




Autoantibodies associated with systemic sclerosis in three autoimmune diseases imprinted by type I interferon gene dysregulation: a comparison across SLE, primary Sjögren's syndrome and systemic sclerosis

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ABSTRACT

Objective SLE, primary Sjögren's syndrome (pSS) and systemic sclerosis (SSc) are heterogeneous autoimmune diseases with a dysregulated type I interferon (IFN) system. The diseases often show overlapping clinical manifestations, which may result in diagnostic challenges. We asked to which extent SSc-associated autoantibodies are present in SLE and pSS, and whether these link to serum IFN- α , clinical phenotypes and sex. Samples with clinical data from patients with SSc and healthy blood donors (HBDs) served as controls. Finally, the diagnostic performance of SSc-associated autoantibodies was evaluated.

Methods Samples from well-characterised subjects with SLE (n=510), pSS (n=116), SSc (n=57) and HBDs (n=236) were analysed using a commercially available immunoassay (EuroLine Systemic Sclerosis Profile (IgG)). IFN- α was quantified by ELISA. Self-reported data on Raynaud's phenomenon (RP) were available.

Results With exceptions for anti-Ro52/SSA and anti-Th/To, SSc-associated autoantibodies were more frequent in SSc than in SLE, pSS and HBDs regardless of sex. IFN- α levels correlated with the number of positive SSc-associated autoantibodies ($r=0.29$, $p<0.0001$) and associated with Ro52/SSA positivity ($p<0.0001$). By using data from SLE, SSc and HBDs, RP was significantly associated with topoisomerase I, centromere protein (CENP)-B, RNA polymerase III 11 kDa, RNA polymerase III 155 kDa and PM-Scl100 whereas Ro52/SSA associated inversely with RP. In SLE, CENP-A was associated with immunological disorder, CENP-B with serositis and Ku with lupus nephritis. By combining analysis of ANA (immunofluorescence) with SSc-associated autoantibodies, the diagnostic sensitivity reached 98% and the specificity 33%.

Conclusions The 13 specificities included in the EuroLine immunoassay are commonly detected in SSc, but they are also frequent among individuals with other diseases imprinted by type I IFNs. These findings are valuable when interpreting serological data on patients with suspected

WHAT IS ALREADY KNOWN ON THIS TOPIC

- ⇒ Although SLE, primary Sjögren's syndrome (pSS) and systemic sclerosis (SSc) constitute different diagnostic entities, organ involvement and disease mechanisms may overlap and result in significant diagnostic challenges.
- ⇒ Autoantibodies are important diagnostic tools in the three diseases.

WHAT THIS STUDY ADDS

- ⇒ Autoantibodies associated with SSc can be found in different diseases imprinted by type I interferons (IFNs).
- ⇒ These autoantibodies show association with separate clinical phenotypes as well as with increased IFN- α .

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- ⇒ SSc-associated antibodies alone are of limited value in the differential diagnosis of patients with new-onset autoimmune disease and must be interpreted together with the clinical picture.
- ⇒ The link between IFN- α and SSc-associated autoantibodies highlights the interplay between type I IFNs and B cells, and motivates further investigation of type I IFN-blocking regimens in SLE, pSS and SSc.

SSc, especially as patients may present with disease manifestations overlapping different rheumatological diseases. In SLE, we observed associations between manifestations and SSc-associated autoantibodies which have not previously been reported.

INTRODUCTION

SLE, primary Sjögren's syndrome (pSS) and systemic sclerosis (SSc) are heterogeneous

chronic autoimmune conditions, which primarily affect women.¹² These diseases can present with clinically separate features but the organ involvement and disease mechanisms may overlap and result in significant diagnostic challenges. In addition, secondary Sjögren's syndrome is estimated to be present in >20% of patients with both established SLE and SSc.^{3,4}

Another common clinical feature of these conditions is the presence of Raynaud's phenomenon (RP). Yet, presence of RP is not restricted to autoimmune disorders. The idiopathic primary form of RP has a prevalence of approximately 5%–15% in the general population (higher in women than in men), whereof the vast majority have a benign course.^{5,6} Secondary RP may develop in association with use of various drugs or presence of hypothyroidism, diabetes, haematological abnormalities or autoimmune diseases.^{7,8} RP is common in rheumatic diseases, including SLE (30%–40%), pSS (15%–25%) and especially in SSc, where at least 95% of patients develop this manifestation. Occasionally, RP may precede the onset of autoimmune diseases and RP is the most common debut symptom of SSc.^{9,10} Because of this, early testing of antibodies associated with SSc/rheumatic disease is recommended in adult-onset of RP, as well as nailfold capillaroscopy.

Of aetiopathogenetic relevance, activation of the type I interferon (IFN) response constitutes a common denominator of SLE, pSS and SSc.¹¹ Recent data from randomised controlled trials show that blocking the type I IFN receptor by anifrolumab decreases global disease activity in SLE.¹² Corresponding studies in pSS are lacking but a phase I open-label study in SSc has been conducted, and recent studies indicate that type I IFN signalling is important also in SSc.^{13–15} Another similarity between the three diseases is the high prevalence of autoantibodies, for example, antibodies targeting cellular antigens referred to as ANA usually detected by immunofluorescence (IF) microscopy on HEp-2 cells and/or antigen-specific assays.^{16–18}

The EuroLine Systemic Sclerosis Profile is a commercial immunoblot test including a panel of 13 target antigens for SSc-associated antibodies and has been launched to aid the identification of patients with recent-onset SSc and to stratify patients into more homogeneous subsets.¹⁹ Some established associations between these autoantibodies with organ involvement and disease progression exist.^{20,21} These autoantibody specificities include centromere protein (CENP) A and B, topoisomerase I (Scl-70) and RNA polymerase III. Others, like Ku, have been described in the context of idiopathic inflammatory myopathies and recently in SLE, whereas autoantibodies against platelet-derived growth factor receptor (PDGFR) and NOR90 are rare and their clinical significance remains uncertain.^{22,23} Nowadays, this panel of SSc-associated antibodies is available for use in clinical practice. Still, the panel has not been systemically evaluated using large control groups of resembling conditions.

Table 1 Characteristics of the included patients with SLE

	SLE: discovery cohort (n=282)	SLE: replication cohort (n=228)
Female, n (%)	243 (86.2)	207 (90.7)
Age (years), mean (range)	48.6 (18–82)	47.8 (17–81)
Caucasian race/ethnicity, n (%)	260 (92.2)	217 (95.2)
SLEDAI, mean (range)	3.2 (0–24)	2.3 (0–24)
Classification criteria (1982 ACR definitions)		
Malar rash, n (%)	114 (40.4)	130 (57.0)
Discoid lupus, n (%)	42 (14.9)	74 (32.5)
Photosensitivity, n (%)	144 (51.1)	136 (59.6)
Oral ulcers, n (%)	34 (12.1)	73 (32.0)
Arthritis, n (%)	217 (77.0)	190 (83.3)
Serositis, n (%)	107 (37.9)	121 (53.1)
Renal disorder, n (%)	81 (28.7)	91 (39.9)
Neurological disorder, n (%)	16 (5.7)	17 (7.5)
Haematological disorder, n (%)	173 (61.4)	154 (67.5)
Immunological disorder, n (%)	147 (52.1)	172 (75.4)
IF-ANA, n (%)	279 (98.9)	227 (99.6)
Other manifestations		
Raynaud, n (%)	75 (28.7)*	75 (44.1)†
PAH, n (%)	4 (1.4)	9 (3.9)

*Data available for 261 of 282.
†Data available for 170 of 228.
ACR, American College of Rheumatology; IF-ANA, ANA detected with immunofluorescence microscopy; PAH, pulmonary arterial hypertension; SLEDAI, SLE Disease Activity Index.

Herein, we investigated to what extent SSc-associated autoantibodies can be found in patients with SLE and pSS, with and without detectable IFN- α , and to whether these antibodies link to clinical phenotypes, including RP. In addition, we employed samples and concomitant clinical data from patients with confirmed SSc as well as from a large group of healthy blood donors (HBDs). Finally, the diagnostic performance of the SSc-associated autoantibodies in relation to SSc diagnosis in a clinical setting was evaluated.

Patients and methods

Clinical characterisation

SLE: discovery cohort

This cohort consisted of samples obtained from 282 patients (243 women, 39 men), mean age 48.6 years, classified with SLE according to the 1982 American College of Rheumatology (ACR) and/or the 2012 Systemic Lupus International Collaborating Clinics criteria as detailed in [table 1](#).^{24,25} All subjects were included in the prospective and observational research programme *Clinical*

Lupus Register in North-Eastern Gothia (Swedish acronym 'KLURING') at the Rheumatology Unit, Linköping University Hospital.²⁶

SLE: replication cohort

This cohort consisted of samples obtained from 228 patients (207 women, 21 men), mean age 47.8 years, classified with SLE according to the 1982 ACR criteria as demonstrated in table 1.²⁴ The patients attended the Department of Rheumatology in Lund, Skåne University Hospital, during 1982–2022 and were consecutively asked to participate in the cohort.²⁷

For both SLE cohorts, none of the subjects fulfilled classification criteria for SSc. Global disease activity was assessed by the SLE Disease Activity Index (SLEDAI) at the time point of sampling.²⁸

Primary Sjögren's syndrome

Samples were obtained from 116 patients (111 women, 5 men), mean age 61.6 years, who fulfilled the American-European Consensus Criteria for pSS.²⁹ The subjects with pSS have previously been described in detail and lived in the same geographical area as those with SLE (discovery cohort).¹⁷ None of the subjects with pSS fulfilled classification criteria for SSc or SLE. The samples were collected and stored at the Rheumatology Unit, Linköping University Hospital.

Systemic sclerosis

Samples were obtained from 57 patients (43 women, 14 men), mean age 55.2 years, meeting the 2013 European Alliance of Associations for Rheumatology/ACR classification criteria for SSc.³⁰ In total, 19 (33.3%) had diffuse SSc (15 women), while 38 (66.7%) had limited SSc (28 women). All patients had RP and three (5.3%) were diagnosed with pulmonary arterial hypertension (PAH). Subjects with SSc lived in the same region as patients with SLE (replication cohort) and the samples were collected at the Department of Rheumatology in Lund, Skåne University Hospital.

Healthy blood donors

Samples were obtained from 236 HBDs (127 women, 109 men), mean age 43.8 years, who lived in the same region as the patients with SLE (discovery cohort) and pSS. Twenty-five of these sera were selected due to known positive IF-ANA, whereas the remaining 211 were IF-ANA negative. Via questionnaire, 45 (19.1%) reported RP.

In SLE and SSc, data on RP were self-reported and/or observed by a physician, whereas in HBDs, RP was self-reported.

Autoantibody assays

The samples were analysed using the commercially available line immunoblot assay (EuroLine Systemic Sclerosis (Nucleoli) Profile (IgG); Euroimmun, Lübeck, Germany), and performed at the accredited laboratory of Clinical Immunology, Linköping University Hospital. All serum samples were stored at -70°C until the time

of testing according to the manufacturer's instruction. The antibody kit enables simultaneous detection of 13 different antibody specificities (Scl-70, CENP-A, CENP-B, RNA polymerase III 11 kDa, RNA polymerase III 155 kDa, fibrillarin (U3-RNP), NOR90, Th/To, PM-Scl100, PM-Scl75, Ku, PDGFR and Ro52/SSA). The samples were analysed using EUROBlotmaster (Euroimmun, Euroimmun Lübeck, Germany), and after drying, the strips' signal intensities (SIs) were read by EuroLineScan. The strength of positive reaction was reported in SI units corresponding to 'weak positive' (11–25), 'positive' (26–50) or 'strong positive' (>50). Borderline results (6–10) were classified as 'negative'.

All positive findings with EuroLine immunoblot assay were evaluated with an immunodot assay (BlueDot Scleroderma¹² IgG; D-tek, Mons, Belgium) and a fluorescence enzyme immunoassay (EliA Phadia 250, ThermoFisher Scientific, Phadia, Uppsala, Sweden). BlueDot Scleroderma¹² assay enables analysis of the following antibody specificities: Scl-70, CENP-A, CENP-B, PM-Scl100, PM-Scl75, Ku, RNA polymerase III (entire complex), U1-RNP, Th/To, fibrillarin, NOR90 and Ro52/SSA. EliA detects the following specificities: Scl-70, CENP-B, RNA polymerase III (entire complex), fibrillarin and PM-Scl100. The analyses were performed according to the manufacturer's instructions and recommended cut-offs were used, that is, >10 arbitrary units for BlueDot Scleroderma¹² and >10 U/mL for EliA. BlueDot Scleroderma¹² strips were analysed using Dr DOT software and scanning system provided by D-tek.

ANA

ANA were detected by IF microscopy on HEp-2 cells (IF-ANA), including interpretation of the staining patterns using the International Consensus on ANA Patterns nomenclature, detailed elsewhere.^{31 32}

IFN- α assay

For the IFN- α assay, samples stored at -70°C until analysis were available only from SLE (discovery cohort; n=282), pSS (n=116) and HBDs (n=226). IFN- α was analysed by ELISA according to the manufacturer's instructions (Human IFN- α (pan-specific) ELISA^{PRO} kit), Mabtech, Nacka Strand, Sweden) and previously detailed.^{33 34} This ELISA detects subtypes 1/13, 2, 4, 5, 6, 7, 8, 10, 14, 16 and 17 of IFN- α with a standard ranging from 5 to 4000 pg/mL.

Statistics

The data were analysed using SPSS statistics software V.27.0 (IBM) and Prism V.9 (GraphPad Software, La Jolla, USA) for construction of graphs. Concordance was estimated by the sum of double-positive and double-negative samples, divided by the total number of samples, multiplied by 100. The diagnostic performances of the detected autoantibodies for SSc and RP as outcomes were examined with analyses of sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive

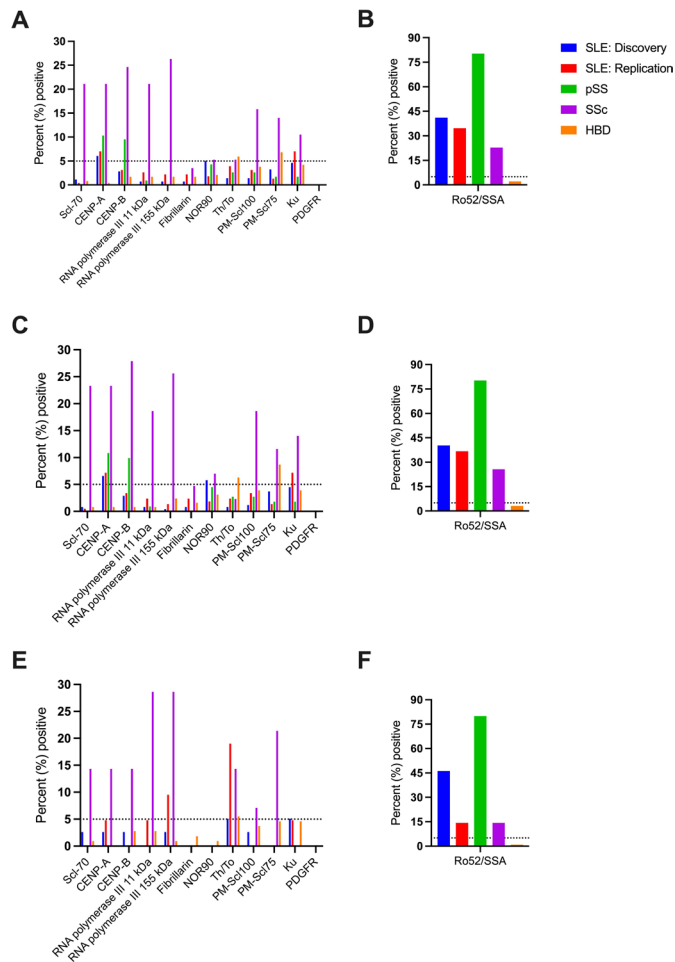


Figure 1 Percentage of positive subjects for each autoantibody specificity in SLE, pSS, SSc and HBDs (A,B). Results are divided with regard to sex, for women (C,D) and men (E,F). The dotted line represents 5% positives. A cut-off of $\leq 5\%$ positives among HBDs is commonly applied for immunoassays. CENP, centromere protein; HBD, healthy blood donor; PDGFR, platelet-derived growth factor receptor; pSS, primary Sjögren's syndrome; Scl-70, topoisomerase I; SSc, systemic sclerosis.

value (NPV), including 95% CIs. Differences between groups were calculated using χ^2 , Mann-Whitney U test or Fisher's exact test (where appropriate). Correlation analyses between antibody specificities and IFN- α levels were performed by Spearman's r . P values of ≤ 0.01 were considered statistically significant.

Patient and public involvement

Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our research.

RESULTS

SSc-associated autoantibodies in SLE, pSS, SSc and HBDs

The prevalence of 12 SSc-associated autoantibodies detected by EuroLine is presented in [figure 1A](#). A significantly higher proportion of subjects with SSc than SLE, pSS and HBDs were positive for Scl-70, CENP-A, CENP-B, RNA polymerase III 11 kDa, RNA polymerase III 155 kDa,

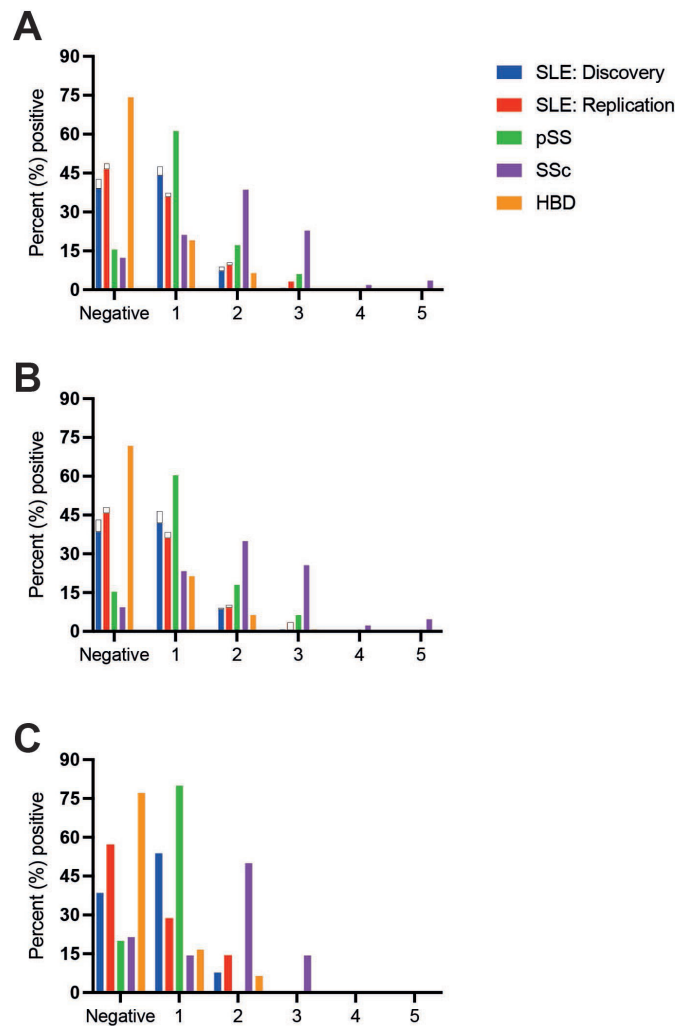


Figure 2 Percentage of individuals (SLE, pSS, SSc and HBDs) positive for multiple autoantibody specificities (A) is shown and divided according to sex (B,C). Non-Caucasian race/ethnicity is illustrated in white (only depicted for SLE). No male patients with SLE had non-Caucasian race/ethnicity (C). HBD, healthy blood donor; pSS, primary Sjögren's syndrome; SSc, systemic sclerosis.

PM-Scl100 and PM-Scl75 but not for Ku, fibrillarin, NOR90, Th/To or PDGFR. As shown in [figure 1B](#), Ro52/SSA was found in a higher proportion of patients with pSS and SLE than in SSc. Autoantibodies against CENP-A were detected in 6%–10% of patients with SLE (both cohorts) and pSS, CENP-B in approximately 10% of subjects with pSS and Ku in 7% of patients with SLE in the replication cohort. Unexpectedly, high proportions of HBDs (28 of 236) were positive for Th/To and/or PM-Scl75, but in most cases (23 of 28) reactions were 'weak positive' (SI 11–25). Autoantibody data were further analysed separately according to sex. As demonstrated, the results essentially remained among both women ([figure 1C,D](#)) and men ([figure 1E,F](#)).

Some individuals were positive for >1 of the antibodies tested. [Figure 2A–C](#) illustrates the percentage of individuals positive for multiple autoantibodies, divided according to sex and Caucasian race/ethnicity. Among

patients with SSc, 12 (21.1%) were positive for one specificity only, while 22 (38.6%), 13 (22.8%), 1 (1.8%) and 2 (3.5%) were positive for two, three, four and five antibodies, respectively. Seven patients with SSc (12.3%) were negative for all tested SSc-associated antibodies.

Among subjects in the SLE discovery cohort, 134 (47.5%) were positive for one specificity only (whereof isolated Ro52/SSA positivity was found in 94 patients), while 25 (8.9%), 2 (0.7%) and 1 (0.4%) were positive for two, three and four antibodies, respectively. Among subjects in the SLE replication cohort, 85 (37.3%) were positive for one specificity only (whereof isolated Ro52/SSA positivity was found in 57 patients), while 24 (10.5%), 7 (3.1%) and 1 (0.4%) were positive for two, three and four antibodies, respectively. The number of positive autoantibodies was not statistically different between Caucasian and non-Caucasian subjects with SLE ($p=0.89$). Global disease activity, assessed by SLEDAI, was significantly higher in non-Caucasian ($p=0.001$) than in Caucasian patients. SLEDAI scores showed a borderline significant correlation with the number of positive SSc-associated autoantibodies ($r=0.11$, $p=0.015$).

Among individuals with pSS, 71 (61.2%) were positive for one specificity only (whereof isolated Ro52/SSA positivity was found in 68 patients), while 20 (17.2%) and 7 (6.0%) were positive for two and three autoantibodies, respectively. Regarding HBDs, 45 (19.1%) were positive for one specificity and 16 (6.8%) positive for ≥ 2 SSc-associated autoantibodies. The number of positive autoantibodies was not statistically different between female and male HBDs ($p=0.36$). Positive nucleolar IF-ANA (AC-8) was detected among four HBDs, of which two also showed an SSc-associated antibody; PM-Scl75 (weak positive) and NOR90 (strong positive), respectively.

Signal intensity of EuroLine results in SLE, pSS, SSc and HBDs
SI values for Scl-70, CENP-A, CENP-B, RNA polymerase III 11 and 155 kDa, PM-Scl100 and PM-Scl75 were highest in patients with SSc (online supplemental table 1). For CENP-A and CENP-B, high SI values were also seen in subjects with pSS. Fibrillarin, NOR90 and Th/To overall showed low and similar SI values across the evaluated groups. For Ku, highest SIs were observed among patients with pSS.

Evaluation of positive EuroLine results with BlueDot and EliA
Positive EuroLine results from subjects with SLE (both cohorts), pSS and HBDs were evaluated with two alternative methods. Samples showing isolated anti-Ro52/SSA positivity were excluded from these analyses. In total, out of 266 positive EuroLine test results, corresponding specificities were confirmed positive in 48 (18%) with BlueDot. EliA was used to evaluate 97 positive EuroLine test results and 18 (19%) of them could be verified as positive. The level of agreement for positive results between EuroLine and the two alternative methods varied between 0% and 57%, depending on the assay and antibody specificity. Overall, the agreement was higher (approximately

75%) at EuroLine SI values >50 , while results in the 11–25 interval showed the lowest agreement (2%–3%) (online supplemental table 2).

In addition, subgroups of the SLE discovery cohort ($n=153$) and the HBD cohort ($n=140$) were analysed with EliA, and the complete SSc cohort ($n=57$) was evaluated with both EliA and BlueDot. With EliA, none of the HBD samples tested positive for any specificity; of the SLE samples, only one (0.7%) sample tested positive for fibrillarin, one (0.7%) for Scl-70 and three (2.0%) for CENP-B. Of the 57 patients with SSc, 51 (89.5%) tested positive with BlueDot. The concordance rates between the three immunoassays in the SSc cohort, in total and for each antibody specificity, were convincingly high as shown in online supplemental table 3.

Immunofluorescence-ANA

The percentages of IF-ANA positivity in the groups were as follows: SLE (discovery cohort) 74.5%, pSS 75.0%, SSc 96.5% and HBDs 10.6%. As demonstrated in table 2, homogeneous staining pattern (AC-1) was the most common in SLE followed by homogeneous/speckled (AC-1+AC-4). In pSS, speckled (AC-4) was the most common pattern followed by AC-1.

In SSc, speckled (AC-4) was the most common followed by centromere pattern (AC-3). AC-3 was rare among the other diseases than SSc as well as in HBDs. Six of the seven patients with SSc (85.7%), who tested negative for all SSc-associated antibodies by EuroLine, were IF-ANA positive (one individual with AC-1 and five with AC-4).

Interferon- α

Detectable levels of IFN- α were found in 178 of 282 patients (63.1%) with SLE (discovery cohort), 77 of 116 (66.4%) pSS and 16 of 226 (7.0%) HBDs (figure 3A). Patients with pSS showed higher levels of IFN- α than those with SLE ($p=0.01$). IFN- α levels were significantly higher in subjects with ≥ 1 detected SSc-associated autoantibody (mean 14.1 vs 2.5 pg/mL; $p<0.0001$) than in negative individuals. In addition, the IFN- α levels correlated with the number of SSc-associated autoantibodies among all ($r=0.29$, $p<0.0001$), as well as in separate analyses of SLE ($r=0.20$, $p=0.0006$) but insignificant in HBDs ($r=0.16$) and pSS ($r=0.16$). Of all the examined SSc-associated autoantibodies, positive anti-Ro52/SSA showed the strongest association with IFN- α (mean 17.8 vs 3.6 pg/mL; $p<0.0001$; figure 3B).

Antibodies versus RP

Data on RP were available for all groups except pSS. None of the autoantibodies associated significantly with RP in SLE or HBDs. None of the HBD subjects reporting RP combined with positive RNA polymerase III 155 kDa and/or PM-Scl100 had been diagnosed with SSc 4 years after sampling. When data from all groups were merged ($n=711$), antibodies against Scl-70 ($p<0.0001$), CENP-B ($p<0.0001$), RNA polymerase III 11 kDa ($p<0.001$), RNA polymerase III 155 kDa ($p<0.0001$) and PM-Scl100

Table 2 IF-ANA staining patterns (HEp-2 cells) according to the ICAP nomenclature for the three diseases and as well as for HBDs

ICAP staining pattern	SLE: discovery cohort (n=282)	pSS (n=116)	SSc (n=57)	HBDs (n=236)
AC-0 (negative)	72 (25.5)	29 (25.0)	2 (3.5)	211 (89.4)
AC-1 (homogeneous)	104 (36.9)	30 (25.9)	8 (14.0)	9 (3.8)
AC-3 (centromere)	2 (0.7)	1 (0.9)	12 (21.1)	0 (0)
AC-4 (speckled)	41 (14.5)	36 (31.0)	17 (29.8)	6 (2.5)
AC-6 (multiple nuclear dots)	2 (0.7)	0 (0)	0 (0)	1 (0.4)
AC-8 (nucleolar)	10 (3.5)	1 (0.9)	1 (1.8)	4 (1.7)
AC-1+4	39 (13.8)	18 (15.5)	7 (12.3)	5 (2.1)
AC-1+4+8	2 (0.7)	0 (0)	2 (3.5)	0 (0)
AC-1+8	9 (3.2)	1 (0.9)	0 (0)	0 (0)
AC-4+7 (speckled+few nuclear dots)	0 (0)	0 (0)	1 (1.8)	0 (0)
AC-4+8	1 (0.35)	0 (0)	7 (12.3)	0 (0)

Percentages are given in parentheses.

HBDs, healthy blood donors; ICAP, International Consensus on ANA Patterns; IF-ANA, ANA detected with immunofluorescence microscopy; pSS, primary Sjögren's syndrome; SSc, systemic sclerosis.

($p=0.002$) were significantly associated with RP, whereas Ro52/SSA ($p=0.004$) associated inversely with RP.

Antibodies versus manifestations of SSc

We observed significant associations between pulmonary fibrosis and presence of Scl-70 (EuroLine, $p=0.008$; EliA, $p=0.009$), whereas antibodies against RNA polymerase III 11 kDa (EuroLine, $p=0.003$) and RNA polymerase III 155 kDa (EuroLine, $p=0.003$) were inversely associated with pulmonary fibrosis. PAH showed a non-significant trend of association with CENP-A (EuroLine, $p=0.05$). None of the investigated SSc-associated antibodies were associated with arthritis.

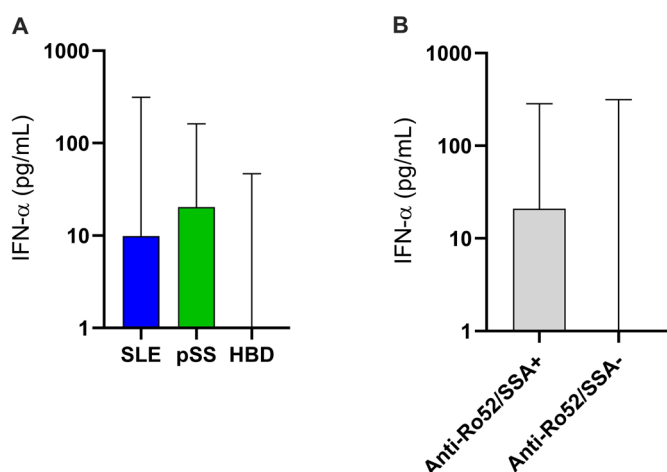


Figure 3 Levels of IFN- α in subjects with SLE, pSS and HBDs (A). The levels were lowest among HBDs, whereas patients with pSS showed significantly higher levels than those with SLE ($p=0.01$). IFN- α levels were significantly associated with positivity for anti-Ro52/SSA ($p<0.0001$) (B). HBDs, healthy blood donors; IFN, interferon; pSS, primary Sjögren's syndrome.

The diffuse type of SSc was associated with antibodies against Scl-70 (EuroLine, $p=0.001$; EliA, $p=0.001$; BlueDot, $p<0.001$), RNA polymerase III 11 kDa (EuroLine, $p=0.01$) and the entire RNA polymerase III complex (BlueDot, $p=0.004$), whereas the limited type of SSc associated with antibodies against CENP-A (EuroLine, $p=0.005$; BlueDot, $p=0.01$) and CENP-B (EuroLine, $p=0.002$; EliA, $p=0.005$; BlueDot, $p=0.005$).

Antibodies versus SLE classification criteria

In SLE, presence of antibodies against CENP-B was significantly associated with serositis in the discovery cohort ($p=0.005$) and in both cohorts merged ($p=0.007$) but did not reach statistical significance in the replication cohort. Similarly, anti-Ku associated significantly with lupus nephritis ($p=0.007$) in the discovery cohort and in both cohorts merged ($p=0.01$) but not in the replication cohort. Finally, anti-CENP-A was significantly associated with immunological disorder in the discovery cohort ($p=0.002$) and in both cohorts merged ($p=0.001$) but was only borderline significant in the replication cohort.

Diagnostic performance of the EuroLine Systemic Sclerosis Profile

The sensitivity, specificity, accuracies, as well as PPV and NPV for SSc diagnosis and presence of RP as outcomes were calculated (table 3). Antibodies included in the 2013 ACR criteria; that is, antibodies targeting Scl-70, RNA polymerase III 11 kDa and RNA polymerase III 155 kDa, achieved best accuracy regarding SSc diagnosis.³⁰ For the entire panel (≥ 1 positive antibody), the diagnostic sensitivity was estimated to 98% and the diagnostic specificity to 33%.

For identifying RP in SLE and HBDs, antibodies against Scl-70, CENP-A, CENP-B, RNA polymerase III 11 kDa, RNA polymerase III 155 kDa, fibrillar and PM-Scl100

Table 3 Diagnostic performance with SSc as outcome based on data from individuals with SLE, pSS, SSc and HBDs

	Sensitivity	Specificity	Accuracy	PPV	NPV
IF-ANA	0.96 (0.88 to 0.99)	0.49 (0.45 to 0.53)	0.66 (0.63 to 0.69)	0.15 (0.11 to 0.19)	0.99 (0.98 to 1)
Ro52/SSA	0.23 (0.14 to 0.35)	0.66 (0.62 to 0.70)	0.77 (0.74 to 0.79)	0.06 (0.03 to 0.10)	0.91 (0.88 to 0.93)
Scl-70	0.21 (0.12 to 0.33)	0.99 (0.98 to 1)	0.96 (0.95 to 0.97)	0.71 (0.47 to 0.87)	0.93 (0.91 to 0.95)
CENP-A	0.21 (0.12 to 0.33)	0.95 (0.93 to 0.97)	0.94 (0.93 to 0.95)	0.29 (0.17 to 0.44)	0.93 (0.91 to 0.95)
CENP-B	0.25 (0.15 to 0.37)	0.96 (0.95 to 0.98)	0.95 (0.94 to 0.96)	0.38 (0.24 to 0.54)	0.93 (0.91 to 0.95)
RNA polymerase III 11 kDa	0.21 (0.12 to 0.33)	0.99 (0.98 to 0.99)	0.96 (0.95 to 0.97)	0.63 (0.41 to 0.81)	0.93 (0.91 to 0.95)
RNA polymerase III 155 kDa	0.26 (0.17 to 0.39)	0.99 (0.98 to 1)	0.96 (0.95 to 0.97)	0.71 (0.50 to 0.86)	0.94 (0.92 to 0.95)
Fibrillarin	0.04 (0.01 to 0.12)	0.99 (0.98 to 1)	0.95 (0.94 to 0.96)	0.25 (0.07 to 0.59)	0.92 (0.90 to 0.94)
NOR90	0.05 (0.02 to 0.14)	0.96 (0.94 to 0.97)	0.94 (0.93 to 0.95)	0.11 (0.04 to 0.28)	0.92 (0.90 to 0.94)
Th/To	0.05 (0.02 to 0.14)	0.97 (0.95 to 0.98)	0.94 (0.93 to 0.95)	0.12 (0.04 to 0.31)	0.92 (0.90 to 0.94)
PM-Scl100	0.16 (0.09 to 0.27)	0.97 (0.96 to 0.98)	0.95 (0.94 to 0.96)	0.36 (0.20 to 0.55)	0.93 (0.91 to 0.95)
PM-Scl75	0.14 (0.07 to 0.25)	0.96 (0.94 to 0.97)	0.94 (0.93 to 0.95)	0.23 (0.12 to 0.39)	0.93 (0.90 to 0.94)
Ku	0.11 (0.05 to 0.21)	0.96 (0.94 to 0.97)	0.94 (0.93 to 0.95)	0.19 (0.09 to 0.36)	0.92 (0.90 to 0.94)
PDGFR	0 (0 to 0.06)	1 (0.99 to 1)	0.96 (0.94 to 0.97)	–	0.92 (0.89 to 0.94)
≥1 antibody specificity	0.98 (0.91 to 1)	0.33 (0.29 to 0.36)	0.49 (0.46 to 0.53)	0.12 (0.09 to 0.15)	1 (0.97 to 1)

Sensitivity, specificity, accuracy, PPV and NPV are detailed including 95% CIs (in parentheses).

CENP, centromere protein; HBDs, healthy blood donors; IF-ANA, ANA detected with immunofluorescence microscopy; NPV, negative predictive value; PDGFR, platelet-derived growth factor receptor; PPV, positive predictive value; pSS, primary Sjögren's syndrome; Scl-70, topoisomerase I; SSc, systemic sclerosis.

performed best (online supplemental table 4). The entire panel (≥1 positive antibody) showed a sensitivity for RP of 71% and a specificity of 41%.

DISCUSSION

The objective of this cross-sectional study was to investigate the prevalence of SSc-associated antibodies in sera from well-characterised patients with three different type I IFN-dependent diseases and HBDs by using the EuroLine Systemic Sclerosis Profile kit. This immunoassay is commercially available and has been thoroughly evaluated in SSc, but to our knowledge, large groups of disease controls have not been included.^{19 35–37} Our study propounds caution when using Euroimmun's immunoassay in the differential diagnosis of patients with recent-onset IFN-mediated rheumatic disease.

We demonstrate that this immunoassay frequently identifies autoantibodies in patients with SLE and pSS (and also in HBDs), but the majority of samples achieving SI values within the 11–25 interval could not be confirmed with alternative methods. In addition, only very few patients with SLE, and none of the HBD sera, tested positive for any specificity with the ELISA assay. Based on these findings, we suggest that Euroimmun's recommended cut-off should be adjusted, and this is important to consider when using this immunoassay in a clinical setting for patients with suspected new-onset rheumatic disease.

Nevertheless, presence of the SSc antibodies was associated with higher levels of IFN- α , which is in line with a recent observation from the USA and previous data from Southern Sweden in patients with early SSc.^{38 39} Furthermore, our findings are consistent with the profound effects of type I IFNs on B cells, increased plasma cell differentiation, isotype switch and enhanced autoantibody production.¹¹ Herein, we quantified IFN- α with an ELISA which has previously shown good concordance with type I IFN activity measured in vitro by Wistar Institute Susan Hayflick (WISH) reporter cell assay.³⁴

For the patients with SSc, we observed similar associations between the SSc-associated antibodies and organ manifestations/involvement (eg, PAH, pulmonary fibrosis, diffuse and limited SSc) as have been described previously.²⁰ As demonstrated in online supplemental table 3, the concordance between the assays appeared to be high among subjects with SSc. Our evaluation of diagnostic performance of the EuroLine Systemic Sclerosis Profile kit combined with IF-ANA resulted in an overall (≥1 autoantibody) excellent diagnostic sensitivity, whereas the specificity was considerably lower.

The prevalence of RP in our study is consistent with frequencies in previous reports.^{5 40 41} We had access to data on RP, which were collected for individuals with SSc, SLE and HBDs. Unfortunately, we had no information on tobacco usage or comorbidities associated with RP, for example, hypothyroidism and diabetes, among the HBDs (individuals with well-controlled hypothyroidism

and non-insulin-treated diabetes are accepted as blood donors in Sweden). The evaluation of RP as outcome indicated that antibodies targeting Scl-70, CENP-A, CENP-B, RNA polymerase III 11 kDa, RNA polymerase III 155 kDa, fibrillarin and PM-Scl100 achieved the best accuracy. These findings are in line with previous observations by Patterson *et al.*¹⁹ However, we acknowledge that our study was cross-sectional and without systematic follow-up of autoantibody-positive subjects.

SLE is known to present with an array of different autoantibodies, and before onset of disease epitope spreading usually occur and increasing numbers of specificities precede clinical diagnosis.^{42–44} Also in pSS, data indicate that autoantibodies may appear several years before onset of sicca symptoms.⁴⁵ Herein, however, apart from autoantibodies against Ro52/SSA, which was significantly more common in pSS and SLE than in SSc, only autoantibodies against CENP-A (SLE both cohorts and pSS), CENP-B (pSS) and Ku (SLE replication cohort) were positive in $\geq 5\%$ indicating that the cut-offs applied by the manufacturer were mostly acceptable.⁴⁶ However, surprisingly, many samples from the HBD and SLE discovery cohorts showed positivity for the rare specificities Th/To and NOR90, respectively, and only few of these could be verified with BlueDot and EliA. This does raise the question of not only the cut-off, but also of the source and selection of antigens included in the Euroimmun assay.

Interestingly, similarly to our current findings in SSc, Alkema *et al* demonstrated high concordance between EuroLine, BlueDot and EliA when a large Dutch SSc cohort (n=347) was analysed.⁴⁷ Yet, that concordance applied to patients with established SSc while our evaluation is based on a small group of patients with SSc, combined with SLE, pSS and HBD individuals generally showing antibodies with lower SI values. Except for Scl-70 that is affinity purified, the antigens of the panel are produced by recombinant techniques that may generate irrelevant epitopes.⁴⁸

Autoantibodies targeting Scl-70, CENP-B, RNA polymerase III 11 kDa and RNA polymerase III 155 kDa have previously been shown to be the most common in SSc.¹⁹ Also in our hands, these antibody specificities had a significantly higher prevalence in SSc compared with pSS, SLE and HBDs. Other SSc-associated antibodies were less common in SLE and pSS (fibrillarin, NOR90 and Th/To). Consistent with a previous report in SLE, we observed an association between anti-Ku and lupus nephritis, but this association could not be confirmed with EliA.⁴⁹ Anti-fibrillarin antibodies have previously been linked to non-Caucasian ethnicity and poor survival in SSc.⁵⁰

A striking finding was that the number of subjects positive for >1 autoantibody specificity (figure 2A) differed between the groups. With Ro52/SSA excluded, patients with SLE, pSS and even HBDs were often positive for one autoantibody while those diagnosed with SSc were frequently positive for two or more autoantibodies. In addition, a clear gender difference was observed with very

few or none of the male subjects with SLE and pSS showing positivity for >1 autoantibody (figure 2C). Fifty of 57 patients (87.7%) with SSc had at least one SSc-associated autoantibody and only two (3.5%) were IF-ANA negative. Historically, however, both these patients had tested IF-ANA positive. The frequency of negative IF-ANA in SSc has previously been estimated to 6%–15%.^{51 52} In SLE and pSS, IF-ANA was positive in approximately 75% of patients. This proportion of IF-ANA positivity may appear low. However, as this study had a cross-sectional design and included patients with different disease duration, it should be emphasised that individuals with SLE may lose ANA over time.^{32 53} It cannot be excluded that this fact may have had an impact on the diagnostic performance tests for SSc and RP herein.

The EuroLine immunoassay includes 13 specificities ranging from established markers with known high diagnostic values for SSc (ie, Scl-70, CENP-A, CENP-B and RNA polymerase III) to rare specificities like Ku, Th/To, NOR90 and PDGFR for which the clinical value is less well known. This underlines that these specificities should not be included for screening purposes but are of value for subtyping individuals with a confirmed, or strongly suspected, diagnosis within the spectrum of systemic scleroderma disorders. The established clinical importance of autoantibodies against Scl-70 and RNA polymerases relies on decades of experience from less sensitive techniques detecting precipitating antibodies.⁵⁴ In recent years, most clinical laboratories have shifted to automated and broad testing of autoantibodies with more sensitive methods with the risk for lower diagnostic specificity. Automation and broad testing have advantages, but interpretation of unexpected 'borderline positive' findings may be challenging and lead to unnecessary investigations as well as patients' worries. Antibodies against Ku, Th/To, NOR90 and PDGFR have been described in small proportions of patients with SSc. Anti-NOR90 have mostly been associated with limited scleroderma, is rarely found in other rheumatic diseases and may be associated with malignancy.²³ Autoantibodies against Ku are also found in other systemic inflammatory diseases, while the diagnostic specificity of Th/To and PDGFR for SSc is reported to be high.^{55 56} However, our finding of autoantibodies against Th/To in HBDs questions that view.

Limitations of this study include the limited number of patients with SSc. Also, data on RP were not available for subjects with pSS. Indeed, the female-to-male ratio among HBDs was different than among the other groups, but the number of positive autoantibody findings was similar. The study had a cross-sectional design and no longitudinal analyses were made, which is a possible limitation. However, follow-up data from Patterson *et al* indicate that the SSc-associated antibodies detected by the EuroLine Systemic Sclerosis Profile kit usually remain stable over time.¹⁹ There are several strengths of the study, for example, the well-characterised disease controls and the large group of HBDs with data on RP. Another advantage

was that all antibody analyses were performed at the same time by an accredited laboratory.

To conclude, we demonstrate that the 13 autoantibodies included in the EuroLine immunoassay are commonly detected among patients with SSc, but they are also frequent among individuals with other diseases characterised by type I IFNs regardless of sex. Positivity for SSc-associated antibodies—especially anti-Ro52/SSA—was linked to higher levels of IFN- α , and among patients with SLE, we observed associations between clinical manifestations and SSc-associated autoantibodies which have not previously been reported. The Systemic Sclerosis Profile kit showed a decent performance regarding diagnostic accuracy, but the diagnostic specificity was lower. An important observation is that weakly positive antibody results could rarely be confirmed when analysed by two alternative assays (especially in samples originating from subjects without SSc), indicating that some of Euroimmun's recommended cut-offs are too low and/or that antibodies against irrelevant epitopes are detected.

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Supplementary Table 1. Number of individuals positive with EuroLine for each specificity among HBD ($n=236$), SLE discovery ($n=282$) and SLE replication ($n=228$) cohorts, pSS ($n=116$) and SSc ($n=57$). SI values for the specificities are presented in median SI units and range.

Specificity	Cohort	Positive individuals, (n)	Signal intensity (SI), median (range)
Scl-70	HBD	2	12.5 (12–13)
	SLE: Discovery	3	16 (12–42)
	SLE: Replication	4	17 (14–58)
	pSS	0	
	SSc	12	76.5 (11–116)
CENP-A	HBD	1	23 (23–23)
	SLE: Discovery	17	23 (11–135)
	SLE: Replication	17	22 (11–142)
	pSS	12	122 (12–145)
	SSc	12	139.5 (48–150)
CENP-B	HBD	4	16.5 (12–37)
	SLE: Discovery	8	19.5 (11–122)
	SLE: Replication	7	36 (13–133)
	pSS	11	106 (14–142)
	SSc	14	141 (28–153)
RNA Pol III 11kDa	HBD	4	35.5 (24–40)
	SLE: Discovery	2	12 (11–13)
	SLE: Replication	6	14.5 (13–40)
	pSS	1	15 (15–15)
	SSc	12	99 (15–130)
RNA Pol III 155kDa	HBD	4	25 (16–37)
	SLE: Discovery	2	20 (16–24)
	SLE: Replication	5	16 (13–29)
	pSS	0	
	SSc	15	78 (15–131)
Fibrillarin	HBD	4	16 (13–53)
	SLE: Discovery	2	15.5 (14–17)
	SLE: Replication	6	14 (11–88)
	pSS	0	
	SSc	2	21 (17–25)
NOR90	HBD	5	13 (11–52)
	SLE: Discovery	14	37.5 (14–94)
	SLE: Replication	4	19.5 (13–31)
	pSS	5	30 (11–138)
	SSc	3	13 (13–95)

Th/To	<i>HBD</i>	14	14.5 (11–60)
	<i>SLE: Discovery</i>	4	13 (11–26)
	<i>SLE: Replication</i>	9	15 (11–38)
	<i>pSS</i>	3	19 (16–21)
	<i>SSc</i>	3	24 (14–27)
PM-Scl100			
	<i>HBD</i>	9	23 (11–62)
	<i>SLE: Discovery</i>	4	14 (11–30)
	<i>SLE: Replication</i>	7	13 (11–29)
	<i>pSS</i>	3	12 (12–36)
	<i>SSc</i>	9	56 (13–86)
PM-Scl75			
	<i>HBD</i>	16	14.5 (11–52)
	<i>SLE: Discovery</i>	9	16 (11–93)
	<i>SLE: Replication</i>	3	26 (12–145)
	<i>pSS</i>	2	13 (11–15)
	<i>SSc</i>	8	62 (11–142)
Ku			
	<i>HBD</i>	10	13.5 (12–19)
	<i>SLE: Discovery</i>	13	20 (11–122)
	<i>SLE: Replication</i>	16	26 (12–132)
	<i>pSS</i>	2	67 (30–104)
	<i>SSc</i>	6	32.5 (11–90)
PDGFR			
	<i>HBD</i>	0	
	<i>SLE: Discovery</i>	0	
	<i>SLE: Replication</i>	0	
	<i>pSS</i>	0	
	<i>SSc</i>	0	

Supplementary Table 2. Comparisons between immunoassays. All samples achieving positive test results with EuroLine (reference assay) were evaluated with BlueDot and EliA™. Results are shown in total and for each specificity in signal intensity (SI) intervals. Highest agreement of positive results was found at SI units >50, and for anti-CENP-B followed by anti-CENP-A.

		Number of positive antibodies and agreement with EuroLine, n (%)	Signal intensity (SI), units		
			11–25	26–50	>50
Total	<i>EuroLine</i>	266	164	49	53
	<i>BlueDot</i>	48 (18)	4 (2)	5 (10)	39 (74)
	<i>EuroLine</i>	97	61	19	17
	<i>EliA</i>	18 (19)	2 (3)	3 (16)	13 (76)
Scl-70					
	<i>EuroLine</i>	9	7	1	1
	<i>BlueDot</i>	0	0	0	0
	<i>EliA</i>	1 (11)	0	0	1
CENP-A					
	<i>EuroLine</i>	44	20	7	17
	<i>BlueDot</i>	13 (30)	0	0	13
CENP-B					
	<i>EuroLine</i>	30	12	5	13
	<i>BlueDot</i>	17 (57)	2	2	13
	<i>EliA</i>	16 (53)	2	2	12
RNA Pol III 11kDa					
	<i>EuroLine</i>	13	9	4	0
	<i>BlueDot*</i>	1 (8)	1	0	0
	<i>EliA*</i>	0	0	0	0
RNA Pol III 155kDa					
	<i>EuroLine</i>	11	7	4	0
	<i>BlueDot*</i>	0	0	0	0
	<i>EliA*</i>	0	0	0	0
Fibrillarin					
	<i>EuroLine</i>	11	9	0	2
	<i>BlueDot</i>	1 (9)	1	0	0
	<i>EliA</i>	0	0	0	0
NOR90					
	<i>EuroLine</i>	27	14	8	5
	<i>BlueDot</i>	3 (11)	0	0	3
Th/To					
	<i>EuroLine</i>	30	24	5	1
	<i>BlueDot</i>	0	0	0	0
PM-Scl100					
	<i>EuroLine</i>	23	17	5	1
	<i>BlueDot</i>	4 (17)	0	3	1
	<i>EliA</i>	1 (4)	0	1	0
PM-Scl75					
	<i>EuroLine</i>	30	22	4	4
	<i>BlueDot</i>	4 (13)	0	0	4
Ku					
	<i>EuroLine</i>	38	23	6	9
	<i>BlueDot</i>	5 (13)	0	0	5

* In contrast to the EuroLine assay, the BlueDot and EliA™ assays detect antibodies against the entire RNA polymerase III complex.

Supplementary Table 3. Concordance of EuroLine results (reference assay) versus (vs.) BlueDot and EliA™ results in patients with SSc. For most specificities, BlueDot and EliA™ showed a high level of concordance with the EuroLine results.

	EuroLine (n=57)	BlueDot (n=57)	EuroLine (n=56)	EliA™ (n=56)	Concordance: EuroLine vs. BlueDot	Concordance: EuroLine vs. EliA™
All positive and negative results					94.4%	96.1%
+	96	63	62	49		
-	531	529	274	274		
ScI-70					98.2%	100.0%
+	12	11	12	12		
-	45	45	44	44		
CENP-A					98.2%	
+	12	11				
-	45	45				
CENP-B					96.5%	98.2%
+	14	12	14	13		
-	43	43	42	42		
RNA Pol III 11 kDa					94.7%*	94.6%*
+	12	9	11	8		
-	45	45	45	45		
RNA Pol III 155 kDa					89.5%*	89.3%*
+	15	9	14	8		
-	42	42	42	42		
Fibrillarin					96.5%	96.4%
+	2	0	2	0		
-	55	55	54	54		
NOR90					93.0%	
+	3	0				
-	54	53				
Th/To					94.7%	
+	3	0				
-	54	54				
PM-ScI100					94.7%	98.2%
+	9	7	9	8		
-	48	47	47	47		
PM-ScI75					91.2%	
+	8	3				
-	49	49				
Ku					91.2%	
+	6	1				
-	51	51				

* Comparisons were done between antibody results of the entire RNA polymerase III complex (BlueDot and EliA™) vs. the 11 and 155 kDa antigens (EuroLine), respectively.

Supplementary Table 4. Diagnostic performance with RP as outcome based on data from individuals with SLE and HBD. Sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) are detailed including 95% confidence intervals (in parentheses).

	Sensitivity	Specificity	Accuracy	PPV	NPV
IF-ANA	0.62 (0.53–0.70)	0.61 (0.56–0.66)	0.70 (0.67–0.74)	0.34 (0.28–0.41)	0.83 (0.78–0.87)
Ro52/SSA	0.33 (0.26–0.42)	0.80 (0.76–0.84)	0.80 (0.76–0.82)	0.36 (0.28–0.45)	0.79 (0.74–0.82)
Scl-70	0.01 (0–0.05)	0.99 (0.97–1)	0.85 (0.83–0.88)	0.20 (0.01–0.62)	0.75 (0.71–0.79)
CENP-A	0.04 (0.02–0.09)	0.97 (0.95–0.98)	0.85 (0.82–0.87)	0.31 (0.14–0.56)	0.75 (0.71–0.79)
CENP-B	0.02 (0–0.06)	0.98 (0.95–0.99)	0.85 (0.82–0.87)	0.18 (0.05–0.48)	0.75 (0.71–0.79)
RNA Pol III 11kDa	0.02 (0–0.06)	0.99 (0.97–1)	0.86 (0.83–0.88)	0.33 (0.10–0.70)	0.75 (0.71–0.79)
RNA Pol III 155kDa	0.01 (0–0.05)	0.99 (0.97–0.99)	0.85 (0.83–0.88)	0.17 (0.01–0.56)	0.75 (0.71–0.79)
Fibrillarin	0.01 (0–0.05)	0.99 (0.97–0.99)	0.85 (0.83–0.88)	0.17 (0.01–0.56)	0.75 (0.71–0.79)
NOR90	0.02 (0–0.06)	0.96 (0.93–0.97)	0.84 (0.81–0.86)	0.12 (0.03–0.34)	0.75 (0.71–0.78)
Th/To	0.02 (0–0.06)	0.96 (0.94–0.98)	0.84 (0.81–0.86)	0.12 (0.03–0.36)	0.75 (0.71–0.79)
PM-Scl100	0.03 (0.01–0.08)	0.98 (0.96–0.99)	0.85 (0.83–0.87)	0.33 (0.14–0.61)	0.75 (0.71–0.79)
PM-Scl75	0.04 (0.02–0.09)	0.95 (0.92–0.97)	0.84 (0.81–0.86)	0.21 (0.09–0.40)	0.75 (0.71–0.79)
Ku	0.03 (0.01–0.08)	0.96 (0.93–0.97)	0.84 (0.81–0.86)	0.20 (0.08–0.42)	0.75 (0.71–0.79)
PDGFR	0 (0–0.03)	1 (0.99–1)	0.86 (0.83–0.88)	–	0.75 (0.71–0.79)
≥1 antibody specificity	0.71 (0.62–0.78)	0.41 (0.36–0.46)	0.55 (0.50–0.59)	0.28 (0.24–0.34)	0.81 (0.75–0.86)