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Original article

Predicting cardiopulmonary involvement in patients with systemic sclerosis: complementary value of nailfold videocapillaroscopy patterns and disease-specific autoantibodies

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Abstract

Objective. To evaluate the prevalence of anti–extractable nuclear antigen (anti-ENA) antibodies in Dutch SSc patients and the predictive power of the combination of specific anti-ENA antibodies and nailfold videocapillaroscopy (NVC) patterns to improve identification of patients with high risk for cardiopulmonary involvement.

Methods. A total of 287 patients (79%) from the Leiden SSc-Cohort had data available on NVC-pattern (no SSc-specific, early, active, late) and anti-ENA antibodies. Associations between anti-ENA/NVC combinations with cardiopulmonary parameters were explored using logistic regression.

Results. Prevalence of ACA was 37%, anti-Scl-70 24%, anti-RNP 9%, anti-RNAPIII 5%, anti-fibrillarin 4%, anti-Pm/Scl 3%, anti-Th/To 0.3% and anti-Ku 1.4%. NVC showed a SSc-specific pattern in 88%: 10% early, 42% active and 36% late. The prevalence of different NVC patterns was equally distributed among specific anti-ENA antibodies, except for the absence of early pattern in anti-RNP positive patients. Fifty-one percent had interstitial lung disease (ILD), 59% had decreased diffusion capacity for carbon monoxide and 16% systolic pulmonary artery pressure >35 mmHg (sPAP↑). Regardless of ENA-subtype, NVC-pattern showed a stable association with presence of ILD or sPAP↑. For ILD, the odds ratios (ORs) were 1.3–1.4 ($P < 0.05$ for analyses with anti-RNAPIII, anti-RNP). For diffusion capacity for carbon monoxide, the OR was 1.5 ($P < 0.05$ for analyses with ACA, anti-Scl-70, anti-RNAPIII, anti-RNP). For sPAP↑, the ORs were 2.2–2.4 ($P < 0.05$ for analyses with anti-RNAPIII, anti-RNP).

Conclusion. In Dutch SSc patients, all SSc-specific auto-antibodies were found, with ACA and anti-Scl-70 being the most prevalent. Strikingly, the association between NVC-pattern and heart/lung involvement was independent of specific anti-ENA antibodies, which might indicate microangiopathy is an important cause of organ involvement.

Key words: systemic sclerosis, autoantibodies, anti-ENA, nailfold videocapillaroscopy, cardiopulmonary involvement, screening

Rheumatology key messages

- In our Dutch population, the most prevalent SSc-specific auto-antibodies were ACA and anti-Scl-70.
- Independent of anti-extractable nuclear antigen antibodies, nailfold videocapillaroscopy pattern can predict cardiopulmonary involvement in SSc.
- Our results indicate microangiopathy might be an important cause of organ involvement in SSc.

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Introduction

SSc can affect multiple internal organs of e.g. the cardiopulmonary, gastrointestinal and vascular systems. The extent and severity of cardiopulmonary involvement notably determines the prognosis of the patient [1]. In order to map the extent of the organ manifestations and to detect progression in an early stage, regular screening is needed [2]. The variability of the cardiopulmonary involvement requires extensive screening, resulting in considerable costs and burden for the patient. Therefore, it is desirable to distinguish subgroups of patients that have a high risk for specific cardiopulmonary involvement.

Specific autoantibodies to extractable nuclear antigens (ENAs; anti-ENA antibodies) are associated with specific clinical features and prognosis, although the pathophysiological role is still unknown [3, 4]. Three anti-ENA antibodies are currently used for the classification of the disease: ACA, anti-topo I (anti-Scl-70) and anti-ribonucleic acid polymerase III (anti-RNAPIII) [5]. ACA is associated with limited cutaneous involvement, gastrointestinal involvement and pulmonary arterial hypertension (PAH), anti-Scl-70 with diffuse cutaneous involvement and interstitial lung disease (ILD) and anti-RNAPIII with diffuse cutaneous involvement and renal crisis [3, 4].

With nailfold videocapillaroscopy (NVC), the morphology of the nailfold dermal papillary vessels can be evaluated [6]. Specific features seen by NVC are associated with microvascular heart involvement [7], severe peripheral vascular disease [8, 9] and lung involvement [10–12]. Moreover, they are related to the ANA staining pattern [13], as well as disease activity [14]. Another study showed more severe nailfold capillaroscopy patterns to be able to predict future severe cardiopulmonary involvement [15].

Early NVC changes representing microangiopathy in SSc are suggested to be caused by endothelial cell activation, inflammation and production of pro-angiogenic factors, followed by vascular regression and angiostasis [16]. This eventually results in loss of capillaries. A severe NVC pattern is associated with worse disease outcome [14]. If a joint pathophysiological mechanism for both microangiopathy and autoantibody production exists, more severe NVC patterns would be determined in patients with autoantibodies (such as anti-Scl-70 and anti-RNAPIII) that are associated with more severe disease. On the other hand, if specific autoantibodies and stage of microangiopathy reflect different processes in the disease, a combination of autoantibody status and NVC could be helpful for identifying patients at highest risk for cardiopulmonary involvement.

In this study, we describe the prevalence of ANAs, a broad panel of SSc-related ENAs and NVC patterns in a Dutch cohort of SSc patients. We evaluated the prevalence of autoantibodies at different stages of microvasculopathy. Furthermore, we set out to determine whether the combination of specific autoantibodies and NVC patterns contribute to a better discrimination of patients at risk for cardiopulmonary involvement, in order to study the role of microangiopathy in the pathophysiology of SSc.

Methods

Study design and patients

For this cross-sectional analysis, data from the Leiden SSc Cohort were used. All SSc patients who are referred to, or diagnosed at, the Rheumatology Department of the Leiden University Medical Center are invited for a 2-day multidisciplinary health care programme. The cohort represents a large region in the South-West of the Netherlands. This programme aims to standardize care for SSc patients, classified according to the 2013 ACR criteria for SSc or the LeRoy criteria [5, 17], and to systemically screen for organ involvement.

A total of 365 patients consecutively recruited from 2009 to 2014 were included for this analysis. The study design was in accordance with the ethics principles of the Declaration of Helsinki and was approved by the Medical Ethics Committee of the Leiden University Medical Center. All patients gave written informed consent.

Study endpoints

At baseline, extensive autoantibody testing was performed. In all patients, ANA (detected by indirect immunofluorescence on HEP-2000 cells, evaluated by two assessors, and an adjudicator in case of disagreement) and ENA screening [measured by fluorescence enzyme-linked immuno sorbent assay, using a Phadia250 system (Thermo Fisher Scientific, Nieuwegein, The Netherlands)] was performed. ENA screening included screening for ACA, anti-Scl-70, anti-U1 RNP (anti-U1RNP), anti-RNP 70 (anti-RNP70) and three other autoantibodies not specific for SSc. For the current analysis, anti-U1RNP and anti-RNP70 were combined as anti-RNP, because they are largely overlapping. If a patient was both ANA and ENA negative, the testing was repeated. If a patient was ANA positive, but no SSc-related autoantibody was detected, further testing using Phadia250® for anti-RNAPIII, anti-fibrillarin (anti-U3RNP) and anti-Pm/Scl antibodies (PM/scleroderma overlap) was performed. Additionally in all patients, antibodies to Th/To RNP (anti-Th/To) and anti-Ku antibodies were determined by a research chemiluminescence immuno assay using the INOVA BioFlash (Werfen/INOVA, San Diego, USA). Since it is known that the autoantibody profile rarely changes over time, no sequential autoantibody testing was undertaken [18–20]. This may not be the situation for patients who have previously undergone an autologous haematopoietic stem cell transplantation, so patients with a previous haematopoietic stem cell transplantation (or with a revised diagnosis) were excluded from this analysis.

NVC to visualize the morphology of the nailfold capillaries could not be performed in all patients at baseline for logistic reasons [6, 7]. Therefore, the first available NVC result was used for this analysis, and following the cross-sectional design, clinical data from the same visit were selected. Three NVC patterns specific for SSc have been described: early, active and late [6, 7, 21]. The early pattern consists of a relatively normal distribution of capillaries with few giant capillaries, few capillary

haemorrhages and limited loss of capillaries. The active pattern entails a mildly disturbed architecture of capillaries, with giant capillaries, capillary haemorrhages and moderate loss of capillaries with neo-angiogenesis (ramified capillaries). The late pattern involves total loss of capillary architecture, neo-angiogenesis, irregularly enlarged capillaries, few or no giant capillaries and capillary haemorrhages.

Digital tip ulcers, puffy fingers, telangiectasia and proximal muscle weakness were assessed during an extensive physical examination, which also included the modified Rodnan skin score [22]. ILD was defined based on assessment of a high-resolution CT of the thorax (HRCT-thorax). Diffusing capacity of the lung for carbon monoxide (DLCO) was determined with a pulmonary function test. Decreased DLCO was defined as $< 70\%$ of predicted (based on age, gender and height). During a cardiopulmonary exercise test, the maximum oxygen uptake (peak VO_2) was measured, denoting the exercise capacity based on pulmonary and cardiac function. Decrease in maximum oxygen uptake (peak $VO_2 < 75\%$ of predicted) is suggested to be an early reflection of PAH [23]. All patients also underwent a transthoracic echocardiography, where the systolic pulmonary artery pressure (sPAP) was estimated, for which a cut-off of 35 mmHg was used [24]. Although right heart catheterization is the gold standard for assessing PAH, it is recommended by the guidelines to start with echocardiography [25]. If the echocardiography result is not suggestive for PAH, an invasive catheterization can be omitted. For N-terminal pro-brain natriuretic peptide (NT pro-BNP), 300 pg/ml was used as the cut-off [26, 27].

Statistical analysis

The prevalence of ENA, NVC patterns and clinical features were described using numbers and percentages.

Test characteristics of NVC were examined by calculating the sensitivity, specificity, positive predicted value (PPV) and negative predicted value (NPV) for each clinical outcome under study. Patients with no SSc-specific or an early pattern were classified as having a negative test result; patients with an active or late pattern were classified as having a positive test result. The NPV for the NVC pattern was considered as the true negative result, that is, the number of patients with no SSc-specific or an early NVC pattern and no clinical feature, as a proportion of all patients with no SSc-specific or an early NVC pattern. No SSc-specific pattern and an early pattern were taken together as one group due to the low number of patients in the first group (resulting in limited power).

The additive value of the NVC pattern in addition to the presence of a specific autoantibody was examined with a logistic regression analysis. The most prevalent autoantibodies were selected for this analysis (ACA, anti-Scl-70, anti-RNP and anti-RNAPIII); the others were excluded due to lack of power. First, univariate logistic regression analyses were performed, with cardiac and lung parameters as dependent variables, and ACA, anti-Scl-70, anti-RNP, anti-RNAPIII, NVC pattern, age, disease duration and use of vasoactive or immunosuppressive medication as independent variables (one by one). These analyses show the raw and unadjusted effect estimates of each determinant on the various outcomes under study. Under the assumption of an exponential relationship within NVC patterns from mild to severe [15], NVC pattern was entered as a continuous variable in the following order: no specific SSc pattern, early pattern, active pattern and late pattern. The selected clinical outcomes under study were based on additional examinations (i.e. not physical examination), focusing on heart and lung parameters, adding burden for the patient and costs for the system. Then, multivariate logistic regression analyses were performed, stratified for each autoantibody and iterated for each clinical outcome under study. Autoantibody and NVC pattern were entered as independent variables, and the clinical feature as a dependent variable. All analyses were adjusted for age, disease duration and the use of immunosuppressive and/or vasoactive drugs. Logistic regression analyses were repeated for other classifications of NVC; no SSc-specific/early pattern compared with active/late pattern; and no SSc-specific pattern compared with early/active/late pattern.

Results

Prevalence

Complete data on autoantibody profile and NVC pattern were available for 287/365 patients (79%). The patients had a mean (S.D.) age of 54 (14), a median (interquartile range) disease duration of 3 (0.6–9) years, and 234/287 patients (82%) were female. The vast majority, 226/287 patients (79%) were both ANA and ENA positive, 42 patients (15%) were ANA positive without a specific nuclear antibody, and 17 patients (6%) were ANA and ENA negative. The prevalence of ACA was 37% ($n = 107$), of anti-Scl-70 24% ($n = 69$), of anti-RNP 9% ($n = 26$), of anti-RNAPIII 5% ($n = 13$), of anti-fibrillarin 4% ($n = 12$), of anti-Pm/Scl 3% ($n = 9$), of anti-Th/To 0.3% ($n = 1$) and of anti-Ku 1.4% ($n = 4$). Two patients were ANA negative but ENA positive, both for anti-Scl-70. Table 1 shows the prevalence and clinical features per autoantibody status. Of 287 patients, 29 (10%) had an early NVC pattern, 121 (42%) an active pattern, and 103 (36%) had a late pattern. The remaining 34 patients (12%) had no SSc-specific NVC pattern (Fig. 1). Table 2 gives details on the distribution of autoantibodies among the various NVC patterns, and Fig. 1 on the distribution of NVC patterns among the autoantibodies. Patients with anti-RNP and anti-Pm/Scl antibodies seemed to more often have an active or late pattern than an early pattern. This difference was, however, not statistically significant ($P > 0.10$ for either comparison), probably due to small numbers.

Predictive value

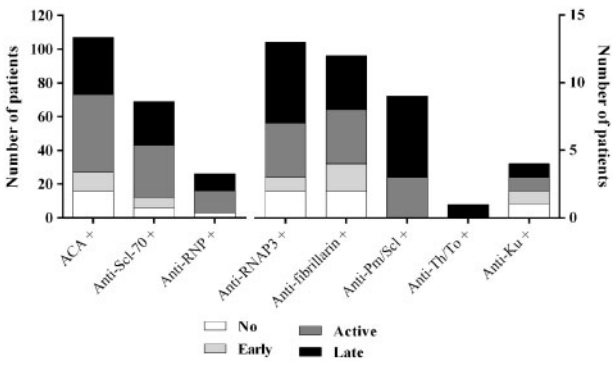
More severe NVC patterns were associated with a higher risk of cardiopulmonary involvement, independent of the presence of a specific autoantibody. Indeed, in ACA-

Table 1 Characteristics of patients with positive tests for SSc-specific autoantibodies

Characteristic	ACA+	Anti-Scl-70 +	Anti-RNP +	Anti-RNAPIII +	Anti-fibrillarin +	Anti-Pm/Scl +	Anti-Th/To +	Anti-Ku +
	n = 107	*n* = 69	*n* = 26	*n* = 13	*n* = 12	*n* = 9	*n* = 1	*n* = 4
Prevalence	37.3	24.0	9.1	4.5	4.2	3.1	0.3	1.4
Age, mean (S.D.)	57 (14)	50 (14)	50 (12)	68 (7)	51 (15)	54 (19)	60[a]	41 (10)
Female, *n* (%)	97 (91)	50 (73)	20	13	9	8	1	4
Caucasian, *n* (%)[a]	77 (83)	38 (64)	15	10	6	6	1	1
Disease duration, median (IQR), years	2 (0.5–6)	3 (0.6–11)	5 (1–12)	4 (2–12)	2 (0.2–11)	1 (0.3–10)	8[a]	5 (1–17)
Type of SSc, *n* (%)								
lcSSc	72 (67)	28 (41)	20	7	6	4	1	3
dcSSc	3 (3)	37 (54)	4	4	5	2	0	1
lSSc	32 (30)	4 (6)	2	1	1	3	0	0
Unclassified	0	0	0	1	0	0	0	0
Medication, *n* (%)								
Vasoactive	22 (21)	15 (22)	8	6	2	2	0	0
Immunosuppressive	9 (8)	16 (23)	4	3	3	2	0	1
Skin								
mRSS, median (IQR)	2 (0–6)	5 (3–9)	4 (2–5)	4 (3–12)	8 (1–9)	4 (2–5)	2[a]	3 (2–12)
Puffy fingers, *n* (%)[a]	17 (20)	9 (16)	3	1	1	1	0	0
Vascular, *n* (%)								
RP	101 (96)	66 (97)	25	13	12	9	1	4
Digital tip ulcers	30 (29)	18 (27)	8	6	2	2	0	1
Telangiectasias[a]	69 (66)	31 (46)	13	10	9	6	1	3
Lung, *n* (%)								
ILD	24 (22)	56 (81)	15	8	5	7	1	2
DLCO <70% of predicted	50 (47)	45 (70)	20	8	5	4	1	2
Muscles, *n* (%)								
Proximal muscle weakness[a]	4 (4)	4 (6)	3	2	4	2	0	1
Increased CPK	7 (7)	16 (23)	1	2	2	3	0	0
Heart								
NT pro-BNP, median (IQR)	88 (56–150)	87 (48–186)	152 (60–333)	192 (65–601)	136 (53–264)	111 (42–401)	455[a]	92 (52–700)
Increased NT pro-BNP, *n* (%)	9 (9)	15 (22)	7	4	2	3	1	1

Only antibody-positive patients were reported, as coexistence of two specific autoantibodies is rare. [a]Some data were missing on these clinical features; never >8.6%, generally <3%. CPK: creatine phosphokinase: cut-off used to define 'increased' was 145 U/l; DLCO: diffusing capacity of the lungs for carbon monoxide; ILD: interstitial lung disease; lSSc: limited subset of SSc; mRSS: modified Rodnan skin score; NT pro-BNP: N-terminal pro-brain natriuretic peptide: cut-off used to define increased was 300 pg/ml.

Fig. 1 Prevalence of SSc-specific autoantibodies, and the distribution of nailfold videocapillaroscopy patterns among the autoantibodies



The left and right *y*-axes denote different ranges to depict the distribution of nailfold videocapillaroscopy patterns among frequent and rare autoantibodies. The break in the *x*-axis shows which frequencies should be read from which *y*-axis. Anti-Ku: antibodies directed against the Ku complex; anti-Pm/Scl: antibodies in PM/scleroderma overlap; anti-RNAPIII: anti-ribonucleic acid polymerase III; anti-Scl-70: anti-topo I; anti-Th/To: antibodies directed against Th/To RNP.

Table 2 Prevalence of SSc-specific autoantibodies, and the distribution of nailfold videocapillaroscopy patterns among the autoantibodies

Autoantibody	Total population *n* = 287	Nailfold videocapillaroscopy pattern			
		No *n* = 34	Early *n* = 29	Active *n* = 121	Late *n* = 103
ACA +	107 (37)	16 (47)	11 (38)	46 (38)	34 (33)
Anti-Scl-70 +	69 (24)	6 (18)	6 (21)	31 (26)	26 (25)
Anti-RNP +	26 (9)	3 (9)	0	13 (11)	10 (10)
Anti-RNAPIII +	13 (5)	2 (6)	1 (3)	4 (3)	6 (6)
Anti-fibrillarin +	12 (4)	2 (6)	2 (7)	4 (3)	4 (4)
Anti-Pm/Scl +	9 (3)	0	0	3 (2)	6 (6)
Anti-Th/To +	1 (0.3)	0	0	0	1 (1)
Anti-Ku +	4 (1.3)	1 (3)	1 (3)	1 (1)	1 (1)

Numbers indicate the number (%) of patients. anti-Ku: antibodies directed against the Ku complex; anti-Pm/Scl: antibodies in PM/scleroderma overlap; anti-RNAPIII: anti-ribonucleic acid polymerase III; anti-Scl-70: anti-topo I; anti-Th/To: antibodies directed against Th/To RNP.

Table 3 Results of multivariate logistic regression analyses

Predictor	ILD OR (95% CI)	DLCO ↓ OR (95% CI)	Peak VO_2 ↓ OR (95% CI)	NT pro-BNP ↑ OR (95% CI)	sPAP ↑ OR (95% CI)
ACA +	0.12 (0.07, 0.22)	0.44 (0.26, 0.75)	0.73 (0.42, 1.27)	0.27 (0.12, 0.63)	0.39 (0.12, 1.29)
NVC pattern	1.33 (0.99, 1.77)	1.52 (1.16, 2.00)	1.57 (1.16, 2.11)	1.68 (1.08, 2.62)	2.31 (0.998, 5.36)
Anti-Scl-70 +	6.66 (3.31, 13.37)	1.96 (1.04, 3.71)	0.83 (0.44, 1.54)	2.46 (1.13, 5.39)	2.12 (0.62, 7.27)
NVC pattern	1.33 (1.01, 1.75)	1.52 (1.16, 1.98)	1.60 (1.19, 2.16)	1.63 (1.05, 2.53)	2.24 (0.99, 5.07)
Anti-RNAPIII +	1.38 (0.40, 4.73)	0.82 (0.23- 2.85)	0.29 (0.06, 1.48)	1.10 (0.28, 4.33)	1.21 (0.19, 7.72)
NVC pattern	1.35 (1.04, 1.74)	1.53 (1.17, 1.99)	1.58 (1.17, 2.14)	1.68 (1.09, 2.60)	2.40 (1.05, 5.53)
Anti-RNP +	1.12 (0.47, 2.64)	2.33 (0.88, 6.21)	1.71 (0.62, 4.75)	3.52 (1.24, 10.01)	2.22 (0.42, 11.84)
NVC pattern	1.34 (1.04, 1.74)	1.52 (1.17, 1.98)	1.58 (1.17, 2.13)	1.70 (1.09, 2.64)	2.33 (1.01, 5.39)

All analyses were adjusted for age, disease duration and the use of vasoactive and/or immunosuppressive drugs. The NVC pattern was entered as a continuous independent variable, in order: no SSc-specific pattern, early, active, late pattern. Since a multivariate model was used, the effect estimate for the autoantibody is independent of the NVC pattern, and the effect estimate for the NVC pattern is independent of the autoantibody. anti-RNAPIII: anti-ribonucleic acid polymerase III; anti-Scl-70: anti-topoisomerase I; DLCO: diffusing capacity of the lungs for carbon monoxide measured with a pulmonary function test: DLCO <70% of predicted was considered impaired; ILD: interstitial lung disease: assessed on a high-resolution CT of the thorax; NVC: nailfold videocapillaroscopy; NT pro-BNP: N-terminal pro-brain natriuretic peptide: higher than 300 pg/ml was considered abnormal; OR: odds ratio; sPAP: systolic pulmonary artery pressure: estimated using transthoracic echocardiography: sPAP >35 mmHg was considered increased; peak VO_2: maximum oxygen uptake: measured during a cardiopulmonary exercise test: peak VO_2 <75% of predicted was considered impaired.

positive patients, among whom prevalence of impaired DLCO is lower, the risk for impaired DLCO increased significantly with more severe NVC patterns (OR = 1.52, 95% CI: 1.16, 2.00). Regarding anti-RNP positivity, there was only a higher risk for increased NT pro-BNP. However, if these patients also had a more severe NVC pattern, the risk for ILD (OR = 1.34, 95% CI: 1.04, 1.74), impaired DLCO (OR = 1.52, 95% CI: 1.17, 1.98) and reduced peak VO_2 (OR = 1.58, 95% CI: 1.17, 2.13), and increased sPAP was raised (OR = 2.33, 95% CI: 1.01, 5.39), in addition to an even higher risk for increased NT pro-BNP (OR = 1.70, 95% CI: 1.09, 2.64). Supplementary Table 1 shows the results of the univariate regression analyses, and Table 3 shows the key finding of this study: the results of the stratified logistic regression analyses. These analyses were repeated for other classifications of NVC, resulting in similar effect estimates and no increase of power (relying on the range of the 95% CIs) (data not shown). The NVC pattern was also associated with the presence of digital ulcers as defined during physical examination (OR = 1.4 in patients who were anti-Scl-70, anti-RNAPIII or anti-RNP positive; OR = 1.5 in patients who were ACA positive, all $P < 0.05$).

Table 4 Test performance of nailfold videocapillaroscopy for cardiopulmonary involvement

	Prevalence, *n/N* (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ILD	147/287 (51)	84	28	55	62
DLCO ↓	165/280 (59)	85	32	64	60
Peak VO_2 ↓	99/256 (39)	88	28	44	79
NT pro-BNP ↑	45/286 (17)	91	24	18	94
sPAP ↑	21/133 (16)	100	21	19	100

DLCO: diffusing capacity of the lungs for carbon monoxide measured with a pulmonary function test: DLCO <70% of predicted was considered impaired; ILD: interstitial lung disease: assessed on a high-resolution CT of the thorax; NPV: negative predicted value; NT pro-BNP: N-terminal pro-brain natriuretic peptide: higher than 300 pg/ml was considered abnormal; sPAP: systolic pulmonary artery pressure: estimated using transthoracic echocardiography: sPAP >35 mmHg was considered increased; peak VO_2: maximum oxygen uptake measured during a cardiopulmonary exercise test: peak VO_2 <75% of predicted was considered impaired; PPV: positive predicted value.

To calculate the NPV, only NVC patterns were taken into account (no SSc-specific and early pattern *vs* active and late pattern), not the autoantibody status. For ILD and a DLCO <70% of predicted, the NPV were disappointing: 62 and 60%, respectively. For decreased peak VO_2, the NPV was 79%; for increased NT pro-BNP it was 94% and for increased sPAP it was 100%. In Table 4, the sensitivity, specificity and PPV are summarized.

Discussion

In this study, we evaluated the prevalence of the main SSc-specific autoantibodies in a Dutch SSc cohort as well as the predictive value of the combination of SSc-specific autoantibodies and NVC patterns for determining patients at risk for severe cardiopulmonary involvement.

Remarkably, a comparable proportion of patients with an active or late NVC pattern was observed in anti-Scl-70–positive patients, as was observed in patients who were ACA, anti-RNP or anti-RNAPIII positive. This suggests that presence of a specific autoantibody is independent of the development of microangiopathy. As expected, ACA was associated with a decreased risk of abnormal lung and heart parameters, while patients with anti-Scl-70 had an increased risk. However, more severe NVC patterns are independently associated with cardiac and pulmonary involvement. Furthermore, in specific situations, severe NVC patterns reveal an increased risk for cardiopulmonary manifestations, even where based on the autoantibody status a low risk would be expected. Therefore, the combination of both contributes to the identification of patients at highest risk for organ manifestations.

When combining the predictive value of both NVC and SSc-specific autoantibodies, encouraging results were generated. For example, prevalence of ILD is generally lower among ACA-positive patients. According to our data, even among ACA-positive patients there was a trend for more ILD being associated with more severe NVC patterns (OR = 1.33). Based on anti-RNP and anti-RNAPIII positivity, patients did not have an increased risk of a sPAP >35 mmHg; however, with a severe NVC pattern, this risk was significantly increased (OR = 2.33). Patients with anti-RNAPIII positivity were at higher risk for digital ulcers, and NVC patterns independently augmented this risk (OR = 1.4). Nonetheless, in some situations, autoantibody profile seems to represent a higher risk for the outcome than NVC pattern. For example, anti-Scl-70 is a stronger predictor (OR = 6.66) than NVC pattern (OR = 1.33) for ILD.

The clinical features and autoantibody profile of patients with various NVC patterns have been described before [28]. The previous finding, that patients with more severe NVC patterns have more severe disease, is confirmed by our results. However, our study is novel in focusing on the additive value of NVC patterns for each of the different SSc-specific anti-ENA antibodies, and also by calculating ORs, rather than denoting percentages.

In addition to the clinical implications, our data suggest that different mechanisms are responsible for causing breaking of tolerance (resulting in autoantibody production) and microvascular damage (resulting in abnormal NVC patterns) in SSc. Therefore, a different pathophysiology for autoimmunity and vasculopathy might exist. Thus, we consider autoantibody detection and NVC as complementary examinations for screening for current cardiopulmonary involvement.

We also examined the prevalence of a broad panel of anti-ENA antibodies in relation to various NVC patterns in a Dutch cohort. In this cohort, ACA (37%) and anti-Scl-70 autoantibodies (24%) are the most prevalent, followed by anti-RNP antibodies (9%). Other autoantibodies, such as anti-RNA polymerase III, anti-fibrillarin, anti-Pm/Scl, anti-Th/To and anti-Ku, are rare (⩽5%). Clinical phenotype for each autoantibody was comparable to previous reports with diffuse cutaneous SSc and ILD being more frequent among anti-Scl-70 positive patients. Similar distributions of NVC patterns among the autoantibodies were demonstrated.

Despite the presence of ANA in almost all SSc patients, the prevalence of ENA varies between different cohorts.

This study is the first to describe the prevalence of ENA in a Dutch cohort (representing the South-West of the country). The prevalence of ACA and anti-Scl-70 are comparable with the prevalences described in other countries [3, 29–31]. However, anti-RNP is more prevalent in the Netherlands than in for example Belgium and Germany, in contrast to the relatively low prevalence of anti-RNAPIII that we found compared with Belgium and Australia [29–31].

In our study, active and late NVC patterns were more prevalent than the early pattern or no SSc-specific results, which could be related to the mean/median disease duration of 6/3 years at inclusion. Although patients with anti-Scl-70 are generally considered to have a more severe disease [3], this could not be reasserted with more severe NVC patterns in our study. Anti-RNP and anti-Pm/Scl autoantibodies seemed to be less often present among patients with an early or no SSc-specific pattern than among patients with an active or late pattern. Probably as a result of the few patients in these categories, these differences did not reach statistical significance.

SSc is a heterogeneous disorder. Including only patients with early SSc would have led to more homogeneity, with a higher proportion of patients having an early NVC pattern. This subgroup of patients was rather small in our population, resulting in limited power to detect statistically significant differences. On the other hand, the heterogeneity of our population might have been beneficial for the generalizability of our results. As regular organ screening is advocated, including in patients with long-standing disease, cross-sectional association with presence of cardiopulmonary involvement is relevant in this population. Besides the clinical outcomes under study, which already represent an important subset of cardiopulmonary involvement, it would be interesting to examine heart and lung involvement in more detail, for example, to measure sPAP during heart catheterization. In addition, abnormalities in the cardiac parameters used in this study might be secondary to pulmonary involvement, rather than present as primary cardiac involvement. This should be distinguished in future analyses, and data on left ventricular ejection fraction should also be taken into account (this data was not available at the time of this study). Outcomes such as gastrointestinal involvement and renal crisis were omitted from this analysis because for gastrointestinal involvement no standard diagnostic testing was performed, and for renal crisis numbers were low. However, in all patients a HRCT of the thorax was performed, regardless of risk factors present for lung involvement. This makes the outcomes based on the HRCT less affected by bias. Obviously, our analyses should be repeated in a larger cohort of patients, to confirm our findings. In addition, previous studies have shown significant associations between cardiopulmonary involvement and specific quantitative NVC features, such as the number of giant capillaries and the extent of loss of capillaries [11, 12]. Future studies evaluating the association between quantitative NVC findings and organ involvement could further add value to the prognostic contribution of NVC in SSc.

In conclusion, the prevalence of ACA and anti-Scl-70 in our Dutch cohort was similar to other international populations, the frequency of anti-RNP was relatively high and of anti-RNAPIII relatively low compared with previous reports. In addition we showed that different NVC patterns are equally distributed among the different autoantibodies. Although specific anti-ENA antibodies and NVC patterns are well-known predictors for disease course in SSc, this study is novel in showing that the combination of both contributes more in identifying patients at risk for organ manifestations than one of these alone. This observation might indicate that not only breaking tolerance (with autoantibody production as a result), but also microvascular damage (as reflected by NVC patterns) are relevant in the pathophysiology of SSc.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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