected patients' group. In our analyses we included a heterogeneous and unselected population of 492 SSc patients in which 202 were incident cases, contributed data had a high rate of completeness and, in our opinion, we thus provide fairly generalizable results.

In conclusion, this study provides unique information about the most important determinants of HRQoL in SSc. Deeper knowledge of factors significantly influencing not only HRQoL, but also changes of HRQoL over time, is of relevance to tailor most appropriate treatment strategies. Moreover, a thorough understanding of HRQoL determinants may help caring physicians to identify the unmet needs of SSc patients and the areas where more vigorous pharmacological or non-pharmacological interventions are indicated. We confirm previous findings about the impact of RP, hand function, skin involvement and GI symptoms on daily life of SSc patients, but we also outline the possibility that these factors may predict further HRQoL deterioration. Our results suggest that major attention should be paid to GI symptoms, Raynaud and DU as possible predictors of worsening HRQoL. In addition, in patients with longstanding disease PAH is importantly influencing HRQoL over time.

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Part II

Disease progression in Systemic Sclerosis

Chapter 3

Disease progression in Systemic Sclerosis

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Published Rheumatology

DEAR EDITOR,

Systemic sclerosis (SSc) is a heterogeneous disease in which vasculopathy and fibrosis affect multiple organ systems such as the skin, gastrointestinal tract, kidneys, heart and the lungs (1). The clinical course of the disease can vary from rapidly progressive resulting in generalized fibrosis of the vital organs to a more indolent form developing over an extended period of time. For both physicians and patients, SSc represents a major clinical challenge, as prediction of the disease course remains difficult. Disease progression has been shown to occur often (2,3). However, many studies have been performed in selected subgroups of patients or have focused on specific organ domains.

In order to improve prediction of the individual disease course, we need accurate and complete information on the occurrence rate of disease progression per organ system during follow-up, preferably in an unselected cohort of SSc patients. This is crucial as in many SSc cohorts mild cases are not extensively followed, making these cohorts ungeneralizable to the entire SSc population. The Leiden Combined Care In SSc Cohort (4) has, from its beginning, included patients in accordance with the ACR/EULAR 2013 criteria (5). All included patients undergo extensive annual assessment irrespective of symptoms, and as such, data of this cohort can provide data on disease progression in an unselected SSc cohort. We analyzed occurrence of disease progression in 492 SSc patients fulfilling ACR/EULAR SSc 2013 criteria who underwent at least two complete assessments for organ involvement (4). Of the included patients, 79% (n= 389) was female; mean age at baseline was 55 years (SD 14); the median disease duration since first non-Raynaud symptom was 3.2 years (IQR 0.9-10.3), 39% (n= 194) were anti-centromere antibody (ACA) positive, 24% (n= 116) anti-topoisomerase antibody (ATA) positive, 6% (n= 27) anti-RNA polymerase III (anti-RNPIII) positive and 12% (n= 61) were anti-nuclear antibody (ANA) negative. Twenty-four percent (n= 118) of the patients had diffuse cutaneous SSc at baseline, and 37% (n= 183) had signs of interstitial lung disease (ILD) at baseline.

Disease progression was defined as progression in one or more organ systems, death, or start of immunosuppressive treatment, and was evaluated annually. For ILD, pulmonary arterial hypertension (PAH), modified Rodnan Skin Score (mRSS) and renal crisis, progression was defined as described previously (6,7) (supplementary file table S1 for detailed explanation). Cardiac progression, gastro-intestinal progression and myositis were each defined using a combination of variables (supplementary file table S1 for detailed definitions).

In 492 SSc patients (2109 timepoints, range follow-up 2-10 years [see supplementary file table S2 for differences in follow-up per subgroup]), disease progression was observed in 52% (n= 257) after a median follow-up duration of 4 years (range 1-8 years), including cardiac progression in 29% (n= 142), lung progression in 23% (n= 114), skin progression in 16% (n= 79), and GI progression in 12% (n= 60). Death (12%, n= 60), development of PAH (4%,n= 20), myositis (3%,n= 14) and renal crisis (1%,n= 5) occurred less frequently. Forty-eight percent of the patients (n= 235) did not show any progression during a median follow-up of 3 years (range 1-9) (82% female, 18% ATA+)

Current literature indicates SSc disease progression occurring most often early in disease course, specifically in ATA positive patients. Therefore, we evaluated disease progression during follow-up stratified for disease duration, autoantibody subgroup and disease subset (Figure 1 A,B,C and Figure S1 supplementary file).

In our cohort, 56% of observed progression occurred within 5 years since first non-Raynaud symptom. While progression in skin involvement occurred more frequently in early disease, proportion of patients with lung, heart or GI progression was relatively stable over time. In total 24% (n= 63/257) of first time organ progression occurred after 10 years since first non-Raynaud. When stratifying for both autoantibody and disease duration, we saw a striking difference in occurrence of skin progression: while in ATA patients this typically occurred early, the proportion of patients with skin progression increased over time in the ACA group. Cardiopulmonary progression was more frequent in ATA patients (58% of ATA showed cardiopulmonary progression), but also frequently observed in ACA patients (26% of ACA showed cardiopulmonary progression), and independent of disease duration in both groups. In ACA positive patients the percentage of progressors was often highest in the subgroup with longstanding disease. Unfortunately, we were underpowered to draw firm conclusions on progression rates in anti-RNPIII positive patients and ANA negative patients (supplementary figure S1). In addition to antibody specificity, also pattern of skin involvement has been identified as important clinical biomarker for risk of disease progression. Of the patients that presented with lcSSc, 17% progressed to dcSSc, most frequently within 5 years since first non-Raynaud. Any progression (excluding progression to dcSSc) occurred in 47% of lcSSc and in 72% of dcSSc (supplementary figure S1). Finally, one might hypothesize that follow-up duration might be different depending on clinical severity of the disease; however, in our cohort clinical follow-up was remarkably comparable for the clinical subgroups we evaluated (supplementary table S2), which supports that patient were evaluated annually independent of disease severity.

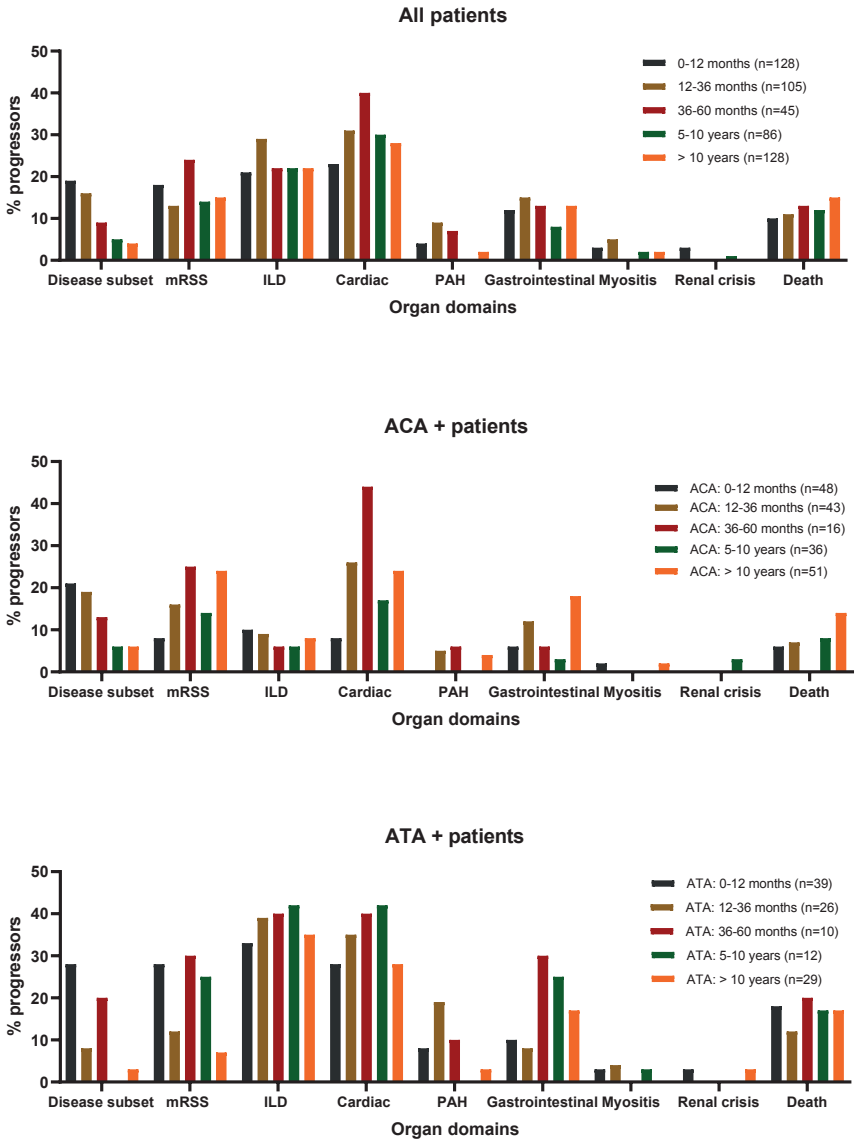

Figure 1. Percentage of progressors per subdomain. A. Stratified for disease duration since first non-Raynaud symptom, B. Stratified for anti-centromere (ACA) positive patients and disease duration, C. Stratified for anti-topoisomerase (ATA) positive patients and disease duration. mRSS= modified Rodnan Skin Score, ILD= interstitial lung disease, PAH= pulmonary arterial hypertension, n= number.

In conclusion, our data confirm that the proportion of SSc patients that experiences disease progression over time is substantial, also when applying ACR 2013 criteria that were designed to diagnose SSc earlier. We show that among ACA positive patients skin progression does occur, typically in longstanding disease. Finally, our data indicate that cardiopulmonary progression can occur at any time during follow-up, independent of disease duration, underlining the importance of identifying biomarkers for risk stratification that can guide follow-up. The challenge remains to identify individual patients with a low risk of progression and in whom annual complete assessment might be redundant. This justifies our ongoing research in identifying autoantibody levels and characteristics that could contribute to personalized risk stratification [work in progress] (7-8).

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SUPPLEMENTARY DATA

Supplementary table S1. Detailed explanation disease progression per organ system

Pulmonary progression	≥ 10% relative decline in forced vital capacity (FVC) with follow-up FVC < 80% predicted or ≥ 5% to < 10% relative decline in FVC and either a ≥ 15% relative decline in diffusing capacity of the lung for carbon monoxide (DLCO) with follow-up DLCO < 80% predicted or increase of the extent of lung involvement (interstitial lung disease (ILD)) as determined by HRCT
Cardiac progression	Based on a combined definition, which included clinical cardiac involvement, decreased left ventricular ejection fraction < 54% (LVEF), arrhythmias (> 2% ventricular extrasystoles, atrial fibrillation), and major cardiac events (including all acute coronary syndromes and pacemaker implantations).
Pulmonary arterial hypertension (PAH)	Mean pulmonary arterial pressure (mPAP) ≥ 25 mmHg at rest as assessed by right heart catheterization (RHC), pulmonary capillary wedge pressure ≤ 15 mmHg and a pulmonary vascular resistance > 3 Wood units, was classified as progression.
Gastro-intestinal	Development of gastric antral vascular ectasia (GAVE), or anemia AND weight loss (> 10% in 1 year).
Skin progression	mRSS increased ≥ 5 points and ≥ 25%
Renal progression	Clinical diagnosis of renal crisis (including hypertension, increase in creatinine, oligo/anuric renal failure)
Myositis progression	Diagnosis of myositis based on muscle complaints and histologic prove of myositis or complaints of myositis and an increased creatine kinase (>150 U/l) not otherwise explained AND muscle weakness.

Supplementary table S2: follow-up duration starting from first non-Raynaud for different clinical subsets in years

Subgroup	Follow-up duration starting from first non-Raynaud symptom, median (IQR)
ACA +	8.9 (4-14)
ATA+	8.4 (3-14)
ANA-	7.6 (4-16)
RNApIII +	9.5 (5-17)
LcSSc	9.2 (4-15)
DcSSc	9.0 (4-15)

ACA= anti-centromere antibody, ATA= anti-topoisomerase antibody, ANA= anti-nuclear antibody, LcSSc= limited cutaneous systemic sclerosis, dcSSc= diffuse cutaneous systemic sclerosis

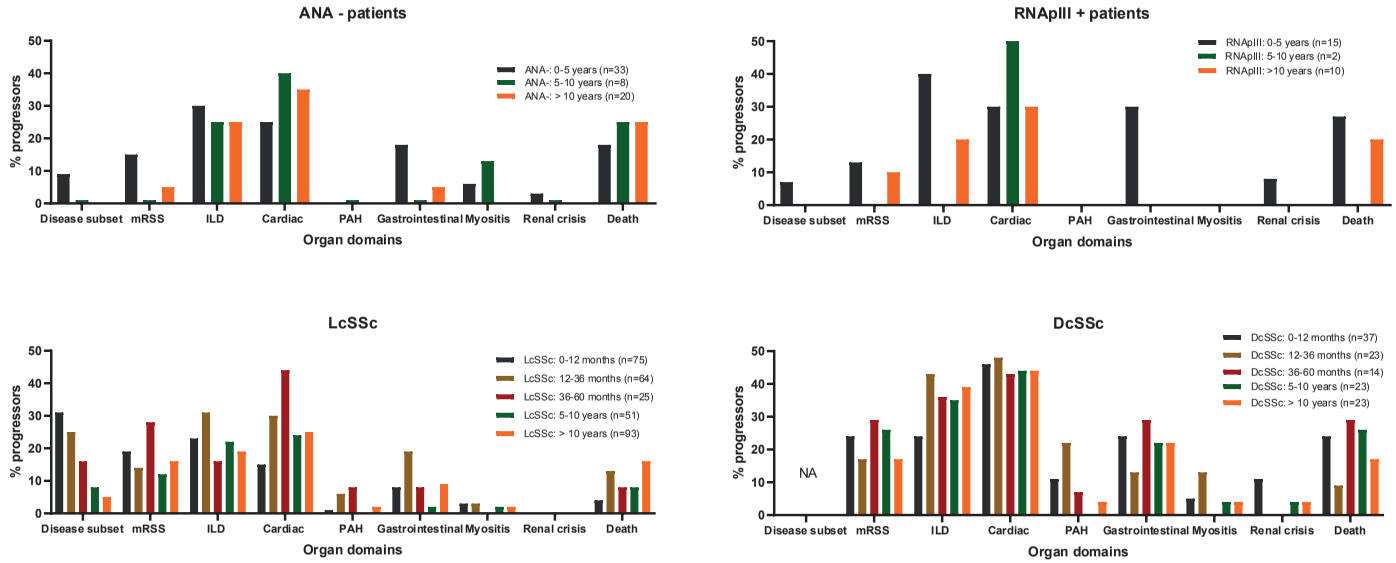

Figure S1. Organ progression stratified for disease duration since first non-raynaud symptoms, for disease subset and for autoantibody profile. ANA= anti-nuclear antibody, lcSSc= limited cutaneous systemic sclerosis, dcSSc= diffuse cutaneous systemic sclerosis, mRSS= modified Rodnan Skin Score, ILD= interstitial lung disease, PAH= pulmonary arterial hypertension.

Chapter 4

Gastro-intestinal symptom severity and progression in systemic sclerosis

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Under revision Rheumatology

Objectives: To evaluate the severity and evolution of patient-reported gastro-intestinal (GIT) symptoms in systemic sclerosis (SSc) patients, assess predictive factors for progression and determine the impact of standard of care treatment.

Methods: SSc patients from the Leiden and Oslo cohorts were included. We assessed clinical data and patient-reported GIT symptoms measured by the validated University of California, Los-Angeles Gastro-intestinal-tract (UCLA-GIT) score at baseline and annually. GIT severity and progression was determined for total GIT score and individual subdomains. Logistic regression was applied to identify risk factors associated with baseline GIT symptom severity. Linear mixed-effect models were applied to assess progression in GIT symptom burden and to identify predictive factors.

Results: We included 834 SSc patients with baseline UCLA GIT scores, 454 from Leiden and 380 from Oslo. Demographics and clinical characteristics at baseline were comparable in the cohorts. At baseline, 28% reported moderate-severe GIT symptoms, with increased risk for severity conferred by anti-centromere antibody (ACA), smoking and corticosteroid use, while use of calcium channel blockers appeared protective. After one and three years follow-up, 27% and 29% of patients reported increased GIT symptom severity, with reflux/bloating as the most prominently progressing symptoms. In the mixed-effect models, female sex and ACA predicted GIT symptom progression, while immunomodulatory therapies seemed to have no major impact.

Conclusion: In this large, multicenter SSc population high GIT symptom burden is present early in the disease course. Over one year, 27% of patients reported worsening of GIT symptoms, occurring independently of baseline GIT symptom severity and disease duration.

INTRODUCTION

Second to skin, the gastrointestinal tract (GIT) is the most commonly afflicted organ system in systemic sclerosis (SSc) (1). All segments of the gastrointestinal tract can be affected resulting in dysmotility and hypomotility of the oesophagus, the small intestine and the colon with possible life-threatening complications (2). GIT symptoms have negative impact on quality of life and severe GIT involvement associates with high mortality (3-8). Molecular mechanisms underlying GIT involvement in SSc are not clear, but the prevailing view is that immune-mediated inflammatory processes and progressive vascular abnormalities contribute to fibrotic changes of the bowel wall leading to disturbed intestinal blood flow, poor microcirculation, and altered contractility (9-12).

Currently, the approved treatment options for GIT involvement in SSc are limited, but this may, at least partially relate to the fact that very few intervention studies have addressed GIT-related outcome measures; and those that exist have focused on SSc patients with advanced GIT disease (2, 13-15). SSc patients are frequently treated with immune-modulating and/or vasodilating treatment regimens. GIT side effects have been reported to be frequent among these therapeutic agents, including corticosteroids, mycophenolate mofetil (MMF), methotrexate, hydroxychloroquine and azathioprine as well as nintedanib (16-22). Knowledge on how SSc-related GIT symptoms develop over time is in general limited, but from the European Scleroderma Observational Study (ESOS) we know that patients with recent-onset diffuse cutaneous SSc (dcSSc), the most severe form of SSc, have increasing cumulative incidence of GIT symptoms over 24 months. But this increase did not appear to be influenced by the immune-modulating therapies used by the patients (23).

From the above, it appears that more high quality evidence is needed to be able to address everyday clinical challenges including; how is the severity of GIT symptoms, how do GIT symptoms behave over time, and can we find predictors for GIT worsening in SSc patients? Lastly, does standard of care treatment in SSc has any influence on GIT symptoms?

To approach these important questions, we need to evaluate GIT symptoms presence and severity at time of diagnosis (before treatment) and over the disease course.

In the current study, we aim to evaluate patient-reported GIT symptoms at baseline and during follow-up, determine predictive factors for GIT symptom progression, and assess associations between standard of care treatment and GIT symptoms using two prospective SSc cohorts with annual standardized recording of GIT symptoms.

METHOD

SSc patient cohorts

All SSc patients from prospective, observational cohorts followed at the Leiden University Medical Center (LUMC) and Oslo University Hospital (OUH) were consecutively recruited and included in the total SSc cohort; if they (1) fulfilled the 2013 American College of Rheumatology classification criteria for SSc and (2) had at least one UCLA GIT score available which assessed the GIT symptoms (see below) (24). Clinical data were retrieved from the research databases "Combined Care in Systemic Sclerosis (CCISS) approved by the local Ethics Committee P09.003/SH/sh" in Leiden, and the "Norwegian systemic CTD and vasculitis registry (NOSVAR) approved by the Regional Committee for Medical&Health Research Ethics South East Norway;2016-119" in Oslo, and supplemented with data from electronic patient files (25, 26). All study patients with disease duration <24 months from disease onset, defined as first non-Raynaud symptom were included in the study inception cohort, allowing for separate analyses of GIT symptom severity at time of diagnosis, the natural evolution and the effect of standard of care treatment on GIT symptom severity and progression. The cohort study was designed in accordance with the ethical principles of the Declaration of Helsinki. All patients gave written informed consent.

Patient reported GIT symptoms, and assessment of GIT symptom severity and progression

In both SSc centers we started to collect the validated patient reported outcome UCLA GIT 2.0 in 2013 on an annual basis to assess GIT symptoms together with registration in the hospital databases (27). The UCLA GIT 2.0 questionnaire is a seven-item scale including reflux, distention/bloating, diarrhea, fecal soilage, constipation, emotional well-being, and social functioning capturing SSc-related GIT symptoms and their severity. All scales are scored from 0 (better) to 3 (worse) except the diarrhea and constipation scales (ranges are 0–2 and 0– 2.5). The total UCLA GIT score is the sum of all scales (except constipation) and ranges from 0.00–2.83 providing an estimation of the severity of GIT involvement (27, 28) (supplementary file data S1). To evaluate progression of GIT involvement the reported and validated minimal clinical important differences (MCID) in the UCLA GIT score between 2 time points (yearly visits) were assessed (MCID values can be found in supplementary file data S1) (29).

Risk factors and treatment assessments for associations with severe GIT symptoms and for progressive GIT symptoms

Candidate baseline variables for associations with severe GIT symptoms and prediction of GIT symptom progression were selected based on reports from literature and expert opinion (supplementary data S2 for included variables). As SSc is a multiorgan disease,(8) general SSc disease severity at baseline was defined based on a composite score based on individual items which are all validated. Patients were classified as having severe SSc

disease in case of presence of one or more of the following: interstitial lung disease (ILD), defined by presence of lung fibrosis on high resolution computed tomography (HRCT) and a forced vital capacity (FVC) <70%; pulmonary arterial hypertension (PAH) with mean pulmonary arteria pressure ≥25mmHg by right heart catheterization; scleroderma renal crisis; digital ulcers; and/or severe skin involvement defined as modified Rodnan Skin Score (mRSS) >15 (30). Information about standard of care treatment for SSc was collected and included immunomodulatory drugs for any indication (cyclophosphamide, methotrexate, MMF, azathioprine, corticosteroids, hydroxychloroquine), vasodilating drugs (calcium channel blocker [CCB], angiotensin-converting-enzyme inhibitors [ACE inhibitors], endothelin receptor antagonist [ET-1 inhibitors], phosphodiesterase 5 inhibitor [PDE-5 inhibitor], prostacyclin analogue), and specific GIT medications (proton pump inhibitor (PPI), H_2 antagonist). Use of immunomodulatory medication was collected at every visit, and at each time of completing the UCLA GIT questionnaire. Vasodilatory and GIT drugs were evaluated as ever used. Detailed explanation on organ involvement screening can be found in supplementary file data S3.

Statistical analysis

Statistical analyses were performed on SPSS version 25 and STATA version 15. Both the total cohort and the inception cohort were analyzed at baseline, the inception cohort (disease duration since first non-Raynaud symptom < 24 months) was analyzed to exclude the effect of longstanding disease, and the total cohort was included as this mirrors the daily clinical practice. For the longitudinal data we only used the inception cohort. Ordinal logistic regressions were used to identify baseline variables associated with baseline GIT disease and were expressed as odds ratios (OR) and the corresponding 95% confidence intervals (CI). Bonferroni-Holm correction was applied to adjust for multiple testing (indicated with * in tables). To adjust for SSc disease severity at baseline as a possible confounder on treatment effect on GIT symptoms, we included the above explained composite variable in the multivariable regression analyses (detailed explanation on generating models supplementary data S4). To assess whether other organ manifestations were associated with GIT symptom severity, we applied binary logistic regression analyses including the separate clinical characteristics that were assessed in the disease severity variable as predictors. To better understand the effect of immunosuppressive treatment on GIT symptoms we determined GIT symptom progression in patients naïve for immunosuppressive treatment and in treatment exposed patients. Linear mixed-effect models were used to assess changes in UCLA GIT score (all domains) over the observation time, to control for repeated measurements, and to identify risk factors predictive for any change in GIT symptoms during follow-up. Time and risk factors were fixed effects in the analysis. Interaction effects between time and fixed factors were checked. All models included random intercept and slope, and an unstructured correlation matrix was used.

RESULTS

Patient populations

The total study cohort included 834 SSc patients, all with baseline UCLA GIT scores. Demographics and clinical characteristics at baseline were comparable in patients from the LUMC and OUH cohorts (supplementary table S1). From the total cohort, 236 patients had disease duration <24 months and were included in study inception cohort (table 1).

Prevalence of patient-reported gastro-intestinal symptoms and baseline characteristics associated with GIT symptom burden

We assessed all baseline values of all the sub-items of the UCLA GIT score separately in the total and inception cohorts and found that the prevalence and distribution of symptoms did not differ, except for significantly lower frequencies of moderate-severe reflux and distension/bloating in the inception cohort (figure 1). At baseline, the total UCLA GIT score was equivalent to none-mild GIT symptoms in most of the patients (72%, n= 601), with 11% of these patients reporting a score of zero. Moderate or severe GIT symptom burden were reported by 28% of patients, with 21% moderate (n= 175) and 7% severe (n= 58). As shown in Figure 1, the frequency of severe symptom burden varied considerably between the UCLA GIT subdomains.

By multivariable analysis on the inception cohort, to control for the influence of longstanding disease, we found that female sex (OR 8.5 (1.1-36.01)) and ever smoking (OR 2.9 (1.2-7.3)) was associated with GIT symptom severity, while none of the standard of care therapies did reach the significance level (table 2). In the total cohort, the association of female sex (OR 1.76 (1.04-2.98)) and ever smoking (OR 1.69 (1.19-2.41)) was confirmed, and presence of anti-centromere antibody [ACA] (OR 2.07 (1.34-3.19)), and corticosteroid use (OR 1.92 (1.18-3.12)) were significantly associated with moderate-severe total GIT symptom burden at baseline, while CCB use (OR 0.55 (0.39-0.83)) seemed to be protective (supplementary file table S2). Other standard of care therapies were not associated with GIT symptom severity at baseline.

Baseline demographic and clinical characteristics of the total systemic sclerosis cohort*, and for patients with disease duration < 2 years since first non-Raynaud (inception cohort)

	Total cohort	Inception cohort	P
Demographic	n=834	n=236	
Female, n (%)	687 (82)	180 (76)	0.57
Age, yrs mean (SD)	55 (14)	54 (13)	0.91
SSc disease duration at inclusion, median (IQR)	5.9 (1.7-11.9)	0.7 (0.3-1.2)	<0.001
Smoking, ever n (%)	420 (50)	122 (62)	0.32
Organ involvement			
Diffuse cutaneous SSc, n (%)	186 (22)	67 (28)	0.24
Severe skin involvement, n (%)	96 (12)	43 (19)	0.32
Myositis, n (%)	51 (6)	18 (8)	0.46
DLCO% < 60% of predicted, n (%)	267 (33)	71 (31)	0.75
FVC% < 70% of predicted, n (%)	65 (8)	15 (7)	0.78
ILD on HRCT, n (%)	305 (37)	71 (30)	0.33
PAH, n (%)	57 (7)	18 (8)	0.67
SSc specific autoantibodies			
Anti RNA polymerase III, n (%)	62 (7)	29 (12)	0.23
Anti-centromere, n (%)	392 (47)	96 (41)	0.34
Anti-topoisomerase, n (%)	165 (20)	56 (24)	0.57
Treatment at baseline			
Calcium Channel Blockers, n (%)	305 (37)	95 (40)	0.67
H2 receptor blocker, n (%)	256 (31)	72 (31)	0.78
ET-1 inhibitors&prostacyclin analogue, n (%)	97 (12)	19 (5)	0.23
Proton Pump Inhibitor, n (%)	298 (36)	83 (35)	0.66
Methotrexate, n (%)	78 (9)	24 (10)	0.71
Mycophenolate mofetil, n (%)	38 (5)	13 (6)	0.81
Azathioprine, n (%)	13 (2)	2 (1)	0.91
Corticosteroids, n (%)	85 (10)	27 (11)	0.81
Cyclophosphamide, n (%)	11 (1)	10 (4)	0.54
Hydroxychloroquine, n (%)	27 (3)	7 (3)	0.68

Table 1. N = 454 patients of the Leiden University Medical Center and N= 380 patients of the Oslo University Hospital; DLCO= single-breath diffusing lung capacity for carbon monoxide, ET-1= endothelin receptor, FVC= forced vital capacity, ILD= interstitial lung disease, HRCT= high resolution computed tomography, PAH= pulmonary arterial hypertension, n= number, SD= standard deviation; IQR= interquartile range. ± based on modified Rodnan Skin Score > 15 points.

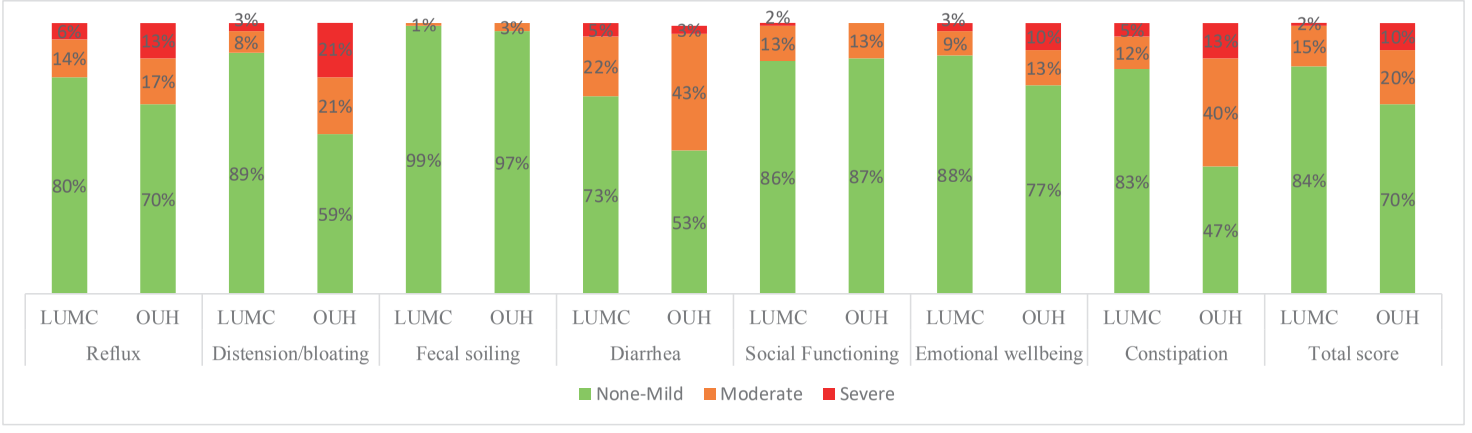

Figure 1. Severity of gastro-intestinal involvement at baseline in the total cohort and the inception cohort. Disease severity at baseline according the UCLA GIT 2.0 score on every subdomain in the total and the inception cohort. This figure shows the percentage of SSc patients with none-mild, moderate and severe gastro-intestinal involvement for each subdomain at baseline.

Table 2: Logistic regression analyses of baseline characteristics associated with moderate/ severe total UCLA GIT symptom score in the 236 systemic sclerosis patients from the Leiden and Oslo inception cohort

	Univariable			Multivariable		
	Moderate / severe total GIT symptom score					
	OR	95% CI	Significance p-value	OR	95% CI	Significance p-value
Female	**3.03**	**1.22-7.5**	**0.01***	**8.5**	**1.1-36.01**	**< 0.001***
Age, Years	1.01	0.99-1.03	0.30	-	-	-
Disease duration, yrs	0.61	0.37-1.01	0.06	0.65	0.43-1.11	0.13
Raynaud duration, yrs	1.01	0.97-1.04	0.68	**-**	**-**	**-**
Smoking ever	**1.73**	**1.13-3.68**	**0.03***	**2.9**	**1.2-7.2**	**<0.001***
Diffuse subset*	0.89	0.56-1.40	0.89	-	-	-
Disease severity#	1.22	0.63-2.45	0.56	1.34	0.78-2.66	0.62
Weight loss (>10% in 1 year)	0.67	0.25-1.82	0.43	-	-	-
Hemoglobine level	0.70	0.41-1.20	0.20	-	-	-
Myositis	1.01	0.32-3.22	0.98	-	-	-
Anti-centromere antibody	1.86	0.98-3.4	0.07	2.01	0.93-5.32	0.11
Anti-topoisomerase antibody	1.52	0.71-3.25	0.28	-	-	-
Anti-RNApIII antibody	0.54	0.24-1.23	0.14	-	-	-
Proton pump inhibitor	0.65	0.25-1.20	0.17	-	-	-
H2 receptor blocker	1.62	0.73-3.56	0.23	-	-	-
ACE-inhibitor	0.89	0.41-1.96	0.78	-	-	-
Calcium channel blocker	1.06	0.57-1.98	0.84	**-**	**-**	**-**
Mycophenolate mofetil	0.38	0.09-1.03	0.06	0.43	0.08-1.05	0.09
Methotrexate	0.70	0.28-1.78	0.45	-	-	-
Azathioprine	0.26	0.22-1.03	0.09	0.43	0.34-1.18	0.23
Corticosteroids	0.65	0.27-1.57	0.33	**-**	**-**	**-**
Cyclophosphamide	1.09	0.22-5.35	0.91	-	-	-
Hydroxychloroquine	0.79	0.15-4.07	0.77	-	-	-
Prostacyclin & ET-1 inhibitor	0.34	0.09-1.28	0.11	-	-	-

*Disease subset: entered as ordinal variable with order: non-cutaneous, limited and diffuse cutaneous subset. CI= confidence interval, OR= odds ratio. # disease severity is a compound variable which included: interstitial lung disease, pulmonary arterial hypertension, renal crisis, severe skin disease or presence of digital ulcers. Medication is entered as yes or no, with a yes if the medication was used in the same year as the GIT questionnaire was completed. ACE= angiotensin-converting-enzyme, ET-1= endotheline receptor. IQR= interquartile range, SD= standard deviation. In bold are the significant associations. * remains significant after Holm-Bonferroni correction.

Next, we evaluated associations of baseline clinical characteristics and use of standard-of care therapies separately for each of the individual subdomains of the UCLA GIT questionnaire in the total cohort with correction for disease duration and disease severity (supplementary table S2-S9). We identified dcSSc as a risk factor for more severe distension/bloating, diarrhea and for severe affection of social functioning. Presence of ACA was a risk factor for severity in every subdomain except for constipation. Regarding standard of care treatments, we found that ever use of corticosteroids associated with more severe fecal soilage (OR 3.91 (1.27-12.08)). The use of CCB was protective against severe distention/bloating (OR 0.56 (0.37-0.83)) and diarrhea (OR 0.61 (0.44-0.85)).

Regarding, reflux medication we found that ever use of PPI associated with more severe reflux (OR 1.39 (1.07-1.80)) and with more severe fecal soilage symptoms (OR 3.07 (1.28-7.49)). Ever use of H2 receptor blockers was also associated with more severe reflux (OR 1.71 (1.28-2.32), but was protective for severe distension/bloating (OR 0.66 (0.47-0.93)). The other therapeutics assessed were not associated with severity in any of the seven sub-items.

To assess whether any of the specific organ manifestations of SSc associate with GIT symptom severity we applied multivariable logistic regression with each of the single organ manifestations. Presence of digital ulcers showed significant associations with total GIT symptom score (OR 1.5 (1.1-2.2)) and with distension/bloating (OR 1.7 (1.2-2.4)).

Progression of GIT symptoms from baseline to follow-up

To account for effects of longstanding disease leading to more organ damage, we evaluated progression of GIT symptoms in the inception cohort. In the inception cohort, after one year of follow-up, 27% of patients had clinically important GIT symptom progression, defined by increase in total GIT score from baseline, with sub-item analysis showing progression of reflux symptoms in 26%, distension/bloating in 29% and constipation in 21%. By evaluating the mean scores in the inception cohort over time our understanding of GIT progression improved, as shown by figure 2 the mean scores vary greatly over time, in all subdomains the scores after 5 years are higher, indicating worse GIT symptoms, compared to the baseline scores. Especially for the subdomains diarrhea, social functioning, emotional wellbeing and distension/bloating the figures show slow progression during follow-up (figure 2).

GIT symptom progression was not associated with GIT symptoms severity at baseline, neither for the total GIT score (OR 0.8 (0.3-2.6)), nor for any of the subdomains.

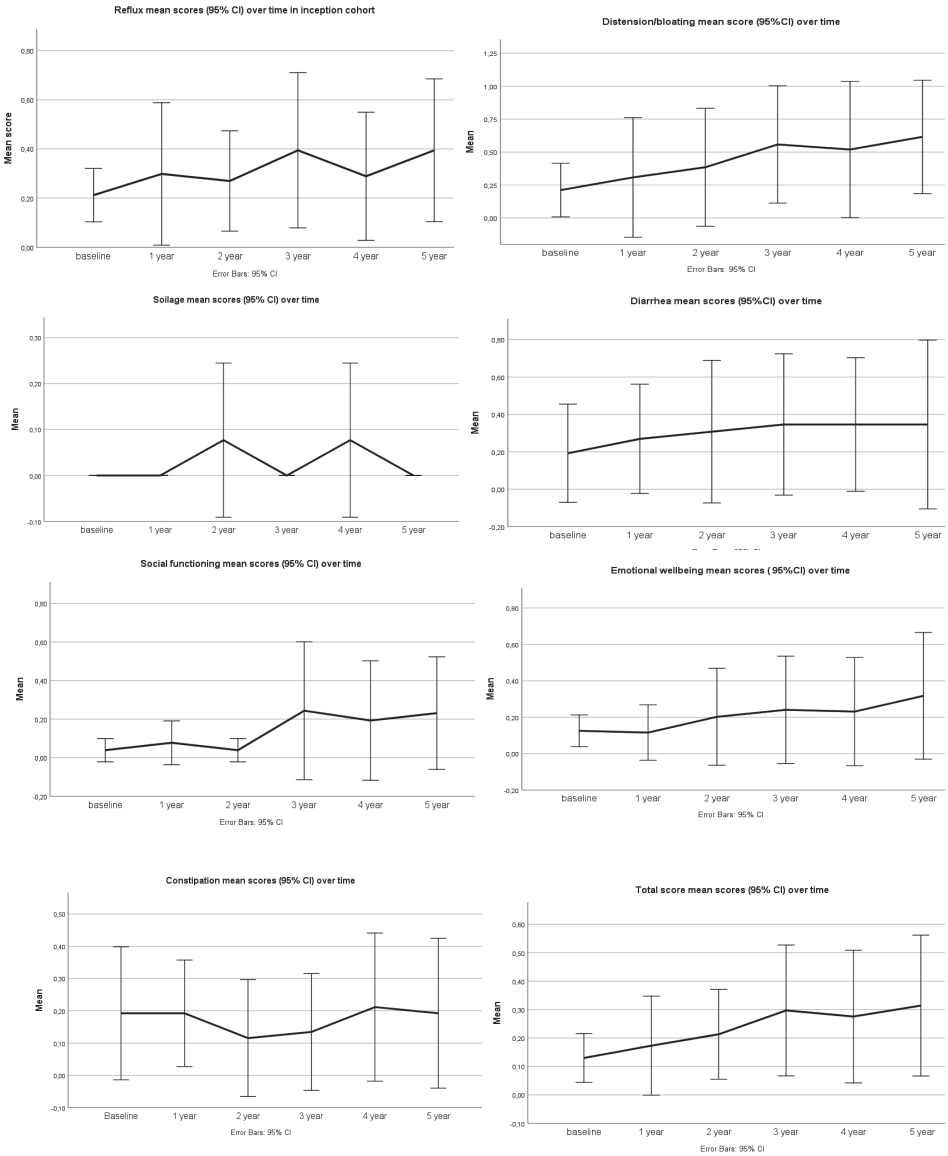

Figure 2. Mean scores per GIT subdomain over the follow-up period in the inception cohort. Higher scores indicates worse GIT symptoms, lower scores indicate less GIT symptoms.

GIT symptom progression in treatment naïve patients and in patients starting with immunosuppressive treatment

To assess natural evolution of GIT symptoms in SSc, we determined change in UCLA GIT scores from baseline to follow-up in patients who were naïve for immunosuppressive treatment across the observation period. In the inception cohort, 81% (n= 192) were treatment naïve at baseline and 48% (n= 72) remained so at follow-up.

We found that more patients at each subsequent visit had worsened since baseline over the first three years in inception patients naïve for immunosuppressive treatment, except for the diarrhea and emotional subdomain (supplementary figure S1).

To evaluate impact of start with immunomodulatory therapies, we determined GIT symptom progression in treatment exposed patients. Typical indications for immunomodulatory therapies in SSc are severe skin and lung disease. Hence, it was not unexpected to find that patients exposed to treatment had more frequently ILD, diffuse cutaneous SSc and positive ATA than the treatment naïve patients, while age and sex were comparable.

We found no significant difference in GIT symptom progression between immunosuppressive treatment naïve patients and patients exposed to methotrexate, MMF, azathioprine, hydroxychloroquine, corticosteroids or cyclophosphamide after one year or follow-up. Numerically, more patients that started with corticosteroids, or MMF had progressive GIT symptoms after one year in the inception cohort (figure 3).

Predictors of GIT symptom progression during the follow-up period

For these multivariable linear mixed-effect model analyses, we focused primarily on the inception cohort, arguing that patients with short disease duration would have more active disease and less structural damage, and therefore be more liable to react to standard-of-care therapies. Of the variables of interest only treatment with CCB was identified as a predictor of marginal worsening of total GIT symptoms, with significant interaction effects between time and CCB treatment . The estimated difference of 0.04 in total GIT symptom score between CCB treated and CCB untreated patients was below the MCID previously defined for UCLA GIT (29).

Supplementary analyses of the total cohort showed that female gender and smoking were predictive for progression of bloating and diarrhoea with significant interaction effects over time. None of the other variables were predictive for time-dependent progression in total GIT or subdomain scores.

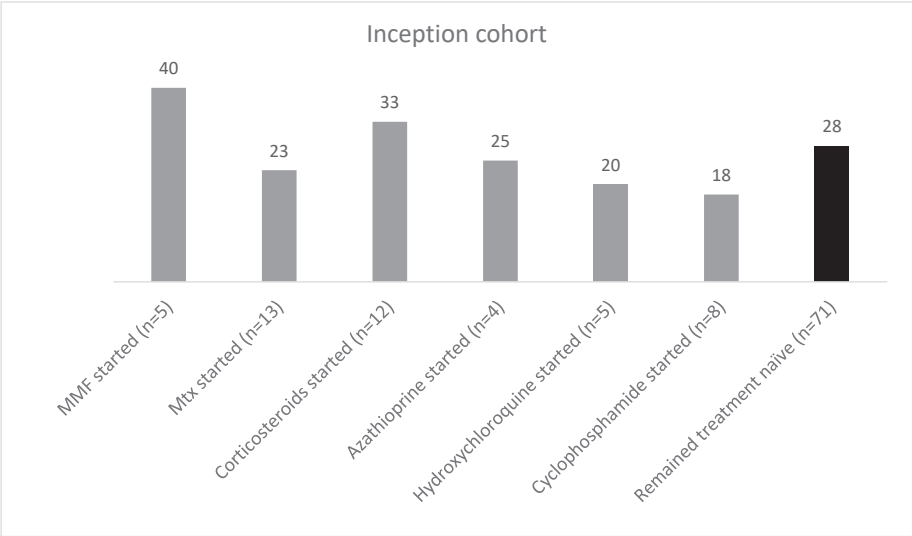

Figure 3. Percentage of progressors (inception cohort) for the total GIT score after 1 year of follow-up stratified for start of immunomodulatory treatment (no treatment are patients who remained treatment naïve). Description: Inception cohort: n= 118 inception patients with available UCLA GIT after one year, n= 71 remained treatment naïve, n= 47 started with immune-modulating therapy.

Results of linear mixed model in the inception cohort of systemic sclerosis patients on total GIT symptom score over a 3 years' time period.

	Univariable			Multivariable		
Predictor variable	**Coeffi-cient**	**95% CI**	***p* value**	**Coeffi-cient**	**95% CI**	***p* value**
Sex	0.02	-0.03-0.06	0.52			
Age	0.0003	-0.001-0.002	0.71			
Disease duration	-0.011	-0.04-0.01	0.37			
Ever smoking	0.04	-0.003-0.08	0.07			
Anti-centromere antibody	-0.004	-0.04- 0.04	0.85			
Anti-topoisomerase antibody	0.006	-0.04-0.05	0.81			
Skin involvement*	0.001	-0.0004-0.003	0.13			
Diffuse disease subset	**0.04**	**- 0.0008-0.09**	**0.05**	0.03	-0.001-0.08	0.12
ESR	-0.0001	- 0.0005-0.0002	0.43			
Myositis	0.04	-0.05 – 0.12	0.41			
PAH	0.05	-0.02-0.13	0.18			
ILD	0.02	-0.02-0.06	0.41			
ACE	0.02	-0.02-0.06	0.33	0.02	-0.02-0.06	0.29
CCB	**0.04**	**0.001-0.08**	**0.04**	**0.04**	**0.0006-0.08**	**0.04**
PPI	0.02	-0.02-0.06	0.34	0.02	-0.02-0.06	0.22
ET-1	0.05	-0.02-0.13	0.22	0.05	-0.03-0.14	0.19
H2 blocker	0.0003	-0.04-0.04	0.99	0.002	-0.04-0.04	0.94
Corticosteroids	0.02	-0.03-0.06	0.48	0.02	-0.03-0.06	0.46
Methotrexate	-0.011	-0.06-0.04	0.68	0.006	-0.04-0.05	0.79
Azathioprine	-0.06	-0.14-0.05	0.18	-0.05	-0.13-0.03	0.22
Hydroxychloroquine	-0.03	-0.10-0.03	0.28	-0.04	-0.10-0.03	0.26
MMF	-0.01	-0.08-0.06	0.76	-0.006	-0.07-0.06	0.87
Cyclophosphamide	0.04	-0.006-0.09	0.090	0.04	-0.006-0.09	0.08

Table 3. Coefficients shown are the interactions terms (predictor * time). Mean between group change during three years of follow-up. Separate multivariable regression analyses with adjustment for time, age, sex and ACA. ILD= interstitial lung disease, PAH= pulmonary arterial hypertension, mRSS= modified Rodnan Skin Score, CCB= calcium channel blockers, PPI = proton pump inhibitors, ET-1= endotheline receptor antagonist, ACE= Angiotensin-converting-enzyme inhibitors, ESR= erythrocyte sedimentation rate, MMF= Mycophenolate mofetil. Time effects of the mixed model can be found in supplementary file.*assessed by the modified Rodnan Skin score. Significant results are not significant after Bonferroni-Holm Correction.

DISCUSSION

GIT involvement is reported as highly frequent in SSc, with major impact on patient's quality of life and survival. Here, we show that about 1/3 of patients in a large, unselected SSc population reports high GIT symptom burden early in the disease course, with reflux and distention/bloating as the most troublesome early symptoms. Over one year observation, 27% of patients reported worsening of GIT symptoms. Interestingly, this worsening occurred independent of baseline GIT symptom severity and disease duration.

To our knowledge, this is the first large, multicenter study prospectively mapping GIT symptom burden in a well characterized SSc cohort. The identification of high symptomatic burden from early in the disease course and the progressive behaviour of the GIT symptoms have impact at several levels. Firstly, knowledge about severity and the natural behaviour of GIT symptoms is a prerequisite to identify patients for inclusion in clinical trials and to assess treatment effects. This is particularly important in times of evolving new therapeutic options, like fecal microbiota transplantation (FMT) (31, 32). Secondly, it is of major clinical relevance for physicians following these patients and argues for the necessity of multidisciplinary team (MDT) assessment including rheumatologists and gastroenterologists in SSc. MDT assessments are important and well-functioning for other diseases, like Crohn's disease and in ILD (33, 34). We strongly advocate to build up MDT for GIT in all SSc expert centers, and initiate work on recommendations for the management of this devastating organ affliction in SSc. The specific need for further development of GIT care was also highlighted in the recently published international standard for longitudinal follow-up of SSc patients, where only reflux symptom assessment and dysphagia, diarrhoea, and weight loss were included (35).

For this study, we performed the first ever systematic assessment of associations between SSc characteristics including standard-of-care therapies and GIT symptoms in two large SSc cohorts mapping the whole SSc population. Interestingly, we found that corticosteroid use might associate with high GIT symptom burden. The associations to corticosteroids was not identified in the inception cohort subset. The smaller sample size could be one explanation for this, another could be that GIT side effects of corticosteroids accumulate over time. ACA was associated with more severe GIT symptoms. We can only speculate about the background of this association; 1) ACA positive SSc patients might have a longer 'prodrome' as a consequence of a more indolent disease course. Indeed, disease duration since RP was significantly longer in ACA positive patients compared to ATA positive patients. On the other hand, the association between ACA and GIT symptoms remained significant after correcting for disease duration 2) ACA are directly implicated in SSc-related vasculopathy and consequently GIT symptom severity (still unknow) (36-39).

In apparent contrast to the association findings, there were no differences in GIT symptom progression between patients who were treatment naïve at all time-points and patients that started standard of care immune-modulating drug treatment during follow-up. These results argue that immunomodulatory treatment for SSc do not seem to have major impact on GIT disease evolution in SSc. Although, the exact etiology of GIT symptoms in SSc is and remains largely unknown and therefore can be influenced by multiple (unknown) factors. Previous data on this subject are highly limited, but it appears that our results are in line with McMahan et al. who showed no effect of immunomodulatory treatment on severe dysmotility assessed by the Medsger activity score (40). Notably, that study did not use a patient reported GIT outcome measure, it did not include treatment naïve patients, and it did not assess effects of vasodilatory treatments.

The mechanisms behind the GIT involvement in SSc are not well understood, but appear multifactorial. Vasculopathy is an important factor in SSc pathogenesis, and some reports indicate associations between GIT symptoms and progressive vasculopathy (41, 42). Interestingly in our study, patients showing progression of GIT symptoms had also more often digital ulcers, while no association was found with other organ manifestations as ILD or skin involvement. Although this is not a mechanistic study, we speculate that the digital ulcer association, and possibly also the effects of CCB in the cross-sectional data set, implicates vasculopathy in the pathophysiology of SSc GIT disease.

Our study is not without limitations. The UCLA GIT is a validated questionnaire, however it remains a self-reported questionnaire and this can always introduce bias. The UCLA GIT captures symptoms in the past seven days, we included annual follow-up which might not capture all short-term changes. Many assessments were performed in this study, by using Bonferroni correction we have reduced the risk of type I errors. A relatively low percentage of patients experienced fecal soilage, which might be underestimation due to recruitment bias and patients' reluctance to talk about this symptom. It should also be mentioned, that using the MCID could still miss patients with clinical relevant GIT development, as there is an inherent uncertainty around MCID estimates (29). Unfortunately, objective measurements are not performed as standard assessment in SSc patients and therefore we were not able to include this in our manuscript. Other than gastroduodenoscopy few assessments are performed at all and for dysmotility evaluation none are even routinely available to date. We suggest that for a better understanding of the etiology this would be a next step to evaluate and this highlights a clear clinical unmet need. We should also be aware of the possibility of confounding by indication in the analyses evaluating GIT treatment and GIT symptom progression as patients with more severe reflux symptoms are also more likely to receive GIT treatment. By collecting patient reported outcome measures not more than once a year, we are not positioned to fully capture medication

driven side effects. In addition, we did not adjust for treatment indication; but we believe this is of minor interest as medication despite indication may have an influence on all organ manifestations. The medication included in this study was based on standard of care therapy in SSc and availability in the database. Unfortunately, GIT medication outside of gastroesophageal reflux disease (GERD) medication has not been collected to incorporate this in the analysis. In addition, only a minority of SSc patients uses other GIT treatments and then often only occasionally or for short-term treatment. In the Leiden cohort ever use of metoclopramide, domperidone and antibiotics is collected since 2018. In 483 SSc patients, 4% used domperidone (n= 20), 4% used metoclopramide (n= 16), 8% laxatives (n= 42), loperamide 1% (n= 4), and 1% used antibiotics for bacterial overgrowth (n= 4). The power the evaluate progression of GIT symptoms in the inception cohort is not sufficient enough to draw firm conclusions, more follow-up data and more patients are needed the validate these data.

Strengths of this study were the large and prospective cohort, and the amount of questionnaires at baseline and over the observation period with a very small amount of missing data (<5%) and a high compliance rate (>90%). Biomarkers for GIT disease activity are still not defined, making it challenging to assess the effects of existing therapies. The UCLA GIT questionnaire allows for a standardized assessment of important clinical response measures in SSc and may play a role for informing both clinical practice and trial design. Based on the results of our analyses we can identify several important points to consider for future SSc trials focusing on GIT involvement : 1) the total UCLA GIT score does not capture all important GIT symptoms, 2) between 10-25% (depending on the domain) of the patients show improvement of GIT symptoms based on the UCLA GIT 2.0; in trial design one should take into account the natural course of GIT symptoms, and 3) validated outcome measures on GIT disease are still lacking; ideally, for clinical trials focusing on treatment of GIT involvement objective measurements should be taken into account. These novel data provide important insights regarding the high frequency of severe GIT symptoms early in the disease course, and the progressive nature of GIT symptoms in patients with SSc. We confirm ACA positive and female patients are specifically at risk for GIT symptoms, but very few other variables can help identify patients at risk of disease progression.

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SUPPLEMENTARY FILE

Gastro-intestinal symptom severity and progression in systemic sclerosis

Data S1. UCLA GIT 2.0

Clinical GIT involvement by the UCLA GIT score was defined as apparent if the patients reported symptoms resulting in a total score or at least one of the seven GIT items ≥0.01 and was segregated into none-mild (including patients with a score of 0, or <0.5 or for fecal incontinence and distention/bloating <1.01), moderate (≥0.5 or for fecal incontinence and distention/bloating ≥1.01) or severe GIT symptoms (> 1.01 or for distension/bloating > 1.61 or for fecal soiling > 2.01) (1).

The minimal clinical important difference (MCID) somewhat worse threshold was used to assess disease progression and cut-offs were defined as: Reflux: 0.19, Distension/bloating: 0.12, Diarrhoea: 0.07, Constipation: 0.13, Fecal Soilage 0.06, Emotional well-being: 0.16, Social functioning: 0.21, Total GIT score 0.12 (2).

Data S2. Candidate variables

The following variables were included as candidate baseline variables for associations with GIT symptoms and prediction of GIT symptom progression: sex, age at registration, disease duration since disease onset, smoking, SSc specific antibodies (anti-centromere antibody [ACA], anti-topoisomerase antibody [ATA], anti-RNA polymerase III antibody [ARA]), SSc subset (diffuse cutaneous and limited cutaneous SSc), myositis (defined as increased creatine kinase AND proximal muscle weakness and/or confirmed with muscle biopsy), weight loss (>10% of body weight in 1 year), and hemoglobin level (3-10).

Data S3. Organ involvement screening

Skin involvement was assessed using the modified Rodnan skin score (mRSS), a validated measure of skin thickening in SSc.(11) Skeletal myopathy was considered present when patients had an abnormal creatinine phosphokinase (CPK) and muscle weakness and/or abnormal electromyography or muscle biopsy. The presence of interstitial lung disease (ILD) was considered present if a high resolution CT scan of the thorax showed ground glass opacifications, honey combing and/or reticular infiltrations interpreted by an experienced radiologist. Pulmonary function tests were performed by every patient, and the percent predicted FVC and DLCO were determined. Pulmonary arterial hypertension was defined as an increase in mean pulmonary arterial pressure (mPAP) ≥ 25 mmHg at rest as assessed by RHC; including presence of pre-capillary PH, defined by a pulmonary capillary wedge pressure (PCWP) ≤15 mmHg and a PVR >3 Wood units (WU) on RHC.

Data S4. Generating univariable and multivariable model

At the first stage, we used subject-specific knowledge to derive a list of independent variables which in principle are relevant as predictors or adjustment variables for the study in question. This list was also based on the availability of variables. With three researchers (NvL, JVB, AMH) we went through the list and critically questioned the role and further properties of each of the variables, such as chronology of measurement collection, quality of measurement, or availability also to the "user" of the model. Not for all independent variables pre-clinical knowledge was available, therefore we carried out an univariable analysis and selected those that reached a certain level of significance ($p < 0.10$). We selected the priori covariates based on biological mechanism, clinical expertise, evidence from published articles, and the univariable model. We checked the assumptions of linearity in logit for each continuous covariate and we checked for interactions. The proportional odds assumption was met. The following covariates were fixed in the model based on their clinical relevance and expert opinion: age, sex, disease duration NR, cutaneous subtype, smoking, autoantibodies and disease severity. The medication covariates were included based on hypothesis testing, frequency of use in clinical practice and availability.

Table S1: Baseline demographic and clinical characteristics of the total systemic sclerosis cohort, stratified by origin (Leiden University Medical Center, LUMC, or Oslo University Hospital, OUH)

	LUMC	OUH	P
Demographic	n=454	n=380	
Female, n (%)	336 (81)	307 (81)	0.9
Age, yrs mean (SD)	55 (14)	56 (13)	0.8
SSc disease duration at inclusion, median (IQR)	3.8 (1.1-10.2)	4.3 (1.8-8.8)	0.6
Smoking, ever n (%)	245 (55)	122 (45)	0.4
Organ involvement			
Diffuse cutaneous SSc, n (%)	88 (21)	84 (22)	0.5
Severe skin involvement, n (%)±	37 (8)	59 (16)	0.2
Myositis, n (%)	13 (3)	48 (13)	0.2
DLCO% < 60% of predicted, n (%)	134 (32)	100 (29)	0.6
FVC% < 70% of predicted, n (%)	36 (9)	26 (8)	0.8
ILD on HRCT, n (%)	180 (39)	120 (32)	0.5
PAH, n (%)	20 (4)	34 (9)	0.6
SSc specific autoantibodies			
Anti RNA polymerase III, n (%)	25 (6)	37 (10)	0.7
Anti-centromere, n (%)	184 (40)	208 (55)	0.3
Anti-topoisomerase, n (%)	117 (26)	48 (13)	0.3
Treatment at baseline			
Calcium Channel Blockers, n (%)	190 (42)	114 (30)	0.4
H2 receptor blocker, n (%)	150 (33)	106 (28)	0.5
ET-1 inhibitors&prostacyclin analogue, n (%)	62 (14)	56 (15)	0.7
Proton Pump Inhibitor, n (%)	179 (39)	121 (32)	0.6
Methotrexate, n (%)	79 (17)	20 (5)	0.2
Mycophenolate mofetil, n (%)	28 (6)	19 (5)	0.5
Azathioprine, n (%)	14 (3)	5 (1)	0.7
Corticosteroids, n (%)	71 (16)	37 (10)	0.6
Cyclophosphamide, n (%)	20 (4)	6 (2)	0.8
Hydroxychloroquine, n (%)	32 (7)	3 (1)	0.7

DLCO= single-breath diffusing lung capacity for carbon monoxide, ET-1= endothelin receptor, FVC= forced vital capacity, ILD= interstitial lung disease, HRCT= high resolution computed tomography, PAH= pulmonary arterial hypertension, n= number, SD= standard deviation; IQR= interquartile range. ± based on modified Rodnan Skin Score > 15 points.

Table S2: Logistic regression analyses of baseline characteristics associated with moderate/ severe total UCLA GIT symptom score in the 834 systemic sclerosis patients from the Leiden and Oslo cohort

	Univariable			Multivariable		
	Moderate / severe total GIT symptom score					
	OR	95% CI	Significance p-value	OR	95% CI	Significance p-value
Female	**1.97**	**1.25-3.10**	**<0.001***	**1.76**	**1.04-2.98**	**0.03**
Age, Years	0.99	0.99-1.004	0.28	0.99	0.98-1.01	0.07
Disease duration, yrs	**1.04**	**1.02-1.06**	**<0.001***	**1.05**	**1.03-1.07**	**<0.001***
Raynaud duration, yrs	**1.01**	**1.00-1.02**	**0.04**	**-**	**-**	**-**
Smoking ever	**1.50**	**1.07-2.08**	**0.02**	**1.69**	**1.19-2.41**	**<0.001***
Diffuse subset*	1.11	0.66-1.87	0.69	1.88	0.94-3.76	0.08
Disease severity#	1.13	0.82-1.54	0.46	1.02	0.70-1.48	0.91
Weight loss (>10% in 1 year)	1.20	0.74-1.97	0.46	-	-	-
Hemoglobine level	1.10	0.88-1.39	0.40	-	-	-
Myositis	1.12	0.64-1.96	0.69	-	-	-
Anti-centromere antibody	**1.77**	**1.30-2.39**	**<0.001***	**2.07**	**1.34-3.19**	**<0.001***
Anti-topoisomerase antibody	**0.57**	**0.37-0.86**	**0.01**	0.78	0.46-1.33	0.36
Anti-RNApIII antibody	0.90	0.41-1.99	0.79	-	-	-
Proton pump inhibitor	1.26	0.95-1.67	0.11	-	-	-
H2 receptor blocker	0.92	0.68-1.24	0.58	-	-	-
ACE-inhibitor	0.75	0.53-1.06	0.10	-	-	-
Calcium channel blocker	0.77	0.56-1.07	0.10	**0.56**	**0.39-0.83**	**<0.001***
Mycophenolate mofetil	1.23	0.64-2.33	0.54	-	-	-
Methotrexate	0.88	0.55-1.42	0.60	-	-	-
Azathioprine	0.53	0.15-1.86	0.32	-	-	-
Corticosteroids	1.25	0.83-1.88	0.10	**1.92**	**1.18-3.12**	**<0.001***
Cyclophosphamide	0.77	0.31-1.93	0.58	-	-	-
Hydroxychloroquine	0.99	0.48-2.09	0.99	-	-	-
Prostacyclin & ET-1 inhibitor	1.57	0.92-2.67	0.09	1.49	0.96-2.33	0.13

*Disease subset: entered as ordinal variable with order: non-cutaneous, limited and diffuse cutaneous subset. CI=confidence interval, OR= odds ratio. # disease severity is a compound variable which included: interstitial lung disease, pulmonary arterial hypertension, renal crisis, severe skin disease or presence of digital ulcers. Medication is entered as yes or no, with a yes if the medication was used in the same year as the GIT questionnaire was completed. ACE= angiotensin-converting-enzyme, ET-1= endotheline receptor. IQR= interquartile range, SD= standard deviation. In bold are the significant associations. * remains significant after Holm-Bonferroni correction.

Table S3. Logistic regression analyses of baseline characteristics associated with moderate or severe reflux symptom score in the 834 systemic sclerosis patients from the Leiden and Oslo cohort

	Univariable			Multivariable		
	Moderate or severe reflux symptom score					
	OR	**95% CI for OR**	**Signifi-cance**	**OR**	**95% CI for OR**	**Signifi-cance**
Female	**1.75**	**1.16-2.64**	**0.01**	**1.60**	**1.00-2.55**	**0.05**
Age, Years	0.99	0.99-1.003	0.72	0.99	0.98-1.01	0.46
Disease duration, years	**1.02**	**1.007-1.040**	**0.01**	**1.02**	**1.004-1.04**	**0.02**
Raynaud duration, years	**1.01**	**1.005-1.034**	**0.03**			
Smoking ever	1.17	0.87-1.58	0.31	1.19	0.86-1.63	0.30
Disease subset*	1.04	0.64-1.68	0.89	1.43	0.78-2.62	0.25
Disease severity#	1.15	0.86-1.55	0.35	1.09	0.78-1.54	0.62
Weight loss (>10% in 1 year)	**1.74**	**1.09-2.77**	**0.02**	**2.20**	**1.33-3.63**	**<0.001***
Hemoglobin levels	0.89	0.72-1.12	0.32	-	-	-
Myositis yes/no	0.63	0.13-3.07	0.60	-	-	-
Anti-centromere antibody	**1.71**	**1.22-2.27**	**<0.001***	**1.76**	**1.10-2.27**	**0.01**
Anti-topoisomerase antibody	**0.51**	**0.34-0.76**	**<0.001***	**0.57**	**0.34-0.90**	**0.02**
Anti-RNApIII	0.72	0.41-1.29	0.28	-	-	-
Proton pump inhibitor	**1.39**	**1.07-1.80**	**0.01**	1.16	0.82-1.66	0.40
H2 receptor blocker	**1.71**	**1.28-2.32**	**<0.001***	**1.71**	**1.23-2.59**	**0.003***
ACE inhibitor	1.17	0.82-1.63	0.38	-	-	-
Calcium channel blocker	1.09	0.84-1.41	0.54	-	-	-
Immunosuppressiva combined	1.34	0.93-1.93	0.12	-	-	-
Prostacyclin & ET-1 inhibitor	1.47	0.89-2.42	0.22	-	-	-

Univariable and multivariable logistic regression. *Disease subset: entered as ordinal variable with order: non-cutaneous, limited and diffuse subset. # disease severity is a compound variable which included: ILD with FVC<70%, PAH, renal crisis, mRSS>20 or presence of digital ulcers. Medication are entered as yes or no, with a yes if the medication was used in the same year as the GIT questionnaire was completed. * significant after Holm-Bonferroni correction.

Table S4. Logistic regression analyses of baseline characteristics associated with moderate or severe distension/bloating symptom score in the 834 systemic sclerosis patients from the Leiden and Oslo cohort

	Univariable			Multivariable		
	Moderate or severe distention/bloating symptom score					
	OR	**95% CI for OR**	**Signifi-cance**	**OR**	**95% CI for OR**	**Signifi-cance**
Female	**2.34**	**1.48-3.67**	**<0.001***	**2.38**	**1.40-4.03**	**<0.001***
Age, Years	0.99	0.99-1.003	0.14	0.99	0.98-1.00	0.06
Disease duration, years	**1.02**	**1.002-1.04**	**0.03**	**1.03**	**1.01-1.05**	**<0.001***
Raynaud duration, years	**1.00**	**1.00-1.02**	**0.04**			
Smoking ever	**1.46**	**1.05-2.01**	**0.02**	**1.86**	**1.32-2.64**	**<0.001***
Disease subset*	1.25	0.75-2.08	0.38	**2.29**	**1.16-4.53**	**0.02**
Disease severity	1.31	0.97-1.78	0.08	1.26	0.88-1.82	0.21
Weight loss (>10% in 1 year)	0.93	0.54-1.61	0.80	-	-	-
Hemoglobin	0.86	0.70-1.06	0.17	-	-	-
Myositis yes/no	1.21	0.73-2.02	0.47	-	-	-
ACA	**1.54**	**1.20-1.97**	**<0.001***	**1.65**	**1.08-2.52**	**0.02**
ATA	0.64	0.47-0.88	0.06	0.84	0.50-1.40	0.50
Anti-RNApIII	1.48	0.88-2.47	0.14	-	-	-
Proton pump inhibitor	1.10	0.85-1.43	0.45	-	-	-
H2 receptor blocker	**0.66**	**0.47-0.93**	**0.02**	0.85	0.56-1.27	0.42
ACE inhibitor	0.58	0.38-0.88	0.01	0.80	0.50-1.28	0.35
Calcium channel blocker	**0.66**	**0.48-0.91**	**0.01**	**0.56**	**0.37-0.83**	**<0.001***
Immunosuppressiva combined	0.96	0.64-1.41	0.82	-	-	-
Prostacyclin & ET-1 inhibitor	1.30	0.83-2.09	0.29	-	-	-

*Disease subset: entered as ordinal variable with order: non-cutaneous, limited and diffuse subset. # disease severity is a compound variable which included: ILD with FVC<70%, PAH, renal crisis, mRSS>20 or presence of digital ulcers. Medication are entered as yes or no, with a yes if the medication was used in the same year as the GIT questionnaire was completed. * Significant after Holm-Bonferroni correction.

Table S5. Logistic regression analyses of baseline characteristics associated with moderate or severe constipation symptom score in the 834 systemic sclerosis patients from the Leiden and Oslo cohort

	Univariable			Multivariable		
	Moderate or severe constipation symptom score					
	OR	**95% CI for OR**	**Signifi-cance P**	**OR**	**95% CI for OR**	**Signifi-cance P**
Female	1.76	1.16-2.67	0.008	1.51	0.94-2.42	0.08
Age, Years	1.001	0.99-1.01	0.48	1.00	0.99-1.01	0.97
Disease duration, years	1.01	0.99-1.03	0.29	1.02	0.99-1.03	0.13
Raynaud duration, years	0.99	0.98-1.02	0.61			
Smoking ever	0.76	0.56-1.04	0.09	0.96	0.67-1.30	0.68
Disease subset*	1.26	0.78-2.02	0.34	1.29	0.65-2.56	0.47
Disease severity	1.30	0.96-1.76	0.09	1.21	0.85-1.73	0.29
Weight loss (>10% in 1 year)	1.26	0.76-2.10	0.37	-	-	-
Hemoglobin	0.86	0.68-1.09	0.21	-	-	-
Myositis yes/no	1.11	0.62-1.99	0.73	-	-	-
ACA	1.18	0.88-1.57	0.28	1.26	0.84-1.89	0.27
ATA	0.67	0.45-0.99	0.05	1.04	0.64-1.67	0.89
Anti-RNApIII	**2.49**	**1.52-4.06**	**<0.001***	**2.43**	**1.31-4.50**	**0.01**
Proton pump inhibitor	0.98	0.75-1.29	0.91	-	-	-
H2 receptor blocker	0.76	0.48-1.08	0.11	-	-	-
ACE inhibitor	0.72	0.51-1.02	0.25	-	-	-
Calcium channel blocker	0.75	0.55-1.03	0.07	0.74	0.52-1.06	0.09
Immunosuppressiva combined	**0.60**	**0.40-0.92**	**0.02**	0.76	0.45-1.09	0.09
Prostacyclin & ET-1 inhibitor	1.34	0.73-2.23	0.58	-	-	-

*Disease subset: entered as ordinal variable with order: non-cutaneous, limited and diffuse subset. # disease severity is a compound variable which included: ILD with FVC<70%, PAH, renal crisis, mRSS>20 or presence of digital ulcers. Medication are entered as yes or no, with a yes if the medication was used in the same year as the GIT questionnaire was completed. * Significant after Holm-Bonferroni correction.

Table S6. Logistic regression analyses of baseline characteristics associated with moderate or severe diarrhea symptom score in the 834 systemic sclerosis patients from the Leiden and Oslo cohort

	Univariable			Multivariable		
	Moderate or severe diarrhea symptom score					
	OR	**95% CI for OR**	**Significance P**	**OR**	**95% CI for OR**	**Significance P**
Female	1.06	0.74-1.53	0.74	1.05	0.70-1.60	0.80
Age, Years	0.99	0.98-1.004	0.21	0.99	0.98-1.01	0.13
Disease duration, years	**1.01**	**1.01-1.04**	**0.01**	1.02	1.01-1.04	0.05
Raynaud duration, years	**1.02**	**1.01-1.06**	**0.02**			
Smoking ever	1.35	1.01-1.81	0.05	1.43	1.05-1.94	0.02
Disease subset*	**2.27**	**1.37-3.76**	**<0.001***	**2.59**	**1.40-4.79**	**<0.001***
Disease severity	**1.39**	**1.05-1.85**	**0.02**	1.33	0.96-1.85	0.08
Weight loss (>10% in 1 year)	1.40	0.87-2.26	0.16	-	-	-
Hemoglobin	1.21	0.95-1.53	0.12	-	-	-
Myositis yes/no	1.72	1.01-2.95	0.05	1.52	0.82-2.84	0.18
ACA	1.27	0.96-1.67	0.09	**1.63**	**1.12-2.36**	**0.01**
ATA	0.76	0.53-1.08	0.12	0.79	0.52-1.22	0.29
Anti-RNApIII	0.92	0.54-1.56	0.76	-	-	-
Proton pump inhibitor	0.97	0.73-1.30	0.84	-	-	-
H2 receptor blocker	0.80	0.59-1.10	0.17	-	-	-
ACE inhibitor	0.82	0.57-1.17	0.26	-	-	-
Calcium channel blocker	0.69	0.52-0.93	0.01	**0.61**	**0.44-0.85**	**<0.001***
Immunosuppressiva combined	0.71	0.49-1.03	0.07	0.73	0.52-1.13	0.11
Prostacyclin & ET-1 inhibitor	1.03	0.61-1.70	0.87	-	-	-

*Disease subset: entered as ordinal variable with order: non-cutaneous, limited and diffuse subset. # disease severity is a compound variable which included: ILD with FVC<70%, PAH, renal crisis, mRSS>20 or presence of digital ulcers. Medication are entered as yes or no, with a yes if the medication was used in the same year as the GIT questionnaire was completed. * Significant after Holm-Bonferroni correction.

Table S7. Logistic regression analyses of baseline characteristics associated with moderate or severe fecal soilage symptom score in the 834 systemic sclerosis patients from the Leiden and Oslo cohort

	Univariable			Multivariable		
	Moderate or severe fecal soilage symptom score					
	OR	**95% CI for OR**	**Significance P**	**OR**	**95% CI for OR**	**Significance P**
Female	2.08	0.48-9.01	0.33	1.39	0.28-6.89	0.69
Age, Years	**1.04**	**1.001-1.08**	**0.02**	1.04	0.99-1.08	0.13
Disease duration, years	**1.06**	**1.02-1.10**	**<0.001***	**1.08**	**1.04-1.13**	**<0.001***
Raynaud duration, years	**1.04**	**1.03-1.06**	**0.02**			
Smoking ever	1.69	0.66-4.33	0.28	1.44	0.52-3.99	0.48
Disease subset*	2.55	0.28-23.08	0.41	13.36	0.89-201.61	0.06
Disease severity	0.92	0.37-2.32	0.89	0.93	0.31-2.80	0.90
Hemoglobin	1.40	0.65-2.99	0.39	-	-	-
Myositis yes/no	0.80	0.11-6.12	0.83	-	-	-
ACA	**3.76**	**1.36-10.35**	**0.01**	**4.28**	**1.08-16.95**	**0.04**
ATA	0.20	0.03-1.46	0.11	0.26	0.02-2.62	0.23
Proton pump inhibitor	**3.07**	**1.28-7.49**	**0.01**	2.90	0.96-8.75	0.06
H2 receptor blocker	2.39	1.01-5.72	0.05	1.60	0.55-4.69	0.39
ACE inhibitor	1.77	0.68-4.64	0.25	-	-	-
Calcium channel blocker	0.89	0.35-2.17	0.76	-	-	-
Immunosuppressiva combined	1,11	0.37-3.33	0.86	-	-	-
Prostacyclin & ET-1 inhibitor	2.23	1.11-8.7	0.04	1.59	0.48-6.01	0.48

*Disease subset: entered as ordinal variable with order: non-cutaneous, limited and diffuse subset. # disease severity is a compound variable which included: ILD with FVC<70%, PAH, renal crisis, mRSS>20 or presence of digital ulcers. Medication are entered as yes or no, with a yes if the medication was used in the same year as the GIT questionnaire was completed. * Significant after Holm-Bonferroni correction.

Table S8. Logistic regression analyses of baseline characteristics associated with moderate or severe emotional wellbeing symptom score in the 834 systemic sclerosis patients from the Leiden and Oslo cohort

	Univariable			Multivariable		
	Moderate or severe emotional wellbeing symptom score					
	OR	**95% for CI OR**	**Signifi-cance P**	**OR**	**95% for CI OR**	**Signifi-cance P**
Female	**1.94**	**1.15-3.27**	**0.01**	**1.98**	**1.08-3.64**	**0.03**
Age, Years	1.04	0.99-1.02	0.43	0.99	0.98-1.01	0.66
Disease duration, years	**1.03**	**1.01-1.05**	**0.02**	**1.04**	**1.02-1.06**	**<0.001***
Raynaud duration, years	**1.01**	**1.00-1.04**	**0.03**			
Smoking ever	**1.68**	**1.15-2.46**	**0.007***	**1.87**	**1.25-2.79**	**<0.001***
Disease subset*	1.04	0.58-1.86	0.90	1.71	0.80-3.67	0.17
Disease severity	1,28	0.91-1.82	0.16	1.12	0.74-1.70	0.59
Weight loss (>10% in 1 year)	1.13	0.63-2.06	0.68	-	-	-
Hemoglobin	1.09	0.81-1.47	0.55	-	-	-
Myositis yes/no	1.03	0.51-2.10	0.93	-	-	-
ACA	**1.57**	**1.11-2.21**	**0.010**	1.60	0.99-2.56	0.05
ATA	**0.55**	**0.34-0.90**	**0.02**	0.72	0.40-1.32	0.29
Anti-RNApIII	1.01	0.53-1.94	0.97	-	-	-
Proton pump inhibitor	1.17	0.83-1.67	0.37	-	-	-
H2 receptor blocker	0.83	0.56-1.22	0.33	-	-	-
ACE inhibitor	0.60	0.37-0.99	0.05	0.63	0.40-1.05	0.11
Calcium channel blocker	0.91	0.64-1.30	0.60	-	-	-
Immunosuppressiva combined	0.80	0.49-1.27	0.33	-	-	-
Prostacyclin & ET-1 inhibitor	1.35	0.66-2.33	0.63	-	-	-

*Disease subset: entered as ordinal variable with order: non-cutaneous, limited and diffuse subset. # disease severity is a compound variable which included: ILD with FVC<70%, PAH, renal crisis, mRSS>20 or presence of digital ulcers. Medication are entered as yes or no, with a yes if the medication was used in the same year as the GIT questionnaire was completed. * Significant after Holm-Bonferroni correction.

Table S9. Logistic regression analyses of baseline characteristics associated with moderate or severe social functioning symptom score in the 834 systemic sclerosis patients from the Leiden and Oslo cohort

	Univariable			Multivariable		
	Moderate or severe social functioning symptom score					
	OR	**95% for CI OR**	**Signifi-cance P**	**OR**	**95% for CI OR**	**Signifi-cance P**
Female	1.57	0.95-2.58	0.08	1.45	0.83-2.54	0.19
Age, Years	1.00	0.99-1.01	0.95	0.99	0.98-1.01	0.35
Disease duration, years	**1.03**	**1.01-1.05**	**<0.001***	**1.04**	**1.01-1.06**	**<0.001***
Raynaud duration, years	**1.01**	**1.01-1.07**	**0.01**			
Smoking ever	**1.48**	**1.02-2.15**	**0.04**	**1.65**	**1.12-2.45**	**0.01**
Disease subset*	1.50	0.80-2.79	0.21	**2.27**	**1.03-5.02**	**0.04**
Disease severity	1.10	0.78-1.58	0.56	1.02	0.67-1.55	0.92
Weight loss (>10% in 1 year)	1.12	0.70-1.81	0.63	-	-	-
Hemoglobin	1.01	0.76-1.35	0.94	-	-	-
Myositis yes/no	1.04	0.51-2.11	0.92	-	-	-
ACA	**1.83**	**1.25-2.14**	**<0.001***	**1.91**	**1.18-3.09**	**0.01**
ATA	0.62	0.38-0.99	0.05	0.87	0.49-1.56	0.64
Anti-RNApIII	0.59	0.27-1.28	0.17	-	-	-
Proton pump inhibitor	1.36	0.96-1.93	0.08	1.33	0.89-1.87	0.12
H2 receptor blocker	0.88	0.60-1.30	0.53	-	-	-
ACE inhibitor	0.94	0.60-1.46	0.77	-	-	-
Calcium channel blocker	1.12	0.85-1.48	0.41	-	-	-
MMF	1.43	0.71-2.90	0.31	-	-	-
Immunosuppressiva combined	1.29	0.84-1.99	0.25			
Prostacyclin & ET-1 inhibitor	1.88	0.88-2.67	0.33	-	-	-

*Disease subset: entered as ordinal variable with order: non-cutaneous, limited and diffuse subset. # disease severity is a compound variable which included: ILD with FVC<70%, PAH, renal crisis, mRSS>20 or presence of digital ulcers. Medication are entered as yes or no, with a yes if the medication was used in the same year as the GIT questionnaire was completed. * Significant after Holm-Bonferroni correction.

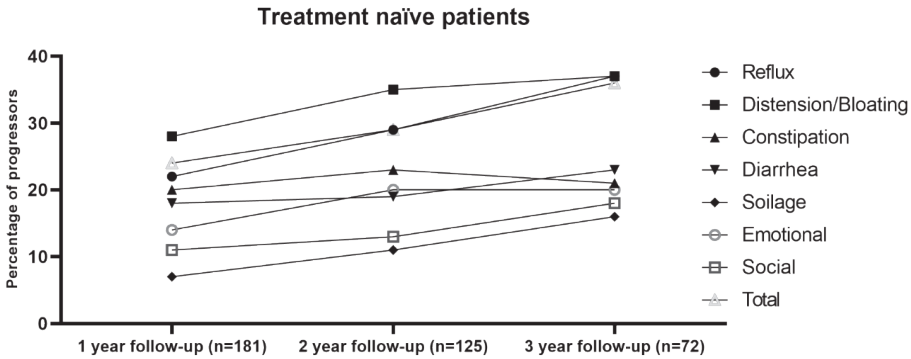

Figure S4. Percentage of progressors per GIT subdomain over the follow-up period in treatment naïve patients in the inception cohort (for immunosuppressive treatment). The line indicate years of follow-up and the numbers (n) the amount of treatment naïve patients at that time point. We calculated the percentage of progressors based on the MCID of the UCLA GIT 2.0 per subdomain after 1,2 and 3 years of follow-up.

Chapter 5

A new risk model is able to identify patients with a low risk of progression in Systemic Sclerosis

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Objectives: To develop a prediction model to guide annual assessment of systemic sclerosis (SSc) patients tailored in accordance to disease activity.

Methods: A machine learning approach was used to develop a model that can identify patients without disease progression. SSc patients included in the prospective Leiden SSc cohort and fulfilling the ACR/EULAR 2013 criteria were included. Disease progression was defined as progression in ≥1 organ system, and/or start of immunosuppression or death. Using elastic-net-regularization, and including 90 independent clinical variables (100% complete), we trained the model on 75% and validated it on 25% of the patients, optimizing on negative predictive value (NPV) to minimize the likelihood of missing progression. Probability cutoffs were identified for low and high risk for disease progression by expert assessment.

Results: Of the 492 SSc patients (follow-up range: 2-10yrs), disease progression during follow-up was observed in 52% (median time 4.9yrs). Performance of the model in the test set showed an AUC-ROC of 0.66. Probability score cutoffs were defined: low risk for disease progression (<0.197, NPV:1.0; 29% of patients), intermediate risk (0.197-0.223, NPV:0.82; 27%) and high risk (>0.223, NPV:0.78; 44%). The relevant variables for the model were: previous use of cyclophosphamide or corticosteroids, start with immunosuppressive drugs, previous gastrointestinal progression, previous cardiovascular event, pulmonary arterial hypertension, modified Rodnan Skin Score, creatinine kinase, and diffusing capacity for carbon monoxide.

Conclusion: Our machine-learning-assisted model for progression enabled us to classify 29% of SSc patients as 'low risk'. In this group annual assessment programs could be less extensive than indicated by international guidelines.

INTRODUCTION

Systemic Sclerosis (SSc) is a heterogeneous disease. The spectrum of the disease ranges from rapidly progressive, with generalized fibrosis of the skin and the vital organs to a more indolent form developing over an extended period of time (1). The amount of patients with progression of SSc is substantial and progression occurs most often in early disease (2, 3). It is important to note that around 50% of patients will never show any signs of progression. To accurately assess the trajectory of the disease, several studies addressed identification of risk factors of future skin and organ progression in different SSc subpopulations (4, 5). Existing prediction models in SSc are often based on a subset of SSc patients, and do not capture the whole population (2, 6). Prediction of the disease course remains challenging in the individual patient which raises the questions whether personalized prediction in the heterogeneous SSc population is actually feasible.

For physicians in clinical practice, it is important to have clear guidance regarding intensity and frequency of follow-up, not only to identify disease progression timely but also to limit excessive diagnostics in mild SSc patients. Currently, no evidence based international guidelines for follow-up of SSc exist, except for the ESC/ERS guideline recommending annual echocardiography for detection of pulmonary arterial hypertension (PAH) (7). In 2019, an international standard for longitudinal follow-up describing points to address in annual assessment of patients with SSc was developed based on Delphi-expert consensus. Overall, 55 items were identified including clinical assessments, laboratory measurements, imaging and functional investigations (8). Whether the identified items are sufficient to identify disease progression timely in all patients is yet to be determined. Moreover, in some patients with mild disease, annual follow-up might even be more concise and assessing 55 tools on an annual basis might not be necessary. Of note, previous prediction studies concercing prevalent SSc cohorts might have underestimated progression in SSc by failing to capture the early rapid progressors (9). On the other hand, with the introduction of the ACR/EULAR 2013 criteria additional cases with less severe disease might be identified (10). Together, these observations provide the rationale for the design of data-driven recommendations that describe tailormade systematic assessments for individual SSc patients in line with their individual disease course.

Our prospective SSc cohort includes both mild and severe patients who undergo annual assessment, as the health care system in The Netherlands is characterized by high accessibility. Starting from 2009 all patients fulfilling Leroy criteria for early SSc have been included (11, 12). In the current study, we included detailed information on disease

progression in our prospective cohort and we addressed an important limitation that is often encountered when searching for predictive factors in large datasets from SSc patient registries: the high incidence of missing data. Therefore, in our prediction model, we only included patients with complete data available on at least three visits.

With this study we aim to develop a tailormade model to guide annual assessment in individual SSc patients, with a special focus on patients with a low risk of disease progression in whom annual extensive investigations may be considered redundant. To address this we: 1) determined the proportion of patients without disease progression, 2) applied machine learning to build a prediction model in the patients with complete data available at ≥ 3 time points to predict lack of progression. Additionally, 3) we evaluated a second prediction model including the variables from the Delphi consensus guideline and compared the performance of this model to the Machine-Learning-Assisted model in order to assess which investigations are minimally needed to identify patients with a low risk of disease progression.

METHOD

Patient selection

In the Leiden University Medical Center (LUMC), all SSc patients, with a range of disease severity from mild to very severe, undergo annual extensive screening during a 1 to 2 day health care program (combined care in systemic sclerosis (CCISS)). This includes a detailed physical examination, modified Rodnan skin score (mRSS) assessment (13), laboratory testing (with autoantibody screening at baseline), electrocardiography (ECG), pulmonary function test, optional echocardiography (mandatory at baseline visit), optional 24-hour Holter ECG monitoring (mandatory at baseline visit), optional cardiopulmonary exercise tests (CPET) and optional high-resolution computed tomography (HRCT) (mandatory at baseline visit). Patients are requested to complete various questionnaires at every visit (14-22). Additionally, at every visit, blood and serum samples are collected and stored in the Leiden Scleroderma Biobank.

For the first part of the study (i.e. numbers of disease progressors), SSc patients who fulfilled the 1) ACR 1980 and/or LeRoy (from 2009-2013) criteria or the 2) ACR/EULAR 2013 and/or Leroy criteria and had at least two assessments were included (12, 23, 24). For the second part of the study, including the Machine-Learning-Assisted prediction model, we analysed SSc patients who fulfilled the ACR/EULAR 2013 criteria and had at least three complete visits (a third visit was necessary for our primary outcome). This ensured that included patients had complete data available of at least three time points. A complete visit consisted of at least a physical examination (including mRSS), laboratory testing, a pulmonary function test, ECG, HRCT and a transthoracic echocardiography. The strict inclusion criteria were necessary to limit the amount of missing data on important organ systems and only patients who underwent complete screening of organ systems were included in the model in order to minimalize the likelihood of missing any important organ involvement.

The cohort study was designed in accordance with the ethical principles of the Declaration of Helsinki. All patients gave written informed consent. Collection and analysis of data have been approved by the local ethics committee (Leiden CME number B16.037).

Outcomes

Disease progression was defined as progression in one or more organ systems; pulmonary, cardiac, gastro-intestinal, skin, renal, and/or myositis (supplementary table S1 for detailed explanation). For pulmonary, PAH, skin and renal crisis, progression was defined as described previously (10, 25, 26). Cardiac progression, gastro-intestinal progression and myositis were each defined using a combination of variables and based on consensus among authors. Use of immunomodulatory medication was recorded at

every visit and included: cyclophosphamide, methotrexate, myocophenolate mofetil (MMF), azathioprine, corticosteroids, hydroxychloroquine and stem cell transplantation. Patients included in clinical trials (Resolve [lenabasum], Senscis [nintedanib], FocuSSced [Tocilizumab], ASTIS [autologous stemcell transplantion), RITIS [rituximab], ASTIS [stem cell transplantation] were also captured. Use of biologicals outside of trials was observed in <0.5% of the patients, therefore these were not depicted separately but were included in the primary outcome (27-31). The primary endpoint in the prediction model was defined as progression in ≥ 1 organ system, and/or start of immunosuppression (IS) or death between the two most recent visits.

Predictors

The included predictors in the Machine-Learning-Assisted model were selected based on the predictors identified by experts (8), additional predictors were selected based on clinical expertise and current literature. In order to prevent exclusion of too many patients due to missingness, we dropped four variables (out of 94) with a missingness percentage > 5% (nailfold videocapillaroscopy, of which annual collection started in 2013, and 3 variables derived from the UCLA GIT questionnaire, namely fecal soiling, diarrhea and distension/bloating, of which annual collection started in 2013). This resulted in 90 independent variables (100% complete in n=248 patients) to predict progression at the final assessment. The 90 variables included in our model -all 100% complete- are described in the supplementary Table S2. A timeline of the study can be found in the supplementary file (Figure S1).

Statistics

For the first part of the study we used descriptive statistics to evaluate the number of disease progressors in SSc during 10 years of follow-up. For the second part of the study, the development of the prediction model, we applied a machine learning approach known as "elastic net regularization". The elastic net performs simultaneous regularization and variable selection in order to reduce variance with minimal risk of bias (32). Independent variables were all the variables collected during follow-up visits [predictors] until the prediction visit [event visit= primary outcome]. Disease progression on any organ system during follow-up was also included as independent variable in different manners: 1) progression between third **to** event visit and the prediction visit (dichotomous), 2) progression developed before the prediction visit (dichotomous), 3) the amount of times progression occurred between baseline visit and the prediction visit (quantitative), and 4) in how many organ systems progression occurred over time (quantitative). Including progression as an independent variable mimics the decision making of the physician in clinical care, where decisions regarding follow-up are made based on previous information (including progression occurring years before the current

visit). Given the extensive amount of information from previous visits we examined whether these data could predict the development of progression at the final [event] visit. The dependent variable in the model was defined as progression in ≥ 1 organ system, and/or start of IS at the last recorded visit, or death after a complete baseline visit. All patients without any progression during follow-up were identified as 'nonprogressors'. In order to preserve the maximum number of patients, we filtered variables on 95% call rate prior to deleting incomplete cases, variables with more > 5% missingness were checked in the EPD and in case of true missingness deleted from the dataset. To develop the model using leave-one-out cross validation and independently validate the final model's performance, the included patients were randomly split in a training (75%) and a test (25%) set. The model was developed and optimized on the larger training set and subsequently applied to the test set with the lambda, alpha and coefficients set to the identified optima. The chosen predictive variables were entirely based on the training data. Risk probability scores and AUC-ROC were created (based on test data) to identify optimal cut-offs for the risk on disease progression.

Due to the detrimental impact of undertreatment in SSc, we opted to maximize negative predictive value (NPV) with a constraint on sensitivity, which minimizes the likelihood of missing progression. However, a benefit of the probabilistic nature of a prediction model is the flexibility of the case cut-off point; by sliding across the ROC curve one can choose to prioritize any preferred performance metric. Cut-offs for risk probability scores were defined based on the test characteristics (maximize NPV) and the distribution of probabilities plots in the test set. After cut-off selection, we ran a post-hoc analysis to assess the missed progressors.

Lastly, using logistic regression we built a prediction model including 51 (out of the 55) variables from the Delphi consensus guideline (8). We had to exclude leg edema, urine analysis, liver function test and New York Heart Association (NYHA) class due to missingness in > 5%. The performance, and the risk probability scores of the model including the Delphi variables as predictors were compared with the model derived from machine learning based on the AUC-ROC curve and the probability plots using descriptive analyses.

All analyses were performed in R version 3.5.0. The "glmnet" package was used for elastic net regularization and leave one out cross-validation was implemented through the "caret" package.

RESULTS

Patient population

For the first part of the study we included 492 SSc patients who completed at least two visits in our cohort (flowchart Figure 1). Seventy-nine percent was female (n= 389) with a mean age of 55 years (SD 14), a median disease duration since first non-Raynaud's symptom of 3.2 years years (IQR 1-10) and the median mRSS was 4 (IQR 0-6) (table 1).

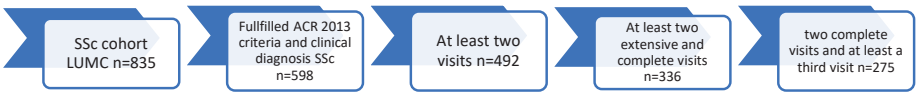

Figure 1. Flowcharts of inclusion process. Cut-off timepoint for inclusion: 01-July-2019. * Ninety-two patients had to be excluded due to missing data/incomplete data even though they had three or more visits. Of these, the majority did not show progression based on clinical and laboratory assessment, 6 minute walking distance and pulmonary function testing.

Progressors versus non-progressors in SSc cohort

In n= 492 SSc patients (2109 timepoints, range of follow-up 2-8 years), disease progression during follow-up was observed in 52% (n=2 57) after a median of 4.9 years (IQR 2-7) (Figure 2). Pulmonary (23%) and cardiac progression (29%) occurred most often, death (all-cause) occurred in 12% of the patients (n= 60). We confirm that patients with dcSSc, ILD and ATA at baseline were more likely to experience disease progression somewhere during the disease course (table 1). Forty-eight percent of the SSc patients (n= 235) did not show progression during follow-up (median 3.5 years (IQR 2-6).

Patient selection Machine-Learning-Assisted model

Of the 248 patients that could be included for development of the prediction model, 80% was female (n=220) with a mean age of 53 years (SD 14), a median disease duration since first non-Raynaud sign or symptom of 3.5 years (IQR 1-9) and median mRSS of 4 (IQR 1-6). The baseline characteristics of these patients are shown in table S3 in the supplementary file. Comparison of baseline demographic and clinical characteristics between these patients and the 492 patients with two assessments available showed that the patients included for the prediction model development were more often ATA positive and had higher prevalence of ILD. Other characteristics were not significantly different between the groups (supplementary file table S4).

Baseline characteristics	Total n=492	Non-Progressors N=235	Progressors N=257
Demographics			
Female, n (%)	389 (79)	193 (82)	196 (76)
Age, mean (SD)	55 (14)	55 (15)	55 (13)
Disease duration nonRP, median (IQR)	3.2 (0.9-10.3)	3.5 (0.8-10.5)	3.6 (1.1-9.3)
- lcSSc, median (IQR)	4.1 (1-11)	3.9 (1-11)	2.4 (1-11)
-DcSSc, median (IQR)	3.0 (1-8)	2.7 (1-7)	4.1 (0.5-9)
Organ involvement			
DcSSc, n (%)	118 (24)	**34 (15)**	**84 (33)**
mRSS, median (IQR)	4 (0-6)	**2 (0-5)**	**4 (1-7)**
DU, n (%)	62 (13)	29 (12)	33 (13)
DLCO% of pred, mean (SD)	66 (18)	69 (18)	64 (17)
FVC% of pred, mean (SD)	98 (23)	96 (24)	97 (21)
ILD on HRCT, n (%)	183 (37)	**66 (28)**	**117 (46)**
PAH, n (%)	26 (5)	10 (4)	16 (6)
GAVE, n (%)	9 (2)	4 (2)	5 (2)
Cardiac involvement, n (%)	28 (6)	14 (6)	14 (5)
Myositis, n (%)	8 (2)	6 (3)	2 (1)
Renal crisis, n (%)	14 (3)	6 (3)	8 (3)
Autoantibodies			
Anti-centromere, n (%)	194 (39)	**118 (50)**	**76 (30)**
Anti-topoisomerase, n (%)	116 (24)	**42 (18)**	**74 (29)**
Medication (current use)			
Corticosteroids, n (%)	42 (9)	16 (7)	26 (10)
Methotrexate, n (%)	68 (14)	34 (15)	34 (13)
Mycophenolate mofetil, n (%)	19 (4)	5 (2)	14 (5)
Hydroxychloroquine, n (%)	22 (5)	7 (3)	15 (6)
Cyclophosphamide, n (%)	11 (2)	4 (2)	7 (3)
Azathioprine, n (%)	14 (3)	2 (1)	12 (5)
ASCT, n (%)	4 (1)	2 (1)	2 (1)

Table 1. RP= Raynaud's phenomenon, dcSSc= diffuse cutaneous systemic sclerosis, mRSS= modified Rodnan skin score, DU= digital ulcers, DLCO= single-breath diffusing capacity of the lungs for carbon monoxide, FVC= forced vital capacity, ILD= interstitial lung disease, HRCT= high resolution computed tomography, PAH= pulmonary arterial hypertension, GAVE= gastric antral vascular ectasia, ASCT= autologous stem cell transplantation. Bold indicates significant differences $p < 0.05$ between progressors versus non-progressors.

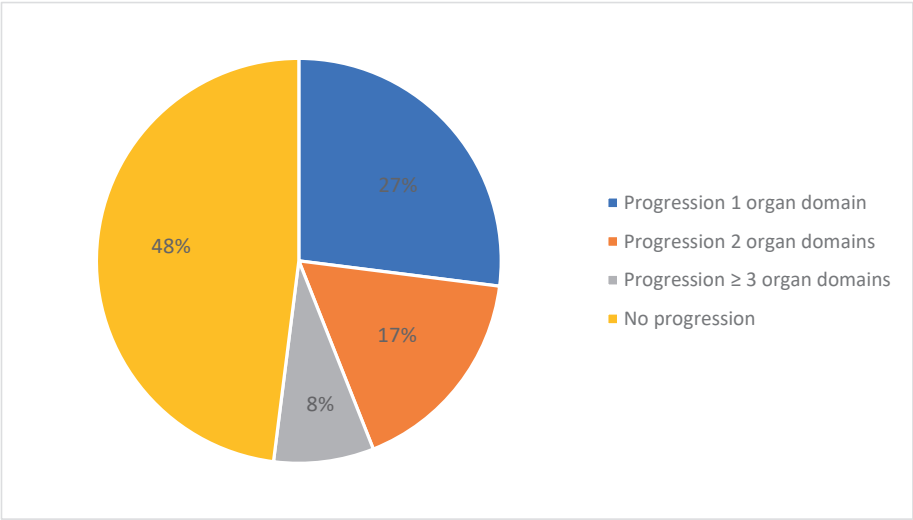

Figure 2. Organ progression in SSc cohort, progression was not always limited to one organ domain. Twenty-five % of the patients showed organ progression on more than one organ domain.

Machine-Learning-Assisted prediction model

After leave-one out cross validation, the final model consisted of 90 variables. The Machine-Learning-Assisted model identified 10 independent variables predictive for disease progression (supplementary table S5). The identified predictors were: previous use of cyclophosphamide (β 0.94) or corticosteroids (β 0.43), previous GI progression (β 0.34), a cardiac event in medical history (β 0.31), PAH (β 0.30), start of immunosuppressives (β 0.21), previous cardiac progression (β 0.08), mRSS (β 0.01), CK (β 0.0006), and DLCO (β -0.004).

The Machine-Learning-Assisted model had an AUC-ROC of 0.77 in the training set (n= 185). The mean (SD) probability score for risk of progression in non-progressors was 0.23 (0.05), and in progressors 0.31 (0.11) (supplementary figure S2). The AUC-ROC of the model in the validation set (n= 63) was 0.66 (figure 3 ROC curve and distribution of probability plot). In this set, the mean (SD) probability score for risk of progression in non-progressors was 0.24 (0.06) and in the progressors 0.29 (0.13). Based on expert opinion, the distribution of probabilities plot and the test characteristics of the validation set (maximize NPV), we identified two cut-offs to identify patients with low (< 0.197), intermediate (0.197-0.223) and high risk (> 0.223) of progression (table 2). A third threshold (> 0.627) corresponding to maximal specificity is also presented (table 2).

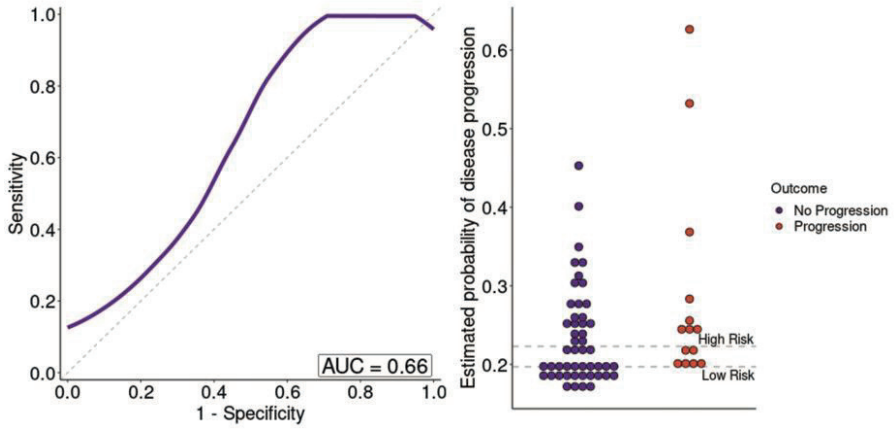

Figure 3. ROC curve and distribution of probability plot of the validation set in progressors and non-progressors.

Threshold	Sensitivity	Specificity	Accuracy	Positive predictive value	Negative predictive value
0.627	0	1	0.78	NaN	0.78
0.223	0.57	0.57	0.57	0.28	0.82
0.197	1	0.37	0.51	0.31	1

Table 2. Test characteristics of data driven prediction model. NaN= not a number.

Disease progression and probability scores in Machine-learning assisted model prediction model

Our primary outcome was progression at the event visit, which occurred in 60 out of 248 patients (24%). Progression was identified in all subdomains: disease subset (n= 3), skin (n= 4), lung (n= 14), cardiac (n= 28), GI (n= 15), renal (n= 2), PAH (n= 4), myositis (n= 6), start of IS therapy (n= 6), and all-cause death (n= 11; detailed overview of cause of death is shown in supplementary file S1). In the validation set (n= 63), 22% (n= 14) showed progression during the event visit, while 78% (n= 49) did not show progression. With guidance of the Machine-learning assisted model prediction model 28 patients were identified as high risk for progression which was correct in 32% (n= 9); 18 patients were identified as low risk which was correct in 100% (due to our strict cut-off), which means that 29% of the patients in the validation set (18 out of 63) had a low risk score and indeed did not show progression. Of the patients with intermediate risk according to our model (n= 17), five showed progression.

Progressors stratified for treatment initiation

To evaluate the clinical relevance of the probability scores we performed an additional analyses in the organ progressors group (validation set) by stratifying them for immunosuppressive treatment initiation after the data collection closure. We hand-searched the electronic patient files of the organ progressors to collect data on IS treatment initiation (started after data collection closure 01.07.2019). Our results showed that patients with organ progression at the most recent visit (primary outcome), for which medication was started, were more likely to score higher on the probability risk score. There was one patient with lung progression who also started treatment with a risk score just above the cut-off for low risk (figure 4). In the non-progressors with a low risk score (n= 18), we identified n= 8 patients who never had any IS treatment during their disease course, n= 9 did use IS medication somewhere during follow-up (HCQ: n= 3 due to arthralgia, polyarthritis, or synovitis, MTX: n= 6 due to limited skin involvement), and 1 patient is still on MMF treatment because of minimal skin (mRSS 2) and lung involvement (minimal interstitial changes on HRCT, without pulmonary function abnormalities).

Delphi score versus Machine-Learning-Assisted model

The most recently published guideline on follow-up in SSc is based on expert opinion by Delphi consensus which advises to yearly measure 55 variables. Based on these 55 independent variables, we built a prediction model [Delphi Model] in order to assess the ability of the Delphi items to identify patients at risk for progression, and compare the performance of the 'Delphi model' with the model derived from machine learning. The AUC-ROC of the Delphi model in the validation set was 0.65 (figure 5 ROC curve and distribution of probabilities plot). The mean (SD) probability score for the risk of progression in non-progressors was 0.14 (0.30), and in the progressors 0.42 (0.46). Of the 54 patients in this validation set, 13 developed progression, of whom 6 patients had a probability risk score below 0.007. The results of the Delphi model, shown in the distribution of probabilities of the validation set, made it difficult to identify cut-offs for low, intermediate and high risk patients on progression. Therefore a cut-off based on an NPV of 1.0, as we used in the Machine-Learning-Assisted model, is not feasible in this model.

The coefficients that were significant in the final model of this prediction set can be found in table S6 supplementary file. The included predictors with the largest coefficients were: previous use of corticosteroids (β 6.66), previous use of iloprost (β 15.1), previous use of bosentan (β -18.0), current use of MMF (β 5.98) or cardiac event in the past (β 5.39).

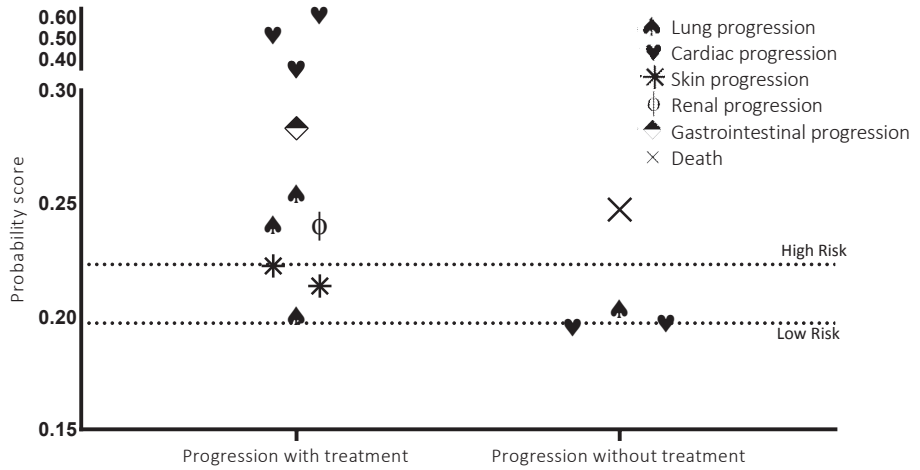

Figure 4. Probability risk scores of the progressors stratified for treatment initiation and organ domain. *Patients with cardiac progression and treatment (n= 3)*: [1] trifascicular block with pauses > 3 seconds for which pacemaker implantation, severe tricuspid insufficiency, [2] new right bundle branch block, decrease in LVEF < 50%, increase dyspnea,[3] clinical cardiac involvement; supraventricular arrhythmias 2%, diastolic dysfunction grade 1, elevated troponin T and CK, progressive dyspnea). *Patients with cardiac progression without treatment (n= 2):* [1] LVEF < 54%, [2] supraventricular arrhythmias > 2% on 24h Holter ECG monitoring . *ILD progression with treatment (n= 3):* [1] mild fibrotic changes with a decrease in FVC (73% to 58%) and in DLCO (97% to 76%), [2] increase in fibrotic changes, decline FVC (52% to 42%) and decline in DLCO (48% to 28%), [3] progressive ILD and decline in FVC and DLCO (n=3). *ILD progression without treatment (n=1)*: [1] presence of ILD with bronchiectasis, honeycombing and an increase in reticular opacities, no clinical symptoms, with FVC decline (101% to 90%). *Skin progression with treatment (n= 2):* [1] mRSS increase from 10 to 17, [2] increase mRSS 10 to 23. *Gastrointestinal progression with treatment (n= 1)*: [1] weight loss > 10% in 1 year AND Hb decline. One patient developed renal crisis, one patient died due to lung carcinoma (also had supraventricular extrasystoles > 2 seconds and in increase in fibrotic changes on HRCT).

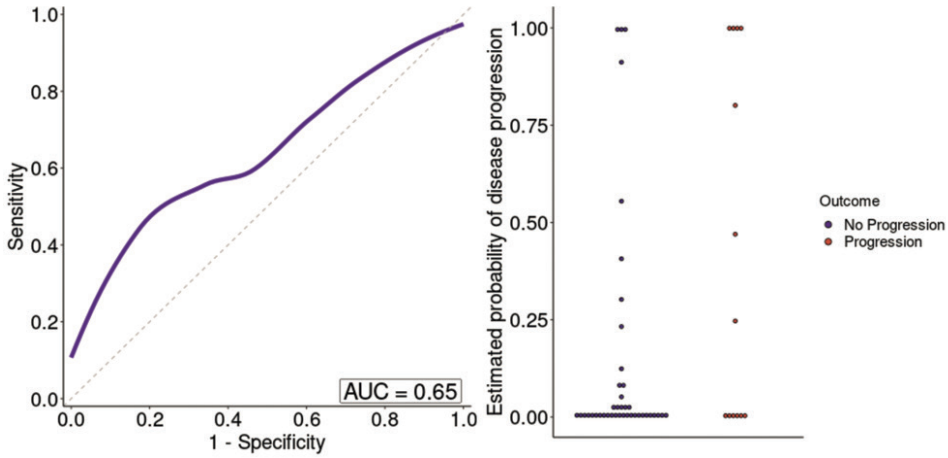

Figure 5. ROC curve and distribution of probabilities plot of the Delphi model stratified for progression.

DISCUSSION

Our newly developed prediction model was able to identify SSc patients with a low risk for disease progression in whom less extensive annual evaluation can be justified. We confirm that SSc is a severe and heterogeneous disease with overall progression occurring in 52% of the patients somewhere during follow-up. In total 235 patients did not experience disease progression during 3.5 years (IQR 2-6) of follow-up.

With the use of machine learning, we developed a prediction model and we managed to include 248 SSc patients with complete data on 90 variables on at least three visits. These patients had a median follow-up of 5.4 years (IQR 3.2-7.5). Although the overall accuracy of the model was moderate, it performed very well in identifying patients with a low risk for disease progression (29% NPV 1.0). For these patients we can adjust annual evaluation using a less extensive diagnostic program.

To identify patients at low risk, we calculated probability scores with the Machine-Learning-Assisted prediction model. The cut-off for low risk patients was very strict, since we did not want to miss any organ progression, with none of the progressors scoring under the low risk cut-off (NPV 1.0). Twenty-nine percent of the SSc patients were identified in the low risk group and extensive follow-up might not be necessary in this patient group. The Machine-Learning-Assisted model could therefore significantly reduce health-care costs without substantial risk to our patients. The assessments that are necessary to identify progression with our model are predominantly: use of IS medication in the past, presence of PAH, mRSS, DLCO and cardiac and GI involvement/ progression. Based on this observation showing a diverse group of characteristics that identify risk of progression, we conclude that in all patients with a new diagnosis of SSc complete organ assessment is necessary to guide future follow-up.

To build the Machine-Learning-Assisted model, we used “elastic-net regularization”, a variable selection method that allows to address multicollinearity. It provides a more reproducible prediction than multiple regression, especially when predictors are highly correlated. Elastic net regularization has been shown to robustly maintain predictive accuracy even with a large number of predictors relative to the number of observations. We note that the variables in the final model are predictors, and can therefore not be interpreted as having a causal relationship with progression. Furthermore, since we used a regularization method, variables that play an important causal role could have been dropped from the model when other variables had a similar or stronger association.

Even though the CCISS care pathway is highly standardized and in accordance with international guidelines, we cannot rule out that factors related to the local health care situation have influenced the results. It is therefore important to validate this model in different health care systems. We did not calibrate the probabilities of the Machine-learning assisted model, whereby the probabilities are slightly different from the real risk. This was acceptable since we used a cut-off to identify patients at low risk for progression and not the full range of probabilities.

One of our secondary aims was to compare the Machine-Learning-Assisted model with a model based on the Delphi guideline including the selected tools, to evaluate if assessment of these 55 tools in every patient on a yearly basis might be redundant for a part of the SSc population. The prediction model based on the Delphi variables (including 51 expert opinion variables in the final model) had a similar AUC-ROC as the Machine-learning assisted model model (with only 10 out of 90 variables in the final model based on data driven selection). By using the identified variables for annual follow-up selected by experts to predict disease progression, the discrimination of probability scores between progressors and non-progressors improved but identification of low risk patients was more difficult, and physicians need to collect 51 variables. Forty-six percent of the patients that exhibited progression had a risk-probability close to zero (<0.007) according to the prediction model based on the Delphi model. The Machine-Learning-Assisted model was very well suited to identify patients at low risk as 29% had a probability below 0.197 and all these patients were non-progressors. The comparison between the two models demonstrates that the combination of all Delphi variables cannot directly be used to predict patients at (low) risk for progression. Clearly, the Machine-Learning-Assisted model as constructed in our study is useful to identify patients who are at low risk for disease progression and who therefore may not need intensive follow-up evaluations. Important sidenote, in both models, only patients who underwent complete evaluation for organ involvement and disease progression at least twice were included. Given the severe and heterogeneous nature of SSc, which is underlined by the fact that 52% of patients experienced disease progression during follow-up, in our opinion, annual extensive evaluation is justified in newly diagnosed SSc patients during the first two years. After two evaluations, our current data show that one could consider to apply the probability scores for risk on progression and identify patients in whom follow-up evaluations can be less extensive.

There are some limitations to be acknowledged. First, the clinical variables collected in this study ideally reflect disease activity, disease status and organ damage, to predict disease outcome. However, in SSc, uniform and validated definitions are lacking for some of the organ systems, which should be taken into account as a general limitation.

Secondly, evaluating progression of GAVE and/or PAH is difficult as in clinical practice RHC and endoscopies are not routinely applied as follow-up assessment. We chose to classify PAH patients as non-progressors based on stable pulmonary function testing (PFT), which might have missed some patients. With respect to GAVE we are reasonably convinced that patients with clinically relevant GAVE are correctly identified based on the fact we included hemoglobin in our dataset. Secondly, the follow-up duration might not be sufficient to capture all progressors. Although median follow-up duration is short (5.4 years), 54% of patients had a disease duration of >10 years since first non-RP at the end of the observation period. The follow-up period between progressors and non-progressors was different; however, the proportion of progressors is similar amongst groups stratified for follow-up duration (data not shown). importantly, we had to exclude 244 patients for our final model, as we defined 100% complete data on at least three visits as a prerequisite (for both independent and dependent variables) to predict as accurate as possible (flowchart figure 1). When building the model, we preferred to overestimate progression instead of missing important organ progression. With that in mind, we used these strict inclusion criteria, and, as a consequence, the possibility of selection bias must be considered. The patients included in the model were more often ATA positive and more often had ILD. Therefore, the population used to build the progression model probably had more severe disease, and as such the observed selection does not interferes with the primary aim. Of the 244 patients that had to be excluded for development of the prediction model, 81% was still in follow-up as part of the CCISS cohort, and the majority (89%) did not show progression based on clinical assessment, including PFT and 6 minute walking test (figure S3). The large time frame of the study might also be a limitation as IS treatment might have changed over the years. We evaluated the use of IS therapy for three time frames (2009-2012, 2013-2016, 2017-2019) and found a similar percentage of patients starting IS medication, which makes a large impact on primary outcome unlikely. Another limitation of the study is intrinsic to the heterogeneity of the disease. Included predictors might act as risk and protective factors at the same time, as our primary outcome was aggregated disease progression. We tried to build different models for every organ system, however the occurrence rate of progression was too low to create single reliable models. Finally, we did not have access to a prospective and independent validation sample with all 90 variables available to further test the model's validity. While a completely held-out test sample is statistically equivalent to a prospective sample from the same population, separate validation from a truly prospective sample could further examine the model's generalizability. Previous studies have looked at predictors individually, but the unbiased, data-driven approach, which is a major strength of our work, could contribute to tailor future directions for research and clinical practice. For instance, accurate prediction of patient outcome can be used to inform treatment planning decisions, where modeling the likelihood of disease progression is a critical outcome of interest to health care

systems, providers, and stakeholders. Future development of these tools with larger training samples can improve prediction of patient outcome, even to the point where differential predictions of outcome for the personalization of monitoring of SSc might be possible.

A next step is to validate this model in clinical practice. Therefore, we have designed the trial 'From a pragmatic model to a pragmatic study: a non-inferiority randomized trial'. We aim to start inclusion in 2021. The aim of this trial is to evaluate whether annual assessment in patients who underwent extensive evaluation at least twice and are categorized as low risk patients based on our machine learning derived model, can be less extensive without jeopardizing health care utilization, quality of life, disease perceptions and disease course. In addition, an online tool will be developed to calculate risk scores for SSc patients (work in progress).

In the end, achieving equality of assessment worldwide will most likely increase the standard care for SSc. However, until now there were no existing evidence based guidelines for standardized follow-up of patients with SSc. This study showed that disease progression somewhere during follow-up occurs in 52% of the SSc patients, with a high variety between organ systems. Without the use of a prediction model these findings justify the annual complete organ assessments, at least for the first 5 years since first non-RP symptom. While identifying SSc patients at risk for progression remains difficult, our prediction model facilitates the stratification of low, intermediate and high risk patients. In conclusion, SSc patients with a low risk at progression can be identified with the use of the Machine-Learning-Assisted model and allows us to confidently identify a subset of patients who can safely reduce their visit frequency.

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Part III

Role of microangiopathy and specific autoantibodies in Systemic Sclerosis

Chapter 6

The contribution of sex and auto-antibodies to microangiopathy assessed by nailfold videocapillaroscopy in systemic sclerosis: a systematic review of the literature

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Objective: Microangiopathy and dysregulation of the immune system play important roles in the pathogenesis of Systemic Sclerosis (SSc). Factors that trigger vascular injury in SSc have not been elucidated so far. To evaluate whether sex or expression of specific antinuclear auto-antibodies might associate with the degree of microangiopathy we performed a systematic review summarizing what is known about these associations.

Methods: Standardized search of PubMed, EMBASE, Web of Science and the Cochrane library were performed to identify studies, that report on auto-antibodies in SSc patients and microangiopathy, and for the second search, that report on sex and microangiopathy.

Result: We included 11 studies that described the relationship between SSc-specific autoantibodies and microangiopathy and 6 studies that reported on the association between sex and microangiopathy. Contradictory results were found on the association between auto-antibodies and microangiopathy, and no association was found between sex and microangiopathy based on the current literature.

Conclusion: Based on this review of the literature, we can conclude that sex does not seem to influence degree of microangiopathy in SSc, while results on association between SSc-specific auto-antibodies and degree of microangiopathy were inconclusive.

INTRODUCTION

Systemic Sclerosis (SSc) is characterised by a triad of microvascular damage, dysregulation of innate and adaptive immunity, and generalized fibrosis that can affect skin and internal organs (1). In SSc, the most frequent symptom of microvascular damage is Raynaud's phenomenon (RP), which is present in up to 96% of patients and often represents the earliest manifestation of the disease. Current concepts indicate that microangiopathy is a key factor in early pathogenesis of SSc. In RP evolving to definite SSc, presence of microvascular damage and SSc specific auto-antibodies indicate a very high probability of developing SSc (2). The frequency of progression is higher with both the presence of SSc auto-antibodies and microvascular damage (79.5%), than with presence of one of these predictors (32.2%) (3). In addition to its diagnostic value, the degree of microangiopathy is also a valuable prognostic marker in SSc patients, as it contributes to predict future organ complications (3-5). The SSc specific auto-antibodies are associated with specific clinical characteristics and therefore are of additional prognostic value. Anti-centromere antibody (ACA) is associated with a decreased risk of lung (OR 0.12) and heart involvement (OR 0.39), while anti-topoisomerase antibody (ATA) + patients have an increased risk for these complications (OR 6.66, OR 2.12) (6, 7). Strikingly, the degree of microangiopathy was comparable between ACA+ and ATA+ patients (late SSc pattern; ACA 33%, ATA 25%). This suggests that presence of a specific antinuclear antibody is independent of the development of microangiopathy.

However, in some studies, an association between microvascular damage and auto-antibodies has been described (8). Antinuclear auto-antibodies (ANA), found in 95% of SSc patients, have been mentioned as one of the possible triggers for vascular injury, by causing acceleration of vascular endothelial cell senescence and therefore inducing RP (9, 10). Other studies suggest that auto-antibody production occurs secondary to vasculopathy, and as such these auto-antibodies should be viewed as a bystander in disease pathogenesis (7, 11, 12).

Vasculopathy in SSc involves all layers of the peripheral blood vessels and is caused by a dysfunction of the endothelium, resulting in an imbalance of vasoactive factors. In particular endothelin-1 plays a prominent role in the regulation of vascular tone through its receptors. RP induces prolonged ischemia-reperfusion injury, which may cause persistent endothelial activation, resulting in apoptosis, microvascular damage, and other toxic stimuli. Recent insights showed that impaired functioning of endothelial progenitor cells could be involved in angiogenic response and in the pathogenesis of SSc. Microvascular tone alterations and cell apoptosis trigger the opening of intercellular junctions in the endothelial barrier. This loss of integrity favors further migration and

homing of inflammatory cells inducing increased microvascular permeability and progressive vascular leak (13). Infective stimuli, environmental exposures, sex, and endocrine disturbances, have all been proposed as contributors to microangiopathy (14, 15).

In SSc there is a marked sex imbalance, with higher prevalence of the disease in females than in males (4:1). Also distribution of ANA is disbalanced with females showing more frequently ACA antibody and males showing more frequently ATA antibody. In general, disease course is more severe in males resulting in lower survival rates (45% vs 23% after 10 years) (16-20). The most frequent disease related causes of death also differ between males and females: interstitial lung disease in males and pulmonary hypertension (PH) in females (21). The higher incidence of PH in females, and the fact that unopposed estrogens replacement therapy has been associated with increased RP, suggests a contribution of hormonal factors to microangiopathic manifestations (22). However, little information is known on the relationship between sex and microangiopathy in SSc.

As microvascular damage is one of the hallmarks of SSc, different imaging techniques have been applied to evaluate structural and functional abnormalities of the finger microcirculation in patients with SSc (23-26) (supplementary file). However, NVC is considered the most reliable tool to distinguish between primary and secondary RP. NVC is widely applied and provides the opportunity to directly visualise the evolving obliterative microangiopathy and nailfold capillary abnormalities characteristic of SSc, that have been classified as scleroderma pattern (27).

Given the role of microangiopathy in the pathogenesis of SSc, insights in the factors responsible for microvascular damage could contribute to our understanding of disease pathophysiology. Therefore, we decided to evaluate and summarize in this comprehensive review what is known about the association between the expression of specific auto-antibodies and microangiopathy, and between sex and microangiopathy in SSc.

METHODS

Literature search

A systematic literature search was performed by J.W.S, including studies published before June 17th 2019. The databases used were Medline (via PubMed), Web of Science, Cochrane and Embase. No restrictions on date were applied and manuscripts published in English or Dutch language were selected. The search strategy intended to include all relevant papers reporting on adult patients with SSc, in which microangiopathy of the hand was evaluated and where association with SSc-specific auto-antibodies was assessed. A second systematic literature search performed the same day intended to include all relevant papers reporting on adult patients with SSc, in which microangiopathy in the hand was evaluated and a comparison between male and female patients was described (see supplementary file for search strategies).

Two reviewers (N.v.L and J.C) independently screened the titles of retrieved articles and, in case one or both reviewers identified a publication as possibly relevant, the study proceeded to abstract screening. In case of discrepancies in agreement, abstracts were reviewed by a third investigator (J.d.V.B). Full text reading was performed for the selected abstracts by N.v.L and J.C.

Screening process and study selection criteria

For the review on auto-antibodies and microangiopathy the following criteria were applied: 1) adult participants (>18 years) with a clinical diagnosis of SSc; 2) fulfilment of either American College of Rheumatology (ACR) 2013, ACR 1980, or LeRoy and Medsger criteria (28, 29); 3) report on prevalence of SSc-related auto-antibodies including at least anti-topoisomerase I antibodies (ATA) or anti-centromere antibodies (ACA) and additionally anti-RNA polymerase III (anti-RNAPIII), anti-RNA polymerase I, anti-fibrillarin, anti-PM/Scl, or anti-Th/To antibodies; 4) assessment of microangiopathy using one or more of the following imaging modalities: nailfold NVC, laser dermoscopy, doppler confocal microscopy, LASCA/video image analysis, and photomicroscopy.

For the review on sex and microangiopathy the following criteria were applied: 1) adult participants (>18 years) with a clinical diagnosis of SSc; 2) fulfilment of either ACR 2013, ACR 1980, or LeRoy and Medsger criteria (28, 29); 3) report on comparison between female and male patients, and with at least n= 3 and 10% males included in the study; 4) assessment of microangiopathy using one or more of the following imaging modalities: NVC, laser dermoscopy, doppler confocal microscopy, LASCA/video image analysis, and photomicroscopy.

Exclusion criteria for both search strategies were: animal studies, editorials, reviews, letters to the editor, unpublished material, case-reports and manuscripts written in languages other than English or Dutch.

Quality assessment

The Newcastle-Ottawa scale was used for assessment of quality of case-control studies, whereas the National Institutes of Health quality assessment tool was used for observational cohort studies (30, 31). Discrepancies in scoring and implications for interpretation of the findings were discussed between N.v.L and J.C.

Evaluation of capillaroscopic descriptions throughout the studies

As in literature a variety of definitions are used to describe NVC. In this review we will report the NVC findings in a standardized way by evaluating the used terminology to describe NVC characteristics per included article. In line with the EULAR recommendations on capillaroscopy, the NVC characteristics can be evaluated quantitatively, qualitatively or semi-quantitively (32). See the supplementary file for a detailed explanation. When available, all these NVC characteristics were extracted throughout the included articles.

RESULTS

Literature search and study description

Figures 1 and 2 show the flowcharts of the systematic review processes. Eleven studies reporting on the association between auto-antibodies and microangiopathy (7, 8, 11, 33-40), and six studies reporting on sex and microangiopathy (33, 37, 40-43), were included. Three studies answered both questions (33, 37, 40). All included articles were cohort or case-control studies, but many were limited by small sample sizes. In the majority of the included articles, except for four (8, 11, 42, 43), the association of interest was not the primary outcome of the study. Characteristics of all included studies are provided in Table 1. In all, these studies reported on 4704 women (83%) and 971 men (17%), with a mean age of 49 years. Subtypes of SSc were specified in all but one article (for diffuse cutaneous SSc (dcSSc) n= 1473, 28%; for limited cutaneous SSc (lcSSc) n= 3746, 72%). Disease duration was defined either as time since onset of RP, as time since onset of first sign or symptom attributable to SSc different from RP, or as time since diagnosis, and ranged between 6 months and 37 years.

Comprehensiveness of reporting

The comprehensiveness of reporting was variable. Although all selected studies used NVC, the parameters to describe microangiopathy and to classify severity of microvascular changes differed between the studies.

Risk of Bias

Study quality is summarized in Table S1 (supplementary file). Three articles were assessed as high quality (7, 8, 33), nine as medium quality (11, 33-39, 42), and two as low quality due to selection bias, performance bias and incomplete outcome data (40, 43). Because of the limited number of studies reporting on the association between auto-antibodies, sex and microangiopathy, we chose to include also medium and low-quality articles.

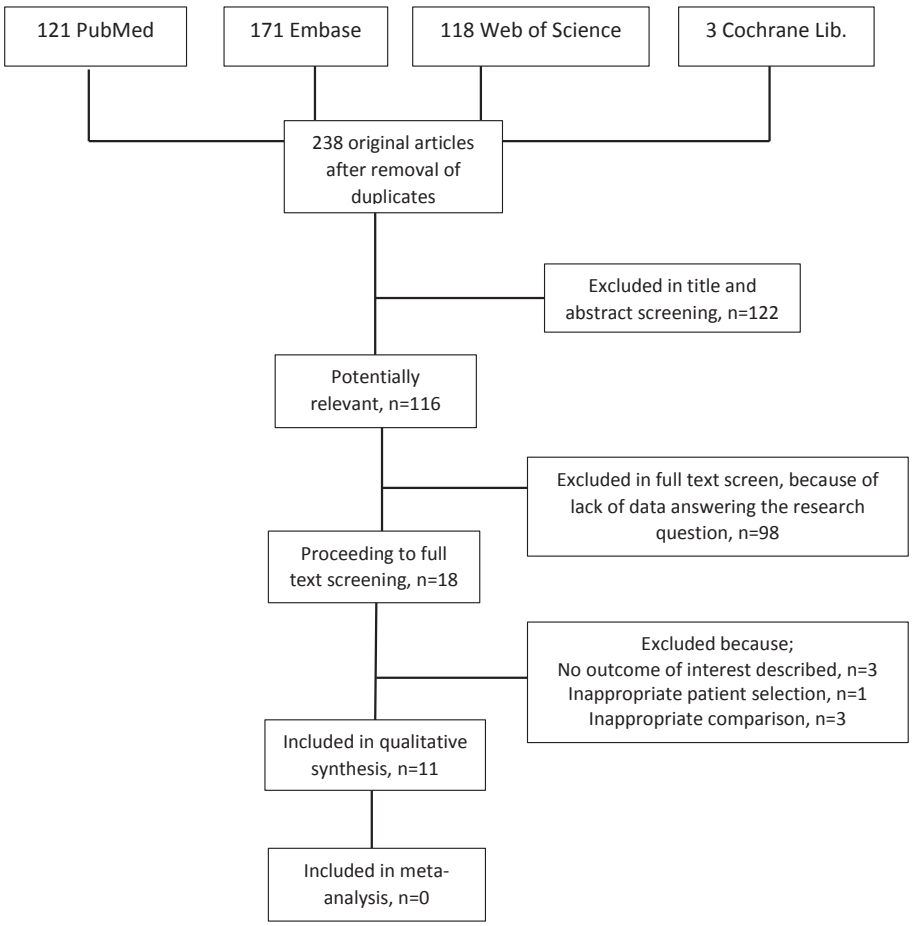

Figure 1. Flowchart association autoantibodies and microangiopathy

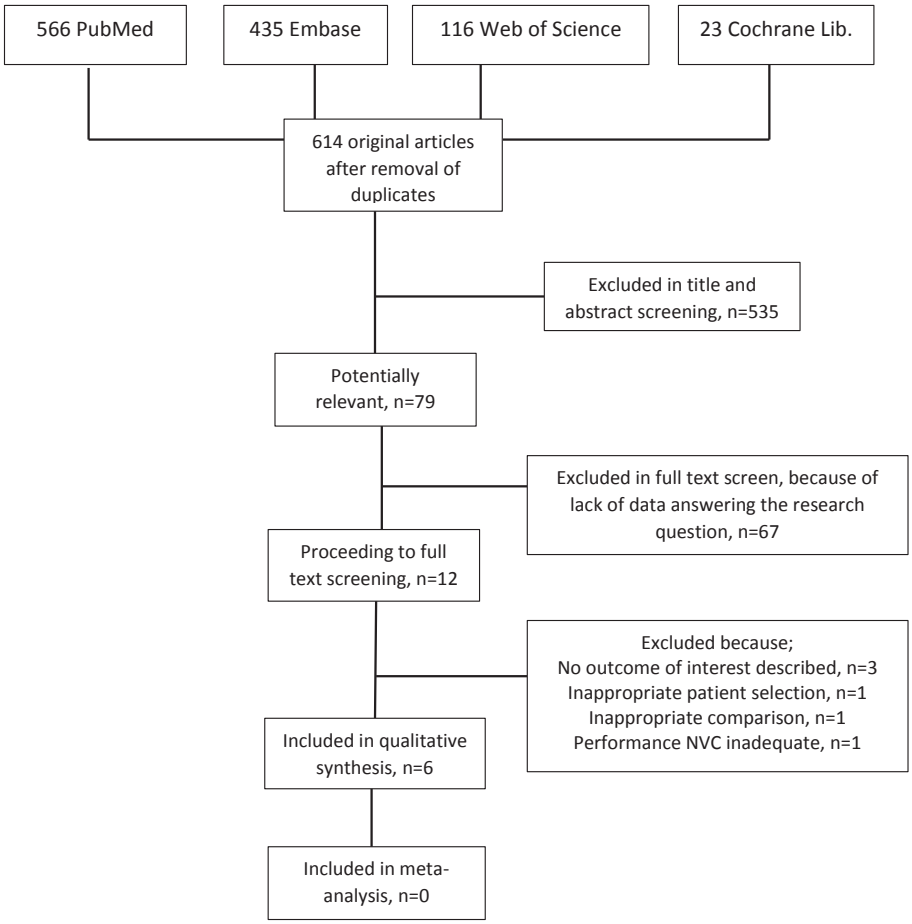

Figure 2. Flowchart association sex and microangiopathy

Baseline characteristics of articles included in the systematic review: association of sex and autoantibodies with the degree of microangiopathy

Study	Country	N	Age, mean years	Sex; f/m	Disease duration*, years since diagnosis
Caramaschi, 2007 (33)	Italy	103	54.3	91/12	7 since diagnosis
De Santis (34)	Italy	44	66	42/2	9 since diagnosis
Fichel (35)	France	88	54.9	81/7	16.5 since onset RP
Ghizzoni (36)	Italy	275	54.9	253/22	36.9 since diagnosis
Markusse (7)	Netherlands	287	53.9	202/85	3.7 since onset RP
Pizzorni (37)	Italy	33	59	28/5	6.6 since diagnosis
Cutolo (8)	Italy	241	57	227/14	5.6 since diagnosis/13.7 since onset RP
Ingegnoli (38)	Italy	2754	54.9	2148/606	7.6 since diagnosis
Sulli (11)	Italy, Belgium	42	47	NA	5 since onset RP
Tieu (39)	Australia	152	43.7	121/31	10.9 since onset RP
Chandran (40)	Australia	148	50	44/8	5 years since onset RP
Simeon (43)	Spain	91	52.5	82/9	6 months and 63 years since RP
Freire (42)	Spain	1506	45.6	1341/165	6.4 since diagnosis
Caramaschi, 2009 (41)	Italy	49	52.4	44/5	8 since diagnosis

Table 1. *Disease duration was defined differently in the articles, either as time since onset RP, time since onset non-RP or time since diagnosis. ANA= anti-nuclear auto-antibody, dcSSc= diffuse cutaneous systemic sclerosis,

	SSc type	Methodological framework	Main topic
	68 lcSSc/ 35 dcSSc	Observation cohort,cross-sectional	NVC pattern and clinical characteristics
	34 lcSSc/ 10 dcSSc	Observational cohort, cross-sectional	Correlation NVC and clinical SSc phenotype
	51 lcSSc/ 15 dcSSc/ 12 non cutaneous	Observational cohort,cross-sectional	Characteristics SSc patients with normal or abnormal NVC
	242 lcSSc/ 33 dcSSc	Observational cohort, longitudinal	Prevalence, evolution of NVC and analysis of characteristics according to capillaroscopic features
	141 lcSSc/ 56 dcSSc	Observational cohort, cross-sectional	Evaluate anti-ENA antibodies in SSc and predictive power of combination of autoantibodies and NVC
	30 lcSSc/ 3 dcSSc	Observational cohort,cross-sectional	Evaluate use of MES assessment with qualitative analysis of NVC and telangiectasia
	148 lcSSc/ 93 dcSSc	Observational cohort,cross-sectional	Relation NVC pattern autoantibodies and subset cutaneous involvement
	1622 lcSSc/ 803 dcSSc	Observatinal cohort,cross-sectional	Frequency of NVC patterns and their disease phenotype
	NA	Observational cohort, longitudinal	Correlation between ANA patterns and NVC stage in SSc
	99 lcSSc/ 30dcSSc	Observational cohort, longitudinal	Investigate possible utility of NVC in predicting survival
	81 lcSSc/ 13 dcSSc	Observational cohort,cross-sectional	Role of NVC in identification and prognostication
	70lcSSc/ 19dcSSc	Observational cohort,cross-sectional	Relationship disease pattern and sex
	1151 lcSSc/ 355 dcSSc	Observational cohort, longitudinal	Influence gender on survival
	31 lcSSc/ 18 dcSSc	Observational cohort, longitudinal	NVC changes after iloprost treatment

ENA= extractable nuclear antigen, lcSSc= limited cutaneous systemic sclerosis, MES= microangiopathy evolution score, NVC= nailfold videocapillaroscopy.

AUTO-ANTIBODIES AND MICROANGIOPATHY

A meta-analysis could not be conducted due to heterogeneity of the studies and use of different outcome measures. In total, 11 studies described associations between auto-antibodies and microangiopathy (Table 2).

Qualitative assessment of NVC

Caramaschi et al. performed NVC in 103 SSc patients and the degree of microangiopathy was defined as early, active or late SSc pattern according to Cutolo et al. (2000) (quality score good) (33) . The distribution of ANA, ACA, and ATA positivity did not differ between patients with early, active, or late SSc pattern. De Santis et al. investigated 44 SSc patients using NVC to identify early, active, or late SSc patterns (quality score medium) (34). No significant differences in the SSc patterns were found between ACA and ATA positive patients. In a study with 287 SSc patients, ACA, ATA, anti-RNP, anti-RNAPIII, anti-fibrillarin, anti-PM/Scl, anti-Th/To and anti-Ku antibodies were evaluated and early, active or late SSc patterns were described on NVC (quality score good) (7). The prevalence of NVC patterns was equally distributed among patients with different specific auto-antibodies. On the contrary, Pizzorni et al. investigated 33 SSc patients and classified the degree of microangiopathy according to the three SSc patterns: early, active or late (quality score medium) (37). ATA positive patients showed more often a late SSc pattern ($p= 0.002$), while in ACA positive patients early or active SSc patterns were more common ($p= 0.03$). Cutolo et al. evaluated NVC patterns and serum auto-antibodies in 241 SSc patients (quality score good) (8). NVC was described as early, active or late SSc pattern. ATA positivity was significantly less frequent in the early (5%) than in the active (25%) or in the late (24%) SSc patterns.

Presence of ATA was shown to be related with earlier expression of the active and late SSc patterns of microvascular damage. On the other hand, ACA positivity was found more frequently, although not significantly, in the early pattern. The authors concluded that specific auto-antibodies do not seem directly linked to the expression of a singular NVC pattern, but that auto-antibodies might be related to the rate of progression of microvascular damage. In a study by Ingegnoli et al. data from the European Scleroderma Trials and Research group (EUSTAR) were used to investigate NVC in 2754 SSc patients (quality score medium) (38). NVC patterns were described as early, active or late SSc pattern. Late pattern was present in 47% of ATA positive and in 28% of ACA positive ($p <0.05$) patients, while early and active patterns were more frequent in ACA positive than in ATA positive patients (44% vs 28%, $p <0.05$). Significant associations were found between ATA positivity and late SSc pattern, and between ACA positivity and early/active SSc pattern ($p= 0.03$). Sulli et al. found that the prevalence of ATA was significantly higher in

patients with the late SSc pattern (n= 42; quality score medium) (11). Fichel et al. described the characteristics of 88 SSc patients with normal, non-specific or SSc-specific NVC pattern (quality score medium) (35). The frequencies of ANA, ACA (p= 0.90) and ATA (p= 0.34) positivity were comparable for normal/nonspecific and SSc-specific NVC patterns. This is in line with the results of Ghizonni et al. who described NVC features, demographic, clinical and serological manifestations of 275 SSc patients (quality score medium) (36). No differences in the percentage of ACA or ATA positivity were found between patients with SSc patterns compared to patients with normal/non-specific NVC patterns (ACA: 15.2% vs 14.6% ATA: 31.8% vs 23.6%; all non-significant).

Quantitative assessment of NVC

Besides the SSc-specific NVC patterns, de Santis et al. also described the amount of giants, neoangiogenesis, avascular areas and the capillary density and compared these characteristics between ACA and ATA positive patients (34). No significant differences were found.

Semi-quantitative assessment of NVC

Tieu et al. included 152 SSc patients and investigated capillary dropout during follow-up (quality score medium) (39). Patients with anti-RNAPIII had a significantly higher nailfold capillary total damage index compared with ACA, ATA and anti-RNP positive patients. Patients with ATA or anti-RNAPIII had greater capillary dropout than patients with ACA, despite a significantly shorter disease duration. Finally, Chandran et al. mentioned that in 52 SSc patients, the ATA positive cases had more severe nailfold changes (quality score low) (40). However, in this study only four ATA positive patients were included and two of them had severe NVC changes, whereas of the 22 ACA positive patients, three had severe NVC changes. Two studies, by Pizzorni et al. and by Sulli et al. (quality score medium) used the MES to semi-quantitatively evaluate the degree of microvascular damage and no significant differences in MES were found between ACA and ATA positive patients (11, 37).

In conclusion, weighing the results of Table 2, the total number of patients in the studies that found an association between auto-antibodies and microangiopathy was 2364, compared to 742 patients in the studies that did not find an association. This would implicate that specific auto-antibodies are associated with the degree of microangiopathy but, when only high-quality studies were evaluated (7, 8, 33), an association was found only in 241 patients, while in 390 patients no association between auto-antibodies and microangiopathy was described.

Association between autoantibodies and microangiopathy

	Study	Patients	Antibodies
Qualitative	Caramaschi, 2007	103	ACA, ATA
	De Santis, 2016 $	44	ACA, ATA
	Markusse, 2017	253	ACA, ATA, RNApol3, RNP, U3RNP, Pm/Scl
	Cutolo, 2004	241	ACA, ATA
	Ingegnoli, 2013	2754	ACA, ATA
	Sulli, 2013 #	42	ACA, ATA
	Pizzorni, 2017 *	33	ACA, ATA
	Ghizzoni, 2014	275	ACA, ATA
	Fichel, 2014	88	ACA,ATA
Semi-quantitative	Tieu, 2018	152	ACA, ATA, RNP, RNApol3
	Chandran, 1995	52	ACA, ATA, RNP
	Pizzorni, 2017 *	33	ACA, ATA
	Sulli, 2013 #	42	ACA, ATA
Quantitative	De Santis, 2016 $	44	ACA, ATA

Table 2. ACA= anti-centromere antibody, ANA= anti-nuclear antibody, ATA= anti-topoisomerase antibody, NVC= nailfold videocapillaroscopy, RNApolIII= anti-RNA polymerase III antibody,

NVC assessment	Significant	Conclusion
Early; Active; Late SSc pattern	Non-significant (not specified)	No significant difference
	P > 0.05	No significant difference
	P > 0.10	No significant difference
	P < 0.01	ATA+ more frequent in Active and Late patterns than in Early
	P < 0.005	ATA more often present in Late pattern compared to Early and Active
	P= 0.03 (OR 8.0 (1.4-47.0))	ATA more frequently present in Late pattern than in Early and Active
	ACA early-active/late p= 0.03, ATA early-active/late p= 0.002	Early-Active pattern is more often present in ACA patients, Late pattern is more often present in ATA patients.
Normal; SSc pattern	Non-significant (not specified)	No significant difference
	ACA normal/ SSc pattern p= 0.90 (OR 0.90 (0.3-2.6)) ATA normal/SSc pattern p= 0.34 (OR 0.50 (0.1-2.6))	No significant difference
Mean capillary damage score; mean capillary dropout score	RNApol3 > capillary damage compared with ACA and RNP (p < 0.001). ATA and RNApol3 > dropout compared with ACA (p= unknown)	Difference found between autoantibodies and capillary damage and capillary dropout.
Moderate loss and enlargement; Extreme capillary dropout; class 1 to 5	Not mentioned	ATA positive patients more severe nailfold changes compared to ACA and RNP+
Microangiopathy evolution score (MES)	ACA MES <6/ > 6 p= 0.72, ATA MES < 6/ > 6 p= 0.43	No significant differences
	ANA vs ACA p= 0.09, ANA vs ATA p= 0.05	No significant differences
Giants, neoangiogenesis, avascular areas, density	P > 0.05	No significant differences

RNP= anti- ribonuclear protein antibody, SSc= systemic sclerosis. $/#/*= same article used two techniques for NVC assessment.

SEX AND MICROANGIOPATHY

In total six studies reported on sex and microangiopathy in patients with SSc (Table 3). A meta-analysis could not be conducted due to heterogeneity of the studies.

Qualitative assessment

Caramaschi et al. investigated 103 SSc patients (12 men, 91 women) and the microvascular alterations were classified as early, active and late SSc patterns (quality score good) (33). In this study no significant differences in NVC patterns were found between male and female patients. Freire et al. studied 1506 SSc patients (165 men, 1341 women) assessing microangiopathy with the use of NVC and describing the degree of microangiopathy as "slow" or "active" pattern (quality score medium) (42). No significant difference in the distribution of patterns was observed between men and women (m/f; 46%-53% for slow pattern and 37% vs 33% for active pattern). Pizzorni et al. evaluated 33 patients, including 5 males, and found no difference in the prevalence of SSc patterns in men or women (37). One out of 6 studies suggested a possible sex difference regarding microangiopathy (41). In 49 SSc patients who were treated with iloprost and underwent two NVC examinations with a 3-year interval, improvement of SSc pattern was found to be associated with male sex (r= 9.07, p= 0.019).

Association between sex and microangiopathy

	Study	Patients	Male/Female
Qualitative	Caramaschi, 2007	103	91 female, 12 male
	Caramaschi, 2009	49	44 female, 5 male
	Pizzorni, 2017*	33	28 women, 5 male
	Freire, 2017	1506	1341 female, 165 male
Semi-quantitative	Simeon, 1996	91	82 female, 9 male
	Chandran, 1995	52	44 female, 8 male
	Pizzorni, 2017*	33	28 women, 5 male

Table 3. NVC= nailfold videocapillaroscopy, SSc= Systemic Sclerosis. * same article used two techniques for NVC assessment.

Quantitative assessment

None of the included studies evaluated the association between sex and quantitative assessment of microangiopathy.

Semi-quantitative assessment

Chandran et al. performed a study on prevalence, subset characteristics and NVC patterns of SSc patients in South-Australia (quality score low) (40). They included 44 females and 8 males, and an equal proportion of males and females had severe capillary changes of class IV (moderate loss of capillaries) and V (extreme capillary dropout). Simeon et al. evaluated 91 SSc patients, of which 9 were men (quality score low). The NVC patterns were described using capillary loss and mega capillaries as parameters. No significant NVC differences were found between male and female patients. In line with these results, Pizzorni et al. compared MES between males and females, and no significant difference was found (37).

In conclusion, of the 6 included articles, 5 studies including 1614 women and 204 men did not show an association between sex and microangiopathy. The only study showing a significant difference included 44 women and 5 men and, importantly, male patients were more often treated with cyclophosphamide, but a multivariate analysis to identify the contribution of sex corrected for the prescribed treatment was not performed (41).

NVC assessment	Significant	Conclusion
Early; Active; Late SSc pattern	Non-significant (not specified)	No significant difference
	P < 0.05	Improvement of NVC was associated with male sex
	P= 0.623	No significant difference
Slow (giants and minimal loss) or Active Pattern (capillary loss and neovascularization)	Slow pattern male/female p= 0.126, Active pattern male/female p= 0.420	No significant difference
Capillary loss and megacapillaries	P= 0.71 for capillary loss, p= 1.00 for megacapilaries	No significant difference
Moderate loss and enlargement; Extreme capillary dropout; class 1 to 5	Not mentioned	No significant difference
Microangiopathy evolution score (MES) score 0 -9, < 6 or > 6 dichotomized	P= 0.625	No significant difference

DISCUSSION

Microangiopathy can be secondary to different causes. Research in different fields shows that many factors can affect microangiopathy, including biological, environmental and socio-economic factors (44, 45). In addition, gender specific factors have been postulated as men and women develop different types of ischemic heart disease with different pathophysiological background (3, 4). Atherosclerosis is more common in men, while in women vasoreactivity prevails, characterized by spasm and endothelial alterations. Microvascular dysfunction with perfusion problems seems to be present more often in women with cardiovascular disease (CVD) and also takotsubo cardiomyopathy, heart failure and stroke are more common in women (46, 47).

Similarly, it has been recognized that there are clinical differences between female and male patients with systemic autoimmune rheumatic diseases in which microangiopathy plays a role, such as systemic lupus erythematosus (SLE) and SSc (48). SLE is rare in men, and males with SLE are more likely to experience cardiovascular complications and myocardial infarction, and less likely to have dermatological manifestations (48). Nevertheless, also for SLE it remains unknown why male SLE differs substantially from SLE in women.

Although there is a growing interest, the exact interplay between auto-antibodies and microangiopathy in autoimmune diseases remains to be elucidated. In SLE, a difference in auto-antibody prevalence has been suggested between men and women. Anticardiolipin antibodies, anti-dsDNA antibodies and lupus anticoagulant were found to be more prevalent in men in a few studies (49). Some studies showed that in lupus nephritis, antiphospholipid antibodies and lupus anticoagulant were more frequently observed in patients with thrombotic microangiopathy of the kidney. In addition, among the auto-antibodies mainly implicated in neuropsychiatric (NP) SLE, anti-β2glycoprotein I (β2GPI) antibodies are preferentially involved in focal NP events which are a consequence of noninflammatory microangiopathy; otherwise, anti-ribosomal P protein antibodies and anti-N-methyl-D-aspartate receptor (NMDAR) antibodies might cause diffuse NP events (49). In dermatomyositis anti-MDa5 auto-antibodies have a strong correlation with vasculopathy (50). Irrespective of these specific cases, little information is available on the association between sex or auto-antibodies and microangiopathy in connective tissue diseases, both for SSc and for other systemic autoimmune diseases.

As the assessment of microangiopathy has an established diagnostic and prognostic role in SSc patients (51), we value possible factors that could influence microangiopathy as relevant. In this review of the literature we focused on the influence of sex and auto-antibodies on microangiopathy in SSc patients. We can conclude that sex does not associate

with degree of microangiopathy in SSc, while the results on association between specific auto-antibodies and degree of microangiopathy were inconclusive. When summarizing the findings of the positive studies for auto-antibodies and microangiopathy, presence of ATA might be associated with more severe microangiopathy as reflected by a late pattern. Indeed, both more severe damage and presence of ATA associate with more severe disease in SSc. However, the degree of microangiopathy can change over time and possible confounders as age, disease duration, comorbidities or medications, were not taken into account in any of the included studies. When evaluating the high-quality studies only, no clear association between ATA and more severe microangiopathy was shown. However, even in these studies the results were not adjusted for confounders. Therefore, we believe that further prospective controlled studies are needed to better explore the association between presence of specific antibodies and the degree of microangiopathy.

Regarding sex and microangiopathy, no clear association was found in the included articles. However, only six studies were retrieved and two evaluated sex differences as primary outcome (42, 43). Besides, a relatively limited number of men was included in the studies. Noteworthy, although several studies focused on sex differences in SSc, a possible difference between males and females in the degree of microangiopathy was disregarded in most studies. To account for the gender gap and disease dissimilarities in SSc, a role of sex hormones has been proposed. Estrogens act as enhancers of the immune system and of cell proliferation, as also demonstrated in cultures of cells harvested from skin biopsies of SSc patients (52-54). A recent study demonstrated a protective effect of estrogens in dermal fibrosis, as estrogens reduce TGF-B dependent activation of dermal fibroblasts, and estrogen inhibition leads to a more severe experimental dermal fibrosis, but their effects on vasculature are largely unknown (55). At macrovascular level, hormone replacement therapy (HRT) might be protective against the risk of pulmonary arterial hypertension, and short- or long-term administration of conjugated estrogens induced flow-mediated dilatation in the brachial artery of SSc patients (56-58). Regarding microvasculature, little is known about the effects of estrogens in patients with SSc (22). A recent study investigated the influence of cumulative endogenous estrogen exposure (CEEE) in patients with SSc on the degree of microvascular damage observed through NVC, and no association between length of CEEE and degree of microvascular impairment was found (59).

We aimed to summarize the available evidence about the association between sex, or specific auto-antibodies, and microangiopathy in SSc, but our review is not without limitations. We could include only a limited number of articles, with variable quality and, due to the heterogeneity of patients and outcomes, a meta-analysis could not be conducted.

Contradictory results were found about the association between auto-antibodies and microangiopathy and no firm conclusions can be drawn. As NVC has prognostic relevance in the global assessment of each single SSc patient, we believe that the identification of factors possibly affecting microangiopathy is of relevance to elucidate the pathophysiology of microangiopathy and also for clinical risk stratification. Therefore, in consideration of the paucity of available data, and especially the lack of data derived from high-quality research, we advocate further prognostic cohort studies to evaluate factors contributing to the degree of microangiopathy in SSc.

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Chapter 7

Degree of vasculopathy in systemic sclerosis patients with anti-U3RNP antibody indicates need for extensive cardiopulmonary screening

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TO THE EDITOR:

In patients with systemic sclerosis (SSc) regular screening is needed to determine the extent and severity of organ involvement(1). Specific autoantibodies are associated with clinical manifestations and are therefore used as predictors for organ involvement(2). Identifying patients who are at risk for organ involvement enables distinguishing between patients with high risk that need extensive screening and follow-up, and patients with most likely mild disease. Antibodies against the U3-ribonucleoprotein (U3RNP or anti-fibrillarin) are detected in 3-8% of SSc patients. Some studies indicate a higher risk for cardiac involvement in U3RNP+ patients, but results are conflicting(3-7). As shown before, the degree of microangiopathy as reflected by nailfold videocapillaroscopy (NVC) is identified as independent predictor for organ involvement in SSc(8). Associations between presence of U3RNP and NVC pattern and disease manifestations have however, not been evaluated thus far. Therefore, we evaluated degree of microangiopathy as shown by NVC and its association with cardiopulmonary involvement in anti-U3RNP + SSc patients.

All patients participating in the Combined Care in Systemic Sclerosis (CCISS) prospective observational cohort study (approved by the Ethics Committee P09.003/SH/sh) of the Leiden University Medical Center gave written informed consent. Eighteen U3RNP+ patients (indicating a prevalence of 3% in the CCISS cohort) were compared with an equal number of age- and sex matched anticentromere+ (ACA) and antitopoisomerase+ (ATA) controls. Although controls were not matched for disease duration, there were no significant differences between the groups. Cardiopulmonary screening was performed routinely in all patients.

Myocardial involvement was defined using a combined value where patients had to have at least two of the following: arrhythmias (>2% ventricular/supraventricular arrhythmia or atrial fibrillation), conduction problems (atrioventricular/bundle branch blocks), left ventricular ejection fraction (LVEF)< 50%, diastolic dysfunction, or pericardial effusion. Pulmonary involvement was defined based on the combination of diffusion capacity for carbon monoxide (DLCO)<60% and forced vital capacity (FVC)<70% and evidence for interstitial lung disease (ILD) on high resolution computed tomography (HRCT). NVC was performed at baseline, and images were classified by a trained observer as early, active or late SSc pattern (9). In addition capillary loss (<7 per mm) was determined.

U3RNP+ patients were most often female (78%), with a high prevalence of diffuse cutaneous SSc (dcSSc 39%) and 22% was of African-American origin (n= 4); compared to 0% among the ACA and ATA+ patients. This confirms the results of other studies in which an association between U3RNP antibody, skin score and African-American race

was found. Myocardial involvement was present in 17% (n= 3) of the U3RNP+ patients compared to 17% (n= 3) in the ACA+ and 33%(n= 6) in the ATA+ patients. Pulmonary involvement was present in 6% (n= 1) of the U3RNP+ patients compared to 6% (n= 1) in the ACA+ and 29% (n= 5) in the ATA+ patients. Based on these data we cannot confirm a higher prevalence of cardiopulmonary involvement in patients with U3RNP antibody compared with ACA+/ATA+ patients. As mean skin scores and pulmonary function test results were clearly worse in U3RNP+ patient as compared to ACA patients, indicating more severe disease with possible higher risk, we consequently evaluated whether degree of microangiopathy could add to distinguish those U3RNP patients at risk for cardiopulmonary involvement. The distribution of SSc patterns among U3RNP+ and ATA+ patients was comparable; in the ACA+ patients a late SSc pattern and capillary loss were less common (figure 1).

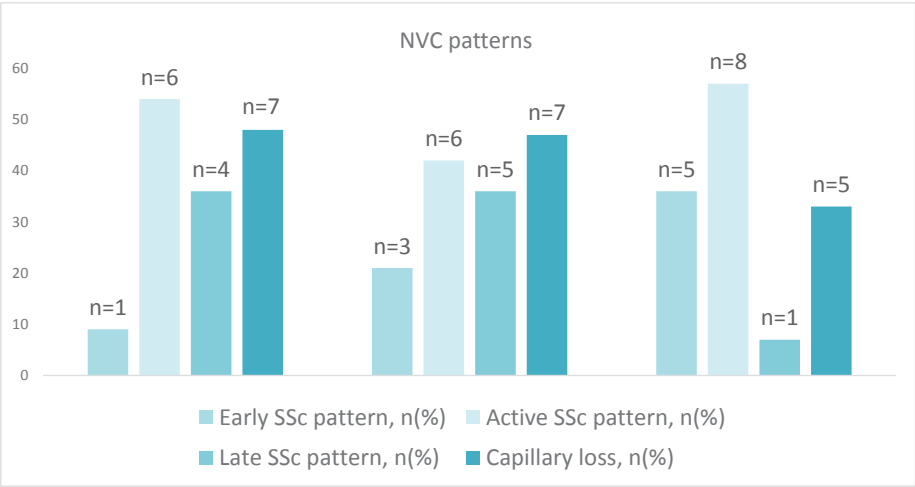

Figure 1. Distribution of nailfold videocapillaroscopy patterns among the autoantibodies. SSc= Systemic Sclerosis. U3RNP= U3-ribonucleoprotein positive patients, ATA= antitopoisomerase positive patients, ACA= anticentromere positive patients. In the U3RNP group 3 patients had secondary pattern and 1 an aspecific pattern, in the ACA and ATA group only 1 patient had a secondary pattern.

For the complete population, with NVC available (n= 45), late NVC pattern was associated with pulmonary involvement (X^2, p= 0.03; table 1) and capillary loss with myocardial involvement (X^2, p= 0.05; table 1). Nevertheless, we do believe that it is the severity of microangiopathy that is associated with both cardiac and pulmonary involvement and do not consider late pattern and capillary loss as different pathophysiological processes . When selecting all U3RNP+ patients (n= 18) for extensive cardiopulmonary screening, cardiopulmonary involvement was detected in 11% (2 out of 18). When only selecting U3RNP+ patients with either late SSc pattern or capillary loss, extensive screening was necessary in 47% of U3RNP+ patients (7 out of 15 patients), and none of the U3RNP+ patients with cardiopulmonary involvement was missed.

Total group and U3RNP+ group, SSc pattern, capillary loss and organ involvement

	Cardiac involvement -	**Cardiac involvement +**	**Pulmonary involvemement -**	**Pulmonary involvement +**
All	n=37	n=8	n=42	n=3
Early, n=9	8 (22%)	1 (13%)	9 (21%)	0 (0%)
Active, n=20	18 (47%)	2 (26%)	20 (58%)	0 (0%)
Late, n=10	6 (17%)	4 (52%)	7 (17%)	**3 (100%)***
Secundary, n=6	5 (14%)	1 (13%)	6 (14%)	0 (0%)
U3RNP+	n=13	n=2	n=15	n=0
Early, n=1	1 (8%)	0 (0%)	1 (6%)	0 (0%)
Active, n=6	6 (46%)	0 (0%)	6 (40%)	0 (0%)
Late, n=4	3 (23%)	1 (50%)	4 (27%)	0 (0%)
Secundary, n=4	3 (23%)	1 (50%)	4 (27%)	0 (0%)
All	n=37	n=8	n=42	n=3
Capillary loss -, n=26	24 (65%)	2 (25%)	26 (62%)	0 (0%)
Capillary loss +, n=19	13 (35%)	**6 (75%)****	16 (38%)	3 (100%)
U3RNP+	n=13	n=2	n=15	n=0
Capillary loss -, n=8	8 (61%)	0 (0%)	8 (53%)	0 (0%)
Capillary loss +, n=7	5 (39%)	2 (100%)	7 (47%)	0 (0%)

Table 1. *Total group and U3RNP+ group, SSc pattern, capillary loss and organ involvement.* * p= 0.009 chi square; late pattern vs non late pattern, ** = p value 0.047 chi square; capillary loss vs no loss, all other comparisons non-significant.Cardiac involvement defined as a Medsger score of 1 or higher, or at least two of the following: decreased LVEF, arrhythmias, conduction abnormalities, diastolic dysfunction or pericardial effusion, pulmonary involvement defined as decreased FVC and DLCO and ILD on HRCT

To our knowledge, this is the first study to describe NVC findings in U3RNP+ SSc patients, and associate NVC patterns with cardiopulmonary involvement in this group. The strength of the presented data lies in the prospective study design with standardized annual follow-up independent of clinical presentation. However, given the low numbers, our findings need replication. As previously shown, U3RNP antibody was more prevalent among African-Americans. We could not confirm previous suggestions of a higher prevalence of cardiac involvement in this group. Our data confirm that in SSc the degree of microangiopathy as reflected by NVC is associated with severity of cardiopulmonary involvement, this is also true in U3RNP+ patients. As such, NVC can serve as biomarker to select U3RNP+ SSc patients with higher risk for cardiopulmonary involvement.

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Chapter 8

Association between centromere and topoisomerase specific immune responses and the degree of microangiopathy in systemic sclerosis

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Objectives

Autoreactive antibody responses, including the use of several isotypes of autoantibodies, have been shown to associate with clinical outcome in several rheumatic autoimmune diseases. The goal of this study was to evaluate whether 1) anti-centromere antibody (ACA) and anti-topoisomerase antibody (ATA) specific isotype expression and 2) organ involvement are associated with the degree of microangiopathy in Systemic Sclerosis (SSc).

Methods

ACA and ATA IgG, IgM and IgA levels were measured in baseline serum samples of ACA IgG+ and ATA IgG+ patients with SSc. The degree of microangiopathy was determined based on nailfold videocapillaroscopy (NVC) images collected at the same time point. Univariable and multivariable logistic regression analyses with autoantibodies, clinical characteristics, isotype expression and ACA and ATA IgG, IgM and IgA levels as independent variables, and NVC pattern as dependent variable were performed.

Results

In 164 patients, isotype levels and degree of microangiopathy were evaluated. Logistic regression confirmed the association of the degree of microangiopathy with the presence of digital ulcers (OR 3.07, 95% CI 1.43-6.60), interstitial lung disease (OR 3.41, 95% CI 1.11-10.61) and pulmonary arterial hypertension (OR 5.58, 95% CI 2.05-17.81). ATA positivity was associated with more severe microangiopathy (OR 2.09, 95% CI 1.05-4.13). Patients that solely expressed ACA IgG showed a trend towards less severe microangiopathy compared to patients also expressing ACA IgM and/or IgA. Levels of ACA IgG and ATA IgM were found to be associated with the severity of microangiopathy.

Conclusions

We observed an association between ACA and ATA responses and the degree of microangiopathy in SSc. These findings might indicate that the breadth of the autoimmune response, as reflected by autoantibody production and microvascular damage, interact in the pathophysiology of SSc.

INTRODUCTION

Systemic Sclerosis (SSc) is characterised by the triad of microvascular damage, dysregulation of innate and adaptive immunity and generalized fibrosis (1). The pathogenesis of SSc has still not been completely elucidated, and the primary cause of SSc remains to be determined (2). Approximately 95% of patients with SSc have antinuclear autoantibodies (ANA). These autoantibodies contribute to the disease classification and are associated with specific clinical manifestations, making them important tools for disease prognostication (3). Anti-centromere antibodies (ACA) and anti-topoisomerase antibodies (ATA) are the two most common ANA in patients with SSc (3). Of these, ACA is associated with a relatively mild disease course, while ATA is associated with more severe disease including diffuse skin and lung involvement. This clear association with a typical clinical phenotype suggests that the immune response, reflected by ATA or ACA production, is closely linked to disease pathophysiology. The exact pathogenicity of ATA or ACA, however, remains unclear (4).

A second important diagnostic and prognostic tool in SSc is nailfold videocapillaroscopy (NVC) which is an investigation that determines the degree of microangiopathy by using standardised magnification to visualize the capillaries in the nailfold. In SSc, specific patterns of capillary changes and the degree of these changes have been defined extensively (5). More severe microangiopathy is associated with worse disease in SSc patients, in recent studies an association between NVC pattern, organ involvement and disease progression was found (6-12). In addition, an association between NVC patterns and specific autoantibodies was also described (13). NVC can therefore be seen as an important biomarker that can be used to predict severe complications in SSc (14).

Some studies suggest that autoantibody production is secondary to vasculopathy, and thus specific autoantibodies can be viewed as bystanders in disease pathogenesis (2). However, other studies suggest that circulating autoantibodies may be directly implicated in the disease process. Higher levels of ATA have been shown to associate with the development of organ involvement (15). An association between autoantibody specific isotypes and disease severity has been found, with higher ATA IgM levels in ATA + SSc patients who showed disease progression, and higher ACA IgG and ACA IgM levels in ACA+ SSc patients who had a more severe disease (16, 17). A study performed by Ahmed et al. (18) demonstrated that SSc sera containing ACA or ATA can trigger fibrillin expression in human dermal endothelial cells and induce cell apoptosis, while Shen et al. (19) concluded that the pathognomonic ACA and ATA in SSc accelerate vascular endothelial cell senescence and functional impairment inducing Raynaud's Phenomenon (RP). Together, these studies implicate a possible association between ACA, ATA, and possibly specific isotype levels and vasculopathy. This association has not yet been evaluated in SSc.

As the presentation of SSc can be very heterogeneous and prediction of the disease course is still very difficult, a better understanding of the interaction between the specific auto-immune response and the degree of microangiopathy could not only improve our insights in disease pathogenesis, but could also contribute to more reliable disease prognostication, which is of utmost importance. In line with this, we hypothesized that an activated immune response, as reflected by higher ATA or ACA IgG levels associate with more severe microvascular damage.

METHODS

Study design and patients

SSc patients at the Leiden University Medical Center (LUMC) are included in an observational cohort study (Combined Care in Systemic Sclerosis; CCISS (12)), which was approved by the Ethics Committee (P09.003). The cohort study is designed in accordance with the ethical principles of the Declaration of Helsinki. All patients gave written informed consent. This standardized annual care pathway comprises extensive screening, including autoantibody testing, electrocardiography (ECG), thoracic echocardiography, high resolution computed tomography (HRCT), pulmonary function test, and NVC. An exercise test, 24 hour Holter ECG or right heart catheterization (RHC) are performed if indicated. In our current study, patients who fulfilled the ACR/EULAR 2013 classification criteria for SSc (20), were positive for IgG either ATA or ACA, and had a clinical diagnosis of SSc were included. Use of current medication (calcium channel blockers, sildenafil, bosentan and Iloprost for vasoactive medication, and corticosteroids, cyclophosphamide, azathioprine, methotrexate, mycophenolate mofetil and azathioprine for immunosuppressive medication) at the time of blood sampling, baseline clinical characteristics and investigations were retracted from the database. Baseline characteristics were considered at time of inclusion in the cohort.

Nailfold videocapillaroscopy

NVC was performed at the same time point as the baseline characteristics and blood samples were collected. All images were obtained in the hospital in a comfortable room with a temperature of 22-25 degree Celsius. All fingers except for the thumbs from both hands were examined using a videocapillaroscope (LUMC: 2009-2015; Videocap 3.0, DS Medica; 2015 -2017, Inspectis pro; 2018 onwards) equipped with a probe with 200x magnification. NVC images were scored by trained observers and classified qualitatively as described previously; 'normal', 'non-specific' and 'scleroderma pattern' (21). A "normal pattern" is defined as a pattern of typical hairpin-like capillaries with a regular distribution. A "non-specific pattern" is defined as a pattern with abnormalities without fulfilling the definition of a 'scleroderma pattern' (22). A 'scleroderma pattern' was defined according to the standards set by Cutolo et al. and categorized in an 'early' 'active' or 'late' pattern (23). The presence of giant capillaries, hemorrhages and avascularity are the main denominators in the definition of a scleroderma pattern. A more severe degree of microangiopathy is defined as a late scleroderma pattern with capillary loss (< 4 capillaries per mm) as its main denominator. For our current evaluation, the images were re-examined by a trained investigator (NvL). The inter-observer agreement was high for qualitative pattern determination (ICC 0.97).

ACA and ATA assay and measurements

Total immunoglobulin ATA, IgG, IgM and IgA and total immunoglobulin ACA, IgG, IgM and IgA levels of all the collected samples were measured in baseline samples by fluorescence enzyme-linked immune sorbent assay (FEIA), using the Phadia250 system (ThermoFisher Scientific, Nieuwegein, the Netherlands). The cut-off levels for ATA and ACA IgG were set at 7 units/ml (U/mL) according to the manufacturer's instructions. Fifty serum samples of non-rheumatic age and sex matched subjects were measured to establish cut-off values (mean + 2SD) for IgM and IgA isotypes of ACA and ATA. A cut-off for ACA IgA was determined at 37 aU/ml, for ATA IgA at 77 aU/ml, for ACA IgM at 13 aU/ml and for ATA IgM at 432 aU/ml. To evaluate the specificity of the assay, 10 SSc patients who were negative for ATA IgG were tested and all had ATA-IgM and -IgA levels below the defined cut-off, in addition 10 SSc patients negative for ACA IgG were tested for ACA IgM and ACA IgA, and these levels were also below the defined cut-off. An 'expressed isotype' was defined as a level above the cut-off value. Outliers were checked and when necessary remeasured.

Organ involvement

Digital ulcers (DU) were present when there was clear visible tissue breakdown, and both ischemic and mechanical (results of microtrauma and increased skin tension) ulcers were included in this definition. Interstitial lung disease (ILD) was defined based on the combination of forced vital capacity (FVC) <70% and evidence for ILD on HRCT. An experienced radiologist evaluated the HRCT for ground glass opacifications, reticulations and honeycombing. We chose to use a combined value including both pulmonary function and HRCT to make sure that we only classify patients with clinically relevant pulmonary involvement as having ILD. Pulmonary arterial hypertension (PAH) was defined as an increase in mean pulmonary arterial pressure (mPAP) ≥ 25 mmHg at rest as assessed by RHC; including presence of pre-capillary PH, defined by a pulmonary capillary wedge pressure (PCWP) ≤15 mmHg and a PVR >3 Wood units (WU) on RHC, in the absence of other causes of precapillary PH such as PH due to lung diseases, chronic thromboembolic pulmonary hypertension, or other rare diseases. To evaluate myocardial involvement, we used different measurements. The Medsger subdomain reflecting myocardial involvement was evaluated, in which grade 0 represents a normal heart function, grade 1 denotes conduction abnormalities and a left ventricular ejection fraction (LVEF) between 44-49%, grade 2 signifies arrhythmias and a LVEF 40-45%, grade 3 indicates severe involvement with a LVEF < 40%. As the Medsger scale mainly relies on the LVEF for determination of myocardial involvement, using only this parameter could lead to underestimation of its presence, since in patients with SSc with myocardial involvement, LVEF is not always below the normal cut-off. Therefore, we additionally used a combined value where patients had to have at least two of the following: arrhythmias (> 2% ventricular or supraventricular arrhythmia, atrial fibrillation), conduction problems, decreased LVEF < 50%, diastolic or systolic dysfunction, pericarditis or pericardial effusion.

Statistical analysis

No sample size calculation was performed due to the explorative character of this study. Analyses were performed by IBM SPSS version 23. All analyses were performed cross-sectionally. NVC patterns and clinical features, at the time of blood sample collection for autoantibody determination, were compared between ACA+ and ATA+ SSc patients using descriptive statistics, and differences were tested for significance as appropriate. Disease duration was defined as duration since onset of Raynaud's Phenomenon (RP), as current SSc pathophysiology indicate that RP is a direct consequence of vasculopathy. We performed a Mann-Whitney U test to calculate the significance of the continuous variables. A Chi-square test was performed for the categorical variables. Fisher exact test was employed when appropriate. Binary logistic regression (univariable and multivariable) was performed, with autoantibodies, NVC pattern, isotype expression and ACA or ATA isotype levels as independent factor and organ involvement as dependent variables. Ordinal logistic regression analyses, with disease characteristics, autoantibodies and isotype expression and ACA or ATA IgG level as independent and NVC SSc pattern as dependent variables were also performed. Since age and disease duration can be a confounder for the association between organ involvement and degree of microangiopathy, we corrected for these variables in the multivariate analyses. In addition, variables with significant association in the univariate analysis were added as indicated. All isotype levels were transformed using log2. To adjust for multiple testing, Bonferroni correction was applied. P values < 0.05 were considered significant.

RESULTS

Study group

A total of 231 SSc patients (129 ACA+ and 102 ATA+) were included. The included patients had a mean age of 55 years (SD 14) and median disease duration from onset first non-RP of 4 years (IQR 1-11). As expected, females represented the majority of the study population (n= 186). ATA+ patients differed from ACA+ in sex (p<0.001), age (p= 0.01), disease duration (p<0.001), diffuse cutaneous subset (p<0.001), and ILD (p<0.001). The main demographic and clinical data of the patients are summarized in Table 1. An important difference between the ATA+ and the ACA+ group that could have an influence on the degree of microangiopathy is the disease duration since onset RP, which is longer in the ACA+ patients compared to the ATA+ patients (16 years vs 6 years). Complete data on NVC patterns were available for 164/231 patients (100 ACA+ and 64 ATA+). The missing NVC were all from patients with baseline visits before 2013, in 2013 the NVC became an annual standard examination.

Organ involvement and the degree of microangiopathy

In the univariable analysis (Table 2), a more severe degree of microangiopathy (late SSc pattern), as shown by NVC, was associated with ILD (OR 3.59, 95%CI 1.75-15.91), PAH (OR 5.85, 95% CI 1.90- 18.65), cardiac involvement (OR 2.95, 95%CI 1.20-7.23) and DU (OR 2.28, 95% CI1.17-4.47). Multivariable analysis showed that a more severe degree of microangiopathy was significantly associated with ILD (OR 3.41, 95%CI 1.11-10.61), PAH (OR 5.58, 95%CI 2.05-17.81) and DU (OR 3.07, 95%CI 1.43-6.60), with correction for age, disease duration and ATA positivity.

NVC patterns in autoantibody subgroups

A late SSc pattern was numerically seen more often in ATA+ patients vs ACA+ patients (31% vs 18%; p= 0.05* after Bonferroni correction; Table 1). The frequency of early (10% ATA, 18% ACA) and active (58% ATA, 68% ACA) SSc pattern were comparable between ATA+ and ACA+ patients. In the multivariable analysis (Table 5 supplementary file), after adjustment for vasoactive medication, age, and disease duration, ATA positivity was associated with more severe microangiopathy (OR 2.97, 95CI 1.41-6.24) compared to ACA positivity.

Baseline characteristics of included ACA+ and ATA+ SSc patients

	Total group	ACA+	ATA+
Demographic	n= 231	n= 129	n= 102
Female, n (%)	186 (81)	116 (90)	70 (69)
Age, mean (SD)	55 (14)	58 (13)	51 (14)
Smoking, ever, n (%)	138 (60)	76 (59)	62 (61)
Disease duration			
Since RP, median (IQR)	10 (4-20)	16 (6-26)	6 (2-13)
Since non RP, median (IQR)	4 (1-11)	5 (1-12)	3 (1-9)
Organ involvement			
DcSSc, n (%)	49 (21)	2 (2)	47 (46)
Puffy fingers, n (%)	74 (33)	38 (30)	36 (37)
Sclerodactylie, n (%)	151 (67)	71 (55)	80 (82)
mRSS, median (IQR)	4 (2-6)	3 (1-5)	6 (2-12)
Pitting Scars, n (%)	106 (46)	59 (46)	47 (46)
Teleangiectasia, n (%)	152 (66)	108 (84)	44 (44)
Digital ulcera, n (%)	43 (19)	30 (23)	13 (13)
DLCO % predicted mean (SD)	65 (17)	70 (17)	62 (17)
FVC % predicted, mean (SD)	92 (21)	92 (21)	91 (20)
ILD on HRCT, n (%)	55 (24)	11 (9)	44 (43)
PAH, n(%)	20 (13)	10 (8)	10 (10)
Nailfold videocapillaroscopy			
NVC early, n (%)	22 (15)	16 (18)	6 (10)
NVC active, n (%)	90 (63)	56 (68)	34 (58)
NVC late, n (%)*	33 (23)	15 (18)	18 (31)
NVC capillary loss < 7mm, n (%)	114 (70)	64 (65)	50 (78)
Medication			
CYC, n (%)	39 (17)	19 (15)	20 (20)
HSCT, n (%)	14 (7)	7 (7)	7 (7)
Iloprost/bosentan, n (%)	17 (12)	14 (11)	13 (13)
Methotrexate ever, n (%)	44 (19)	23 (18)	21 (20)
ACA or ATA characteristics			
IgA positivity, n (%)	-	95 (74)	100 (98)
IgA level (aU/mL), median (IQR)	-	73 (34-146)	2778 (933-8368)
IgM positivity, n (%)	-	95 (74)	66 (65)
IgM level (aU/mL), median (IQR)	-	65 (4-561)	822 (286-2162)
IgG level (U/mL), median (IQR)	-	478 (186-1031)	484 (170-934)

Table 1. Baseline characteristics. ACA= anti-centromere antibody, ATA= anti-topoisomerase antibody, CYC= cyclophosphamide, dcSSc= diffuse cutaneous SSc, DLCO= carbon monoxide diffusing capacity, FVC= forced vital capacity, HSCT= stem cell transplantation, ILD= interstitial lung disease, mRSS= Modified Rodnan Skin Score, NVC= nailfold video capillaroscopy, PAH= pulmonary arterial hypertension, RP= Raynaud phenomenon.

ATA and more severe microangiopathy is associated with organ involvement

	Univariable OR (95% CI)		
	Interstitial lung disease	Cardiac involvement	PAH
Male	1.71 (0.84-3.48**)**	0.59 (0.26-1.34)	1.04 (0.33-3.27)
Age	1.01 (0.99.1.04)	**1.07 (1.03-1.10)**	**1.08 (1.03-1.13)**
Disease duration since RP	0.99 (0.95-1.01)	1.04 (0.98-1.03)	1.00 (0.99-1.05)
Disease duration since NR	1.03 (0.99-1.06)	1.01 (0.97-1.05)	1.01 (097-1.07)
ATA	**6.68 (1.87-23.94)**	0.96 (0.47-1.95)	1.29 (0.52-3.24)
NVC SSc pattern	**3.59 (1.75-15.91)**	**2.95 (1.20-7.23)**	**5.85 (1.90-18.65)**
Immunosuppressiva	**4.64 (1.57-13.66)**	1.08 (0.50-2.31)	0.53 (0.26-1.99)

Table 2. In the multivariable logistic regression autoantibody, age, disease duration (since onset RP) and variables with significant association in univariate analysis were included. Interstitial lung disease (ILD) was defined as ILD on HRCT and a FVC < 70% of predicted. NVC pattern was entered as ordinal variable in the following order: early, active or late.

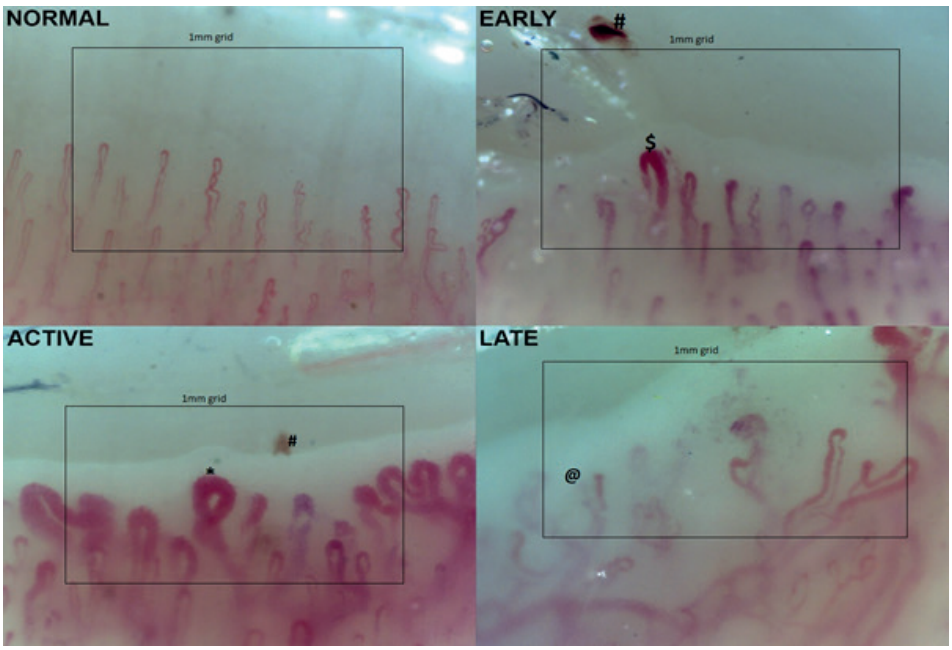

Figure 1. Nailfold videocapillaroscopy image made with INSPECTIS pro**.** Examples of NVC images with a 1 mm grid. # hemorrhages, $ dilation > 30uM, * giant (dilation > 50um), @ neoangiogeneses.

	Multivariable OR (95% CI)			
DU	Interstitial lung disease	Cardiac involvement	PAH	DU
1.32 (0.55-3.20)	-	-	-	-
0.99 (0.97-1.02)	1.01 (0.95-1.07)	**1.06 (1.01-1.11)**	**1.09 (1.02-1.18)**	0.98 (0.95-1.01)
1.01 (0.99-1.03)	0.98 (0.89-1.07)	1.01 (0.97-1.05)	1.02 (0.94-1.07)	0.99 (0.96-1.03)
1.04 (1.01-1.08)	-	-	-	-
2.07 (1.02-4.22)	**13.34 (2.87-52.61)**	1.28 (0.38-4.31)	3.92 (0.68-22.46)	0.36 (0.14-0.92)
2.28 (1.17-4.47)	**3.41 (1.11-10.61)**	2.17 (0.86-5.48)	**5.58 (2.05-17.81)**	**3.07 (1.43-6.60)**
0.46 (0.20-1.07)	1.79 (0.45-7.01)	-	-	-

ATA= anti-topoisomerase, CI= confidence interval, DU= digital ulcera, NR= non-Raynaud, NVC= nailfold videocapillaroscopy, OR= odds ratio, PAH= pulmonary arterial hypertension, RP= Raynaud Phenomenon, SSc= systemic sclerosis .

Isotype expression

In ACA IgG+ patients, 74% (n= 95) were ACA IgA+ and 74% (n= 95) ACA IgM+. Of the ACA + patients, 11% expressed solely ACA IgG, 16% were positive for IgG and IgM, 16% of the ACA patients were positive for IgG and IgA, and 58% were positive for ACA IgG, IgM and IgA. All ATA IgG + patients expressed more than one ATA specific isotype: ATA IgA+ was found in 98% (n= 100), ATA IgM+ was found in 65% (n= 66). Two percent of ATA IgG+ patients expressed also ATA IgM, in the absence of detectable IgA ATA, 33% of ATA IgG+ patients expressed also ATA IgA in the absence of detectable IgM ATA, and 65% expressed all three ATA isotypes.

Association ACA and ATA isotype expression with the degree of microangiopathy

As shown in Figure 2, the ACA IgG+ patients that expressed only ACA IgG, and no other ACA isotypes more frequently showed an early pattern and less frequently a late pattern when compared to ACA IgG+ patients expressing two or three ACA isotypes. The differences between the groups were not statistically significantly. The ATA IgG+ patients that concurrently expressed only ATA IgA showed a late pattern less often than ATA IgG+ patients expressing all three ATA isotypes. These differences were not statistically significant.

Numerically, ACA IgG levels were higher in ACA IgG+ patients with a late SSc pattern than in ACA IgG+ patients with an early SSc pattern (median: 630 U/mL vs 200 U/mL). ATA IgM levels were higher in ATA IgG+ patients with a late SSc pattern compared to ATA IgM in patients with an early pattern (median: 1515 aU/mL vs 691 aU/mL). These results were not statistically significant (supplementary file). In the multivariable analysis with adjustment for disease duration and use of vasoactive medication, antibody isotype levels were associated with degree of microangiopathy (Table 3 and 4). For ACA IgG levels (OR 2.49, 95%CI 1.04-5.83) and for ATA IgM levels (OR 2.70, 95%CI 1.06-4.22) the associations were significantly different.

ACA IgG levels are associated with more severe microangiopathy after adjustment for disease duration and vasoactive medication.

	Univariable OR (95% CI)	**Multivariable OR (95% CI)**
	NVC SSc pattern	NVC SSc pattern
Male	0.69 (0.17-2.77)	-
Age	1.02 (0.99-1.05)	-
Disease duration since RP	1.01 (0.97-1.04)	1.01 (0.98-1.05)
Disease duration since NR	1.01 (0.95-1.07)	
Autoantibody specific IgM positive	1.21 (0.42-3.52)	-
Autoantibody specific IgA positive	1.13 (0.45-2.81)	-
Autoantibody specific IgG levels, U/ml	2.24 (0.99-5.05)	**2.46 (1.04-5.83)**
Autoantibody specific IgM levels, aU/ml	0.99 (0.60-1.65)	-
Autoantibody specific IgA levels, aU/ml	1.51 (0.67-3.37)	-
Autoantibody specific isotype expression	0.80 (0.33-1.91)	-
Immunosuppresive medication	0.64 (0.22-1.88)	-
Vasoactive medication	**3.33 (1.03-12.11)**	**1.81 (1.07-2.87)**

Table 3. Univariable and multivariable logistic regression for **anti-centromere positive** patients. NVC pattern was entered as ordinal variable, in order: early, active or late. ACA= anti-centromere antibody, ATA= anti-topoisomerase antibody, CI= confidence interval, NR= non-Raynaud, NVC= nailfold videocapillaroscopy, OR= odds ratio, RP= Raynaud Phenomenon, SSc= systemic sclerosis.

ATA IgM levels are associated with more severe microangiopathy after adjustment for age and disease duration.

	Univariable OR (95% CI)	**Multivariable OR (95% CI)**
	NVC SSc pattern	NVC SSc pattern
Male	2.20 (0.64-7.53)	-
Age	**1.06 (1.02-1.10)**	1.05 (1.01-1.10)
Disease duration since RP	**1.06 (1.01-1.11)**	1.05 (1.0-1.11)
Disease duration since NP	**1.21 (0.88-1.67)**	-
Autoantibody specific IgM positive	1.29 (0.43-3.82)	-
Autoantibody specific IgA positive	1.00 (1.00-1.00)	-
Autoantibody specific IgG levels, U/ml	1.24 (0.41-3.77)	-
Autoantibody specific IgM levels, aU/ml	2.24 (0.97-5.18)	**2.70 (1.06-4.22)**
Autoantibody specific IgA levels, aU/ml	0.96 (0.42-2.18)	-
Autoantibody specific isotype expression	1.29 (0.43-3.82)	-
Immunosuppresive medication	1.65 (0.56-4.86)	-
Vasoactive medication	1.33 (0.30-5.96)	-

Table 4. Univariable and multivariable logistic regression for **anti-topoisomerase positive** patients. NVC pattern was entered as ordinal variable, in order: early, active or late. ACA= anti-centromere antibody, ATA= anti-topoisomerase antibody, CI= confidence interval, NR= non-Raynaud, NVC= nailfold videocapillaroscopy, OR= odds ratio, RP= Raynaud Phenomenon, SSc= systemic sclerosis.

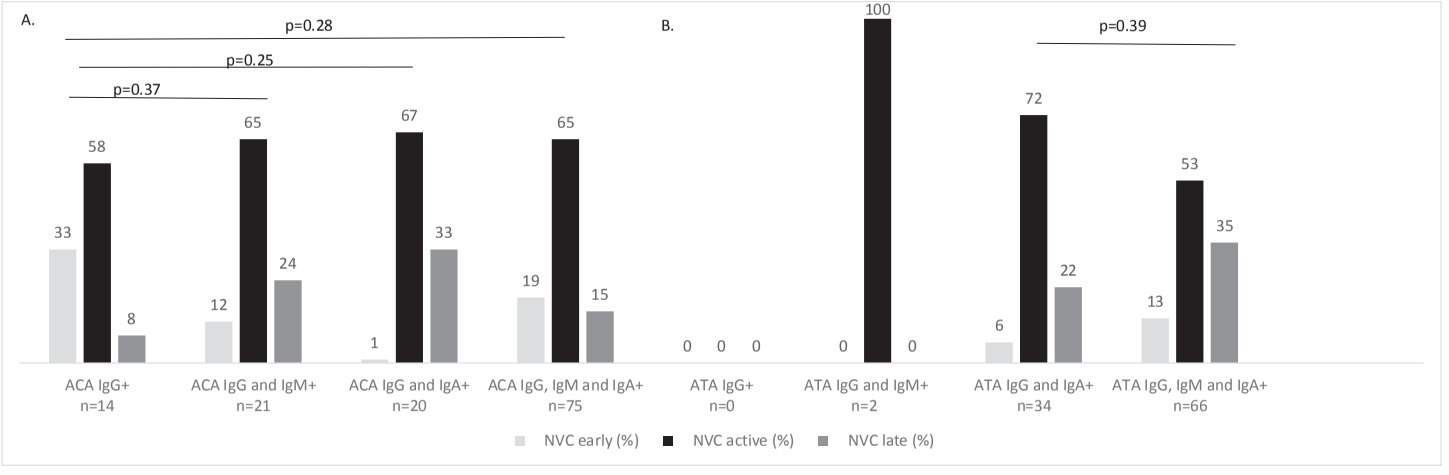

Figure 2. Presence of one, two or three autoantibody specific isotypes and the distribution of early, active or late SSc pattern. The significant values are on group level; 1A:group ACA IgG+ vs. ACA IgG+ and IgM+ p= 0.37 group ACA IgG + vs ACA IgG+ and IgM+ p= 0.25, group ACA IgG+ vs ACA IgG+, IgM+ and IgA+ p= 0.28 (no significant difference in NVC pattern prevalence). 1B; All ATA IgG+ patients expressed at least one additional ATA isotype. Two patients expressed ATA IgG+ and ATA IgM+ both with an active NVC pattern. Prevalence of NVC patterns was not significantly different between ATA IgG+ and IgA+ vs ATA IgG+, IgM+ and IgA+ patients (p= value 0.39). ACA= anti-centromere antibody, ATA= anti-topoisomerase antibody, NVC= nailfold videocapillaroscopy.

DISCUSSION

In our study we evaluated the association between specific ATA and ACA responses and the degree of microangiopathy in a Dutch SSc cohort. We first confirmed the association between more severe microangiopathy and organ involvement including ILD, PAH and DU in patients with SSc. Second, we showed that ATA+ SSc patients more often had severe microangiopathy compared to ACA+ patients. Finally, our results indicate a possible association between characteristics of specific antibody responses and the degree of microangiopathy. After adjustment for possible confounders, we observed a significant association between ACA IgG and ATA IgM levels and a more severe degree of microangiopathy.

In the association analysis we observed a trend for higher ATA IgM among ATA + patients with a late SSc pattern and higher ACA IgG among ACA+ patients with late SSc pattern on NVC. Only after correcting for possible confounders (including disease duration) did these associations become significant, with an association between a more severe degree of microangiopathy and levels of ACA IgG, and between a more severe degree of microangiopathy and levels of ATA IgM. The rationale behind these findings is not fully understood and we can only hypothesize about possible explanations. As ATA+ and ACA+ patients display clearly different clinical phenotypes, one might hypothesize that behaviour of ATA and ACA specific isotypes differs which might impact on their roles in pathophysiology. Further, ATA and ACA might bind to different cells or antigens which may be one reason for the differences between the two group. ATA bind to DNA topoisomerase I expressed by e.g. fibroblasts, and it may be that ATA is only pathogenic in case there is insufficient clearance of apoptotic bodies of endothelial cells containing DNA topoisomerase I. This would also fit with the observation that a more severe degree of microangiopathy is associated with organ involvement (24). ACA, can react against six different centromeric nucleoproteins, and could have a different ability in e.g. recruiting immune effector- or clearance mechanisms (25). Although ACA and ATA have been reported to react with endothelial cells, no data is published on differences between isotype binding and the effects on endothelial cells (18, 26). Another important factor in the pathogenesis of SSc including the endothelial cell damage, is the complement system. IgM and IgG have the ability to induce inflammation by activating complement, however, IgA is a weak activator for complement, this may be one of the explanations why no association between specific IgA and degree of microangiopathy was found (27).

In general, the production of IgM against protein-antigens is driven by short-lived plasmablasts derived from recently stimulated B cells and therefore presence of ATA and ACA- specific IgM suggests an ongoing active immune response. How IgM production is sustained in the presence of IgG against the same antigen is not fully understood, but

similar observations have been reported for anti-citrullinated protein antibodies (ACPA) in rheumatoid arthritis (28). Production of IgM could also result from a failure of class-switching resulting in prolonged survival of IgM- secreting plasmablasts (29). In both the ATA+ patients (65%) and the ACA+ patients (74%) continuous IgM expression next to IgG is observed, but proportionally IgM expression is more frequent in ACA+ patients.

Our results are partly in line with those reported by Markusse et al. and Caramaschi et al., who reported a relationship between organ involvement and more severe microangiopathy as assessed by NVC (12, 30). However, in these studies it was suggested that the presence of a specific IgG autoantibody is independent of the development of microangiopathy. Relations between ATA/ACA isotype profile and isotype levels and disease severity have not yet been evaluated in large SSc cohorts. ATA IgG and ATA IgA levels, and presence of ATA IgM have previously been described to correlate with skin scores and with disease severity in small cohorts (15, 31). We are currently working on verifying these results in a multicentre study.

Our study has some limitations that should be considered. As not all HRCTs were evaluated according to Goh, we were not able to discriminate between limited and extensive ILD. Therefore, we decided to apply a combined definition including presence of ILD on HRCT and FVC < 70% to make sure that only patients with clinically relevant ILD are classified as having ILD. Secondly, we only included patients positive for ATA IgG or ACA IgG at baseline we cannot fully exclude that there might be patients only positive for ACA IgM, ATA IgM, ACA IgA or ATA IgA that we did not include. This seems however unlikely as ATA IgA and IgM isotypes and ACA IgA and IgM isotype were absent in ANA+ SSc patients lacking a SSc specific antibody. To determine whether relevant ATA IgM or ATA IgA can be expected in SSc patients negative for ATA IgG, and vice versa for ACA IgM and ACA IgA, we have additionally measured expression of ATA IgM and ATA IgA in n= 38 samples of ACA IgG positive and ATA IgG negative patients, and measured expression of ACA IgM and ACA IgA in n= 46 samples of ATA IgG positive and ACA IgG negative patients. This showed that relevant expression of ATA/ACA IgM and ATA/ACA IgA is very rare in ATA/ACA IgG negative patients. Likewise, no conclusions can be drawn for the remaining antibody subgroups in SSc.

The current data were derived from a cohort study in a tertiary center where patients with a (preliminary) diagnosis of SSc were included at presentation at the out-patient clinic and therefore treatment prior to inclusion was uncontrolled. Furthermore, we do not know how (previous) immunosuppressive medication might have influenced our results. However, in our study, ACA and ATA-specific isotype levels were not different for SSc patients who used immunosuppressive medication compared to SSc patients who

did not use medication, and at baseline, the use of immunosuppressive medication was comparable between the ACA+ and ATA+ patients. Strikingly, in the multivariable analysis current use of vasoactive medication was associated with worse microangiopathy. Possibly, this association is reflecting confounding by indication, with patients with more severe microangiopathy more often suffering from DU. Finally, we decided not to include outcomes such as gastro-intestinal involvement and renal crisis in this analysis as endoscopies were not performed routinely, and the numbers for renal crisis was too low. In all patients, an HRCT and transthoracic echocardiography were performed routinely, regardless of risk factors for specific organ involvement.

In conclusion, we observed associations between specific ATA and ACA responses and the degree of microangiopathy in patients with SSc, indicating that dysregulated B cell responses and microvascular damage interact with each other in the pathophysiology of SSc. Further research is needed to confirm these observations, and to identify the possible mechanism behind this association.

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SUPPLEMENTARY FILE

ATA positivity is associated with degree of microangiopathy

	Univariable OR (95% CI)	Multivariable OR (95% CI)
	SSc pattern	SSc pattern
Male	1.54 (0.62-3.84)	-
Age	1.03 (1.00-1.05)	-
Disease duration since RP	1.01 (0.98-1.04)	1.01 (0.98-1.04)
Disease duration since NR	1.03 (0.99-1.08)	-
ATA antibody	**2.09 (1.05-4.13)**	**2.97 (1.41-6.24)**
Immunosuppresive medication	1.66 (0.81-3.39)	-
Vasoactive medication	**3.06 (1.09-8.60)**	**3.88 (1.31-11.54)**

Table 5. Logistic regression of all the patients together, not stratified for autoantibody. ATA= anti-topoisomerase antibody, SSc= systemic sclerosis, RP= Raynaud phenomenon, OR= odds ratio, CI= confidence interval.

Isotype levels compared between NVC SSc patterns in ACA+ and ATA+ SSc patients

NVC available baseline ACA+	n=99	IgG ACA level	IgM ACA level	IgA ACA level
Early SSc pattern, median (IQR)	n=12	200 (114-858)	49 (0-208)	63 (33-103)
Active SSc pattern, median (IQR)	n=56	430 (184-1067)	122 (22-602)	67 (17-152)
Late SSc pattern, median (IQR)	n=15	630 (181-1094)	50 (23-1210)	102 (23-178)
		NS	NS	NS
NVC available baseline ATA+	n=64	IgG ATA level	IgM ATA level	IgA ATA level
Early SSc pattern, median (IQR)	n=6	170 (77-969)	691 (432-871)	1848 (1285-4971)
Active SSc pattern, median (IQR)	n=34	578 (277-999)	802 (325-7288)	2782 (784-10254)
Late SSc pattern, median (IQR)	n=18	394 (164-641)	1515 (492-7718)	1810 (890-6868)
		NS	NS	NS

Table 6. ACA= anti-centromere antibody, ATA= anti-topoisomerase antibody, IgG levels U/mL, IgA and IgM aU/mL, IQR= inter quartile range, NS= non-significant, NVC= nailfold videocapillaroscopy, SSc= systemic sclerosis.

Chapter 9

Analyses of anti-centromere antibody levels and isotypes in development of systemic sclerosis

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Objectives

Little is known on the disease course of very early systemic sclerosis (SSc). It is unknown whether anti-centromere antibody (ACA) isotype levels can serve as biomarkers for future SSc development and for organ involvement. We aim to evaluate whether ACA-IgG, -IgM and -IgA levels in ACA-IgG positive patients associate with disease severity and/or progression from very early SSc to definite SSc.

Methods

ACA-IgG positive patients with very early SSc and ACA-IgG positive patients fulfilling the 2013 ACR/EULAR criteria for SSc from five different cohorts were included. A diagnosis of very early SSc was based on the presence of ACA-IgG AND Raynaud and/or puffy fingers and/or abnormal nailfold capillaroscopy but not fulfilling the 2013 ACR/EULAR criteria. Multivariable regression analyses were performed to determine the association between baseline isotype levels and progression to SSc and organ involvement.

Results

Six hundred twenty-five ACA-IgG positive patients were included of whom 138 (22%) fulfilled very early SSc criteria and 487 (78%) had definite SSc. ACA-IgG (Odds Ratio (OR) 2.5, 95% CI 1.8-3.7) and ACA-IgM (OR 1.8, 95%CI 1.3 -2.3) levels were significantly higher in definite SSc patients. Of 115 very early SSc patients with follow-up, 48 (42%) progressed to definite SSc within five years. Progression to definite SSc was associated with higher ACA-IgG levels at baseline (OR 4.3, 95% CI 1.7-10.7).

Conclusion

ACA isotype levels might serve as a biomarker to identify very early SSc patients at risk for progression to definite SSc.

INTRODUCTION

Systemic Sclerosis (SSc) is a heterogeneous autoimmune disease with high mortality and morbidity (1, 2). As early intervention has been shown to improve disease course and outcome, it is very important to detect SSc at an early stage, when therapeutic interventions can prevent progression of organ damage (3, 4). The 2013 American College of Rheumatology/ European League Against Rheumatism (ACR/EULAR) classification criteria for SSc have a high sensitivity for classifying patients correctly as SSc patients (5). However, there are still patients, who do not fulfil these criteria, despite showing early signs of SSc (6). Currently, no biomarkers to identify which patients with very early signs of SSc will progress to definite SSc exists. Identification of this subgroup of very early SSc patients, with more precise insights in their disease course, is crucial for early therapeutic interventions (7).

SSc specific anti-nuclear autoantibodies (ANA) are commonly used for disease and risk stratification. Anti-topoisomerase-1 antibodies (ATA) and anti-centromere antibodies (ACA) are the most prevalent autoantibodies in SSc (8). The presence of ACA is associated with limited skin involvement, higher prevalence of calcinoses, and gastro-intestinal involvement (GI) (9-11). Presence of ACA generally carries a better prognosis than most other SSc associated autoantibodies with respect to survival (10, 12). The major reactive antigen of ACA has been identified as CENP-B, which is therefore suggested as the primary target driving a selected B cell response characterized by ACA-IgG production (13, 14). Based on the observation that the generation of disease specific ANA antibodies is closely linked to disease development and clinical phenotype, we hypothesize ANA specific antibodies to be implicated in disease pathogeneses (15-17). However, the exact role of these disease specific ANA and their underlying antigenic triggers in SSc remain unclear.

In rheumatoid arthritis (RA), an autoimmune disease characterised by polyarthritis and by presence of rheumatoid factor (RF) and anti-cyclic citrullinated antibodies (ACPA), an extended ACPA repertoire has been shown to associate with disease development and disease severity, while the effector function of ACPA is still not elucidated (18-20). At present, little information is available regarding ACA isotype levels in SSc. Detailed information on the ACA isotype distribution of ACA positive SSc patients can contribute to a better understanding of the characteristics and dynamics of the underlying, auto-reactive B-cell response. In line with the observations in RA, we hypothesize that in ACA-IgG positive SSc, the expansion of specific ACA isotype responses associates with SSc development and severity, as reflected by organ involvement.

By taking advantage of five independent and well described SSc cohorts (Leiden, Oslo, Zurich, Ghent, Bordeaux) with prospective and comprehensive clinical data available, we aimed to evaluate whether the levels of individual ACA isotype levels associate with disease severity in ACA-IgG positive SSc patients, and whether these levels can identify very early SSc subjects that will progress to definite SSc.

METHOD

Patient population

The SSc cohorts in Leiden, Oslo, Ghent, Bordeaux and Zurich are prospective cohorts including all consecutive SSc patients (21-26). Patients in these cohorts undergo annual extensive screening which includes complete physical examination, laboratory testing, pulmonary function testing, thoracic echocardiography, high resolution computed tomography (HRCT), 24-hours electrocardiography (EKG), nailfold capillaroscopy (NC) evaluation, and, optional, cardiopulmonary exercise testing (CPET). At every visit, blood samples are collected and stored in respective Biobanks (27).

Included ACA-IgG positive patients had to fulfil either the ACR/EULAR 2013 SSc criteria or had to fulfil criteria for very early SSc (VEDOSS criteria) (5, 28, 29). Patients were classified as very early SSc, if additional to being ACA-IgG positive, had Raynaud Phenomenon (RP) and/or puffy fingers and/or abnormal NC, but did not fulfil ACR/EULAR 2013 criteria for SSc (28, 29). This study was performed with the use of a prospectively collected dataset from routine practice with post-hoc analyses. Patients entering the cohorts before March 2019 were selected for the present study. Details of all cohorts are described elsewhere (21, 23-25, 30-32).

Collection and analysis of biomaterial and their clinical associations have been approved by the local ethics committee (Leiden CME number B16.037, Switzerland: PB 2016-02014 02014 and BASEC-Nr. 2018-01873, Norway: No.2006/119, Ghent: 2008/385, Bordeaux: 2012-A00081-42). All participants provided written informed consent.

Clinical characteristics

At baseline visit clinical data and blood samples (including autoantibody testing) were collected for all included patients. Baseline was defined as the first visit at the SSc care pathway which includes screening for SSc. The SSc patients fulfilling the ACR/EULAR 2013 criteria were categorized as definite SSc without organ involvement and definite SSc with organ involvement (explained in the paragraph below). For analyses, patients were categorized as: 1.very early SSc, 2. definite SSc without organ involvement and 3. definite SSc with organ involvement. Follow-up data were only collected for the very early SSc group, as knowledge on this group of patients is of particular interest due to the possibilities to interfere early in their disease course. Follow-up consisted of an annual assessment in the SSc care pathway to monitor the course of the disease including evaluations of the organ systems (skin, lung, heart, gastrointestinal, renal, musculoskeletal). Follow-up duration was the time duration calculated from the first baseline visit to the most recent visit. Disease duration was defined as time from onset of Raynaud Phenomenon (RP), since

in very early SSc time since onset non-RP was missing in patients without puffy fingers. We collected the required clinical characteristics to evaluate the disease status of the patients (very early SSc, SSc with or SSc without organ involvement). The modified Rodnan Skin Score (mRSS), sclerodactyly, puffy fingers, peripheral vascular involvement including pitting scars, digital ulcers (DU) and telangiectasia were evaluated and reported by the physician during evaluation. NC was considered abnormal if a scleroderma pattern was present, according to the definitions consented by the EULAR study Group on Microcirculation in Rheumatic Diseases and Scleroderma Clinical Trials Consortium (SCTC) (33, 34). Use of immunosuppressive treatment at baseline was recorded including: hydroxychloroquine, mycophenolate mofetil, methotrexate, cyclophosphamide, azathioprine and corticosteroids. Use of biologicals at baseline was only present in approximately 0.5% of the patients, therefore this was not taken into account.

Organ involvement

DU were defined as an area with a visually discernible depth and a loss of continuity of epithelial coverage and included both ischemic and traumatic ulcers. Interstitial lung disease (ILD) was present when there was evidence for ILD on HRCT. Myocardial involvement was assessed using a modified Medsger score,(35) which consists of at least two of the following: arrhythmias (> 2% (supra)ventricular extrasystoles, atrial fibrillation), conduction problems [bundle branch block], decreased left ventricular ejection fraction <54%, diastolic/systolic dysfunction, pericarditis or pericardial effusion. Pulmonary arterial hypertension (PAH) was defined as an increase in mean pulmonary arterial pressure ≥25 mmHg at rest as assessed by right heart catheterization (RHC); including presence of pre-capillary pulmonary hypertension (PH), defined by a pulmonary capillary wedge pressure ≤15 mmHg and a PVR >3 Wood units, in the absence of other causes of precapillary PH such as PH due to lung diseases, chronic thromboembolic PH or other rare diseases (36). Renal crisis was based on clinical expertise (including increase in blood pressure, increase in creatinine, oligo/anuric renal failure). GI involvement was defined based on a composite variable: as presence of confirmed gastric-antral vascular ectasia (GAVE, available for all patients), presence of fecal incontinence (data available in 413 patients), and/or malabsorption syndrome (data available in 317 patients), and or weight loss >10% in one year (data available in 309 patients). Very early SSc patients were considered progressors to definite SSc when they developed ILD, DU, PAH, renal crisis, myocardial involvement, or GI involvement and if they met the ACR/EULAR 2013 classification criteria during follow-up.

Anticentromere assay and measurement

Storage, collection and processing of blood samples was performed in line with the European League Against Rheumatism Scleroderma Trials and Research group (EUSTAR) biobank recommendations (27). All baseline samples were assessed in the clinical

chemistry department of the Leiden University Medical Centre (LUMC). Total ACA-IgG, ACA-IgA and ACA-IgM levels (CENP-B) of all the samples collected were measured by fluorescence enzyme-linked immune sorbent assay (FEIA), using Phadia250 system by J.B (Thermo Fisher Scientific, Nieuwegein, The Netherlands). Immunofluorescent patterns (IF) were evaluated at baseline and centromere anti-nuclear antibody patterns (speckled) were found. Commercial labs usually measure ACA-IgG (CENP-B IgG). The cut-off level for ACA-IgG positivity was set at 7 units/ml, according to the manufacturer's instructions. ACA-IgM and ACA-IgA were defined as research only parameters by the manufacturer. To define cut-off values for these parameters sera from fifty healthy subjects were measured and the cut-off values for the presence of IgM and IgA were defined as the mean plus 2SD for serum samples. Cut-off for ACA-IgA was determined at 37 AU/ml and for ACA-IgM at 13 AU/ml.

Statistical analysis

Analyses were performed by IBM SPSS version 23, and GraphPad Prism 7 was used for creating graphs. Descriptive statistics were used to summarize clinical and serological features and differences were tested as appropriate. For comparison of the continuous independent variables Mann- Whitney U test was used (for two groups), for more than two groups Kruskal –Wallis test with correction for multiple comparisons was used. Chi square test was used for categorical variables. To evaluate cross-sectional associations between isotype levels and disease status we used binary logistic regression with adjustment for age and disease duration (predictor: isotype levels [continuous, each isotype was tested in a separate model], outcome: disease status i.e. very early SSc, definite SSc without or definite SSc with organ involvement). Longitudinal analyses included the clinical evaluation of very early SSc patients over time, and progression of organ involvement in definite SSc. For clinical differences between progressors and non-progressors Mann Whitney U test and Chi Square test were used. Multivariable logistic regression was used to assess the independent association between isotype levels and disease progression (predictor: isotype levels [continuous, all isotypes tested in separate models], outcome: progression yes/no) and ROC-curves were evaluated (supplementary file). The possibility to predict progression to SSc based on ACA-IgG level was evaluated. Last observation carried forward was applied in case of missing data during follow-up for ILD, PAH, and ejection fraction in case of clinically stable disease with stable pulmonary function test results and no additional testing available. As the individual components of the composite GI involvement variable were not complete for all included patients, the validity of the parameter was checked in a sensitivity analysis using the subgroup with complete data (supplementary table S2).

RESULTS

Clinical characteristics

In total, 625 ACA-IgG positive patients were included. Ninety percent were female (n= 558) with a mean age of 58 years, a median disease duration since non-Raynaud of 6 years (IQR: 2-9). The baseline characteristics of the three clinical groups are shown in table 1. There were 138 patients with very early SSc (22%), 240 SSc patients without organ involvement (38%), and 247 SSc patients with organ involvement (40%).

ACA IgG, IgM and IgA levels

Of all the ACA-IgG positive subjects, 437 (76%) were ACA-IgA positive at baseline; and 522 (89%) were ACA-IgM positive at baseline. A non-cutaneous disease subset was more common in patients positive for both ACA-IgG and ACA-IgA compared to patients positive for ACA-IgG and ACA-IgM and patients positive for all three isotypes (respectively 47% vs 33%vs 27%). No other clinical differences were observed between subgroups defined by numbers of expressed isotypes (data not shown). In the very early SSc group, the ACA-IgG and ACA-IgM levels were significantly lower compared to patients in the definite SSc group (figure 1, figure S3). Using logistic regression, with adjustment for age and disease duration since RP, we found a significant association between ACA-IgG (OR 2.54 (1.75-3.69)) and ACA-IgM levels (OR 1.77 (1.34-2.34)) and disease status, with higher levels in the SSc patients (SSc with and without organ involvement combined) compared to the very early SSc patients (table 2). No significant associations were found between ACA-IgG, ACA-IgM or ACA-IgA isotypes and definite SSc with or without organ involvement (table 2). In the supplementary file results per included centre are shown, confirming the same trend across all SSc centres (supplementary table S1, figure S1 and figure S2).

To assess a possible effect of immunomodulatory treatment on ACA isotype levels, we performed a logistic regression. No significant associations were found for use of immunosuppressive treatment and ACA-IgG (OR 1.4 (0.91- 2.10)), ACA-IgM (OR 0.91 (0.68-1.22)) or ACA-IgA levels (OR 0.74 (0.43-1.29)).

Baseline characteristics and ACA isotype expression and levels in patients with very early SSc and SSc

	Very early SSc n=138	SSc without organ involvement n=240	SSc with organ involvement n=247	Data available in n. patients
Female, n(%)	125 (91)	225 (91)	208 (87)	625
Age, median (IQR)	52 (40-62)	57 (49-66)	62 (52-69)	625
Since RP, median(IQR) in years	5 (1-12)	10 (3-19)	8 (2-18)	622
Since non RP, median(IQR) in years ¥	NA	5 (2-11)	6 (2-12)	465
lcSSc, n(%)	NA	202 (84)	187 (78)	482Ω
dcSSc, n(%)	NA	14 (6)	27 (11)	482Ω
mRSS, median (IQR)	0 (0-0)	3 (0-5)	4 (0-6)	589
Digital ulcers, n(%)	0	0	81 (33)	616
FVC % predicted, mean (SD)	107 (17)	107 (17)	107 (19)	585
DLCO % predicted, mean (SD)	81 (15)	74 (14)	67 (18)	596
ILD on HRCT, n(%)	0	0	86 (36)	625
PAH, n(%)	0	0	52 (21)	625
Myocardial involvement, n(%)	0	0	42 (22)	563
Renal crisis, n(%)	0	0	3 (1)	625
GI involvement, n(%)	0	0	120 (49)	625
Puffy fingers, n(%)	21 (16)	71 (39)	36 (23)	548
Abnormal NC, n(%)€	69 (55)	160 (84)	149 (86)	488
Immunosuppressive treatment, n(%)	25 (18)	48 (20)	112 (46)	625
ACA characteristics				
IgA positivity, n(%)	88 (72)	177 (78)	172 (75)	617
IgM positivity, n(%)	106 (86)	209 (91)	207 (90)	617
IgG levels, U/ml, median (IQR)	274 (93-662)	480 (197-990)	619 (263-1077)	617
IgM levels, aU/ml, median (IQR)	101 (41-363)	183 (55-907)	251 (63-965)	617
IgA levels, aU/ml, median (IQR)	69 (35-103)	78 (39-166)	86 (37-187)	617

Table 1. Baseline characteristics of the three groups, SSc= systemic sclerosis, RP= Raynaud phenomenon, NA= not applicable; lcSSc= limited cutaneous, dcSSc= diffuse cutaneous SSc, FVC= forced vital capacity, DLCO= diffusing capacity for carbon monoxide, GAVE= gastric antral vascular ectasia, mRSS= modified Rodnan Skin Score, ILD= interstitial lung disease, IQR= interquartile range, HRCT= high resolution computed tomography, n= number, NC= nailfold capillaroscopy, PAH= pulmonary arterial hypertension, SD= standard deviation. ¥ n= 22 missing in definite SSc , € n= 137 missing (started with NC in 2013/2014 and not routinely performed in all cohorts). Medication in very early SSc group: n= 7 corticosteroids, n= 12 methotrexate, n= 5 hydroxychloroquine. Ω maximum available in n= 487 (since very early SSc cannot be categorized in a disease subset).

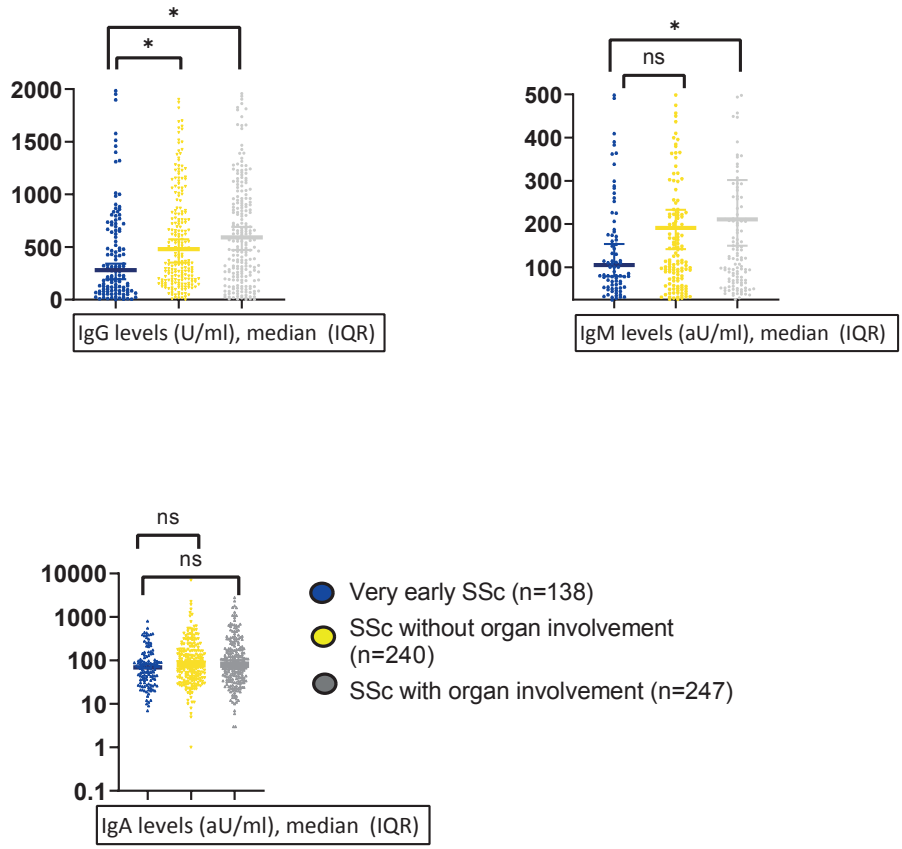

Figure 1. ACA isotype levels in very early SSc, definite SSc without and definite SSc with organ involvement**.** ACA-IgG, ACA-IgM and ACA-IgA levels between the three groups. ACA-IgG and ACA-IgM levels are significantly higher in definite SSc patients compared to very early SSc patients. * significant $p < 0.05$

ACA-IgG and ACA-IgM levels are associated with definite SSc, ACA-IgG levels are associated with disease progression towards definite SSc.

	SSc patients vs. very early SSc	SSc with organ involvement vs. SSc without organ involvement	Very early SSc Progression
	OR 95% CI	OR 95% CI	OR 95% CI
ACA IgG U/ml	**2.54 (1.75-3.69)**	1.09 (0.77-1.53)	**4.27 (1.70-10.71)**
ACA IgM aU/ml	**1.77 (1.34-2.34)**	1.11 (0.83-1.26)	1.75 (0.97-3.14)
ACA IgA aU/ml	1.40 (0.90-2.17)	0.96 (0.67-1.38)	1.36 (0.47-3.96)

Table 2. * adjusted for age and disease duration. ACA-IgG, -IgM and -IgA were log2 transformed to overcome skewness in the data. Very early SSc: ACA isotype levels available in n= 115 very early SSc patients.

Very early SSc evolving to definite SSc

Of the 138 patients classified as very early SSc, 23 were lost to FU (supplementary table S3). In total 48 (42%) progressed to definite SSc during a median follow-up period of 2 years (range 1-4). Of these progressors, 22 (46%) developed vital organ involvement which consisted of ILD (n= 10, 21%), cardiac involvement (n= 5, 10%) or GI involvement (n= 7, 16%). Seventy-seven percent of progressors developed skin involvement including an increase based on the minimal clinical important difference in mRSS (n= 11, 23%),(37) development of telangiectasia (n= 31, 65%) or sclerodactyly (18, n= 38%). Both DU and pitting scars occurred in 17% of the progressors. The remaining 67 patients did not develop organ involvement nor progressed to fulfilling ACR/EULAR 2013 criteria after median FU of 2 years (range 1-5).

Compared to non-progressors, very early SSc that progressed to definite SSc were older and had a longer follow-up duration (table 3). At baseline, ACA-IgG levels were significantly higher in progressors compared to non-progressors, also when adjusted for follow-up duration (figure 2). In logistic regression analyses with correction for age and follow-up duration ACA-IgG levels were significantly associated with progression to definite SSc (table 2 OR 4.27 (1.70-10.71)); puffy fingers were significant in the univariable analysis (OR 2.95 (1.31-6.62)), and abnormal NC did not show a significant association with progression to definite SSc (supplementary table S4). The ROC curves for ACA-IgG and ACA-IgM on progression can be found in the supplementary file figure S4, table S6-S7 and figure S5.When applying a threshold with optimal sensitivity and NPV, ACA IgG of 81 U/ml together with presence of puffy fingers can be applied (figure S5). With this cut of 84% of progressors and 49% non-progressors were classified correctly at baseline. To further evaluate the predictive value of ACA isotype levels for progression in SSc we evaluated association with disease progression in the patients with definite SSc at baseline and complete clinical follow-up data available (n= 93, table S5). In this subgroup ACA-IgG (OR 2.79 (1.08-7.26)) and ACA-IgM (OR 2.06 (1.18-3.61)) were independently associated with disease progression.

Very early SSc patients with follow-up data			
	Progressors n=48	Non progressors n=67	p value
Demographic			
Female, n(%)	43 (90)	61 (91)	0.52
Age, mean (SD)	**53 (15)**	**48 (13)**	**0.03**
Disease duration			
Since RP, median (IQR)	5 (2-11)	6 (2-14)	0.69
Follow-up duration in years, median (IQR)	**5 (3-7)**	**2 (1-5)**	**<0.001**
Clinical characteristics			
Puffy fingers, n(%)Ω	7 (15)	8 (12)	0.55
Abnormal NC, n(%)€	26 (54)	34 (51)	0.45
ACA characteristics			
IgM positivity, n(%)	36 (86)	51 (81)	0.36
IgA positivity, n(%)	31 (76)	43 (68)	0.28

Table 3. Differences between very early SSc patients that progressed during follow-up and very early SSc patients that did not show disease progression. No clinical follow-up data available n= 23 very early SSc patients. Missing data for ACA isotype levels in patients with follow-up; n= 8 very early SSc patients (5 in the progressor group, 4 in non progressors). Ω data available for n= 105 patients, € data available in n= 113 patients.

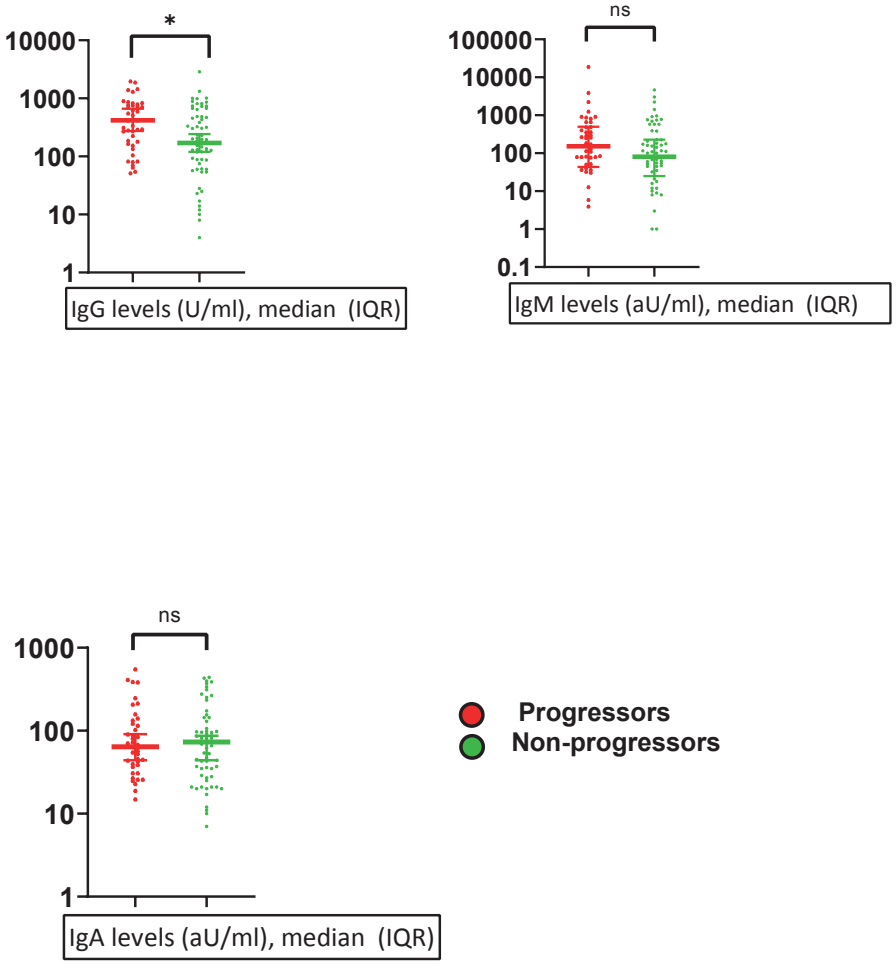

Figure 2. ACA-IgG, ACA-IgM and ACA-IgA levels between very early SSc patients that progress to definite SSc and patients that do not show progression. ACA-IgG levels are higher in patients that progress.

DISCUSSION

In this study, we analysed ACA isotype levels in patients with very early SSc and in patients with definite SSc to evaluate whether disease severity within ACA positive patients is associated with characteristics of the ACA immune response. Secondly, we evaluated the clinical course of patients with very early SSc and assessed whether ACA isotype levels can identify subjects that will progress to definite SSc. We show that definite SSc patients express higher ACA-IgG and higher ACA-IgM levels compared to very early SSc patients. Moreover, we show that in very early SSc, higher levels of ACA-IgG associate with progression to definite SSc within two years.

The lower ACA-IgG and ACA-IgM levels in the very early SSc group might indicate a less pronounced immune response compared to the patients with definite SSc. We found the highest ACA-IgG, ACA-IgM and ACA-IgA levels to be present in the SSc patients with organ involvement, and within the group of definite SSc patients baseline ACA-IgG levels and ACA-IgM levels associated with future disease progression. These findings are in line with our hypothesis that the immune response in very early SSc patients is less pronounced compared to patients with established disease. As shown by our data, and also by others,(30) although the classification might suggest short disease duration, some patients classified as 'very early SSc' in fact show similar disease duration as patients with definite SSc. This indicates that patients fulfilling classification criteria for very early SSc in fact consist of a heterogeneous group of patients: patients that will eventually progress to definite SSc and patients that will persist as 'very early' SSc and do not progress to more severe disease (30). Our data shows that the levels of the ACA specific immune response discriminates between those two subgroups (supplementary file). Similar observations have been made in other rheumatic diseases including RA (18, 38). The observation that ACA-IgG levels are numerically higher in ACA positive SSc patients with organ involvement compared to levels in ACA positive SSc patients without organ involvement is in line with our hypothesis. However, for this comparison observed differences were not statistically significant. We presume that the absence of broadly validated outcome measures for SSc might at least partially explain this lack of significance. Moreover, commonly accepted definitions for severe organ involvement like ILD and diffuse skin involvement might be less sensitive in ACA positive SSc, as severe fibrotic disease complications are less frequent in ACA patients than in ATA positive patients.

Until now the effects of disease duration on isotype levels is not fully understood. One could hypothesize that isotypes levels decline over time as antigenic triggering diminishes (39). Whether and for how long ACA specific response occur before clinical disease development is unknown. In one study, a median duration since onset RP of 4.6

years was found from early SSc to definite SSc (40). No data about initiation of specific antibody expression nor data about different autoantibodies are available from this study. In our very early SSc group, 48 patients (42%) developed definite SSc over a median time of 5 years.

We observe the strongest associations between ACA-IgG and disease subset (very early SSc vs definite SSc). In line with our hypothesis, it is tempting to speculate that either ACA-IgG, and/or the B-cell responses underlying ACA production are involved in the disease pathogenesis. As both microangiopathy, clinically shown by RP, and dysregulated immunity reflected by presence of specific ANA, are among the earliest features of SSc one could speculate that specifically ACA-IgG and ACA-IgM contribute to endothelial cell damage, possibly by activating complement. Indeed, ACA positive sera have been shown to affect endothelial cells (41). In the Leiden cohort, we recently demonstrated an association between ACA-specific immune response and degree of microangiopathy (42). Finally, the implication of the association between both ACA-IgM and ACA-IgG with disease progression can be speculated about. In adaptive immune responses IgM is the first isotype to appear after a vaccination or an infection. In normal adaptive immune responses IgM disappears rapidly, due to isotype switching where IgG will take over, and, secondly, antibodies of the IgM isotype have a short life time ($T_{½}$= 8 days). The ongoing presence of ACA-IgM next to ACA-IgG and its association with disease progression points at ongoing immune activation accompanied by continuous production of IgM which is most likely caused by recently activated B cells. As there is no evidence regarding the nature origin of ACA-IgA in SSc pathogenesis we can only speculate about the implication of high prevalence of ACA-IgA. IgA is mostly found in mucous membranes, particularly the respiratory tract and the gastrointestinal tract; as such expression of disease specific ACA-IgA might implicate involvement of these mucous membranes in SSc pathogenesis. The frequent pulmonary and gastro-intestinal involvement in SSc patients supports this hypothesis, but how and where ACA-IgA is triggered is currently unknown.

Puffy fingers or abnormal NC were found to be predictive for the diagnosis of very early SSc in a population with RP and no diagnosis (yet) of very early SSc (28). Randone et al.(43) identified SSc specific autoantibodies, puffy fingers and NC abnormalities to be predictive for disease progression in patients with RP and/or ANA positivity. We identified ACA characteristics to be predictive for progression, however we were not able to confirm the association between abnormal NC with progression. One explanation could be the differences in included patients, in our study the majority of the very early SSc patients already had 8 points on the ACR/EULAR 2013 classification criteria; in the study performed by Randone et al. the majority of the patients scored <6 points on the ACR/EULAR 2013 classification criteria at baseline. Interestingly progression rates

between patients with 8 points at the ACR/EULAR criteria and patients with < 8 points were comparable. Secondly, we only included ACA positive patients while Randone and colleagues included RP patients that could be ANA negative, or ANA positive with different specificities. Strikingly, the amount of progressors among the very early SSc patients was comparable between the studies (41% vs 42%), which underlines the necessity of biomarkers to adequately identify the patients at risk. Although not the scope of the present study, evaluating ACA-IgG level as possible predictive biomarker in clinical practice showed that, in combination with PF 84% of progressors and 49% non-progressors could be identified correctly at baseline. However, this finding needs to be further evaluated and confirmed in independent cohorts

Previous results on association between disease severity and ACA specific responses have been conflicting. Two longitudinal studies with a small sample size (n= 13 and n= 15) did not provide conclusive results on associations between clinical characteristics and ACA isotypes, they did observe fluctuating levels of ACA isotypes over time (44, 45). These studies were limited by small sample sizes, the use of invalidated outcome measurements and older techniques to measure specific isotypes. In conclusion, to our knowledge, our study is the first that performed complete evaluation of ACA isotype responses in patients with SSc, and specifically evaluated ACA isotype response in association with clinical progression to SSc in the very early SSc group. This study might be helpful to provide more evidence for evaluating a possible pathogenetic role of ACA in SSc disease course by answering one of the Witebsky's postulates (46). We believe that the ACA isotypes can be seen as biomarker for the underlying immune response, and the presence and levels of the different isotypes can be used as a marker for 'the breadth of the immune response. In addition, we hypothesize that the breadth of the immune response is a proxy for the intensity of the immune response, i.e. continuous expression of more isotypes indicates more active triggering of the adaptive immune response, which is also supported by data in other auto-immune diseases (18, 19, 47). This study has some limitations. We included patients with baseline positivity of ACA-IgG. We cannot completely exclude that SSc patients positive for ACA-IgM or -IgA solely have been missed. As a sensitivity check, we additionally measured expression of ACA-IgA and ACA-IgM in 46 ACA-IgG negative SSc patients (negative in both Phadia FEIA and in IF assay) with various disease durations, which confirmed that clear expression of ACA-IgM and/or IgA in ACA-IgG negative patients is not to be expected, since this was very rare (results not shown). Likewise, no conclusions can be drawn for the remaining antibody subgroups in SSc. As no longitudinal samples were analyzed the effect of starting or stopping immunosuppressive medication remains unclear, although we did not find an association between immunosuppressive medication and ACA isotype levels. Another limitation is the difference in follow-up duration in the very early SSc group,

however we performed two additional sensitivity checks, 1) including patients with a long follow-up duration and 2) including patients with a short disease duration, which both confirmed the significant association between ACA-IgG and progression to definite SSc (supplementary file table S8 and S9). GI involvement was assessed based on available parameters including GAVE; this could have led to underestimation of prevalence of GI involvement and therefore we performed a sensitivity check in a subgroup with additional data available (supplementary table S2). Even with this broader definition for GI involvement, patients with organ involvement still showed the highest ACA-IgG and ACA-IgM levels. To strengthen these results the next step would be to evaluate ACA isotypes longitudinal and at the time of progression.

In conclusion, we show for the first time and in a large multicentre ACA positive SSc cohort that ACA-IgG and ACA-IgM levels are significantly higher in definite SSc patients compared to very early SSc patients. Moreover, we show that 42% of ACA positive patients with very early SSc progresses to definite SSc within 5 years and that progression is associated with higher ACA-IgG levels. Both observations indicate CENPB-specific IgG levels as a novel biomarker in SSc and as potentially contributive to disease development.

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Part IV

Appendices

Appendix

Summary, conclusions and future perspectives

SUMMARY, CONCLUSIONS AND FUTURE PERSPECTIVES

The studies described in this thesis contribute to the identification of biomarkers for risk stratification in systemic sclerosis (SSc). Luckily, nowadays many SSc prospective cohorts have been set up worldwide which allows high-quality research. Given the rarity and the heterogeneity of the disease, relatively large cohorts are needed to draw valuable conclusions. For the studies described in the current thesis, I was able to incorporate data from the Leiden prospective SSc cohort and data from other prospective cohorts in Europe, which made it possible to strengthen the data. In this final chapter, I summarize the main findings of the studies presented in this thesis, put our findings in a broader perspective, discuss future perspectives and formulate research questions that are relevant to assess in the years ahead of us.

PART I: IMPACT OF SYSTEMIC SCLEROSIS

Diagnosis of Systemic Sclerosis

SSc can be difficult to diagnose due to its rarity and heterogeneity. In most patients, receiving the diagnosis of SSc takes years as it is often not immediately recognized at time of first symptoms (1, 2). The new ACR/EULAR 2013 criteria and the very early diagnosis of SSc (VEDOSS) classification criteria help us with early diagnosis (3-5). Earlier diagnosis in SSc is necessary to manage and treat patients at a point in their disease where damage of skin and internal organs is still reversible. On the other hand, physicians should be aware that even in patients with early disease, without any organ complications, receiving a diagnosis of SSc can have a major impact on daily life (**chapter 1**)(6).

Patient perception of disease burden

Since early diagnosis lengthens the time window of patients in which the prognosis is unclear, it is necessary to know how this affects the patients (7, 8). A focus group study (**chapter 1)** among 7 patients with a recent diagnoses of SSc showed that patients indeed worry, mainly about the chronic nature, the unpredictable disease course and the negative consequences of the disease (9-13). It is known that these illness perceptions have an impact on physical health, mental health and illness behavior in SSc (14) (15). As illness perceptions influence illness behavior, it is important for physicians to be aware of the decoupling of patients perceptions of disease and objectified disease activity (**chapter 1**). Moreover, these perceptions are associated with the disease course, since a patient's own personal beliefs and emotional responses to symptoms affect disease management. We did not only analyze illness perceptions during the focus group but we also asked patients to make a drawing of their disease. In contrast to the illness perceptions evaluated during the focus group and the questionnaires, which were predefined, drawings can provide an unbiased image of patients' perceptions. The most common features displayed in the drawings were; experienced symptoms, emotional functioning and social functioning. The images drawn by the patients gave us some insights in patients thoughts and concerns and highlighted the importance of psychosocial support. Finally, we learned from this study that patients experienced negative thoughts caused by internet based information and little understanding of the disease in their personal environment. This indicates the importance of patient education after receiving a diagnosis. Patient-centered care that encompasses strategies to promote self-esteem, self-efficacy, and open communication may help to decrease the SSc disease burden.

SSc-related quality of life

Improving patients' quality of life should be one of the main goals for every physician. Health related Quality of Life (HRQoL) includes both physical and mental health, and several validated questionnaires have been developed to assess HRQoL (16-19). HRQoL in SSc patients can vary greatly, independent of disease severity. Even in SSc patients without organ manifestations HRQoL is lower when compared to patients with other chronic diseases (20-22).

Clinical manifestations associated with SSc-related quality of life

Due to the chronic nature of SSc and it's heterogeneity, it is important to know which disease manifestations have the largest impact on HRQoL. A thorough understanding of HRQoL determinants may help treating physicians to identify the unmet needs of SSc patients and the areas where more effective pharmacological or non-pharmacological interventions are indicated. We confirm, in **chapter 2,** that gastrointestinal (GIT) symptom burden, Raynaud phenomenon (RP) symptoms, and digital ulcers are associated with lower HRQoL (13, 23-25). In addition, in SSc, patients with organ involvement experience a lower HRQoL compared to patients without organ involvement. During the disease course and during follow-up, an increase of GIT symptoms and/or RP symptoms were found to be predictive for a decrease in HRQoL in all SSc patients. Skin involvement changes specifically impact HRQoL in patients with early disease (since first non-RP symptom < 24 months), whereas pulmonary arterial hypertension (PAH) had a significant impact on HRQoL in patients with long standing disease. Functional impairments, as shown by a decrease in six-minute-walking distance (6MWT) and hand function, were also associated with impaired HRQoL over time, meaning that loss of function significantly impacts HRQoL. Remarkably, quality of life in SSc patients is significantly affected by troublesome and difficult to control symptoms including RP and GIT, even more than by life threatening complications such as ILD and cardiac involvement. In contrast, treating physicians often focus on life-threatening manifestations. Our results suggest that despite the non-life threatening nature these burdensome disease manifestations deserve attention as these are highly important for the patient. In my opinion, HRQoL assessment measures should play a prominent role also in clinical trials investigating the efficacy of novel therapies for SSc. Future studies should focus on how to address functional impairment, RP and GIT. By improving treatment and care for these symptoms, HRQoL can significantly improve and, consequently, this can have a positive influence on illness and risk perceptions which will also improve disease management and will have a positive effect on the disease course.

PART II: DISEASE PROGRESSION IN SYSTEMIC SCLEROSIS

Disease progression

Clear guidance regarding follow-up is very important for clinicians, not only to identify progression in time but also to limit overdiagnosis and overtreatment in mild patients. Pulmonary, cardiac, gastro-intestinal (GIT), renal, musculoskeletal, functional, and vascular symptoms are annually investigated in our multidisciplinary SSc care pathway, however no evidence based guidelines on the extent and frequency of follow-up exists. Currently, only an expert consensus based guideline exists which describes 55 tools/ measurements to assess on an annual basis in every SSc patient (26). Whether these items are sufficient to identify progression timely is yet to be determined. Approximately 50% of the SSc patients never show any signs of progression and annual follow-up might be redundant in this group of patients (**chapter 3**). On the other hand, also approximately 50% of the patients experiences relevant progression somewhere during their disease course. The most common cause of progression was cardiopulmonary deterioration, which is also the number one cause of death in SSc (27). Although research and guidelines often mention disease progression is most likely to occur in early disease, our data showed that in 24% of the patients progression occurred after a disease duration of > 10 years since first non-RP symptom. We observed that skin progression occurred more frequently in early disease. The proportion of patients with lung, heart or GIT progression was relatively stable over time. Anti-topoisomerase antibody (ATA) positive patients showed progression most often in early disease, while in anti-centromere antibody (ACA) positive patients the proportion of patients with progression seemed to increase over time. This study indicates the importance of follow-up, as half of the patients experience disease progression. The fact that progression can occur both early and late demonstrates why it is difficult to design guidelines on the extent and frequency of follow-up. The fact that half of the patients never show any signs of progression indicates at the same time the importance to identify biomarkers to predict progression in order to enable tailormade follow-up of the individual SSc patient.

Gastrointestinal symptoms and progression; underestimated in SSc

GIT symptoms hamper SSc patients' HRQoL and, after skin involvement, are the most common complications in SSc (28). Approved treatment options for GIT involvement in SSc are very limited, but this may, at least partially, relate to little knowledge and focus. Little is known on GIT progression, and the potential effects on GIT symptom burden by the standard of care treatments in SSc (immune modulating and/or vaso-dilating treatment). Therefore, in **chapter 4**, we performed a multicenter study (Oslo and Leiden) with longitudinal data on patient-reported GIT symptoms and standard of

care treatment. We identified that GIT symptoms are very common in SSc, and a high symptomatic burden from early in the disease course predicted progressive behavior of the GIT symptoms over time. Many patients reported severe reflux and distension/bloating symptoms already at time of SSc diagnosis. All GIT symptoms progressed during the observation period, with highest progression rates for reflux and distension/bloating. Female sex and presence of ACA were significantly associated with total GIT symptom burden and with GIT progression, no other predictors could be identified. With the exception of corticosteroids, standard of care therapies for SSc seem to have little impact on GIT symptoms. This study shows that clinicians should be aware of the high GIT symptom burden at time of SSc diagnosis and should identify patients at risk for progressive GIT disease early to tailor disease management. In addition, more knowledge on the correlation between GIT patient-reported-outcome measurements and objective measurements is necessary. In a very recent study performed by Zampatti et al.(29) the correlation between the UCLA GIT 2.0 score (reflux domain) and esophago-gastro-duodenoscopy (EGD) were investigated, and the reflux subscale was able to discriminate patients with SSc who had an indication for EGD, but this did not correlate with the findings in the EGD. Therefore, this study concluded that the UCLA GIT 2.0 is helpful but should not be used as a stand-alone instrument to identify an indication of EGD (29). We showed that all gastro-intestinal domains can be affected and therefore evaluation of all the different gastro-intestinal domains should be taken into account and should be included in the guideline for follow-up in SSc patients. However, the definition for GIT involvement in SSc patients remains very difficult. Kaniecki et al.(30) published an extended review on the practical approach to the evaluation and management of GIT symptoms in SSc which is of major clinical relevance for physicians following SSc patients (30). Knowledge about severity and the natural behavior of GIT symptoms is a prerequisite to identify patients for inclusion in clinical trials targeting GIT involvement, and to assess treatment effects. This is particularly important in times of evolving new therapeutic options, such as fecal microbiota transplantation. This study helped us to gain knowledge on the severity and evolution of GIT involvement in SSc. A next step would be to identify biomarkers for GIT disease activity or GIT disease progression.

Prediction model

Over 50% of patients with SSc showed disease progression over time, disease progression was very diverse and occurred in a heterogeneous group. To identify which patients were at risk for overall disease progression we developed, with the use of machine learning, a prediction model including 90 variables (100% complete, **chapter 5**). With this model we were able to stratify patients for low, intermediate or high risk of disease progression. Twenty-nine percent of the patients had a low risk of disease progression (negative predictive value [NPV] 1.0), and annual follow-up could be less extensive in these patients.

The Machine-Learning-Assisted prediction model could therefore significantly reduce health-care costs without substantial risk to our patients, and we might be able to reduce the amount of worry in some of the patients. In addition, we compared the Machine-Learning-Assisted driven model with the expert opinion model (26). Where the Machine-Learning-Assisted driven model included 10 variables in the final model, the expert model included 51 variables. Interestingly, the ROC of both models was comparable (ROC of 0.68), however, cutoffs for low, intermediate and high risk of progression were only identifiable in the Machine-Learning-Assisted driven model. This model allows us to confidently identify a subset of patients who can safely reduce their visit frequency. Preferably, in the future we will be able to predict the risk for progression on each specific organ system in every individual patient. We were not able to accomplish this in our cohort due to the sample size and the heterogeneity of the disease. To evaluate organ systems separately in both a test and training set we need a large amount of patients followed over a long period. Besides, predictors for organ progression on one certain domain might be protective for progression on another domain, which means a large amount of predictors are needed in the model. To be able to develop a model with this goal we need to collect data from a few international SSc cohorts (preferable also outside of Europe) and identify models for each organ system and externally validate these models. To be able to accomplish this it is important to identify the most important predictors and evaluate if these predictors are internationally collected in the same manner. If we will be able to develop a model that predicts accurately enough to identify the low and high risk patients we can develop an online tool where physicians can calculate the risk score of every individual patient, and this will help us to provide tailormade follow-up and treatment for every individual patient.

PART III: MICROANGIOPATHY AND SSC SPECIFIC AUTOANTIBODIES

Biomarkers in systemic sclerosis

A better understanding of SSc is the best way to identify important biomarkers, and these biomarkers are the key to improve prediction of the disease course. Currently, the classification criteria for SSc consists out of clinical characteristics, laboratory findings and microvascular abnormalities. Ideal biomarkers are indicators of SSc that can be measured accurately, easily, cheap, and preferably with non-invasive techniques. They are not only helpful for early diagnosis and understanding distinct pathophysiological processes of the disease but are also useful for patient care in terms of prediction of prognosis, and treatment decision-making (31). SSc specific autoantibodies and nailfold capillaroscopy (NC) patterns are used as biomarkers based on significant and sometimes exclusive associations with the disease itself or certain clinical phenotypes of the disease and in this thesis we evaluated these two biomarkers more extensively.

Degree of microangiopathy

Vasculopathy plays an important role in the pathophysiology of SSc (32). The factors that trigger vascular injury in SSc have not been elucidated so far. Antinuclear auto-antibodies (ANAs) have been mentioned as one of the possible triggers for vascular injury (33, 34). In addition, hormonal factors also have been suggested as trigger for microangiopathic manifestations (35). To gain more evidence on this subject we performed a systematic review in **chapter 6** to evaluate whether sex or expression of specific ANA might associate with the degree of microangiopathy in SSc patients. Eleven studies were included that report on the relationship between SSc specific auto-antibodies and microangiopathy, and six studies were included that report on the association between sex and microangiopathy in SSc. The number of included articles already indicates that limited evidence was available for our review. Contradictory results were found on the association between auto-antibodies and microangiopathy, with a trend towards more severe degree of microangiopathy in ATA positive patients. No association was found between sex and microangiopathy based on the current literature. Due to limited evidence on the association between autoantibodies and degree of microangiopathy we decided to evaluate this in our own cohort. In **chapter 8** we demonstrated an association between ACA and ATA specific immune response and degree of microangiopathy (36). We confirmed the association between more severe microangiopathy and organ involvement in SSc patients. Secondly, we showed that ATA positive SSc patients more often have severe microangiopathy compared to ACA positive patients. Finally, and completely novel, was the significant association between ACA-IgG and ATA-IgM levels with a more severe degree of microangiopathy. As ATA positive and ACA positive patients

display clearly different clinical phenotypes, one might hypothesize that behavior of ATA and ACA specific isotypes differs which might impact on their roles in pathophysiology. The continuous presence of IgM indicates a constant trigger of the immune system which might be more obvious in ATA positive patients compared to ACA positive patients. Currently, one of the ideas is that autoimmunity occurs early in the disease course and the auto-immune response is targeting other cell types (endothelial cells). ATA and ACA probably target different cells / pathways which might explain the clinical differences between the two groups. The observation between higher ACA-IgG and ATA-IgM levels and a more severe degree of microangiopathy might indicate that dysregulated B cell responses and microvascular damage interact with each other in the pathophysiology of SSc. To confirm these observations and to identify the possible mechanism behind this association further research is needed. For example, in clinical setting the effect of immunosuppressive therapy affecting B cells on microvascular damage can be evaluated, and a next step would be to isolate the (autoreactive) B cells from SSc patients to be able to evaluate their effect on an in vitro endothelial cell model.

Anti-U3RNP SSc specific autoantibody

Anti-fibrillarin (anti-U3RNP) is a SSc specific autoantibody, which has been described in small subgroups. Previous studies suggested an association with cardiovascular complications, however due to its rarity clear clinical associations remains to be confirmed (37, 38)**.** We evaluated if NC could be of contributive value to identify the anti-U3RNP positive patients with cardiopulmonary involvement (**chapter 7**). We did not observe a higher prevalence of cardiopulmonary involvement in anti-U3RNP positive patients compared to ATA or ACA positive patients, but we did confirm the association between degree of microangiopathy and cardiopulmonary involvement for all the antibody subgroups, which is in line with our results in **chapter 8**. As such, NC can also serve as biomarker in anti-U3RNP positive SSc patients for risk of cardiopulmonary involvement.

SSc specific autoantibodies

ACA generally carries a better prognosis than most other SSc autoantibodies, still 35% of the ACA positive SSc patients develop organ involvement. This already demonstrates that even within one autoantibody group the disease course is very heterogeneous. Therefore, in **chapter 9** we analyzed the ACA isotype levels in ACA-IgG positive SSc patients in relation to clinical disease progression using data from five large and well defined SSc cohorts from European centers of expertise in SSc. We described the ACA isotype levels in patients with very early SSc and in patients with definite SSc, including the association with disease severity and disease progression. Using autoantibodies for risk stratification is not new (33, 39-42), however, evaluating the specific isotypes has never been performed extensively in SSc. Our study showed that in ACA positive SSc patients,

higher ACA-IgG and ACA-IgM levels associated with more severe disease. Moreover, higher ACA-IgG levels associated with disease progression over time in both established SSc and very early, pre-clinical SSc. The continuous presence of ACA-IgM suggests that there is ongoing immune activation triggering continuous production of IgM which is most likely caused by recently activated B cells. Also more mechanistic explanations could be considered. For example, IgM and IgG have the ability to induce inflammation by activating complement, where IgA is a weak complement activator, and therefore might not be involved at the same level. As there is no evidence regarding the nature of ACA-IgA in SSc pathogenesis it is intriguing to hypothesize about its origin and implication. IgA is mostly found in mucous membranes, particularly the respiratory tract and the gastrointestinal tract; as such expression of disease specific ACA-IgA might implicate involvement of these mucous membranes in SSc pathogenesis. The frequent pulmonary and gastro-intestinal involvement in SSc patients supports this hypothesis. We conclude that the ACA isotypes can be seen as biomarker for the underlying immune response, and the presence and levels of the different isotypes can be used as a marker for 'the breadth of the immune response'. In very early SSc disease [pre-disease], two features are present in > 90% of the patients; microangiopathy as clinically shown by RP symptoms, and dysregulated immunity reflected by presence of SSc specific autoantibodies. It is possible that these SSc specific autoantibodies contribute to microangiopathy by endothelial cell damage (34). Taking this in mind it is tempting to speculate that either ACA-IgG, and/or the B-cell responses underlying ACA production are involved in the disease pathogenesis. Based on these data, ACA isotype levels might be considered as a biomarker to predict which SSc patients are at risk for disease progression, and to predict which patients with very early SSc are at risk for future SSc. We believe that the results are therefore highly relevant for clinicians in rheumatology as these findings contribute to risk stratification in SSc using a simple biomarker. Additionally, our findings encourage further evaluation of the contribution of ACA antibodies to the pathogenesis of SSc.

SUMMARY

Can we identify patients at risk for decreased health-related quality of life?
By improving patients' physical functioning, RP symptoms and GIT burden we will be able to influence HRQoL. In order to achieve this, assessment of these symptoms on a regular basis must be performed and pharmacological and non-pharmacological treatment options should be followed according to the EULAR guidelines (43). In addition, further research is needed to increase the treatment options for especially GIT involvement. In SSc, illness perceptions have an important influence on quality of life, independent of disease severity. As physicians we have the possibility to address these perceptions with clear guidance, monitoring and information.

Can we predict SSc disease course?
Unfortunately, prediction of the disease course remains difficult, but we were able to develop a prediction model by means of which patients with a low risk of progression, can be identified in whom annual follow-up can be less extensive. Applying this model to provide tailor made care can influence illness perception, illness behavior and quality of life in SSc importantly. In the future, we need to develop models that can also identify patients with a high risk on specific organ involvement in whom we should interfere timely with the disease course before irreversible damage is done.

Can we identify biomarkers for patients at high risk?
In this thesis we confirmed that the degree of microangiopathy is associated with more severe disease, a next step would be to investigate the predictive role more extensively in a large subset of patients followed over time. We found ACA isotypes and isotype levels to be associated with the disease course which indicates that ACA isotype levels can be used as biomarkers to predict disease progression. Confirmation in larger cohorts with very early SSc patients and established SSc patients is necessary to confirm our observation and to confirm the relevant cut-offs. To be able to investigate this, early recognition of SSc is very important. New biomarkers are identified on a yearly basis and more and more research is and will be performed in the coming years.

FUTURE PERSPECTIVES

Health care

The multidisciplinary approach in SSc patients remains very important and to improve health care outcomes benefiting both patients and health professionals it is necessary to break down barriers and bridge professional boundaries. Ideally a multidisciplinary team involving a rheumatologist, a pulmonologist, a cardiologist, a gastroenterologist and a specialized nurse should determine the best care for every individual SSc patient. To be able to impact as much as possible on HRQoL we also need more information on a patients' illness perceptions, this allows us to individualize our health care system in combination with a patients' need. Illness perceptions should always be taken into account, as all patients are behaving and responding differently on receiving a diagnosis. In my opinion, in the future, SSc care pathways need to be based on the individual risks of a patients stratified for different organ systems; some patients need screening of the pulmonary system where others are more prone for vascular abnormalities. To change or improve the care of patients we need to expand our knowledge on SSc disease course and in order to do that we need to gain more insight on the pathophysiology of SSc. This is one of the examples of how research has a direct influence on health care (see figure). In the end, this will improve the quality of care and will reduce the health care costs.

Research

Despite many advances made in elucidating the pathogenesis of SSc, the exact mechanism remains unsolved. Based on the above background, it is important both clinically and pathologically to elucidate the relationship between microvasculopathy and the immunological heterogeneity of SSc. I would argue for more longitudinal research on the associations between the clinical characteristics and the laboratory findings as these associations might be able to help us with risk stratification in this heterogeneous disease. The most ambitious goal still remains to identify the key elements, in particular in the earlier phases, for targeted intervention and to disease progression and prevent organ complications. Besides targeted therapies, in the future, individualized treatment options may also be dependent of a patients' biomarker profile. This will not only influence daily care, but will also improve the selection of patients for clinical trials. That is how research and healthcare are continuously influencing each other (figure).

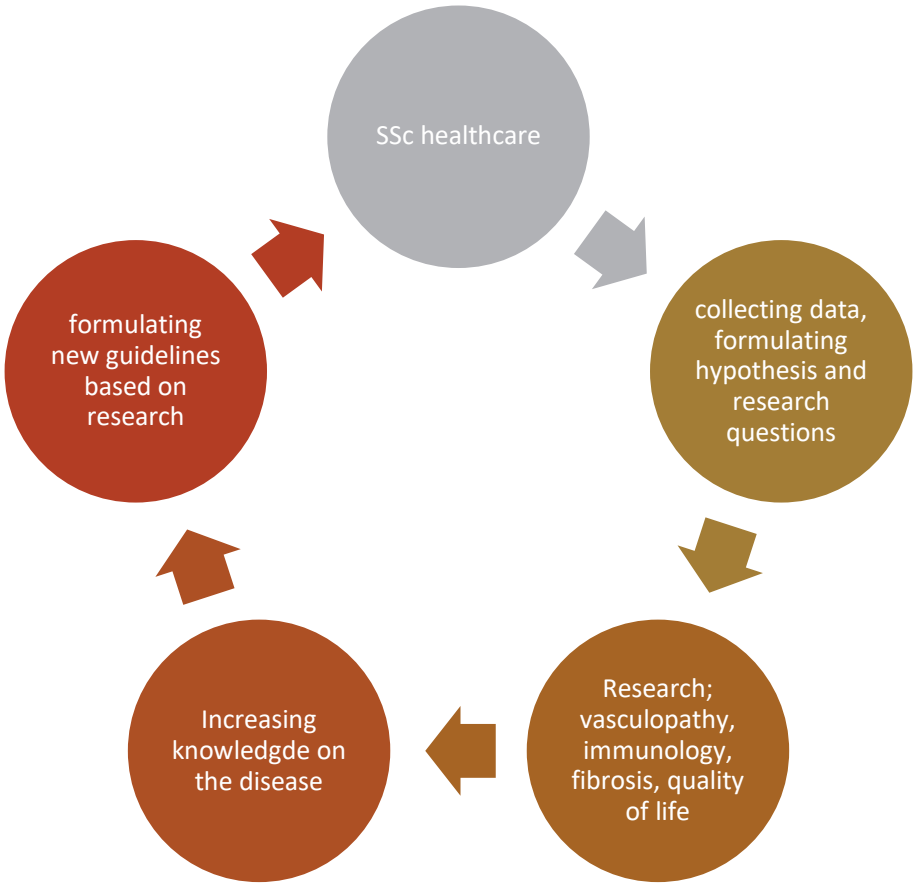

Figure 1. Research and healthcare

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Appendix

Nederlandse samenvatting

SYSTEMISCHE SCLEROSE

Systemische sclerose (SSc) is een zeldzame reumatische auto-immuunziekte. De ziekte is zeer heterogeen, het kan snel progressief verlopen met de dood tot gevolg maar ook zeer mild met een stabiele ziekteactiviteit over jaren. Het aantal geregistreerde patiënten met SSc in Nederland ligt rond de 3000.

De exacte pathogenese van SSc is nog onbekend. De drie belangrijkste pijlers zijn een disregulatie in het immuunsysteem, vasculopathie en gegeneraliseerde fibrose. Deze pathologische veranderingen leiden tot macro- en microvasculaire schade, inflammatie en fibrose van de interne orgaansystemen. Er is een bepaalde interactie tussen de endotheel cellen, immuun cellen en de mediators die resulteren in fibroblasten. We weten echter nog niet welke van deze drie factoren de belangrijkste factor is in de ziekte pathogenese, of hoe ze gerelateerd zijn tijdens de ontwikkeling en de progressie van de ziekte. Het blijft momenteel onduidelijk of de activatie van het immuunsysteem de pathogenese aanstuurt of eerder een poging representeert om de extracellulaire matrix deposities en de progressieve vasculopathie tegen te gaan. Wereldwijd wordt er gewerkt aan onderzoek om de definitieve hiërarchische structuur tussen deze drie kenmerken uit te zoeken.

Het diagnosticeren van SSc gebeurt met behulp van internationale classificatie criteria, de ACR/EULAR 2013 criteria. Desondanks wordt er met deze criteria nog steeds een deel van de zeer vroege of zeer milde SSc patiënten gemist. Daarom zijn sinds een aantal jaar de 'very early diagnosis of systemic sclerosis' (VEDOSS) criteria in het leven geroepen. Beide criteria bevatten onder andere het fenomeen van Raynaud, de aanwezigheid van (SSc specifieke) antistoffen (anti-centromeer antistoffen [ACA], anti-topoisomerase antistoffen [ATA], anti-RNApolymerase III antistoffen [ARA]) en vaatafwijkingen die beoordeeld worden middels een nagelriemscopie.

Het fenomeen van Raynaud, vasospasme van de arteriën in de vingers en/of tenen, is in de meeste gevallen van SSc het eerste symptoom. Dit kan vaak jarenlang de enige klacht zijn. Doordat er bij Raynaud veroorzaakt door SSc (secundair Raynaud) ook daadwerkelijk sprake is van micro- en macro vasculopathiëen kan de mate van vasculopathie beoordeeld worden middels nagelriemscopie. Bij patiënten zonder een SSc specifieke antistof en zonder vasculaire afwijkingen bij nagelriemscopie (primair Raynaud), is de kans op het ontwikkelen van SSc 2%. Echter als er wel sprake is van een SSc specifieke antistof in het bloed OF van een afwijkende nagelriemscopie (secundair Raynaud), is deze kans verhoogd naar 26-35%. Indien zowel een SSc specifieke antistof EN vaatafwijkingen voorkomen, is de kans verhoogd naar 78%.

Op grond van de mate van huidbetrokkenheid worden patiënten geclassificeerd als non-cutaan, gelimiteerd of diffuse SSc. Orgaanbetrokkenheid zien we in wisselende mate bij patiënten met SSc. Betrokkenheid van het maagdarmstelsel zien we in circa 60% van de patiënten, waarbij er sprake kan zijn van een verscheidenheid aan klachten. Longbetrokkenheid, en dan voornamelijk interstitiële longziekten (ILD), treedt in circa 40% van de patiënten op. Pulmonale arteriële hypertensie (PAH) komt in 10% van de patiënten voor en later in het ziekteproces treden vaak klachten op van rechtszijdig hartfalen. Cardiale betrokkenheid, meestal uitend in pericarditis, linker ventrikel disfunctie, of ritme-en geleidingsstoornissen, komt frequent voor, maar geven vaak pas in een laat stadium klachten. Renale crisis komt door het gebruik van ACE-remmers nog maar bij ongeveer 5% van de patiënten voor.

De SSc specifieke antistoffen zijn elk geassocieerd met andere ziekte manifestaties. ACA gaat gepaard met gelimiteerde SSc met daarbij vasculaire en/of gastro-intestinale complicaties. Deze ziektevorm is vaak heel geleidelijk progressief, en wordt gezien als de mildere vorm van SSc. De diffuse vorm wordt meer gezien bij ATA positieve patiënten, hierbij komt orgaanbetrokkenheid veel vaker voor en is het beloop ernstiger en progressiever. De SSc specifieke antistoffen en de mate van vasculopathie helpen ons met het stellen van de diagnose, maar kunnen dus ook behulpzaam zijn bij het voorspellen van de prognose. Ondanks deze biomarkers blijft het erg lastig om het ziektebeloop voor de individuele patiënt te voorspellen.

De overlevingspercentages van SSc zijn de afgelopen jaren verbeterd. Dit komt deels door eerdere herkenning van de symptomen en het eerder stellen van de diagnose. Overleving op 1, 5 en 10 jaar na ziektepresentatie zijn 94%, 80% en 66% respectievelijk. Pulmonale betrokkenheid is de nummer één oorzaak van overlijden in SSc. De behandeling van SSc is nog steeds erg lastig door de complexiteit en de heterogeniteit van de ziekte. Veel behandelingen zijn gericht op symptoom bestrijding en op behandelingen die ingrijpen op een van de drie ziekte pijlers: inflammatie, fibrose en/of vasculopathie. Zo is methotrexaat vaak de eerste keus bij huid betrokkenheid, en moet cyclofosfamide worden overwogen bij ILD, voornamelijk in patiënten met progressieve ILD. Bij patiënten met snelle progressie van de ziekte, inclusief huid en long betrokkenheid, kan stamcel transplantatie de beste behandeling zijn.

Er lopen meerdere internationale trials naar nieuwe medicamenten voor SSc, en elk jaar starten er nieuwe studies. De laatste jaren zijn medicamenten die aangrijpen op de fibrose pathways of de vasculopathie enorm belangrijk geworden in de onderzoekswereld, helaas niet altijd succesvol. Meerdere trials (Resolve, FaSScinate, FASST, ASSET) voornamelijk gericht op anti-fibrotische of anti-inflammatoire therapie zijn verricht

waarbij de primaire uitkomstmaat helaas niet altijd gehaald is. Dit kan deels komen door een verkeerd gekozen medicament, maar het gebruik van een verkeerde primaire uitkomstmaat kan hier ook aan bijdragen. Tot op heden is er nog geen behandeling voor alle manifestaties van SSc.

OPZET VAN DIT PROEFSCHRIFT

Het doel van dit proefschrift is om biomarkers te identificeren die we kunnen gebruiken in de risico classificatie van SSc patiënten. In het eerste gedeelte (**deel I**) van het proefschrift hebben we onderzocht wat voor impact SSc heeft op de patiënt en welke klinische karakteristieken de meeste invloed hebben op de kwaliteit van leven van patiënten met SSc. Het tweede gedeelte (**deel II**) van het proefschrift gaat over ziekteprogressie in SSc patiënten, waarbij de focus eerst ligt op ziekte progressie op alle orgaan systemen en we ons later focussen op gastro-intestinale klachten bij SSc. Als laatste hoofdstuk in **deel II** hebben we een predictie model ontwikkeld om de laag risico patiënten te kunnen identificeren en de zorg te individualiseren. In de derde en laatste sectie (**deel III**) van dit proefschrift zijn we ingegaan op de eerder in de literatuur beschreven biomarkers namelijk de SSc specifieke antistoffen en de mate van vasculopathie. We hebben onderzocht of we bepaalde triggers voor de mate van vasculopathie konden identificeren, of er een associatie tussen de antistoffen en de mate van vasculopathie bestaat, en of we de antistof response kunnen gebruiken als biomarker in het voorspellen van het ziektebeloop.

DE IMPACT VAN SYSTEMISCHE SCLEROSE

Door vooruitgang in kennis over SSc, wordt de diagnose SSc steeds vroeger in het ziekteproces gesteld. Helaas blijft het voor artsen lastig om patiënten informatie te geven over het beloop van de ziekte, doordat de ziekte zo heterogeen is. Daarnaast is er tegenwoordig veel informatie te vinden op het internet, wat de gedachten van patiënten over de ziekte kan beïnvloeden en kan bijdragen aan een patiënt zijn of haar onzekerheid. De ideeën, die patiënten ontwikkelen over hun ziekte en hun prognose, kunnen van invloed zijn op het gedrag van een patiënt. Gedachten en emoties in relatie tot de ziekte en de prognose van de ziekte kunnen een grote impact hebben op het omgaan met de ziekte, het omgaan met medische hulp en ook op het beloop van de ziekte. Het doel van het onderzoek in **hoofdstuk 1** was om de gedachten van patiënten - met een recent gediagnosticeerde SSc- over hun ziekte en over het risico dat zij lopen als gevolg van de ziekte (ziekte- en risicopercepties) verder te onderzoeken. We hebben patiënten geïnterviewd, vragenlijsten afgenomen en tekeningen laten maken. Het krijgen van een diagnose als SSc blijkt meteen al een enorme impact op het leven van patiënten te hebben, zelf als er nog geen ziektecomplicaties zijn. Patiënten ervaren weinig persoonlijke controle, weinig begrip van de omgeving en veel zorgen. Deze zorgen komen veelal voort uit de onzekerheid over de toekomst. Opvallend genoeg zagen wij dat de ziekte percepties niet geassocieerd zijn met de ziekte-ernst, iets wat voor iedere behandelend arts goed is om zich te realiseren. Wij verwachten dat we betere zorg en voorlichting kunnen geven en zo de kwaliteit van leven van patiënten kunnen verbeteren door de risico- en ziektepercepties van SSc patiënten in acht te nemen. Goede educatie voor de patiënt, maar ook voor de omgeving, en de juiste hulp is enorm belangrijk in dit proces.

KWALITEIT VAN LEVEN

In **hoofdstuk 2** werd onderzocht welke karakteristieken van SSc de meeste invloed hebben op de kwaliteit van leven van patiënten, en welke kenmerken er verantwoordelijk zijn voor veranderingen in de kwaliteit van leven over de tijd. Gedurende de jaren van follow-up zagen we dat de ontwikkeling en/of verslechtering van Raynaud, digitale ulcera en gastro-intestinale klachten geassocieerd waren met veranderingen van kwaliteit van leven, waarbij de kwaliteit van leven verslechterde. Een toename van huidbetrokkenheid was geassocieerd met een verslechtering in kwaliteit van leven in patiënten met een korte ziekteduur, terwijl PAH juist geassocieerd was met een verslechtering van kwaliteit van leven in patiënten met een langere ziekteduur. Ook de functionele beperkingen blijken enorm belangrijk te zijn voor patiënten, waarbij een slechtere hand functie, verminderde 6-minuten looptest en een kleinere mondopening geassocieerd waren met een achteruitgang van kwaliteit van leven. Hoewel onderzoek zich veelal focust op ziektecomplicaties met een hoge mortaliteit zoals long en hart betrokkenheid, lijken juist voor patiënten klachten met minder destructieve gevolgen en die moeilijk te hanteren zijn ook veel invloed op het dagelijks leven te hebben. Deze uitkomsten zijn belangrijk om mee te nemen bij het opzetten van trials, want juist medicamenten die ook deze symptomen kunnen verbeteren zijn veelbelovend.

ZIEKTE PROGRESSIE IN SYSTEMISCHE SCLEROSE

Ziekteprogressie is lastig te voorspellen, in ons eigen cohort zagen we dat 52% van de patiënten wel ergens gedurende het ziektebeloop progressie liet zien op één van de orgaansystemen (**hoofdstuk 3**). Progressie van cardiopulmonale en huid betrokkenheid komen het meest voor. Opvallend genoeg zien we dat een toename in huidbetrokkenheid vaker vroeg in de ziekte optreedt, terwijl cardiopulmonale betrokkenheid ook nog later in het ziekteproces kan ontwikkelen. Dit laat wel zien dat goede screening zelfs na 10 jaar ziekteduur nog belangrijk is en dat de ontwikkeling van biomarkers voor betere risico classificatie ons hier zeker in kan helpen. Er is weinig bekend over gastro-intestinale klachten bij SSc, terwijl deze klachten wel belangrijk zijn voor de kwaliteit van leven van patiënten (**hoofdstuk 2**). In **hoofdstuk 4** beschrijven we het voorkomen van gastro-intestinale klachten, het beloop hiervan, mogelijke risicofactoren voor gastro-intestinale progressie en het effect van medicatie op de deze klachten in SSc patiënten. Gastro-intestinale klachten zijn lastig om te objectiveren, aangezien we niet bij iedereen invasieve diagnostiek willen verrichten zoals een scopie. In dit onderzoek hebben we gebruik gemaakt van de gevalideerde vragenlijsten UCLA GIT 2.0 om klachten te evalueren. Gastro-intestinale klachten komen veel voor, zeker bij 1/3 van de patiënten, voornamelijk reflux en een opgeblazen gevoel lieten een hoge prevalentie zien. Drieëndertig procent van de patiënten had een hoge ziekte last als het gaat om gastro-intestinale klachten en 24% liet progressie zien van klachten over de tijd. Behandeling bij SSc bestaat grotendeels uit immunosuppressieve medicatie, hiervan zagen we weinig effect op de gastro-intestinale klachten behalve dat patiënten met gebruik van corticosteroïden meer klachten noteerden. We vonden daarnaast ook dat aanwezigheid van ACA en het vrouwelijke geslacht geassocieerd waren met meer gastro-intestinale klachten. Kennis over de ernst en het beloop van klachten zijn belangrijk om een beter inzicht te krijgen in welke patiënten geïncludeerd kunnen worden in trials. Dit wordt ook steeds belangrijker doordat er meer trials worden gestart waarbij deze klachten een belangrijke rol spelen; zoals de studie naar fecale transplantatie in SSc. Gezien de hoge frequentie van gastro-intestinale klachten in SSc, pleiten wij voor een multidisciplinaire aanpak gezamenlijk met de maagdarmlever artsen. Helaas bestaan er op dit moment geen adequate biomarkers om de gastro-intestinale ziekteactiviteit te meten, de symptomen van de patiënten blijven hierbij voor nu de belangrijkste graadmeter. De exacte oorzaak van gastro-intestinale klachten in SSc zijn nog niet bekend. In ons onderzoek zagen we dat calcium kanaal blokkers en digitale ulcera geassocieerd zijn met gastro-intestinale klachten, een interessante gedachtegang zou kunnen zijn dat vasculopathie een rol speelt in de pathogenese van de gastro-intestinale klachten bij SSc patiënten.

PREDICTIEMODEL IN SSC

Met behulp van machine learning, hebben we onderzocht of het mogelijk is om op basis van 'evidence based medicine' te beoordelen welke SSc patiënten kunnen volstaan met een minder intensieve follow-up doordat zij als laag risico patiënten geclassificeerd kunnen worden (**hoofdstuk 5**). Momenteel zijn er enkel follow-up richtlijnen ontworpen op basis van expert opinie, in deze richtlijn moet je jaarlijks bij iedere patiënt 55 karakteristieken nalopen en onderzoeken, terwijl we weten dat het een zeer heterogene ziekte is. Natuurlijk doen we dit omdat we geen ziekte complicaties willen missen en het voorspellen van het ziektebeloop nog erg lastig is. In **hoofdstuk 5** werden patiënten, met behulp van een nieuw ontwikkeld predictie model, gestratificeerd in drie groepen; laag, medium en hoog risico op ziekteprogressie. We hebben dit gedaan omdat we graag meer "value based health care" willen leveren gericht op de individuele patiënt en het daarbij passende ziektebeloop. Als we patiënten met een laag risico op progressie kunnen identificeren zou voor deze groep de zorg hierop aangepast kunnen worden, waardoor de zorg efficiënter en effectiever voor zowel arts als patiënt wordt. De cutoffs voor laag, medium en hoog risico zijn bepaald door het nemen van een negatieve predictieve waarde (NPV) van 1, we willen namelijk geen patiënten missen met mogelijke complicaties. Negen-en-twintig procent van de patiënten viel in de lage risico klasse, gezien de NPV van 1.0 wil dit zeggen dat geen één patiënt in de laag risico klasse progressie van de ziekte liet zien. Deze patiëntengroep zou dus in principe minder intensief vervolgd hoeven worden. Dit zou kunnen leiden tot minder over diagnostiek, kosten besparing, en kwaliteit waarborging. Ondanks dat het voorspellen van het ziektebeloop lastig blijft door de heterogene populatie zou risico stratificatie al een stap in de juiste richting zijn. Met het ontwikkelde model kunnen we een groep patiënten met een laag risico op progressie identificeren. Dit kan ons helpen om de zorg individueler gericht te maken.

MICROANGIOPATHIE EN SSC SPECIFIEKE AUTOANTISTOFFEN

Sommige studies beschrijven SSc specifieke antistoffen als één van de mogelijke triggers voor vasculaire schade, antistoffen veroorzaken dan mogelijk acceleratie van de vasculaire endotheel cel veroudering en dit kan het fenomeen van Raynaud induceren. Er zijn echter ook studies die zeggen dat de antistoffen juist meer bijstanders zijn in het ziekteproces en secundair ontstaan naar aanleiding van de ontwikkelde vasculopathie. Als trigger voor de vasculopathie worden meerdere mogelijke oorzaken voorgesteld; infecties, omgevingsfactoren (blootstelling schadelijke stoffen), hormonen en/of geslacht. Er is een duidelijke sekse verschil in SSc, waarbij de prevalentie in vrouwen een stuk hoger is. Daarnaast verloopt het ziekteproces tussen mannen en vrouwen vaak ook anders, waarbij vasculaire problematiek een hogere prevalentie in vrouwen heeft. In **hoofdstuk 6** vonden we na literatuur onderzoek echter geen associatie tussen sekse en mate van vasculopathie. We vonden in deze systematische review wel een mogelijke associatie tussen antistoffen en microangiopathie, waarbij we voornamelijk zagen dat ATA positieve patiënten vaker een ernstige mate van microangiopathie hebben en patiënten die ACA positief zijn juist minder schade aan de vaten laten zien. Deze resultaten komen ook overeen met onze bevindingen in **hoofdstuk 8**. In **hoofdstuk 7** hebben we ons verdiept in een zeldzame antistof die geassocieerd is met SSc, namelijk anti-U3RNP (anti-fibrillarin). In de literatuur wordt deze antistof vaak geassocieerd met cardiopulmonale betrokkenheid, echter gezien de zeldzaamheid van deze antistof weten we dit niet helemaal zeker. In ons onderzoek hebben we alle SSc patiënten, die positief voor anti-U3RNP antistof zijn geselecteerd en gematched met ACA en ATA positieve patiënten. Daarnaast hebben we bij alle geïncludeerde patiënten ook de mate van vasculopathie bekeken middels de nagelriemscopie. In dit onderzoek vonden wij in U3RNP positieve patiënten geen hogere frequentie van cardiopulmonale betrokkenheid in vergelijking met de controle groepen (ACA positief en ATA positief). Daarnaast zagen we inderdaad dat een ernstige mate van vasculopathie geassocieerd is met cardiopulmonale betrokkenheid, derhalve zou je de mate van vasculopathie kunnen gebruiken om patiënten met cardiopulmonale betrokkenheid te identificeren. In **hoofdstuk 8** onderzochten we of er een associatie bestaat tussen de ACA en ATA immuunreactie en de mate van microangiopathie. Uit onze eerdere onderzoeken kwam naar voren dat de SSc specifieke antistof wel degelijke geassocieerd was met de mate van microangiopathie. Zo lieten ATA positieve patiënten vaker een ernstige mate van vasculaire schade zien. Om hier dieper op in te zoomen, waren wij benieuwd naar de associatie tussen de antistof response en dan in het specifiek de antistof specifieke isotypes (ACA-IgG, ACA-IgM, ACA-IgA, ATA-IgG, ATA-IgM en ATA-IgA) en de mate van microangiopathie. In onze resultaten zagen we dat de ACA-IgG levels en de ATA-IgM levels geassocieerd zijn met de mate van microangiopathie.

Deze bevinding suggereert dat deze twee belangrijke pijlers in SSc een interactie met elkaar hebben in de ziektepathogenese. Waarom we bij ATA positieve patiënten een associatie vonden tussen ATA-IgM en microangiopathie en bij ACA positieve patiënten tussen ACA-IgG en microangiopathie is nog niet helemaal duidelijk. Mogelijk spelen er toch bij beide antistoffen hele andere factoren een rol in de pathogenese, dat zou ook verklaren waarom de kliniek zo verschilt tussen deze twee antistoffen. Los van deze onderzoeksvraag, bevestigden we ook in deze studie dat ATA positieve patiënten vaker een ernstigere mate van microangiopathie laten zien, en ernstige microangiopathie ook geassocieerd is met orgaanbetrokkenheid. We weten nu dat zowel de antistoffen als de mate van microangiopathie gebruikt kunnen worden voor het stellen van de diagnose en om iets meer over de prognose te kunnen zeggen van patiënten. Op dit moment blijft het echter nog moeilijk om het ziektebeloop van patiënten te voorspellen. Het liefst zouden we patiënten zo vroeg mogelijk diagnosticeren en het ziektebeloop bepalen zodat er ook zo vroeg mogelijk gestart kan worden met de gewenste en meest effectieve behandeling. Uit eerder onderzoek is gebleken dat de SSc specifieke antistoffen al vroeg in het ziekteproces aantoonbaar zijn, echter is er nooit gekeken of de isotype levels van de antistoffen ook iets zeggen over de ziekte-ernst of over het beloop van de ziekte. In enkele studies is er gekeken naar ACA isotypes waarbij er wel aanwezigheid van ACA-IgG, ACA-IgM en ACA-IgA wordt aangetoond, maar er is nooit goed gekeken naar een associatie met de kliniek. Enkel bij ATA positieve patiënten zijn er enkele studies verricht waarbij er ook associaties gevonden worden tussen ATA-IgG, ATA-IgM levels en de huidscore en PAH. In **hoofdstuk 9** hebben we gekeken naar de ACA isotype reactie in SSc patiënten. We hebben ACA-IgG, ACA-IgM en ACA-IgA gemeten in patiënten met definitieve SSc en in patiënten die aan de very early SSc criteria voldoen. Patiënten die aan de very early SSc criteria voldoen hebben het Raynaud fenomeen EN ACA EN een afwijkende nagelriemscopie OF puffy fingers. Om te beginnen zagen wij dat de ACA isotype levels inderdaad geassocieerd zijn met de ziekte ernst, waarbij we zagen dat patiënten met definitieve SSc en met orgaan betrokkenheid hogere isotype levels hadden in vergelijking met de andere patiënten. Daarnaast zagen we ook dat de definitieve SSc patiënten met progressie gedurende hun ziektebeloop hogere levels hadden in vergelijking met definitieve SSc patiënten zonder progressie. In de very early SSc groep ontwikkelde 39% definitieve SSc in de loop van de tijd en juist deze patiënten lieten ook hogere ACA-IgG levels tijdens hun eerste bezoek zien. Deze bevindingen laten zien dat de antistof response mogelijk wel meespeelt in de pathogenese van SSc en dat het wellicht in de toekomst als biomarker gebruikt kan worden om patiënten die progressie zullen vertonen eerder te identificeren.

TOEKOMSTPERSPECTIEVEN

De bestaande zorgpaden voor SSc patiënten zouden zich in de toekomst nog meer kunnen richten op zelfredzaamheid en zouden meer strategieën kunnen ontwikkelen om patiënten te helpen met coping mechanismen. De multidisciplinaire zorg blijft enorm belangrijk, maar ook hier is nog ruimte voor verbetering waarbij de samenwerking tussen de specialisten en de paramedische zorg nog beter op elkaar afgestemd kan worden. We hopen dat er ergens in de komende jaren een internationale 'evidence based' guideline voor SSc patiënten wordt ontwikkeld die ons leert wat voor onderzoeken en hoeveel bezoeken een bepaalde patiënt met de aan- of afwezigheid van bepaalde biomarkers moet doorlopen per jaar. In een ideale situatie lukt het ons dan ook om patiënten in een bepaalde risico groep te classificeren, zodat we de zorg individueler kunnen inrichten. Ondanks de vooruitgang die de afgelopen jaren is geboekt, is er nog steeds een gebrek aan begrip van de pathogenese van SSc. De belangrijkste pijlers zijn al langere tijd geïdentificeerd maar de interactie tussen deze pijlers moet nog grondiger bestudeerd worden. Pas als we dit beter begrijpen, kunnen we ook gerichtere therapeutische studies opzetten. Daarnaast zou de definitie van ziekte activiteit en ziekte progressie uniformer moeten worden vast gesteld zodat deze definities in alle internationale studies aangehouden kunnen worden. Hierdoor kunnen we studies en cohorten beter vergelijken met elkaar.

TOT SLOT

De studies in dit proefschrift hebben geleid tot meer inzicht over de impact van SSc op gezondheidsuitkomsten en over ziektepercepties. Daarnaast hebben we meer inzicht gekregen in het ziektebeloop en de frequentie van ziekteprogressie, de rol van de SSc specifieke antistoffen en de mate van microangiopathie. Het is belangrijk de patiënt centraal te houden waarbij we moeten kijken naar de meest invaliderende manifestaties voor de patiënt (deel 1). Door de uitkomsten van deze onderzoeken mee te nemen in nieuwe trials kunnen we wellicht de kwaliteit van leven verbeteren waarbij we zowel de ziekte percepties als het ziekte beloop verbeteren. Het herkennen en identificeren van gastro-intestinale klachten, huidverstrakking en Raynaud fenomeen, een vroege diagnose, en een beter voorspel model van het ziektebeloop zullen hierbij een belangrijke rol spelen (deel 1 en 2). De eerste twee jaar na het krijgen van een diagnose zullen patiënten jaarlijks gescreend moeten worden op alle orgaan systemen gezien de heterogeniteit van de ziekte. Met het ontwikkelde voorspelmodel kan een arts daarna evalueren in welke classificatie groep de patiënt past (laag, medium of hoog risico op progressie). Op basis hiervan zou een deel van de patiënten minder intensief vervolgd kunnen worden (deel 2). Van belang hierbij is om ook naar de ziekte percepties van de individuele patiënt te kijken (deel 1). We hebben gezien dat de mate van microangiopathie en de antistoffen belangrijke rol spelen in ziekte stratificatie (deel 3). De SSc specifieke isotype levels en de mate van microangiopathie zijn geassocieerd met ziekte ernst en mogelijk ook met ziekte progressie. Voordat we dit echt in de kliniek kunnen toepassen moeten er nog een grote validatie studie plaatsvinden waar er gekeken worden naar de SSc specifieke isotypes en de mate van microangiopathie over de tijd. Hopelijk wordt dit binnen enkele jaren bereikt.

Appendix

Curriculum Vitae

CURRICULUM VITAE

Nina van Leeuwen werd geboren op 18 november 1991 in Wormerveer. Vanaf jongs af aan wist zij al dat ze als arts wilde gaan werken en in 2009 besloot zij vrijwilligerswerk in India te doen gedurende de zomervakantie in onder andere een lepra kliniek. In 2010 behaalde zij haar VWO diploma aan het Dongemond College in Raamsdonksveer waarna zij startte met de studie geneeskunde aan de Vrije Universiteit in Amsterdam. Tijdens haar studie was ze een aantal jaren als student-lid verbonden aan de medische faculteit studievereniging (MFVU). Voor aanvang van de coschappen in 2014-2015 besloot Nina om haar wetenschappelijke stage in Melbourne, Australië te verrichten op het gebied van cerebrale parese. Na het behalen van het artsexamen in oktober 2016 ging zij in december 2016 als arts-assistent aan de slag op de afdeling Interne Geneeskunde in het Groene Hart Ziekenhuis, Gouda. Deze periode heeft zij als ontzettend leerzaam ervaren, maar het onderzoek begon toch ook te trekken. In Maart 2018 kwam er een Phd plek vrij onder leiding van Jeska de Vries-Bouwstra op het gebied van systemische sclerose. Gedurende deze tijd heeft Nina zich ook ingezet als lid van De Jonge Dokter, een organisatie waarin een groep jonge dokters het onbespreekbare bespreekbaar probeert te maken door onder andere het organiseren van verschillende events; foutenfestival, geldzorgen, E-health, en peer support sessies.

Per december 2020 is zij aangenomen als AIOS reumatologie in het Erasmus Medisch Centrum (opleider dr. Dolhain). Onderdeel hiervan is een stage Interne Geneeskunde in het Sint Franciscus Ziekenhuis (opleider dr. Schrama), welke reeds gestart is.

OTHER PUBLICATIONS OUTSIDE OF THIS THESIS

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Appendix

Woord van dank

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