Prevalence and clinical profiles of *autoantibody-negative* systemic sclerosis subjects

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**See page S131 for a list of the investigators of the Canadian Scleroderma Research Group.*

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ABSTRACT

Objective. *To determine the prevalence of autoantibody negative systemic sclerosis (SSc) and to identify the clinical correlates thereof.*

Methods. *Clinical data and sera from 874 SSc subjects were collected and autoantibodies were tested in a central laboratory using 1) indirect immunofluorescence (IIF), 2) commercially available ELISA, addressable laser bead immunoassay (ALBIA), and line immunoassay (LIA), and 3) a sensitive immunoprecipitation (IP) assay.*

Results. *Fifteen (15; 1.7%) subjects were autoantibody negative by IIF, ELISA, ALBIA, LIA and IP, and 16 (1.8%) were antinuclear antibody (ANA) positive by IIF but otherwise negative by ELISA, ALBIA, LIA and IP. Thirty-seven (37; 4.2%) were ANA positive by IIF, autoantibody negative by commercially available immunoassays, but had autoantibodies identified by IP (including Th/To in 20). Autoantibody-negative subjects had generally less severe disease than positive subjects.*

Conclusion. *Autoantibody-negative SSc is rare (<2%) and appears to be associated with a favourable prognosis.*

Introduction

Systemic sclerosis (SSc) is a chronic autoimmune rheumatic disease characterised by endothelial and fibroblast dysfunction. Immune dysregulation is a prominent feature and autoantibodies are present in approximately 90–95% of SSc subjects (1-4). Anti-centromere, anti-topoisomerase I and anti-RNA polymerase III antibodies are relatively specific for SSc and tend to be mutually exclusive (5, 6). Other SSc-associated antibodies, including anti-PM-Scl, anti-U1RNP and anti-Ro52/TRIM21 antibodies, are often markers of overlap disease (7). The clinical associations of these autoantibodies have been studied in considerable detail and they can be used in the diagnosis and prognosis of the disease (8).

Autoantibodies can be detected by indirect immunofluorescence (IIF) using HEp-2 cells and a variety of sensitive immunoassays using cellular extracts and/or recombinant antigens, including enzyme-linked immunosorbent assays (ELISAs), addressable laser bead line assays (ALBIA), line immunoassays (LIA) and immunoprecipitation (IP). Autoantibodies detected by IIF are often referred to as antinuclear antibodies (ANA) while those detected by other immunoassays as extractable nuclear antigens (ENA), although the targets can be nuclear, nucleolar, cytoplasmic, cell membrane or extra-cellular and not all intra-cellular targets are extractable nuclear antigens in the original meaning of the term. Thus, although contemporary nomenclature lacks precision and is somewhat of a misnomer, for the purposes of this study, we will adopt this nomenclature.

Whereas approximately 5–10% of SSc subjects have been reported to be ANA negative (1, 3), little is known about these subjects. The objectives of this study were first, to determine the prevalence of ANA/ENA negative and ANA positive/ENA negative SSc subjects using a battery of commercially available immunoassays, second, to use a sensitive IP assay that detects native autoantigens that are not included in commercially available kits and to identify the serological features of these subjects, and third, to describe the clinical correlates of the truly ANA/ENA negative and ANA positive/ENA negative subsets.

Methods

Design

This was a cross-sectional study of a cohort of SSc subjects.

Study subjects

The study subjects consisted of those enrolled in the Canadian Scleroderma Research Group (CSRG) registry. Subjects in this registry are recruited by rheumatologists across Canada. The subjects must have a diagnosis of SSc confirmed by a rheumatologist, be ≥18 years of age, be fluent in English or French, and likely to be compliant with study procedures and visits. Approximately 87% of subjects enrolled in the CSRG registry fulfill the 1980 American College of Rheumatology preliminary criteria for SSc (9), which are known to be poorly sensitive in particular to subjects with limited SSc (10). The subjects included in this study were those whose baseline visit was between September 2004 and August 2009. Ethics committee approval for the CSRG data collection protocol was obtained at McGill University (Montreal, Canada) and at all participating study sites. All subjects provided informed written consent to participate in the data collection protocol.

Measurement of autoantibodies

Serum was collected on all subjects recruited by the CSRG at their baseline registry visit and sent to a central laboratory, Mitogen Advanced Diagnostics Laboratory, University of Calgary. Aliquots of sera were stored at -80° C until needed. Antinuclear antibodies (ANA) were detected by indirect immunofluorescence (IIF) performed on HEp-2 substrate (HEp-2000; ImmunoConcepts, Sacramento, CA, USA) that included fluorescein-conjugated goat antibodies to human IgG (H+L). IIF patterns were detected at serum screening dilutions of 1:160 and 1:640 on a Zeiss Axioskop 2 plus (Carl Zeiss, Jena, Germany) fitted with a 100-watt USHIO super-high-pressure mercury lamp (Ushio, Steinhöring, Germany) by two experienced technologists with more than 7 years of experience. Topoisomerase I, chromatin, Sm, U1-RNP, ribosomal P, Jo-1, SSA/Ro60, SSB-La were assayed by an addressable laser bead immunoassay (ALBIA) using a commercially available kit (QUANTAPlex ENA 8, INOVA Diagnostics Inc., San Diego, CA; FIDIS Connective 13, BMD, Paris, France) in a Luminex 200 (Luminex Corp., Austin, TX) platform according to protocols previously described (11). Antibodies to RNA polymerase III were detected by ELISA (INOVA Diagnostics) (11) as were antibodies to PM/Scl (PM1 alpha: Dr Fooke Laboratorien GmbH, Neuss, Germany) (12). CENP-A, CENP-B, fibrillarin, NOR-90, Th/To, PM/Scl-75, PM/Scl-100, Ku, platelet derived growth factor (PDGFR) and Ro52/TRIM21 were detected by a line immunoassay (LIA: EUROLINE, Euroimmun, Lubeck, Germany). Sera that were negative on these immunoassays were tested by IP. Protein components were analysed by IP using K562 (human erythroleukaemia) metabolically radiolabelled with ^{35}S-methionine. RNA components of the autoantigens recognised by sera were analysed by IP using unlabelled K562 cell extract and RNA analysis by urea-PAGE and silver staining as described.

The subjects were divided into 3 groups:
1) ANA/ENA negative, if ANA was negative by IIF and ENA was negative by ELISA, ALBIA, LIA and IP;
2) ANA positive/ENA negative, if ANA was positive by IIF but ENA was negative by ELISA, ALBIA, LIA and IP; and
3) others, if ANA was positive by IIF and any autoantibody was detected by ELISA, ALBIA, LIA or IP in assays as described above.

Study measures

The subjects recruited into the Registry undergo an extensive medical evaluation with standardised reporting of history, physical evaluation, and laboratory investigations. Demographic information regarding age and sex was collected by subject self-report. Disease duration, determined from the onset of the first non-Raynaud's disease manifestation to the baseline study visit, was recorded by the study physician. Skin involvement was assessed using the modified Rodnan skin score (13), a widely used clinical assessment where the examining rheumatologist records the degree of skin thickening ranging from 0 (no involvement) to 3 (severe thickening) in 17 areas (total score range 0–51). Subjects were classified into limited and diffuse cutaneous subsets, based on the definition of Leroy *et al*. (14), whereby those with skin involvement distal to the elbows and knees (with or without facial involvement) were identified as having limited cutaneous disease and those with skin involvement proximal to the elbows and knees (with or without truncal involvement) were classified as having diffuse cutaneous disease. The presence of Raynaud's phenomenon, sclerodactyly, calcinosis, oesophageal dysmotility, telangiectasias, digital pits and digital ulcers was recorded by the study physician. History of inflammatory myositis, polyarthritis, scleroderma renal crisis, and overlap with SLE, Sjögren's syndrome, rheumatoid arthritis, and mixed connective tissue disease was recorded by the study physician. Nailfold capillaroscopy, which has been shown to predict outcome in SSc (15), was performed by a physician using a handheld Dermlite® and the presence of dilated or giant capillaries and dropout areas was recorded (16). Global assessment of disease severity, as well as activity and damage, were reported by study physicians using 0–10 numerical rating scales, with 0 representing least and 10 most disease.

The presence of interstitial lung disease (ILD) was determined by high resolution computed tomographic (HRCT) scans of the chest, when available, or by a combination of chest x-ray findings and the presence of typical "velcro-like crackles" on lung auscultation, using a recently published algorithm (17). Pulmonary function tests were obtained in accordance with American Thoracic Society standards. Percent predicted forced vital capacity (FVC) and single breath diffusing capacity for carbon monoxide (DLCO) were recorded. Systolic pulmonary artery pressure (SPAP) was measured using the Doppler flow measurement of the tricuspid regurgitant jet on echocardiography and pulmonary hypertension (PH) was defined as an estimated SPAP ≥45 mmHg (an estimate that correlates strongly with right heart catheter studies (18)).

Statistical analysis

Descriptive statistics were used to

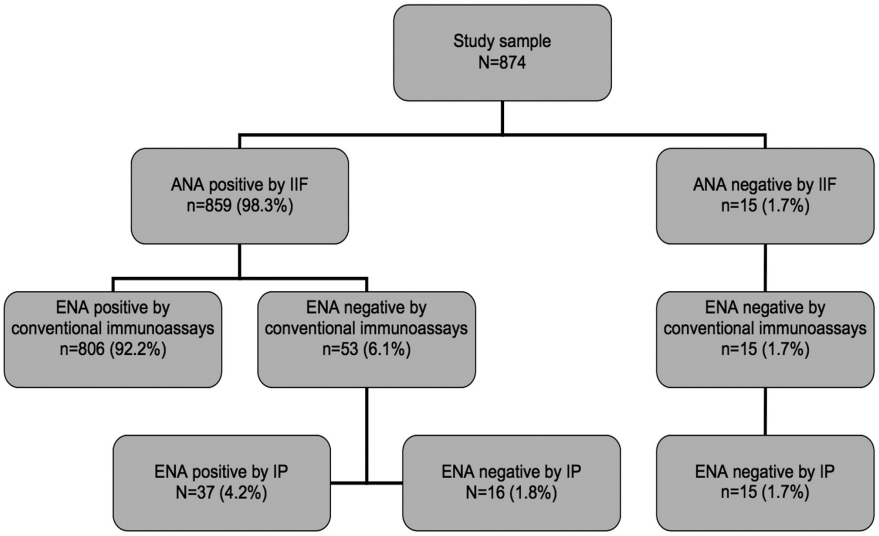

Fig. 1. Serological analyses of sera from subjects in the Canadian Scleroderma Research Group cohort. All sera were tested by indirect immunofluorescence (IIF) and using conventional, commercially available immunoassays, including ELISA, ALBIA and LIA. ANA negative/ENA negative and ANA positive/ENA negative sera by conventional immunoassays were further tested by immunoprecipitation.

summarise the baseline characteristics of the subjects. Comparisons of clinical features among various antibodies profile of the subjects were done using logistic regression, ANOVA and Kruskal-Wallis test, where appropriate. *p*-values <0.05 were considered statistically significant. Further statistical analysis was not performed due to the exploratory nature of the study. All statistical analyses were performed with SAS v. 9.2 (SAS Institute, USA).

Results

This study included 874 SSc subjects who had serum autoantibodies screened by IIF, of which 859 (98.3%) were ANA positive by IIF (Fig. 1). Of these, 806 (92.2%) were ENA positive and 53 (6.1%) were ENA negative by conventional commercially available assays (ELISA, ALBIA and LIA). These 53 samples were further tested by IP and a number of autoantibodies were identified in 37 (4.2%), including Th/To (n=20), fibrillarin (n=5), U1-RNP (n=2), Su/Argonaute2 (Ago2) (n=2), topoisomerase I (n=2), RNAPI/III (n=1), RNAPII (n=1) and NOR90 (n=1). Thus, 16 (1.8%) subjects were ANA positive by IIF but ENA negative by ELISA, ALBIA, LIA and IP and 15 (1.7%) were ANA negative by IIF and ENA negative by conventional commercially available assays (ELISA, ALBIA and LIA) and by IP (*i.e.* no identifiable/known autoantibodies).

The clinical profiles of truly ANA negative/ENA negative (n=15) and ANA positive/ENA negative (n=16) subjects were examined in detail (Tables I and II). Among the ANA negative/ENA negative subset, 12/15 met ACR classification criteria for SSc. Among the 3 who did not, all had Raynaud's phenomenon and 2 had limited cutaneous disease. The subject without skin involvement had oesophageal dysmotility, telangiectasias, abnormal nailfold capillaroscopy, digital ulcers and inflammatory myositis. Among the ANA positive/ENA negative subset, 12/16 met ACR classification criteria for SSc. Among the 4 who did not, all had Raynaud's phenomenon and 2 had limited cutaneous disease. Of the 2 subjects without skin involvement, one had oesophageal dysmotility, telangiectasias, digital ulcers, abnormal nailfold capillaroscopy, ILD and PH, and

Table I. ANA-/ENA- subjects (n=15).

Patient	ACR	Raynaud's Phenomenon	Disease subset	Skin score	Sclero-dactyly	Oesophageal dysmotility	Telangi-ectasias	Digital ulcers	Capillaro-scopy	ILD	PH	Myositis	Arthritis	SRC
1	1	1	Limited	4	1	0	0	0	1	0	0	0	0	0
2	1	1	Limited	4	1	0	0	1	1	1	0	0	0	0
3	1	1	Limited	8	1	1	1	1	1	0	0	0	0	0
4	1	1	Limited	0	1	0	1	1	0	0	0	0	0	0
5	1	1	Limited	8	1	1	1	1	1	1	0	0	0	0
6	1	1	Limited	4	1	1	1	1	1	0	0	0	0	0
7	1	0	Limited	24	1	0	0	0	0	0	0	0	1	0
8	1	1	Diffuse	13	1	0	0	0		0	0	0	0	0
9	1	1	Limited	5	1	1	1	1	1	0	0	0	0	0
10	1	0	Limited	15	1	1	0	0	1	1	0	0	0	0
11	1	1	Diffuse	4	1	0	0	1	0	0	0	0	0	0
12	1	1	Diffuse	16	1	1	1	1	0	0	0	0	0	0
13	0	1	Limited	0	1	0	1	1	1	0	0	0	1	0
14	0	1	Limited	1	1		0	0	0	0	0	0	1	0
15	0	1	Sine	0	0	1	1	1	1	0	0	1	0	0
Total	12	13	Limited 11 Diffuse 3 Sine 1	Mean 7.1 SD±7	14	7	8	10	9	3	0	1	3	0

ANA: antinuclear; ENA: extractable nuclear antigen; ACR: American College of Rheumatology; ILD: interstitial lung disease; PH: pulmonary hypertension; SRC: scleroderma renal crisis; NA: not available.

Table II. ANA+/ENA- positive subjects (n=16).

Patient	ACR	Raynaud's Phenomenon	Disease subset	Skin score	Sclero-dactyly	Oesophageal dysmotility	Telangi-ectasias	Digital ulcers	Capillaro-scopy	ILD	PH	Myositis	Arthritis	SRC
1	1	1	Diffuse	24	1	1	1	1	1	0	0	1	0	0
2	1	1	Diffuse	10	1	0	1	0	0	0	0	1	0	0
3	1	1	Diffuse	34	1	0	0	0	0	0	NA	0	0	0
4	1	1	Diffuse	16	1	1	1	0	1	0	0	0	0	0
5	1	1	Limited	7	1	0	1	1	NA	0	0	0	0	1
6	1	1	Limited	2	1	1	0	0	1	0	0	0	0	0
7	1	1	Limited	4	1	1	1	0	1	1	0	1	0	0
8	1	1	Limited	12	1	0	1	1	1	0	0	0	1	0
9	1	1	Limited	3	1	0	0	0	1	0	0	0	0	0
10	1	1	Limited	0	0	1	1	1	0	0	0	0	1	0
11	1	0	Diffuse	37	1	0	1	0	0	0	0	0	1	0
12	1	0	Limited	11	1	0	0	0	0	0	0	1	1	0
13	0	1	Limited	0	1	1	0	0	0	0	0	0	0	0
14	0	1	Limited	2	0	1	1	0	0	0	0	0	0	0
15	0	1	Sine	0	0	1	1	1	1	1	1	0	0	0
16	0	1	Sine	0	0	1	1	0	1	0	1	0	0	0
Total	12	14	Limited 9 Diffuse 5 Sine 2	Mean 10.1 SD±12	12	9	11	5	8	2	2	4	4	1

ANA: antinuclear; ENA: extractable nuclear antigen; ACR: American College of Rheumatology; ILD: interstitial lung disease; PH: pulmonary hypertension; SRC: scleroderma renal crisis; NA: not available.

Table III. Clinical profiles of ANA-/ENA- and ANA+/ENA- subjects, compared to ANA+/ENA+ subjects in the CSRG (n=874).

	ANA-/ENA- (n=15)		ANA+/ENA- (n=16)		ANA+/ENA+ (n=843)		*p*-values ANA-/ENA- *vs.* ANA+/ENA+	*p*-values ANA+/ENA- *vs.* ANA+/ENA+
	Mean or n.	SD or %	Mean or n.	SD or %	Mean or n.	SD or %		
Female	12	80.0%	14	87.5%	724	85.9%	NS	NS
Age	57.18	11.56	54.93	12.75	55.50	12.24	NS	NS
Meet ACR classification	12	80.0%	12	75.0%	746	88.6%	NS	NS
Disease duration	12.54	11.20	6.40	6.02	10.90	9.36	NS	0.0480
Diffuse disease, %	3	20.0%	5	31.3%	321	38.5%	NS	NS
Modified Rodnan skin score (0-51)	7.07	7.05	10.13	12.01	10.20	9.46	NS	NS
Raynaud's phenomenon	13	86.7%	14	87.5%	820	97.7%	0.0171	0.0214
Sclerodactyly	14	93.3%	12	75.0%	776	92.7%	NS	0.0148
Calcinosis	3	20.0%	3	18.8%	263	31.4%	NS	NS
Oesophageal dysmotility	7	50.0%	9	56.3%	528	71.2%	NS	NS
Telangiectasias	8	53.3%	11	68.8%	608	76.9%	0.0419	NS
Abnormal nailfold capillaroscopy	9	64.3%	8	53.3%	634	77.8%	NS	0.0327
Digital pits	6	42.9%	5	33.3%	404	48.8%	NS	NS
Digital ulcers	10	66.7%	5	31.3%	475	56.7%	NS	NS
Interstitial lung disease	3	20.0%	2	12.5%	311	37.6%	NS	NS
Pulmonary hypertension	0	0.0%	2	13.3%	87	12.1%	NS	NS
Cardiac involvement								
% with ejection fraction <50%	1	7.1%	1	7.7%	15	2.2%	NS	NS
% currently on drugs for heart failure	0	0.0%	1	6.3%	43	5.1%	NS	NS
% currently on anti-arrythmics	0	0.0%	0	0.0%	20	2.4%	NS	NS
FVC, % predicted	93.86	16.37	90.81	14.47	90.54	19.27	NS	NS
DLCO, % predicted	73.54	27.33	65.06	19.39	69.26	20.60	NS	NS
History of scleroderma renal crisis	0	0.0%	1	6.3%	39	4.7%	NS	NS
Proteinuria							NS	NS
0	13	92.9%	13	92.9%	678	92.4%		
1-2	1	7.1%	1	7.1%	47	6.4%		
>3	0	0.0%	0	0.0%	9	1.2%		
History of inflammatory myositis	1	6.7%	4	25.0%	96	11.5%	NS	NS
History of inflammatory arthritis	3	20.0%	4	25.0%	263	32.5%	NS	NS
Joint contractures	3	20.0%	2	12.5%	158	18.9%	NS	NS
CRP (mg/L)	14.28	21.33	5.95	9.40	9.66	18.94	NS	NS
Overlap with other CTD	0	0.0%	1	6.3%	127	15.17	NS	NS
Global assessment of severity (0-10)	1.93	1.44	3.81	3.31	2.74	2.25	NS	NS
Global assessments of activity (0-10)	1.60	1.45	2.63	2.87	2.34	2.09	NS	NS
Global assessments of damage (0-10)	2.13	1.64	3.69	3.22	3.36	2.33	NS	NS

ANA: antinuclear; ENA: extractable nuclear antigen; SD: standard deviation; ACR: American College of Rheumatology; FVC: forced vital capacity; DLCO: diffusion capacity of the lung for carbon monoxide; CRP: C-reactive protein; CTD: connective tissue disease.

the other had oesophageal dysmotility, telangiectasias, abnormal nailfold capillaroscopy and PH.

The clinical profiles of truly ANA negative/ENA negative and ANA positive/ENA negative subjects were compared to ANA positive/ENA positive subjects (Table III). Notable differences included the following: ANA negative/ENA negative had generally less organ involvement than ANA positive/ENA positive subjects (including skin, oesophageal, lung, muscle and joint disease) and less overall disease severity, activity and damage. ANA positive/ENA negative subjects had less CREST symptoms and lung disease than ANA positive/ENA positive subjects, but comparable overall disease severity, activity and damage.

Discussion

In this large cohort of well-characterised SSc subjects, we found that the vast majority of subjects had a positive ANA (98.3%) and an identifiable autoantibody using a battery of commercially available immunoassays (92.2%). An additional 4.2% had identifiable autoantibodies using a sensitive IP assay. Thus, after an exhaustive approach to serology, only a small proportion had an ANA but no identifiable autoantibody using sensitive immunoassays (*i.e.* ANA positive/ENA negative, 1.8%) and even less were ANA negative and had no identifiable autoantibody (1.7%). In addition, we also found that the vast majority of autoantibody-negative SSc subjects had features that were highly consistent with the classification and diagnosis of SSc, although their disease appeared to be generally milder compared to that of autoantibody replete subjects. These data emphasise the fact that the immune diathesis is among the most common manifestation of SSc, even more common than Raynaud's manifestation. Whether the autoantibody profiles of autoantibody-negative subjects are more labile or whether the absence of detectable autoantibodies persists and is to be viewed as a favourable prognostic factor are questions to be addressed by future longitudinal studies, which are considered within the design and ongoing efforts of the CSRG.

Our study was specifically designed to characterise the clinical and serological profiles of SSc subjects without autoantibodies detected by a battery of commercially available kits. In agreement with a previous study, many had autoantibodies to the Th/To system as detected by IP (19). Very few SSc subjects with positive ANA by IIF still remain with undetectable autoantibodies after an exhaustive search (less than 2%).

The German network for systemic scleroderma recently analysed the autoantibody profiles of their subjects, using a detailed approach that included IIF, LIA, immunodiffusion and IP (2). In that study, sera that were negative for ANA by IIF on HEp-2 cells, but exhibited cytoplasmic fluorescence and/or a positive signal in any of the other assays were grouped together as ANA-negative. Sera without any positive signal, neither defined nor undefined, in all immunoassay systems used were listed as autoantibody-negative. They reported 50/863 (5.8%) ANA-negative SSc subjects and 38/863 (4.4%) autoantibody-negative subjects. They provided some clinical correlates for the ANA-negative subjects, including significantly *less* Raynaud's phenomenon, digital ulcers and PH compared to ANA-positive subjects, and more joint contractures. Our findings are generally consistent with these, with our data also suggesting that ANA negative/ENA negative and ANA positive/ENA negative subjects have less organ involvement and disease severity. EUSTAR also recently reported on their subjects without ANA or Raynaud's phenomenon (4). In their large sample, ANA was negative in approximately 8% of subjects. However, only 12/5378 (0.2%) lacked both ANA and RP, which is identical to our results. Differences in the studies may be attributed, among other things, to somewhat different definitions of ANA negativity, different sets of immunoassays used, or differences in the study cohorts.

In conclusion, we showed that, in a disease as heterogeneous as SSc, autoantibodies are almost universally present. Autoantibody-negative SSc is rare and, if it is to be considered as a separate entity, appears to be associated with a favourable prognosis.

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References

1. STEEN VD: Autoantibodies in systemic sclerosis. *Semin Arthritis Rheum* 2005; 35: 35-42.
2. MIERAU R, MOINZADEH P, RIEMEKASTEN G *et al*.: Frequency of disease-associated and other nuclear autoantibodies in patients of the German Network for Systemic Scleroderma: correlation with characteristic clinical features. *Arthritis Res Ther* 2011; 13: R172.
3. MEHRA S, WALKER J, PATTERSON K, FRITZLER MJ: Autoantibodies in systemic sclerosis. *Autoimmun Rev* 2012.
4. SCHNEEBERGER D, TYNDALL A, KAY J *et al*.: Systemic sclerosis without antinuclear antibodies or Raynaud's phenomenon: a multicentre study in the prospective EULAR Scleroderma Trials and Research (EUSTAR) database. *Rheumatology* (Oxford) 2012.
5. REVEILLE JD, SOLOMON DH: Evidence-based guidelines for the use of immunologic tests: anticentromere, Scl-70, and nucleolar antibodies. *Arthritis Rheum* 2003; 49: 399-412.
6. HEIJNEN IA, FOOCHAROEN C, BANNERT B *et al*.: Clinical significance of coexisting antitopoisomerase I and anticentromere antibodies in patients with systemic sclerosis: a EUSTAR group-based study. *Clin Exp Rheumatol* 2013; 31 (Suppl. 76): S96-S102.
7. HUDSON M, POPE J, MAHLER M *et al*.; CANADIAN SCLERODERMA RESEARCH GROUP (CSRG): Clinical significance of antibodies to Ro52/TRIM21 in systemic sclerosis. *Arthritis Res Therapy* 2012; 14: R50.
8. BALDINI C, MOSCA M, DELLA ROSSA A *et al*.: Overlap of ACA-positive systemic sclerosis and Sjögren's syndrome: a distinct clinical entity with mild organ involvement but at high risk of lymphoma. *Clin Exp Rheumatol* 2013; 31: 272-80.

9. SUBCOMMITTEE FOR SCLERODERMA CRITERIA OF THE AMERICAN RHEUMATISM ASSOCIATION DIAGNOSTIC AND THERAPEUTIC CRITERIA COMMITTEE: Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum* 1980; 23: 581-90.
10. HUDSON M, TAILLEFER S, STEELE R *et al*.: Improving the sensitivity of the American College of Rheumatology classification criteria for systemic sclerosis. *Clin Exp Rheumatol* 2007; 25: 754-7.
11. SANTIAGO M, BARON M, HUDSON M, BURLINGAME RW, FRITZLER MJ: Antibodies to RNA polymerase III in systemic sclerosis detected by ELISA. *J Rheumatol* 2007; 34: 1528-34.
12. MAHLER M, RAIJMAKERS R, DAHNRICH C, BLUTHNER M, FRITZLER MJ: Clinical evaluation of autoantibodies to a novel PM/Scl peptide antigen. *Arthritis Res Ther* 2005; 7: R704-713.
13. CLEMENTS P, LACHENBRUCH P, SIEBOLD J *et al*.: Inter and intraobserver variability of total skin thickness score (modified Rodnan TSS) in systemic sclerosis. *J Rheumatology* 1995; 22: 1281-5.
14. LEROY EC, BLACK C, FLEISCHMAJER R *et al*.: Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988; 15: 202-5.
15. KAYSER C, SEKIYAMA JY, PROSPERO LC, CAMARGO CZ, ANDRADE LE: Nailfold capillaroscopy abnormalities as predictors of mortality in patients with systemic sclerosis. *Clin Exp Rheumatol* 2013; 31 (Suppl. 76): 103-8.
16. BARON M, BELL M, BOOKMAN A *et al*.: Office capillaroscopy in systemic sclerosis. *Clin Rheumatol* 2007; 26: 1268-74.
17. STEELE R, HUDSON M, LO E, BARON M: A clinical decision rule to predict the presence of interstitial lung disease in systemic sclerosis. *Arthritis Care Res* (Hoboken) 2011; 64: 519-24.
18. HSU VM, MOREYRA AE, WILSON AC *et al*.: Assessment of pulmonary arterial hypertension in patients with systemic sclerosis: comparison of noninvasive tests with results of right-heart catheterization. *J Rheumatol* 2008; 35: 458-65.
19. CERIBELLI A, CAVAZZANA I, FRANCESCHINI F *et al*.: Anti-Th/To are common antinucleolar autoantibodies in Italian patients with scleroderma. *J Rheumatol* 2010; 37: 2071-5.