Immunopathologia Persa
DOI:10.34172/ipp.2022.24238
http www.immunopathol.com

Correlation between autoantibodies and internal organs involvement in Iranian systemic sclerosis patients

Peyman Mottaghi, Marzieh Daneshbodi*, Mansoor Karimifar

Department of Rheumatology, Alzahra Hospital, Isfahan University of Medical Sciences, Isfahan, Iran

***Correspondence to**
Marzieh Daneshbodi, Email; marziehdaneshbodi@yahoo.com, marzieh@resident.mui.ac.ir

Received 4 Mar. 2021
Accepted 11 Aug. 2021
Published online 11 Dec. 2021

Keywords: Scleroderma (systemic sclerosis), Internal organ involvement, Autoantibody

Abstract

Introduction: Scleroderma is a rare connective tissue disease that leads to fibrosis of the skin and different internal organ. Several autoantibodies can predict disease course and differences among various races.
Objectives: The current study aimed to investigate the correlation between autoantibodies and scleroderma organ involvement among the population living in the central region of Iran.
Patients and Methods: This is a cross-sectional study in which we reviewed the medical records of patients in the Al-Zahra hospital systemic sclerosis (SSc) clinic in Isfahan. We used the EUROLINE Systemic Sclerosis panel test kit to detect antibody profiles of patients in blood samples. The pertinent physical findings and laboratory data showing organ involvement were extracted from the hospital medical records.
Results: Forty-six patients were enrolled in our study (40 patients were women). We found a significant correlation between Scl-70 and diffuse scleroderma, interstitial lung disease (ILD) and cardiac involvement. Anti-centromere antibodies CENP-A correlated with limited scleroderma and digital ulcers. PM/Scl75, PM/Scl100, Ku, and Ro52 autoantibodies correlated with the presence of tendon friction rub. Anti-Th/To is associated with a higher prevalence of myopathy. Cardiomyopathy was more common among patients with higher levels of NOR-90, anti-Th/To, PM/Scl100, Ku, and Ro52 autoantibodies. The remained autoantibodies showed no significant correlation with the clinical features of SSc.
Conclusion: Some autoantibodies in this study predict organ damage in Iranian scleroderma patients.

Citation: Mottaghi P, Daneshbodi M, Karimifar M. Correlation between autoantibodies and internal organs involvement in Iranian systemic sclerosis patients. Immunopathol Persa. 2022;x(x):e24238. DOI:10.34172/ipp.2022.24238.

Introduction

Systemic sclerosis (SSc) is a rare-orphan connective tissue disease, classically characterized by the tightness of the skin. It is not necessarily limited to the skin and can involve other organs too. The clinical picture of the disease based on the extent of skin involvement is generally divided into limited and diffuse SSc forms according to extend of the skin involvement (1). The researchers reported the association between serum autoantibodies and the subtypes of the disease in 1988, for the first time. It brings up the importance of these autoantibodies for the assessment of SSc. (1). Thereafter, new autoantibodies have been described in SSc that a number of which are shown to have clinical relevance (2). The combined clinical-serologic view to SSc and 2013 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria for SSc considerably helps physicians detect patients in the early stages of the diseases with high sensitivity and specificity (2-4). Evidence provides that 75%-95% of SSc patients have a positive test for autoantibodies, specific or strongly associated with SSc (2). Three phases are usually seen throughout the course of this disease; (a) Autoimmunity and inflammation (b) Functional and structural changes in small blood vessels and (c) Widespread fibrosis of the skin and different internal organs (3). Clinical cues pointing at SSc are subtle and few at early stages. Therefore, autoantibodies are considered as means for an early diagnosis (or suspicion) of SSc. Additionally, evidence shows that patients who test positive for these autoantibodies in the early stages of the disease, usually stay antibody-positive over the course of the disease (4). Different studies

Key point

This study evaluates the predictive value of autoantibodies for internal organ involvement in sera of Iranian patients with scleroderma. This correlation helps to improve the care of patients with scleroderma.

have shown an association between certain autoantibodies and clinical manifestations such as hand deformity, Raynaud's phenomenon, pulmonary fibrosis, overlaps, and an overall severity rate of the disease (5,6). Various studies have previously shown that the distribution of SSc-related autoantibodies and their clinical correlations vary across the populations living in different geographical areas (6,7). This makes it essential to assess patients' serologic profiles in view of their genetic background for a more practical and personalized clinical perspective towards each patient.

Objectives

This study aims to evaluate the correlation between various SSc-related autoantibodies and organ involvements among Iranian SSc patients.

Patients and Methods

Study design

This is a retrospective cohort study of patients with SSc diagnosed according to the 2013 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria. All eligible patients with SSc followed in the scleroderma clinic of Al-Zahra hospital in Isfahan (Isfahan province, Iran) from March 2012 to December 2020 were enrolled in the study.

Inclusion and exclusion criteria

We included SSc patients with at least four years since their diagnosis. The exclusion criteria were smoking, malignancy, overlap syndrome, and irregular follow-ups.

Data collection

A rheumatologist visited the patients in the special clinic of scleroderma in Al-Zahra hospital (the biggest referral hospital in the central region of Iran) and we reviewed the charts for clinical and para-clinical data. The history of clinical course, organs involvement, and para-clinical tests showing the state of organ function (e.g., echocardiography, high-resolution CT, and endoscopy) were recorded. We defined organs involvement based on the details presented in Table 1.

Serology

The plasma was collected from blood sample of each patient stored in a -18°C degrees centigrade freezer. The scleroderma autoimmune profile kit from EUROIMMUN Corporation© (via immunoblot method) was used for the detection of the specific autoantibodies. This kit is designed for detection of the following autoantibodies: Scl 70 (anti-topoisomerase-1), centromere antigen subunits (CENP-A and CENP-B), RNA Polymerase III subunits (RP11, RP155), fibrillarin, nucleolus-organizing regions (NOR 90), anti-Th/To, polymyositis PM/Scl 100, PM/Scl 75, Ku, platelet-derived growth factor receptor (PDGFR) and Ro-52. Details of the serologic testing are shown in Figure 1.

Statistical analysis

Descriptive statistics were reported as means and standard deviations for continuous variables and frequencies and percentages for categorical variables. The antibodies were recorded as dichotomous variables based on their positivity and were assessed for their correlation with clinical findings using Fisher's exact test and two by two tables. A *P* value equal to or less than 0.05 was considered significant. SPSS software version 19 was used for statistical analysis.

Results

Forty-six patients including 40 (87%) women and 6 (13%) men were enrolled in our study. Twenty-eight (58.3%) patients had the diffuse, and 18 (37.5%) patients had the limited type of scleroderma. The mean age of patients was 43±10.9, and the mean disease duration was 10.1 ± 4.9 years. Raynaud phenomenon (100%) and gastroesophageal reflux (91.7%) were the most common, but renal crisis (2.1%) and pericardial effusion (6.3%) were the rarest forms of organ involvement in our study.

The most commonly found autoantibodies were anti-topoisomerase-1 (77.1%) and anti-Th/To (33.4%), respectively. Anti-Scl 70 was significantly more prevalent in the diffuse SSC group (*P*= 0.011) and CENPA in the limited type of patients (p= 0.046). Other auto-antibodies did not differ significantly between the two types of SSc. Anti-RP 11 was the least commonly detected autoantibody in our study, and aside from that, anti-PDGF (platelet-derived growth factor) was not detected in our participants. Results of comparative frequencies of autoantibodies among different clinical types of our SSc patients show in Table 2.

Anti-topoisomerase-1 antibody showed an association with interstitial lung disease (ILD), tendon friction rub and cardiomyopathy. Autoantibodies NOR-90, anti-Th/To, PM/Scl 100, Ku and Ro52 were associated with heart disease and Ro52 with pericardial effusion. The autoantibodies to Scl 70, PM/Scl 75, PM/Scl 100, Ku, and Ro52 were found significantly associated with tendon friction rub and anti-Th/To was associated with the presence of myopathy. CENPA was correlated with digital ulcers. We did not find any significant correlation between SSc-associated organs and the remaining autoantibodies tested in our study. Table 3 summarizes these correlations and predictive values of autoantibodies for organs involvements.

Discussion

Interestingly, all of our patients have at least one specific autoantibody whilst in other studies; the prevalence of these autoantibodies was less than ours (2). We found a correlation between anti-topoisomerase-1 antibodies and diffuse scleroderma as well as the development of ILD and cardiac disease. Anti CENP-A correlated with limited scleroderma and digital ulcers. Anti-PM/Scl75, PM/Scl100, anti-Ku and anti-Ro52 autoantibodies were

Table 1. The definitions and measurement of organ involvement

Organ Involvement	Indicators of involvement	Measurement /definition	Source of data
Cutaneous involvement	Skin stiffness	Thickness and hardening of dermis (8)	Physical examination
	Microstomia	Defined as an ability of opening their mouth limited to the patient's three middle fingers (5)	Physical examination or documented in patients' records
	Raynaud's phenomenon	A history of excessive cold sensitivity and recurrent events of sharply demarcated pallor and/or cyanosis of the skin of the digits (9)	Existence or a history of as expressed by the patients or documented in patients' records
	Telangiectasia	Erythematous matted skin lesions of vascular origin, thus they blanch after local pressure (9)	Found through physical examinations, formerly recorded in the patient's records
	Digital ulcerations	Active ulcers were defined as denuded areas with defined borders and loss of epithelialization, loss of epidermis, and dermis distal to the proximal interphalangeal joint on the volar aspect of a finger (10)	Found through physical examinations, formerly recorded in the patient's records
	Calcinosis	Calcium deposition that cluster around joints (9)	Found by physical examination, or through radiologic study
MSK involvement	Synovitis	Arthralgia, joint swelling, or morning stiffness (11)	By history, detected in physical examination, or a former patient's records
	Myopathy	Decrease proximal muscle power	Physical examination
	Tendon friction rub	The examiner to place the palmar aspect of his or her fingers across the tendon area being examined, and ask the patient to actively move the underlying joint through as full a range of motion as possible. A leathery, rubbing, "squeaking" sensation will be noted if a tendon friction rub is present (12)	Examination or recorded in the patient's records
	Digital contracture	A distance of 1.9 cm or more between the patient's palm and their fingertip at maximum flexion (5)	Detected through examination or recorded formerly
Pulmonary involvement	Interstitial lung disease	NSIP, UIP, Honeycombing and fibrosis	Seen on x-ray radiogram or high-resolution chest CT scan. A related pulmonary involvement confirmed by a pulmonologist.
	Pulmonary fibrosis		
Cardiac involvement	Pulmonary hypertension	Right atrial pressure of 40 water-mms or higher (5)	Transthoracic echocardiography
	Cardiomyopathy	An ejection-fraction of less than 45%, arrhythmias that require treatment, or complete heart block (5)	Electrocardiogram, echocardiography
	Pericarditis	Pericardial effusion	Confirmed by echocardiography
Renal involvement	Renal crisis	A sudden onset of hypertension (above 140/90 mm Hg, a 30 mm Hg increase in systolic blood pressure, or a 20 mm Hg increase in diastolic blood pressure) and associated disorders, including an increase of more than 50% in serum creatinine or above 120% of normal range, proteinuria (above +2 in urine qualitative assessment and urine protein / creatinine ratio above normal range); Microscopic hematuria, thrombocytopenia, hemolysis, or hypertensive encephalopathy (13)	Existence or a history of as expressed by the patients or documented in patients' records
GI involvement	GE reflux disease	Heart burn and regurgitation or esophagitis	History expressed by the patient, recorded in their files, or confirmed by endoscopic studies
	Chronic constipation	Fewer than three bowel movements a week more than 3 month (14)	
	Chronic diarrhea	Persistence alteration of stool consistency from the norm with loose stool and increase frequency of greater than 4 weeks duration (15)	

NSIP, nonspecific interstitial pneumonitis; UIP, usual interstitial pneumonitis; MSK: musculoskeletal, GI: gastrointestinal, GE, gastroesophageal.

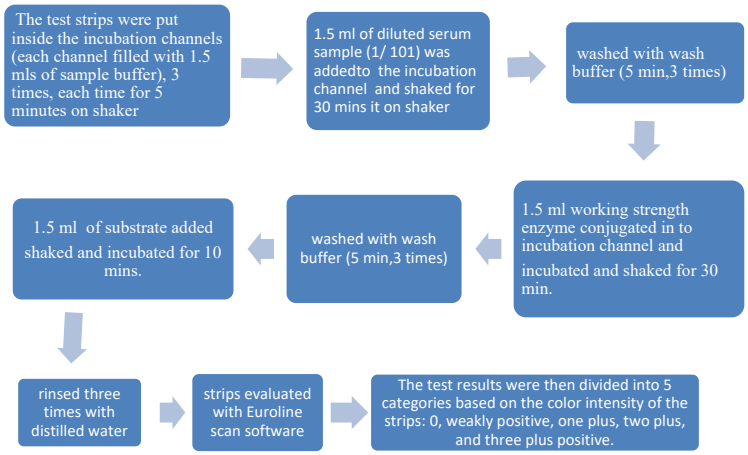

Figure 1. Serologic testing.

correlated with presence of tendon friction rub. Anti-Th/To was associated with a higher rate of myopathy. Cardiomyopathy was more common among patients with higher levels of NOR-90, anti-Th/To, PM/Scl100, anti-Ku and Ro52 autoantibodies. The latter was also associated with pericardial effusion.

A study of 212 SSC anti-Scl 70 positive patients, similar to our study, found a relationship with a higher rate of heart disease followed by the development of ILD (16). Another study involving 28 SSc patients showed that lower SCL 70 antibody levels were associated with more desirable clinical outcomes (17). In a cohort of 460 patients with SSc, the most frequent organ involvement was ILD and was more common in patients with diffuse SSc (18). They also reported a greater probability that the limited type progresses toward the diffuse type with an anti-topoisomerase I positive serum. Anti-topoisomerase-I was formerly associated with diffuse SSc (EUSTAR), ILD (in Belgium), digital ulcers (DU) in Germany, and cardiac involvement in Malaysia (5).

Anti-centromere antibodies are widely accepted to be associated with limited cutaneous SSc, development of pulmonary hypertension, less severe course of the disease, and with a lower prevalence of lung fibrosis, cardiomyopathy, or renal involvement. We found an association between anti-CENP-A and CENP-B with digital ulcers. However, no association between anti-centromere antibodies and other involved organs was detected. Likewise, Volpe et al (18) reported no significant association between the clinical severity of the illness and higher levels of anti-CENP-B. In a multicenter cohort of 802 SSc patients, Perosa et al also reported anti-CENP-A and CENP-B to be linked with a lower rate of clinical symptoms and lower clinical severity, since unlike what we found, pulmonary hypertension was more common in patients with positive anti-CENP (19).

Anti-PM/Scl antibodies were found to be associated with overlap syndromes in Germany, with myositis and ILD in Canada (2). The results from a cohort of 280 SSc patients showed that patients with a positive anti-PM/Scl have a higher likelihood of muscle involvement and lung fibrosis, but little gastrointestinal (GI) involvement was seen (20).

Table 2. Comparative frequencies of autoantibodies among patients with different clinical types

Autoantibody	Limited No. (%)	Diffuse No. (%)	Total No. (%)	*P* value for between-type difference (Fisher's exact test)
Anti-topoisomerase I (Anti-Scl-70)	12 (66.7)	28 (100)	41 (77.1)	0.011 (Cramer's V=0.492)
Anti-centromere A (CENPA)	5 (27.8)	3 (10.7)	8 (16.7)	0.046 (Cramer's V=0.417)
Anti-centromere B (CENPB)	5 (27.8)	3 (10.7)	8 (16.7)	1
Anti-RNA polymerase 11 (RP-11)	0 (0)	2 (7.1)	2 (4.1)	1
Anti-RNA polymerase 155 (RP-155)	2 (11.1)	7 (25)	9 (19.8)	0.4
Anti-U3RNP (fibrillarin)	1 (5.6)	3 (10.7)	3 (6.25)	1
Anti- nucleolus organizer region-90 (Anti-NOR90)	1 (5.6)	3 (10.7)	4 (8.33)	0.6
Anti-Th/To ribonucleoprotein (Anti-Th/To)	4 (22.2)	13 (46.4)	17 (33.4)	0.3
Anti-PM/Scl-75	1 (5.6)	4 (14.3)	5 (10.7)	0.3
Anti-PM/Scl-100	1 (5.6)	3 (10.7)	4 (8.4)	0.6
Anti-Ku	3 (16.7)	5 (17.9)	8 (16.4)	1
Anti-PDGFR	0	0	0	-
Anti-Ro-52	6 (33.4)	6 (20.4)	12 (25)	1

Table 3. The correlation and predictive values of autoantibodies for organs involvement

Autoantibody	Organ involvement	Cramer's V(φc)	*P* value
SCL 70	ILD*	0.426	0.039
	Tendon friction rub	0.450	0.026
	Cardiomyopathy	0.485	0.013
CENPA	Ulcers	0.417	0.046
CENPB	None*	-	-
RP 11	None*	-	-
RP 155	None*	-	-
Fibrillarin	None*	-	-
NOR-90	Cardiomyopathy	0.433	0.034
Th/To	Myopathy	0.436	0.033
	Cardiomyopathy	0.479	0.015
PM/Scl 75	Tendon friction rub	0.435	0.008
PM/Scl 100	Tendon friction rub	0.475	0.006
	Cardiomyopathy	0.475	0.006
Ku	Tendon friction rub	0.444	0.011
	Cardiomyopathy	0.430	0.014
Ro 52	Tendon friction rub	0.490	0.026
	Pericardial effusion	0.475	0.034
	Cardiomyopathy	0.467	0.040

Other studies have also confirmed the correlation of PM/Scl antibodies with muscle involvement (4,5). Both PM/Scl 75 and PM/Scl 100 have been associated with the tendons friction rub and cardiomyopathy in our study.

In a study of 393 SSc patients, individuals positive for this autoantibody were more likely to develop ILD (45%) or pulmonary artery hypertension (25%) (21). The presence of anti-Th/To has also been reported to be correlated with limited skin involvement. However, more severe visceral involvement (kidneys, pulmonary arterial hypertension, and lung fibrosis) was detected along with presence of anti-Th/To (18). Our study does not confirm the latter by showing a significantly higher rate of positive anti-Th/To among patients with diffuse scleroderma. We found more prevalence of myopathy and cardiomyopathy among our patients with anti-Th/To.

In a multi-national cohort consisting of SSc patients, anti-Ro52 was reported as the second most prevalent antibody among SSc patients and was strongly associated with ILD (22). Except for anti-topoisomerase-1, we did not find any significant correlation between the other autoantibodies and ILD. However, in our study, for the first time, anti-Ro 52 was observed to be associated with tendon friction rub, cardiomyopathy, and pericardial effusion. These differences may be due to geographical differences and various genetic backgrounds of our patients.

Anti-NOR90 exists in several rheumatic diseases, including SSc, however it is a rare autoantibody (23). In a Japanese study on anti-NOR90 among autoimmune rheumatic diseases, only nine patients out of 91 included subjects were positive for this autoantibody, only three of them had SSc (24). As well, another study reported, "no correlation" between anti-NOR90 and SSc (25). This autoantibody has been postulated to be related to a mild internal organ involvement and the limited type of cutaneous SSc. In our study, the prevalence of anti-NOR90 was low among patients with limited scleroderma, but it was associated with cardiomyopathy.

Anti-fibrillarin (U3 RNP) antibodies are significantly more prevalent in Americans of African origin, and men (23). Anti-U3 RNP was associated with overlap syndromes, myositis, joint involvement, and pulmonary hypertension in a former study (26). This autoantibody is associated with multi-organ involvement and is more frequent in the diffuse type of SSc with severe pulmonary involvement (2). We found no significant association between this autoantibody and clinical features of SSc. Although our study shows a higher rate of autoantibody among patients with diffuse disease, the overall prevalence of this autoantibody was low, and this difference was not significant.

Anti-RNA polymerase III antibodies have a highly varying prevalence among different studies; ranging from 0 to 41% (27). Geographical factors were suggested for this matter, but the variety is not well explained yet. A systematic review on over 8,000 patients shows an overall prevalence of 11% (27). These autoantibodies are shown to be associated with severe skin thickening and the occurrence of renal crisis (27,28). Our study showed a very low-frequency of these autoantibodies and their presence was not related to any clinical symptoms.

The prevalence of Anti-Ku as a rare autoantibody ranges from 1.5% to 5% (5). In a cohort on 625 SSc patients, only 2.2% were positive for anti-Ku (29) and related to skeletal involvement (particularly myositis). Another study in Italy reported a prevalence of 2% (8/379) for anti-Ku in a cohort of 7239 patients with autoimmune disease (30). They also found association of the antibody with muscle involvements. In our study, the prevalence of anti-Ku was more than reported literature (21%), since no correlation was found between anti-Ku and muscle involvement. However, we found a significant association of these autoantibodies with cardiomyopathy and tendon friction rub.

Conclusion

Our study shows that certain SSc-related autoantibodies are associated with specific clinical findings among Iranian SSc patients, while some of them differ from literature findings. It may be because of the different genetic backgrounds of our patients in this geographical region. Some of the mentioned autoantibodies in this study might predict organ involvement, especially in early diagnosed patients. These biomarkers may predict which patients are more likely to develop organ-specific damage and to make a diagnostic or therapeutic plan individually.

Authors' contribution

PM, MD and MK were included in preparing the concept and

design. MD, and MK revisited the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The institutional ethical committee at the Isfahan University of Medical Sciences approved all study protocols (IR.MUI.MED.REC.1399.419). We took written informed consent from all participants before any intervention. This study was extracted from the fellowship thesis of rheumatology by Marzieh Daneshbodi at this university (Thesis#1399419). Accordingly, ethical issues (including plagiarism, data fabrication and double publication) were completely observed by the authors.

Funding/Support

This study was supported by vice chancellor for research affairs, Isfahan University of Medical Sciences, Isfahan, Iran (Grant# 399143).

References

1. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol. 1988;15:202-5.
2. Domsic, RT, Medsger, TA Autoantibodies and their role in scleroderma clinical care. Curr Opin Rheumatol. 2016;2:239-251.
3. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, et al. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. Ann Rheum Dis. 2013;72:1747-55. doi: 10.1136/annrheumdis-2013-204424.
4. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, et al. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. Ann Rheum Dis. 2013;72:1747-55. doi: 10.1136/annrheumdis-2013-204424.
5. Domsic RT. Scleroderma: the role of serum autoantibodies in defining specific clinical phenotypes and organ system involvement. Curr Opin Rheumatol. 2014;26:646-52. doi: 10.1097/BOR.0000000000000113.
6. Janardana R, Nair AM, Surin AK, Prakash JAJ, Gowri M, Danda D. Unique clinical and autoantibody profile of a large Asian Indian cohort of scleroderma-do South Asians have a more aggressive disease? Clin Rheumatol. 2019;38:3179-87. doi: 10.1007/s10067-019-04659-2.
7. Hamaguchi Y, Hasegawa M, Fujimoto M, Matsushita T, Komura K, Kaji K, et al. The clinical relevance of serum antinuclear antibodies in Japanese patients with systemic sclerosis. Br J Dermatol. 2008;158:487-95. doi: 10.1111/j.1365-2133.2007.08392.
8. Barnett AJ, Miller MH, Littlejohn GO. A survival study of patients with scleroderma diagnosed over 30 years (1953-1983): the value of a simple cutaneous classification in the early stages of the disease. J Rheumatol. 1988;15:276-83.
9. Khimdas S, Harding S, Bonner A, Zummer B, Baron M, Pope J; Canadian Scleroderma Research Group. Associations with digital ulcers in a large cohort of systemic sclerosis: results from the Canadian Scleroderma Research Group registry. Arthritis Care Res (Hoboken). 2011;63:142-9. doi: 10.1002/acr.20336.
10. Firestein GS, Budd RC, Gabriel SE, McInnes IB, O'Dell JR. Kelley and Firestein's textbook of rheumatology e-book: Elsevier Health Sciences; 2016.
11. Steen VD, Medsger TA Jr. The palpable tendon friction rub: an important physical examination finding in patients with systemic sclerosis. Arthritis Rheum. 1997;40:1146-51. doi: 10.1002/1529-0131(199706)40:6<1146::AID-ART19>3.0.CO;2-9 13.
12. Bose N, Chiesa-Vottero A, Chatterjee S. Scleroderma renal crisis. Semin Arthritis Rheum. 2015;44:687-94. doi: 10.1016/j.semarthrit.2014.12.001.
13. Sandler RS, Drossman DA. Bowel habits in young adults not seeking health care. Dig Dis Sci. 1987;32:841-5. doi: 10.1007/BF01296706.
14. Arasaradnam RP, Brown S, Forbes A, Fox MR, Hungin P, Kelman L,et al. Guidelines for the investigation of chronic diarrhoea in adults: British Society of Gastroenterology, 3rd edition. Gut. 2018;67:1380-1399. doi: 10.1136/gutjnl-2017-315909.
15. Perera A, Fertig N, Lucas M, Rodriguez-Reyna TS, Hu P, Steen VD,et al. Clinical subsets, skin thickness progression rate, and serum antibody levels in systemic sclerosis patients with anti-topoisomerase I antibody. Arthritis Rheum. 2007;56:2740-6. doi: 10.1002/art.22747.
16. Kuwana M, Kaburaki J, Mimori T, Kawakami Y, Tojo T. Longitudinal analysis of autoantibody response to topoisomerase I in systemic sclerosis. Arthritis Rheum. 2000;43:1074-84. doi: 10.1002/1529-0131(200005)43:5<1074::AID-ANR18>3.0.CO;2-E.
17. Kranenburg P, van den Hombergh WM, Knaapen-Hans HK, van den Hoogen FH, Fransen J, Vonk MC. Survival and organ involvement in patients with limited cutaneous systemic sclerosis and anti-topoisomerase-I antibodies: determined by skin subtype or auto-antibody subtype? A long-term follow-up study. Rheumatology (Oxford). 2016;55:2001-2008. doi: 10.1093/rheumatology/kew298.
18. Volpe A, Ruzzenente O, Caramaschi P, Pieropan S, Tinazzi I, Carletto A, et al. Clinical associations of anti-CENP-B and anti-Scl70 antibody levels measured by multiplexed fluorescent microsphere immunoassay in systemic sclerosis. Rheumatol Int. 2009;29(9):1073-9. doi: 10.1007/s00296-009-0868-9.
19. Perosa F, Favoino E, Favia IE, Vettori S, Prete M, Corrado A, et al. Subspecificities of anticentromeric protein A antibodies identify systemic sclerosis patients at higher risk of pulmonary vascular disease. Medicine (Baltimore). 2016;95:e3931. doi: 10.1097/MD.0000000000003931.
20. Mitri GM, Lucas M, Fertig N, Steen VD, Medsger TA Jr. A comparison between anti-Th/To- and anticentromere antibody-positive systemic sclerosis patients with limited cutaneous involvement. Arthritis Rheum. 2003;48:203-9. doi: 10.1002/art.10760.
21. Wodkowski M, Hudson M, Proudman S, Walker J, Stevens W, Nikpour M, et al. Monospecific anti-Ro52/TRIM21 antibodies in a tri-nation cohort of 1574 systemic sclerosis subjects: evidence of an association with interstitial lung disease and worse survival. Clin Exp Rheumatol. 2015;33:S131-5.
22. Dagher JH, Scheer U, Voit R, Grummt I, Lonzetti L, Raymond Y, Senécal JL. Autoantibodies to NOR 90/hUBF: longterm clinical and serological followup in a patient with limited systemic sclerosis suggests an antigen driven immune response. J Rheumatol. 2002;29:1543-7.
23. Fujii T, Mimori T, Akizuki M. Detection of autoantibodies to nucleolar transcription factor NOR 90/hUBF in sera of patients with rheumatic diseases, by recombinant autoantigen-based assays. Arthritis Rheum. 1996;39:1313-8. doi: 10.1002/art.1780390808.

24. Foocharoen C, Watcharenwong P, Netwijitpan S, Mahakkanukrauh A, Suwannaroj S, Nanagara R. Relevance of clinical and autoantibody profiles in systemic sclerosis among Thais. Int J Rheum Dis. 2017;20:1572-81. doi: 10.1111/1756-185X.13060.
25. Aggarwal R, Lucas M, Fertig N, Oddis CV, Medsger TA Jr. Anti-U3 RNP autoantibodies in systemic sclerosis. Arthritis Rheum. 2009;60:1112-8. doi: 10.1002/art.24409.
26. Sobanski V, Dauchet L, Lefèvre G, Lambert M, Morell-Dubois S, Sy T,et al. Prevalence of anti-RNA polymerase III antibodies in systemic sclerosis: New data from a French cohort and a systematic review and meta-analysis. Arthritis Rheumatol. 2014;66:407-17. doi: 10.1002/art.38219.
27. Hamaguchi Y, Kodera M, Matsushita T, Hasegawa M, Inaba Y, Usuda T,et al . Clinical and immunologic predictors of scleroderma renal crisis in Japanese systemic sclerosis patients with anti-RNA polymerase III autoantibodies. Arthritis Rheumatol. 2015;67:1045-52. doi: 10.1002/art.38994
28. Rozman B, Cucnik S, Sodin-Semrl S, Czirják L, Varjú C, Distler O,et al . Prevalence and clinical associations of anti-Ku antibodies in patients with systemic sclerosis: a European EUSTAR-initiated multi-centre case-control study. Ann Rheum Dis. 2008;67:1282-6. doi: 10.1136/ard.2007.073981.
29. Cavazzana I, Ceribelli A, Quinzanini M, Scarsi M, Airò P, Cattaneo R, et al. Prevalence and clinical associations of anti-Ku antibodies in systemic autoimmune diseases. Lupus. 2008;17:727-32. doi: 10.1177/0961203308089442.