Clinical Reviews in Allergy & Immunology (2022) 63:210–239
https://doi.org/10.1007/s12016-022-08931-3

Clinical Significance of Antinucleolar Antibodies: Biomarkers for Autoimmune Diseases, Malignancies, and others

Minoru Satoh[1] · Angela Ceribelli[2,3] · Tomoko Hasegawa[1] · Shin Tanaka[4]

Accepted: 19 February 2022 / Published online: 8 March 2022

Abstract

Nucleolar staining is one of the standard patterns in immunofluorescence antinuclear antibodies (ANA), seen in 5–9% of ANA in various conditions. Antinucleolar antibodies (ANoA) are classified into 3 patterns in the International Consensus on ANA Patterns (ICAP) classification; AC-8 homogeneous pattern, AC-9 clumpy pattern, and AC-10 punctate pattern. Specificities known to show AC-8 include anti-Th/To, -PM-Scl, -nucleophosmin/B23, -nucleolin/C23, -No55, and others. AC-9 is seen by anti-fibrillarin/U3RNP and AC-10 by anti-RNA polymerase I and hUBF/NOR-90. ANoA has been classically known to be associated with scleroderma (SSc) and the characterization of nucleolar antigens identified several autoantigens recognized by SSc autoantibodies. The clinical association of anti-Th/To, PM-Scl, fibrillarin/U3RNP, and RNA polymerase I with SSc or SSc-overlap syndrome is well established, and commercial assays are developed. Anti-hUBF/NOR90, nucleophosmin/B23, and nucleolin/C23 are known for decades and reported in systemic autoimmune rheumatic diseases (SARDs), malignancies, graft versus host disease (GVHD), and others; however, their clinical significance remains to be established.

Keywords Antinuclear antibodies · Antinucleolar antibodies · Anti-Th/To · Anti-U3RNP/fibrillarin · Anti-PM-Scl · Anti-NOR90 · Anti-RNA polymerases

Abbreviations

ACA	Anticentromere antibodies
AFP	Alpha fetoprotein
AH	Autoimmune hepatitis
ALCL	Anaplastic large cell lymphoma
ALK	Anaplastic lymphoma kinase
AMA	Anti-mitochondria antibodies
ANA	Antinuclear antibodies
ANoA	Antinucleolar antibodies
APS	Antiphospholipid syndrome
BC	Breast cancer
BMT	Bone marrow transplant
BPH	Benign prostatic hypertrophy
CADM	Clinically amyopathic dermatomyositis
cGVHD	Chronic graft versus host disease
CH	Chronic hepatitis
CI	Confidence interval
CIA	Chemiluminescence immunoassay
CL	Cardiolipin
CLD	Chronic liver disease
CTDs	Connective tissue diseases
CTNE	Calf thymus nuclear extract
dcSSc	Diffuse cutaneous SSc
DB	Dot blot
DID	Double immunodiffusion
DLE	Discoid lupus erythematosus
DM	Dermatomyositis
ELISA	Enzyme-linked immunosorbent assay
ENA	Extractable nuclear antigen
GAVE	Gastric antral vascular ectasia
GVHD	Graft versus host disease
HCC	Hepatocellular carcinoma
HR	Hazard ratio

✉ Minoru Satoh
satohm@health.uoeh-u.ac.jp

[1] Department of Clinical Nursing, School of Health Sciences, University of Occupational and Environmental Health, 1-1 Isei-gaoka, Yahata-nishi-ku, Kitakyushu, Fukuoka 807-8555, Japan

[2] Division of Rheumatology and Clinical Immunology, IRCCS Humanitas Research Hospital, Rozzano (Milan) 20089, Italy

[3] Department of Biomedical Sciences, Humanitas University, Via A. Manzoni 56, Pieve Emnuele (Milan) 20089, Italy

[4] Department of Human, Information and Sciences, School of Health Sciences, University of Occupational and Environmental Health, 1-1 Isei-gaoka, Yahata-nishi-ku, Kitakyushu, Fukuoka 807-8555, Japan

hUBF	Human upstream binding factor
ICAP	International Consensus on Antinuclear Antibody Patterns
IIF	Indirect immunofluorescence
IIM	Idiopathic inflammatory myopathy
ILD	Interstitial lung disease
IP	Immunoprecipitation
IP-NB	Immunoprecipitation-northern blot
IPF	Idiopathic pulmonary fibrosis
ITP	Idiopathic thrombocytopenic purpura
lcSSc	Limited cutaneous SSc
LC	Liver cirrhosis
LIA	Line immunoassay
mAbs	Monoclonal antibodies
MCTD	Mixed connective tissue disease
NCM	Nailfold capillary microscopy
NHANES	National Health and Nutrition Examination Survey
NHC	Normal human controls
NHS	Normal human serum
NOR	Nucleolar organizer regions
NPM	Nucleophosmin
NSCLC	Non-small cell lung cancer
OA	Osteoarthritis
OL	Overlap syndrome
PAH	Pulmonary arterial hypertension
PCa	Prostatic carcinoma
PH	Pulmonary hypertension
PM	Polymyositis
PMR	Polymyalgia rheumatica
PSA	Prostate-specific antigen
RA	Rheumatoid arthritis
RD	Rheumatic diseases
RHA II	Nucleolar RNA helicase II
RIA	Radioimmunoassay
RNAP	RNA polymerases
RNP	Ribonucleoproteins
RP	Raynaud's phenomenon
SARDs	Systemic autoimmune rheumatic diseases
SclMy	Scleromyositis
SjS	Sjögren's syndrome
SLE	Systemic lupus erythematosus
snoRNP	Small nucleolar RNP
SRC	Scleroderma renal crisis
SRD	Systemic rheumatic diseases
SSc	Scleroderma, systemic sclerosis
ssSSc	Systemic sclerosis sine scleroderma
TCAD	Transplant-related coronary disease
TnT	In vitro transcription and translation
TSS	Total skin score
UCTD	Undifferentiated connective tissue disease
WB mice	(NZW × BXSB) F1 mice
WB	Western blot
β2GPI	Beta2 glycoprotein I
2D-WB	Two-dimensional western blot

Introduction

Following the development of the ANA test by indirect immunofluorescence (IIF), the first case of ANoA staining was described in 1961 [1]. Three cases of SSc with ANoA were described [2]. Beck et al. reported results of a large number of patients in 1962 [3]. Eleven sera were found from 358 patients with various connective-tissue diseases, while none of the unselected 490 patients with diseases other than "autoimmune" had ANoA. Six of the 11 patients had scleroderma (SSc, systemic sclerosis), 2 Sjögren's syndrome (SjS), one each of systemic lupus erythematosus (SLE), discoid lupus erythematosus (DLE), and pernicious anemia. ANoA was most common in SSc (19%, 6/32) and found in 5% (2/43) in SjS, 1.5% (1/67) in SLE, 0.67% (1/152) in DLE, 2% (1/52) in pernicious anemia, and none in 90 rheumatoid arthritis (RA). Rodent tissues including rat liver, mouse kidney, and other animal tissues have been used commonly as a substrate of ANA in earlier studies. Animal and human culture cell slides have become commercially available in the late 1970s, and the use of human HEp-2 (laryngeal cancer cell line) has become the standard substrate of immunofluorescence ANA during the late 1980s. Meanwhile, several nucleolar autoantigens were identified and characterized, mainly as cancer-associated antigens.

The use of the ANA slide of human-cultured cells was a major breakthrough in the ANA test. Currently, the HEp-2 ANA slide has been sold by many companies, and virtually, all ANA tests in the laboratory are based on HEp-2 or its variant of cell substrates worldwide. The standardization of methods and interpretation of the ANA test has been an important but difficult issue due to so much variability in the ANA slide, serum dilution, protocol, reagent, in particular, fluorochrome-conjugated secondary antibodies, and equipment used, in addition to the subjectivity of interpreting immunofluorescence reactivity by human eyes.

There have been activities to standardize and harmonize the names and descriptions of the HEp-2 cell ANA patterns. The International Consensus on ANA Patterns (ICAP) was established and a consensus was reached originally on 28 HEp-2 patterns with an alphanumeric code of AC-1 to AC-28 [4]. After the initial classification, the patterns AC-29 [5] and AC-0 (negative) [6] were added (http://www.ANApatterns.org). Historically, ANoA has been recognized and classified into 3–5 fine staining patterns in the literature. In the current ICAP classification, ANoA are defined in 3 AC patterns including AC-8 (homogeneous nucleolar), AC-9 (clumpy nucleolar), and AC-10 (punctate nucleolar) in ICAP classification as shown in Fig. 1.

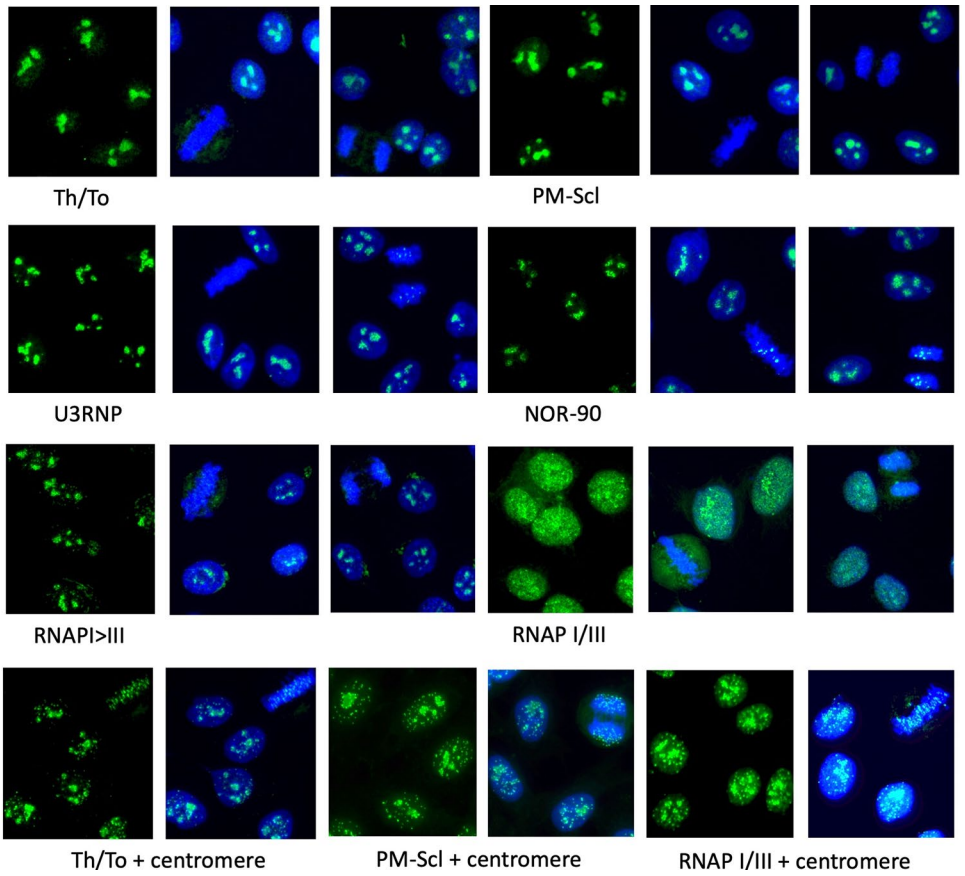

Fig. 1 Immunofluorescence antinuclear antibodies. HEp-2 ANA slides (MBL Inc., Japan) were stained with 1:80 diluted sera from patients followed by incubation with Dylight 488 goat antihuman IgG (γ-chain specific) F(ab)′2. Images with blue color are merged images of Dylight 488 (green) and DAPI staining (blue) of the nuclei to clearly show the staining pattern of mitotic cells. Staining by prototype sera with anti-Th/To, -PM-Scl, -U3RNP, -NOR-90, -RNA polymerases (I > III, anti-RNAP I is strong with weak anti-RNAP III), and -RNAP I/III are shown. Staining by sera with coexistent anti-centromere and -Th/To, PM-Scl, or RNAP I/III is shown

There have been many excellent review articles and textbook chapters on specific autoantibodies based on each disease such as autoantibodies in SLE, SSc, inflammatory myopathy, and others; however, review articles based on specific ANA patterns are limited. Clinical relevance of HEp-2 ANA patterns based on ICAP classification was published recently [7]. This review will focus on the clinical relevance of ANoA and specific antibodies that show ANoA.

Detection of Antinucleolar Antibodies by IIF

Currently, the only method to detect ANoA is IIF using human HEp-2 cells. Since the definition of ANoA is based on the physical location of the areas in the cells recognized by human autoantibodies, other methods that have been used to test ANA, including beads assay or enzyme-linked immunosorbent assay (ELISA), are not suitable for the detection of ANoA. In fact, a high prevalence of negative ANA by methods to detect ANA other than IIF has been reported and thought this was because nucleolar autoantigens were not included in the set of recombinant proteins used in these beads assay. The prevalence of ANoA in the ANA-positive population is not high compared with those of nuclear or cytoplasmic staining. Nevertheless, ANoA has been reported in various autoimmune and non-autoimmune conditions and also in healthy individuals like ANA.

Prevalence of Antinucleolar Antibodies by IIF

Selected data on the prevalence of ANA and ANoA using the HEp-2 slide are summarized in Table 1. The prevalence of ANA in the general population in the USA (NHANES, National Health and Nutrition Examination Survey) was ~ 12 to 16% and ANoA was ~ 6 to 7% of the ANA [8, 9]. In a cohort study in Japan, ANoA was 4.7% of ANA positives [10]. Based on these numbers, 0.5–1% of the general population has ANoA. ANoA among ANA positives in regional or hospital laboratories was not so different than those in the general population and 5–9% in various countries [11–14].

ANoA has been classically known to be associated with SSc, reflecting its association with several SSc-specific or -associated autoantibodies including anti-RNA polymerase I (RNAP I), U3RNP/fibrillarin, Th/To, and PM-Scl. The prevalence of ANoA in SSc was relatively higher than those in other conditions and ~ 20 to 40%, though the prevalence appears to be different depending on race/ethnicity and disease subset [15–20]. ANoA in CTD was 9.1% [21], and a study reported 20.5% of ANA in RA was ANoA [22]. In patients with interstitial cystitis, ANA was positive in 36%

Table 1 Prevalence of anti-nucleolar antibodies

	N = cutoff	All	Speckled	Homo	Nucleolar	Disc sp	Cyto
General population							
Satoh 2012 [8] USA NHANES 1999–2004	4754 1:80 (manual)	13.8%	-	-	*6.1%*	-	*21.8%*
Dinse 2020 [9] USA NHANES	1:80 (NOVA View)	-	-	-	-	-	-
1988–1991	4727	13.6%	*73.8%*	*5.9%*	*6.5%*	*1.6%*	*22.2%*
1999–2004	4749	12.2%	*74.2%*	*3.3%*	*5.7%*	*3.2%*	*19.0%*
2011–2012	4735	15.9%	*70.9%*	*7.3%*	*7.2%*	*1.4%*	*22.6%*
Terao 2014 [123] Japan	9575 1:40 1:80 1:160	45.2% 12.5% 2.8%	*43.7%*	*25.3%*	*4.7%*	*0.9%*	*2.0%*
Laboratory							
Roberts-Thomson 2003 [11] Australia	20,205 1:80 HEp-2000 (Ro60 transfected)	28.3%	*20.1%*	*39.1%*	*8.4%*	*4.3%*	-
Vermeersch 2013 [124] Belgium	9268 1:80	-	*36.5%*	*21.4%*	*17.0%*	*3.2%*	20.5%
Sener 2014 [12] Turkey	19,996 1:100 12,148 SRD 7848 non-SRD	21.9%	*18.7%*	*54.7%*	*5.5%*	*9.1%*	*4.1%* *Others 3.0%*
Mengeloglu 2014 [13] Pakistan	3127 1:100	15.8%	*56.7%*	*4.1%*	*18.0%*	*4.7%*	-
Gauderon 2020 [14] Switzerland	15,728 1:160	22.1%	*69.2%*	*41.7%*	*8.3%*	*3.4%*	-
CTDs							
Sharmin 2014 [21] Bangladesh	152 1:40	72.3%	*50.9%*	*18.2%*	*9.1%*	-	*Peripheral* *21.8%*
SSc							
Ferri 1991 [16] Italy	151	94% 91% 100% 91%	All Limited Intermed diffuse	-	*27%* *26%* *17%* *24%*	*21%* *32%* *16%* *8%*	-
Bunn 1998 [17] UK	735 1:100	97%	26.5%	42.3%	17.3%	25.0%	2.9%
Reveille 2001 [18] USA	Hispanic Af Am White	86% 84% 79%	-	-	34% 34% 23%	18% 4% 32%	-
Hesselstrand 2003 [19] Sweden	276 1:160	84.1% (232/276)	25% (69/276)	41.3% (114/276)	23.9% (66/276)	18.5% (51/276)	-
Sulli 2013 [20] Italy	42	80.9%	28.6%	7.2%	4.8%	40.5%	Neg 19.1%
Nandiwada 2016 [15] USA	1000	93.9%			18.1 + % (in ACA, RNAP, TopoI neg)	*22.0%*	Neg 6.1%
RA							
Nishimura 1996 [22] Japan	104 1:20	37.5%	*64.1%*	*48.7%*	*20.5%*	*10.3%*	-
Interstitial cystitis							

Table 1 (continued)

	N = cutoff	All	Speckled	Homo	Nucleolar	Disc sp	Cyto
Ochs 1994 [23] USA	1:40	36% (35/96)	Fine sp 25% (24/96) Coarse sp	-	7% (7/96)	-	AMA 3% (3/96)
Chronic liver disease							
Daschakraborty 2012 [125] India	175 1:80	*20% (35/175)*	*82.9% (29/35)*	*2.9% (1/35)*	*14,3% (5/35)*	-	-
Liver transplant							
Wu 2011 [126] China	94 LT 94 LDC 1:100	20.2% 12.8%	-	-	*63.2%* *16.7%*	-	-

ACA, anticentromere antibodies; *Af Am*, African American; *AMA*, anti-mitochondria antibodies; *CTDs*, connective tissue diseases; *cyto*, cytoplasmic; *Disc sp*, discrete speckled; *homo*, homogeneous; *LT*, liver transplant patients; *LDC*, liver disease control; *NHANES*, National Health and Nutrition Examination Survey; *RA*, rheumatoid arthritis; *neg*, negative; *RNAP*, RNA polymerases; *sp*, speckled; *SRD*, systemic rheumatic diseases

% shown in regular font, % in total population; % shown in italics, % in ANA positives

and 7% (20% of ANA positives) were ANoA [23]. ANoA in chronic liver disease, in particular, a high prevalence (63.2% of ANA) in liver transplant patients, were reported.

ICAP Classification of Nucleolar Patterns

The following classification and description are from the ICAP website (www.anapatterns.org) and from the publication [7]. Representative immunofluorescence images for each AC pattern using human autoimmune sera are shown (Fig. 1).

AC-8 Homogeneous Nucleolar

Description: diffuse fluorescence of the entire nucleolus, while the metaphase plate shows no staining.

Antigen association: PM/Scl-75, PM/Scl-100, Th/To, B23/nucleophosmin, nucleolin/C23, and No55/SC65.

AC-9 Clumpy Nucleolar

Description: irregular staining of the nucleoli and Cajal bodies with peri-chromosomal staining at the metaphase plates, e.g., anti-fibrillarin.

Antigen association: U3-snoRNP/fibrillarin.

AC-10 Punctate Nucleolar

Description: densely distributed but distinct grains seen in the nucleoli of interphase cells. In metaphase cells, up to 5 bright pairs of the nucleolar organizer regions (NOR) can be seen within the chromatin body. The cytoplasm of mitotic cells may be slightly positive, e.g., anti-NOR-90, anti-RNA polymerase I.

Antigen association: RNA polymerase I, hUBF/NOR-90.

Homogeneous Nucleolar (AC-8): Anti-Th/To and Anti-PM/Scl Antibodies

1. Anti-Th/To antibodies

Anti-Th/To antibodies were first described as anti-Th antibodies using serum from a patient with SLE [24]. Target antigens of anti-To [24] antibodies using serum from a patient with ANoA-positive SSc and those of anti-Th [25] confirmed that they have the same specificity and recognize RNA–protein complex containing 7-2RNA and 8-2RNA. Since then, this specificity has been called anti-Th/To antibodies.

Th/To Antigens

The Th/To antigen is a multiprotein-RNA complex (human RNase mitochondrial RNA processing (MRP) complex) that contains 7-2RNA and 8-2RNA and at least 9 protein subunits, Rpp14, Rpp20, Rpp21, Rpp29 (hPop4), Rpp25, Rpp30, Rpp38/40, hPop1, and hPop5 [26].

Anti-Th/To Detection Methods

Immunoprecipitation (IP) analysis and the detection of RNA components, 7–2 and 8-2RNA is the original method of identification of anti-Th/To antibodies and has been considered the gold standard (Fig. 2). Originally, HeLa cells metabolically labeled with ^{32}P-orthophosphate were used; however, later studies used IP of non-radiolabeled cell extract followed by urea-PAGE and silver staining to identify RNA components. The IP analysis of protein components

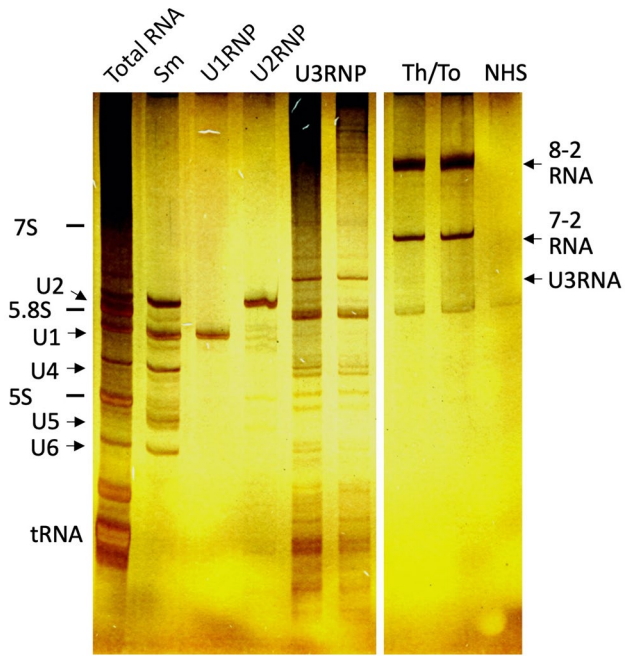

Fig. 2 RNA components of anti-U3RNP and Th/To antibodies by immunoprecipitation. Cell extract from K562 cells was immunoprecipitated by human autoimmune sera. RNA components of the immunoprecipitates were analyzed by urea-PAGE and silver staining

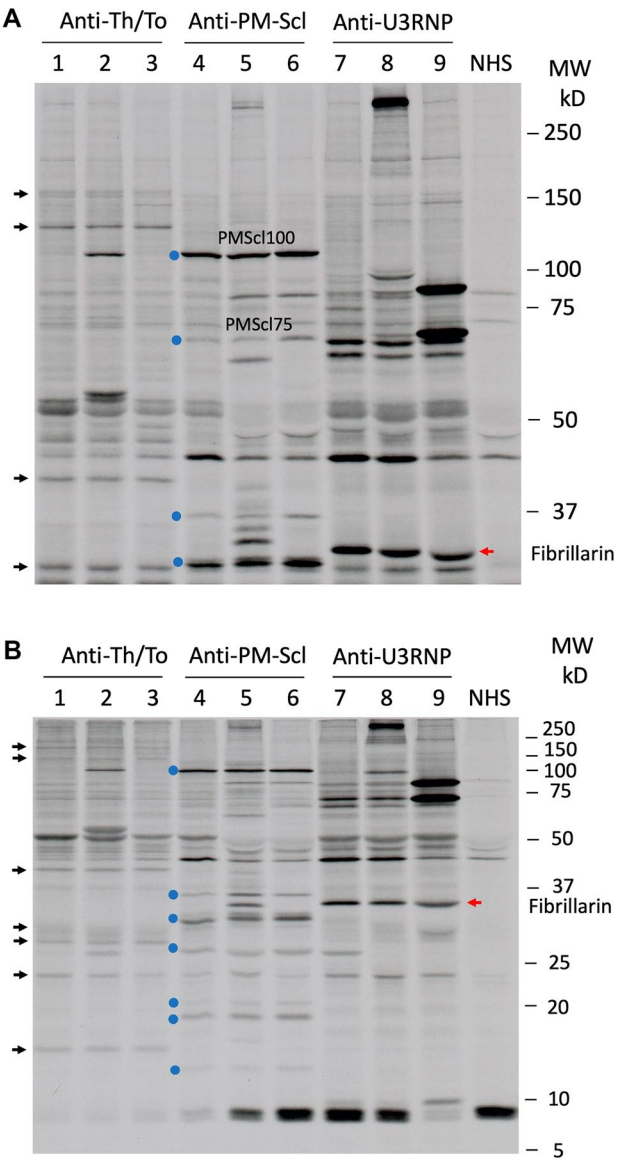

Fig. 3 Immunoprecipitation analysis of protein components of anti-Th/To, -PM-Scl, and -U3RNP antibodies. **A** 8% SDS-PAGE and **B** 13% SDS-PAGE. ^{35}S-methionine-labeled K562 cell extract was immunoprecipitated by anti-Th/To, -PM-Scl, or U3RNP human autoimmune sera, and protein components were analyzed by SDS-PAGE and autoradiography. Arrows, components of Th/To complex; blue dots, components of PM-Scl complex; red arrow, fibrillarin; NHS, normal human serum. Anti-U3RNP serum in lane 9 is also positive for anti-Ku (two strong bands of 70–90 kD)

using ^{35}S-methionine labeled cells had limited significance, though a ~ 40 kD protein named Th40 was considered a major target and utilized in some studies. One study reported that the ~ 40 kD antigen consists of Rpp20 and/or Rpp25 associated with a nuclease-resistant RNA fragment [27]. Protein IP of the ^{35}S-methionine labeled cell extract has not been much used for the detection of anti-Th/To, and components of Th/To complex have not been clearly shown. Nevertheless, a set of proteins immunoprecipitated by anti-Th/To sera can be recognized (Fig. 3 arrows). Which protein band seen in IP corresponds to which known subunit has not been clarified.

Anti-Th/To Antibodies Clinical Significance

It has been believed that anti-Th/To antibodies are relatively specific for the diagnosis of SSc; however, studies reporting disease specificity of anti-Th/To among various systemic rheumatic diseases by immunoprecipitation are very limited, and only a few studies from the USA [28] and Japan [29, 30] are available (Table 2).

It appears that the detection of anti-Th/To is relatively specific for SSc (~ 4%) while the number of patients with anti-Th/To and SARDs other than SSc are small compared with those of SSc. Some of these patients have Raynaud's phenomenon [28, 29, 31–34], maybe considered sine SSc [35–37], early stage of SSc, or undifferentiated connective tissue disease (UCTD) that may evolve into SSc. However, not all anti-Th/To-positive patients have Raynaud's phenomenon or other signs suggesting they are at the early stage of SSc [29]. Almost all studies reported a few percent prevalence of anti-Th/To in SSc, and the prevalence appears to be somewhat different between different countries (Table 3). When the SSc subsets were compared, virtually, all studies reported a much higher prevalence of anti-Th/To in limited

Table 2 Disease specificity of antinucleolar antibodies in various rheumatic diseases

	SSc	SLE	PM/DM	UCTD	Primary Raynaud's	RA	Others
Anti-Th/To							
Okano 1992 [28] USA (IP)	4.0% (15/371)	0% (0/118)	0% (0/29)	0% (0/13)	3.7% (3/81)	-	-
Hirakata 1992 [30] Japan (IP)	4.4% (5/113)	0.8% (1/126)	0% (0/52)				PM + OL 1/39 PM(−)OL 0/15
Kuwana 2002 [29] Japan (IP)	4.6% (14/303)	0.2% (1/392)	2.5% (1/40)	-	-	1.4% (2/148)	SjS 1.4% (1/72) ITP 1.1% (1/93)
Anti-U3RNP							
Okano 1990 [98] USA (IP)	5.8% (24/416)	0% (0/118)	0% (0/29)	0% (0/13)	1.2% (1/81)	-	-
Hirakata 1992 [30] Japan (IP)	7.1% (8/113)	0.8% (1/126)	0% (0/52)				PM + OL 0/39 PM(−)OL 0/15
Katsuri, 1995 [99] USA (ELISA,WB)	55.3% (31/56) CREST 54.5% (6/11)	37.5% (30/80)	-	MCTD 59.1% (13/22)	-	57.9% (22/38)	SjS 86.7% (13/15)

CREST, CREST syndrome; *DM*, dermatomyositis; *ELISA*, enzyme-linked immunosorbent assay; *IP*, immunoprecipitation; *ITP*, idiopathic thrombocytopenic purpura; *MCTD*, mixed connective tissue disease; *OL*, overlap syndrome; *PM*, polymyositis; *RA*, rheumatoid arthritis; *SjS*, Sjogren's syndrome; *SLE*, systemic lupus erythematosus; *SSc*, scleroderma, systemic sclerosis; *UCTD*, undifferentiated connective tissue disease; *WB*, western blot

cutaneous SSc (lcSSc) compared with diffuse cutaneous SSc (dcSSc) and the majority of patients had lcSSc regardless of the country of the studies. Also, one study compared the association of anti-Th/To and SSc subsets in African American, Caucasian, and Hispanic to show its association with lcSSc in all races [15].

The prevalence and subset association of anti-Th/To by line immunoassay (LIA) are summarized (Table 4). The prevalence and association with lcSSc appear similar to the results by IP, though the detection in dcSSc may be somewhat more common. The commercial LIA using hPop1 has been increasingly used worldwide due to the limited availability of IP assay; however, the data will need to be carefully interpreted because LIA has not been fully validated compared with a gold standard IP and many anti-Th/To-positive sera do not recognize hPop1 [29, 33]. One study reported that a significant number of anti-Th/To positives (19 in 873 SSc patients) detected by IP were missed by LIA [38]. Thus, although some anti-Th/To antibody immunoassays are commercially available, technical issues relating to the limited sensitivity and the specificity of these immunoassays should be taken into consideration [7, 26, 39].

Several studies reported the prevalence of anti-Th/To by chemiluminescence immunoassay (CIA) or ELISA using Rpp25 recombinant protein, CIA using Rpp38 peptide, and other assays (Table 5) [39–44]. Immunoassays using a single component would show a lower prevalence compared with IP, which would detect antibodies to all components of multiprotein-RNA complex of Th/To antigen in native form. The relative specificity of SSc, a few percent prevalence, and association with lcSSc of these antibodies were reported. Some studies were reported to show a good correlation with IP results and may become available in the future.

Reported anti-Th/To-positive cases by IP in diseases other than SSc or reports on selected patients are summarized (Table 6). Some studies selected patients based on ANoA to find a few cases of anti-Th/To in patients with SLE, polymyositis (PM), Raynaud's disease, and others [32, 33]. Fischer et al. reported that 25 of 285 idiopathic pulmonary fibrosis (IPF) patients had ANoA and the majority (13/25) of ANoA were anti-Th/To [37]. Four of them were lcSSc, and 9 could be considered sine scleroderma (ssSSc).

The prevalence of anti-Th/To antibodies from the studies with a clear description of the race of the subjects is summarized in Table 7. It appears possible that the prevalence of anti-Th/To in African Americans is lower compared with Caucasian or Latin based on 3 studies directly comparing this point.

In IP assay, a whole RNA–protein complex of Th/To is immunoprecipitated regardless of which components are recognized by serum autoantibodies. To examine which components are directly recognized and whether the fine specificity of anti-Th/To is associated with clinical features, several studies reported the reactivity with recombinant

Table 3 Prevalence of anti-Th/To antibodies identified by IP in various CTDs

Author, year, country	SSc	lcSSc	dcSSc	SSc	Others
Okano 1990 [28] USA	4.0% (15/371)	8.4% (14/167)	0.6% (1/167)	-	Non-SSc 1.2% (3/244) All 3 primary RP
Kipnis 1990 [31] USA	11.6% (13/112)	19.3% (6/31) CREST	10.5% (4/38)	6.7% (1/15)	RP 7.1% (2/28)
Kuwana 1994 [127] Japan	1.8% (5/273)	3.6% (4/112)	0% (0/71)	1.1% (1/92)	-
Harvey 1997 [128] UK, USA, Russia	5.2% (3/58)	8.6% (3/35)	0% (0/23)	-	-
Jacobsen 1998 [50] Denmark	2.2% (5/230) 229 Caucasian	-	-	-	-
Falkner 2000 [102] USA	9.6% (28/292) 94% Caucasian	-	-	-	-
Jacobsen 2001 [129] Denmark	2.3% (4/174)	-	-	-	-
Gunduz 2001 [51] USA	4.7% (11/232)	-	-	-	-
Kuwana 2002 [29] Japan	4.6% (14/303)	11 cases	3 cases	-	0.9% (7/745) non-SSc 1/7 RP+
Reveille 2003 [130] USA	5.6% (10/177)	-	-	-	-
Steen 2005 [49] USA	5.0% (72/1432)	67 cases?	5 cases	-	-
Meyer 2007 [131] France, USA	France 0.8% (1/127) USA 3.6% (9/247)	-	-	-	-
Hamaguchi 2008 [48] Japan	3.4% (7/203)	5.4% (6/112)	1.1% (1/91)	-	-
Ceribelli 2010 [132] Italy	3.7% (8/200)	-	-	-	Only ACA, topo I, RNAP III negative sera were tested
Krzyszak 2011 [133] USA	7.6% (8/105)	-	-	-	-
Van Praet 2011 [134] Bonroy 2012 [135] Belgium	2.1% (3/145) All 3 were SSc without skin involvement 7.3% (3/41)	0% (0/84)	0% (0/20)	-	-
Mahler 2014 [136] Canada	2.2+% (19+/873)	-	-	-	19/53 of ANA+ENA− samples
Nandiwada 2016 [15] USA	3.9+% (39+/1000) Ab neg ANoA+160	-	-	-	-
Nandiwada 2016 [15] USA	African American 1.8+% (4+/228)	4.1+% (3+/73)	0.7+% (1+/151)	-	-
Nandiwada 2016 [15] USA	White 4.5+% (25+/555)	6.4+% (22+/342)	1.4+% (3+/210)	-	-
Nandiwada 2016 [15] USA	Hispanic 4.0+%(8+/196)	7.5+% (6+/73)	1.8+% (2+/112)	-	-

Ab, antibody; *ACA*, anticentromere antibodies; *ANA*, antinuclear antibodies; *dc*, diffuse cutaneous; *ENA*, anti-extractable nuclear antigen antibodies; *lc*, limited cutaneous; *RNAP*, RNA polymerase; *RP*, Raynaud's phenomenon; *SSc*, scleroderma, systemic sclerosis

proteins (Table 8). Nine components were tested, and Rpp25, Rpp30, and hPop1 were recognized by more than half of the sera [29, 33]. One study correlated the reactivity of the serum with Rpp25 and Rpp38 with AC patterns in immunofluorescence (Table 9) [42]. The majority of patients (43/51) was classified as having AC-8 pattern, consistent with the immunofluorescence staining by anti-Rpp25 antibodies. Overall, only 3 of 51 anti-Rpp25 were without the AC-8 pattern. The prevalence of anti-Rpp38 peptide was lower, but all 8 cases had the AC-8 pattern.

Clinical association of anti-Th/To antibodies based on studies where the specificity was defined by IP (Table 10). It is somewhat difficult to define clinical features associated with anti-Th/To antibodies because the methods of

Table 4 Prevalence of anti-Th/To antibodies in various CTDs detected by LIA

Author, year, country	SSc	lcSSc	dcSSc	SSc OL	Others
Rodriguez-Reyna 2011 [137] Mexico	0% (0/139)	-	-	-	-
Graf 2012 [138] Australia	6.2% (8/129)	6	1	1	-
Villalta 2012 [139] Italy	3.3% (7/210)	4.1% (6/146)	1.6% (1/64)	-	-
Mehra 2013 [140] Australia	2.8% (15/528)	-	-	-	-
Poormoghim 2013 [141] Iran	4.6% (4/87)	-	-	-	-
Mahler 2014 [136] Canada	0% (0/873)	-	-	-	-
Wielosz 2014 [142] Poland	3.4% (3/87)	3.8% (2/52)	2.9% (1/35)	-	-
Chang 2015 [143] New Zealand	1.7% (1/59)	2.4% (1/41)	0% (0/18)	0% (0/4)	-
Patterson 2015 [144] Australia	3.0% (15/505)	-	-	-	-
Liaskos 2017 [145] Greece	0% (0/131)	0% (0/82)	0% (0/49)	-	-
Marou 2017 [146] Greece	0% (0/84)	-	-	-	-
Liaskos 2018 [147] Greece	1.3% (2/153)	0% (0/95)	3.1% (2/63)	-	-
Liu 2019 [148] China	1.6% (5/320)	-	-	-	Non SSc CTD 1.0% (1/100) HC 0% (0/30)
Gauderon 2020 [14] Switzerland	-	-	-	-	ANoA (≥ 1:320) or SSc susp 1.6% (6/386)
Mendes 2020 [149] Brazil	Af Br 1.8% (1/57)	2.8% (1/36)	0% (0/21)	-	-
	White 5.4% (11/203)	6.7% (9/135)	2.9% (2/68)	-	-
Tahiat 2020 [150] Argeria	1/150	3/103	0% (0/42)	-	-

Af Br, African Brazilian; *ANoA*, antinucleolar antibodies; *CTD*, connective tissue disease; *dc*, diffuse cutaneous; *HC*, healthy controls; *lc*, limited cutaneous; *LIA*, line immunoassay; *OL*, overlap; *SSc*, systemic sclerosis, scleroderma; *susp*, suspected

comparison in the studies are quite heterogeneous, and how and what should be compared is arguable. Some studies compared the prevalence of features between anti-Th/To positive and anti-Th/To negative in all SSc patients, while others compared between patients with several different SSc autoantibodies. There are studies comparing within lcSSc because anti-Th/To is mainly seen in lcSSc, and other studies compared between anti-Th/To vs ACA because both are mainly associated with lcSSc.

In anti-Th/To-positive SSc, the majority of patients with anti-Th/To had lcSSc and low skin score [15, 28, 29, 45–49] is a consistent finding. Anti-Th/To was associated with a high prevalence of puffy fingers, small bowel involvement, hypothyroidism [28], and a low prevalence of joint involvement [28, 45, 50]. Anti-Th/To-positive patients had reduced survival due to pulmonary hypertension (PH) [28]. One study reported anti-Th/To was detected in four of 11 patients who had both scleroderma renal crisis (SRC) and PH but none in 23 SRC only patients and 1/15 in PH only patients [51].

By comparing clinical features of patients with two autoantibodies associated with lcSSc, anti-Th/To vs ACA, anti-Th/To-positive patients were younger and more frequently had ILD and restrictive lung disease (reduced % FVC), SRC, and reduced survival [34, 52]. Anti-Th/To patients had a lower prevalence of vascular involvement (pitting scars, digital tip ulcers, digital gangrene), esophageal dysmotility, telangiectasia, and sicca symptoms [34].

Regarding non-SSc patients with anti-Th/To, Okano reported 1.2% (3/244), and all 3 patients had primary Raynaud's phenomenon [28], and similarly, Kipnis detected anti-Th/To in 2 of 28 patients with Raynaud's phenomenon

Table 5 Prevalence of anti-Th/To antibodies in various CTDs by CIA and other methods

Author, year, country	SSc	lcSSc	dcSSc	SSc OL	others
QUANTA Flash Rpp25 (CIA)					
Mahler 2013 [39] USA	2.9% (2/70)	-	-	-	SLE 0% (0/67) RA 0.7% (1/141)
Mahler 2014 [136] Canada	1.4 + % (12 + /873)	-	-	-	12/50 of ANA + ENA- samples
Markusse 2017 [41] The Netherlands	0.3% (1/365)	1	0	0	0 in lSSc
Koenig 2019 [42] Canada	20.9% (42/201)	25.7% (38/148)	4.1% (2/49)	-	Sine SSc 50% (2/4) RP 38.5% (5/13) Other CTD 0% (0/47) Infection 1.7% (1/58) Others 11.1% (4/36)
ELISA (Rpp25)					
Mahler 2014 [136] Canada	1.1 + % (10 + /873)	-	-	-	10/50 of ANA + ENA- samples
Rpp38 peptide (CIA)					
Koenig 2019 [42] Canada	4.0% (8/201)	5.4% (8/148)	0% (0/49)	-	Sine SSc 0% (0/4) RP 0% (0/13) Other CTD 0% (0/47) Infection 0% (0/56) Others 0% (0/36)
IP of 40 kD protein + ANoA + WB (unknown rec protein)					
Mierau 2011 [43] Germany	0.2% (2/863)	0.2% (1/513)	0% (0/173)	0.9% (1/108)	Undifferentiated SSc 0/64
Dots assay					
Muller 2020 [44] France	-	6 cases	-	-	6/64 pos ANA, SSc susp, no topo I, ACA, RNAPIII abs all 6 lcSSc

ACA, anticentromere antibodies; *ANoA*, antinucleolar antibodies; *CTDs*, connective tissue disease; *CIA*, chemiluminescence immunoassay; *dc*, diffuse cutaneous; *IP*, immunoprecipitation; *lc*, limited cutaneous; *lSSc*, limited subset of SSc; *pos*, positive; *RP*, Raynaud's phenomenon; *SSc*, systemic sclerosis, scleroderma; *rec*, recombinant; *WB*, western blot

[31]. Though anti-Th/To has been mainly reported in SSc or primary Raynaud's phenomenon, detection in 7 patients with SLE, RA, polymyositis (PM), SjS, and idiopathic thrombocytopenic purpura (ITP) with only one of 7 with Raynaud's phenomenon, was reported [29]. Other studies also reported a small number of anti-Th/To-positive patients in various diseases including SLE, PM, dermatomyositis (DM), autoimmune hepatitis (AH), and others [33, 34, 53]. One study reported 3 cases of anti-Th/To in 70 patients with localized scleroderma [54].

In a study on patients with IPF who had ANoA, clinical features of 13 anti-Th/To patients were compared with 12 ANoA-positive anti-Th/To negative patients [37]. Anti-Th/To was associated with female sex, Raynaud's phenomenon, telangiectasia, digital edema, calcinosis, and PH. They stated that 4 of their anti-Th/To-positive patients could be classified as lcSSc, and the remaining 9 were considered ssSSc [37].

In a prospective study on patients with Raynaud's phenomenon without definite connective tissue disease, anti-Th/To was identified as a predictive factor of progression to definite SSc (HR 5.9, 95% CI 3.2–10.98 by univariate analysis, HR 3.56, 95% CI 1.5–5.3) and an independent factor predictive of progression to microvascular damage by nailfold capillary microscopy (NCM), HR 2.4 (95% CI 1.14–5.06) [55].

In summary, anti-Th/To is one of the ANoA specificity detected in patients with SSc. In SSc, most anti-Th/To-positive patients have lcSSc and less severe internal organ involvement. ILD may be relatively common within lcSSc compared with ACA-positive patients. It can also be detected in patients with ssSSc, idiopathic interstitial lung disease (ILD), Raynaud's phenomenon, and UCTD but may also occasionally be seen in other diseases or patients without Raynaud's phenomenon.

2. Anti-PM-Scl Antibodies

Specific autoantibodies in PM/DM were first described as a new precipitin line in double immunodiffusion (DID) using calf thymus nuclear extract (CTNE) as antigen, named

Table 6 Additional anti-Th/To-positive cases (selected patients, non-SSc patients, etc.)

Author, year, country	Methods	Subjects/selection	Anti-Th/To positive	Notes
Hardin 1982 [53] Hashimoto 1983 [25] USA	IP	260 various 1/260	1 SLE	
Reddy 1983 [24] USA	IP. IF	24 ANoA + SSc 1/24	1 SSc	
Verheijen 1994 [32] Denmark	IP-NB, IF	66 ANoA sera (rheumatology, dermatology)	5 SSc 2 possible SSc 1 RP 2 unknown	3/10 anti-Th/To + U3RNP
Harvey 1997 [128] UK, USA, Russia	IP, IF	58 SSc, 219 1st degree relatives, 24 spouses	3 SSc	
Poormoghim 2000 [35] USA	IP, IF	16 ssSSc 6.7% (1/16)	1 ssSSc	
Yamane 2001 [54] Japan	IP, IF	70 localized scleroderma 4.3% (3/70)	3 localized scleroderma	
Kuwana 2002 [29] Japan	IP	SSc 4.6% (14/303) non-SSc 0.9% (7/745)	14 SSc (11 lcSSc, 3 dcSSc) 3 RA, 1 PM, 1 SLE, 1 pSS, 1 ITP	Only 1/7 non-SSc RP +
Van Eenennaam 2002 [33] The Netherlands	IP, IP-NB, IF	172 ANoA 8.1% (14/172) From ~ 4500 pts	7.0% (7/100) in diagnosed pts 1 SLE, 2 SSc, 1 PM, 2 RP, 1 other	
Mitri 2003 [34] USA	IP, IF	Unknown	107 total 96 SSc (5 dcSSc, 89 lcSSc, 2 unclassified) 8 RP, 1 DM, 1 AH, 1 ILD	
Fischer 2006 [36] USA	IP, IF	235 ILD 5/6 ssSSc	5 ILD/ssSSc	
Fischer 2006 [37] USA	IP, IF	285 IPF 25/285 ANoA 13/25 anti-Th/To	4 lcSSc 9 ssSSc	
Koenig 2008 [55] France	IP, IF	696 RP normal NCM 15/696 Progressed to SSc 13/74	-	
Mahler 2014 [136] Canada	IP	873 SSc Only sera without anti-ENA were tested 35.8% (19/53)	-	

AH, autoimmune hepatitis; *dc*, diffuse cutaneous; *DM*, dermatomyositis; *ENA*, extractable nuclear antigen; *IF*, immunofluorescence; *ILD*, interstitial lung disease; *IP*, immunoprecipitation; *IPF*, idiopathic pulmonary fibrosis; *IP-NB*, immunoprecipitation-northern blot; *ITP*, idiopathic thrombocytopenic purpura; *lc*, limited cutaneous; *NCM*, nailfold capillary microscopy; *PM*, polymyositis; *pSS*, primary Sjogren's syndrome; *RP*, Raynaud's phenomenon; *ssSSc*, systemic sclerosis sine scleroderma

anti-PM-1 [56]. Anti-PM-1 was detected in 17 of 28 patients with PM/DM (PM 9/14, DM 1/6, PM-SSc overlap 7/8) but not in 460 patients with other diseases. The following study pointed out the heterogeneity of the anti-PM-1 system and described anti-PM-Scl antibodies, which were detected in 12.5% of PM/DM (21/68) but none in SLE, SSc, RA, SjS, or normal human controls (NHC) [57]. Among the 21 anti-PM-Scl positive cases were 7 SSc-PM and 2 SSc-DM overlap syndrome, suggesting anti-PM-Scl antibodies are associated with SSc-PM/DM overlap syndrome. The target antigen of anti-PM-Scl was characterized by immunoprecipitation in 1986 [58]. RNA component was not observed and 11 polypeptides of molecular weight 110 to 20 kD, named p1–p11 were identified. PM-Scl is a human exosome complex and functions as exoribonucleases during RNA processing. Among the many components of the complex, PM-Scl 100 (~ 110 kD) and PM-Scl75 are considered major targets of autoantibodies and commercial immunoassays utilize these antigens [59].

The prevalence of anti-PM-Scl antibodies in patients with rheumatic diseases by DID or immunoprecipitation is summarized (Table 11). Generally, in US and European countries, anti-PM-Scl antibodies were reported in 2–5% of PM/DM or SSc, and the prevalence is higher in SSc-PM/DM overlap syndrome or scleromyositis than pure SSc or PM/DM. When anti-PM-Scl positive patients had SSc, most of

Table 7 Prevalence of anti-Th/To antibodies identified by IP and race

	Caucasian	African American	Latin	Asian
Kuwana 1994 [127] Japan	-	-	-	1.8% (5/273)
Jacobsen 1998 [50] Denmark	2.2% (5/230) Includes one Asian	-	-	-
Yamane 2001 [54] Japan	-	-	-	6.7% (2/30)
Reveille 2003 [130] USA	5.7% (5/88)	0% (0/30)	8.5% (5/59)	-
Hamaguchi 2008 [48] Japan	-	-	-	3.4% (7/203)
Ceribelli 2011 [132] Italy	3.7 + % (8 + /213)	-	-	-
Krzyszak 2011 [133] USA	9.3% (7/75)	4.3% (1/23)	0/5	-
Nandiwada 2016 [15] USA	4.5 + % (25 + /555)	1.8 + % (4 + /228)	4.0 + % (8 + /196)	-

IP, immunoprecipitation

them had lcSSc. In one study on 23 cases of anti-PM-Scl, 8/10 SSc-PM/DM overlap patients had lcSSc, and 5/6 SSc patients had lcSSc [60].

In contrast to an association of anti-PM-Scl antibodies with SSc-PM/DM overlap syndrome in the USA and Europe, this specificity was reported absent in Japan until recently, probably related to the low prevalence of HLA-DR3 that is tightly linked with the production of this autoantibodies [30]. Muro et al. reported anti-PM-Scl in 2.3% (3/133) of PM/DM and 0.9% (2/223) of SSc and 25% (4/16) of UCTD [61]. Other cases of anti-PM-Scl positive DM or SSc-DM overlap have been reported in Japan [62, 63]. Thus, anti-PM-Scl also appears to be detected in Japanese patients though at a low prevalence and with a less clear association with overlap syndrome.

In a study on ANoA in SSc, anti-PM-Scl was found in 8 of 37 ANoA positive SSc and associated with SSc-PM/DM overlap syndrome and renal crisis [64].

A strong association of anti-PM-Scl with scleromyositis was reported from Poland. One study reported 83% of the 108 cases with anti-PM-Scl had scleromyositis [65]. Another study reported 19 of 20 anti-PM-Scl positive patients were scleromyositis [66]. In scleromyositis, 90.5% (19 of 21) were anti-PM-Scl positive.

Table 8 Autoantibodies to components of Th/To complex in sera defined by anti-Th/To IP positives

	Kuwana 2002 [29] **Japan**			**Van Eenennaam 2002** [27]	**Mahler 2013** [39]	**Mahler 2014** [136]
				The Netherlands	**USA**	**Canada**
					CIA	CIA
	All	SSc	Non-SSc			
	21	14	7	12	14	19
Component						
Rpp14 (14 kD)				58% (7/12)		
Rpp20 (16 kD)				8% (1/12)		
Rpp21 (18 kD)				42% (5/12)		
Rpp25 (22 kD)				67% (8/12)	78.6% (11/14)	63.1% (12/19) ELISA 52.6% (10/19)
Rpp29/hPop4 (24 kD)				58% (7/12)		
Rpp30 (30 kD)	85.7% (18/21)	85.7% (12/14)	85.7% (6/7)	67% (8/12)		
Rpp38/Th40 (34 kD)	38.1% (8/21)	42.9% (6/14)	28.6% (2/7)	17% (2/12)		
hPop1 (115 kD)	66.7% (14/21)	92.9% (13/14)	14.3% (1/7)	92% (11/12)		
hPop5 (18 kD)				42% (5/12)		

CIA, chemiluminescence immunoassay

Table 9 Prevalence of antibodies to Rpp25 and Rpp38 by ICAP AC pattern [42]

	N =	Anti-Rpp25	Anti-Rpp38
AC-01	31	3.2%	3.2%
AC-03	17	17.6%	17.6%
AC-04	30	6.9%	0%
AC-05	3	0%	0%
AC-08	129	33.3%	3.1%
AC-09	13	0%	0%
AC-10	12	6.1%	0%
AC-12	24	0%	0%
AC-20	2	0%	0%
AC-21	4	0%	0%
AC-22	1	0%	0%
With AC-08	165	29.1%	4.8%
Without AC-08	133	2.3%	0%

In non-rheumatic diseases, in one study on GVHD, 2 of 19 chronic GVHD (cGVHD) with SSc were positive for anti-PM-Scl and anti-Scl-70 [67].

In summary, anti-PM-Scl antibodies are mainly detected in patients with SSc-PM/DM overlap syndrome [57, 60, 68] or scleromyositis [65, 66]; however, it also can be detected in PM/DM or SSc without overlap syndrome at a 3–5% prevalence. When detected in patients with SSc, most of them have lcSSc.

3. AC-8 Pattern ANoA with Unclear Clinical Significance

There are several other ANoA specificities that show the AC-8 pattern but have unclear clinical significance; anti-nucleophosmin/B23, nucleolin/C23, nucleolar RNA helicase II/Gu, No55, Nop52, and others. They have been reported in various conditions including SARDs, malignancies, GVHD, and others. It is possible that some of these specificities have clinical significance and are useful in diagnosis or predicting clinical features; however, standard immunoassay and systematic screening studies are currently missing, and these autoantibodies are not widely utilized.

Nucleophosmin (NPM)/B23

Anti-NPM Antibodies in Rheumatic and Immune-Mediated Diseases

Nucleophosmin (also called B23, numatrin, No38) is a 37-kD abundant nucleolar phosphoprotein that has roles in preribosomal RNA processing and ribosome assembly.

In a study analyzing autoantibodies in sera from 164 patients (120 provisional diagnoses of SARDs, 44 SSc) with ANoA, 7 sera positive for anti-NPM were detected [69]. Six out of seven anti-NPM positive had anti-cardiolipin (CL) antibodies, and anti-NPM was associated with clinical features of SLE ($N=2$) or variants of SLE in 5 out of 7 patients (Table 12).

Ulanet detected antibodies to NPM in 10 of 92 SSc (10.9%) patients and compared clinical features with 82 anti-NPM-negative SSc patients [70]. A total of 70% of anti-NPM positive patients had limited SSc and anti-NPM was associated with low % FVC and a higher prevalence of anticentromere antibodies (ACA) (60% vs 24%), with severe PH. Though anti-NPM has been reported in various malignancies (Table 12), none of the anti-NPM-positive SSc patients had malignancy. Anti-NPM frequently coexisted with anti-U3RNP/fibrillarin (anti-NPM + vs −, 80% vs 18%, $P=0.0002$), and 20% had anti-Scl70, while 40% had anti-U1RNP, but none had ACA.

In SLE, anti-NPM antibodies were positive in 28% of sera and significantly associated with anti-cardiolipin (CL) antibodies but not associated with ANA, anti-dsDNA, anti-β2GPI (beta2 glycoprotein I), and anti-β2GPI-dependent CL antibodies [71]. The interaction of CL to NPM in vitro was shown and suggested a possible role in the concomitant production of anti-NPM and anti-CL antibodies. In a mouse study using (NZW × BXSB) F1 mice (WB mice), a model of SLE, anti-NPM antibodies were positive by ELISA in more than 75% of sera from male WB mice [71]. Anti-NPM in female WB mice was less frequent, detected in 25% at 3 months and 40% at 4–6 months. Anti-NPM antibodies in WB mice were associated with anti-CL antibodies.

In a study on allogeneic bone marrow transplant (BMT), 12/19 of patients with extensive GVHD had ANoA and 10/19 were positive for anti-NPM antibodies [72]. Anti-NPM was found in one out of 10 ANoA-positive SSc but not in 8 autologous BMT patients or 48 healthy controls.

Anti-NPM Antibodies and Malignancy Association

In a study on breast cancer patients, levels of anti-NPM antibodies by ELISA were not different between recurrent and nonrecurrent patients at the time of diagnosis or recurrence; however, anti-NPM levels increased significantly between diagnosis and 6 months before a recurrence in recurrent patients [73]. The degree of changes in the levels of anti-NPM antibodies between diagnosis and 6 months before recurrence was associated with the risk of recurrence. Mojtahedi et al. reported anti-NPM antibodies by 2-dimensional western blot (2D-WB) in 44% of HER2-positive or -negative breast cancer patients [74]. Imai et al. reported anti-NPM antibodies in only one out of 184 hepatocellular carcinomas (HCC) patients by WB [74]. In the other two studies, the prevalence of anti-NPM in HCC was 22.4% and 10.5–21.4% [75, 76]. Anti-NPM was also detected in one of 37 lung

Table 10 Clinical associations of anti-Th/To antibodies detected by IP

	Prevalence in SSc lcSSc vs dcSSc	Increased in anti-Th/To (+)	Reduced/low in anti-Th/To (−)
SSc			
Okano 1990 [28] USA	4.0% (15/371) 8.4% vs 0.6% *N* = 15	Puffy fingers Small bowel involvement Hypothyroidism	Arthralgia/arthritis survival (due to PH)
Falkner 1998 [45] USA	*N* = 23	-	Gastrointestinal (joints/tendons, lung)
Jacobsen 1998 [50] Denmark	2.2% (5/230) *N* = 5	-	SSc joint deformity
Falkner 2000 [102] USA	9.6% (28/292) *N* = 28	-	No individual statistics Skin score Joints, esophagus Heart 0/28 Kidney 0/28
Steen 2005 [49] USA	5.0% (72/1432) *N* = 72	-	Reduced survival among lcSSc (vs U1RNP, PM-Scl, ACA positives)
Hamaguchi 2008 [48] Japan	3.4% (7/203) 5.4% vs 1.1% *N* = 7	-	Rodnan TSS Esophagus 2/7 Joint 1/7
Subset of SSc			
Gunduz 2001 [51] USA	232 SSc 4.7% (11/232)	Anti-Th/To positive SRC + PH 4/11 (36%) (increased) SRC only 0/23 (0%) PH only 1/15 (7%)	*P* < 0.003, SRC-PH vs total *P* < 0.008, SRC-PH vs SRC
Mitri 2003 [34] USA	lcSSc 87 anti-Th/To(+) vs 306 ACA(+) *N* = 87	Younger Shorter disease duration ILD (radiographic) Restrictive lung disease SRC	Vascular involvement (pitting scars, digital tip ulcers, digital gangrene) Esophageal dysmotility Sicca findings
Steen 2003 [151] USA	106 SSc with PH vs 106 without PH	Anti-Th/To was not increased in PHT	-
Ceribelli 2010 [132] Italy	3.7% (8/200) Only ACA, topo I, RNAP III (−) sera were tested 8 anti-Th/To(+) vs 67 ACA(+) *N* = 8	Male (3/8 vs 1/67) Younger (54.5 vs 66.6) Pericarditis (25% vs 4.5%) ILD (38% vs 4.5%)	Telangiectasia FVC
Interstitial lung disease			
Fischer, 2006 [37] USA	ANoA(+) idiopathic pulmonary fibrosis (IPF) 13 Anti-Th/To (+) vs 12 anti-Th/To (−) *N* = 13	Female (8/13 vs 2/12) Raynaud's (9 vs 1) Telangiectasia (5 vs 1) Digital edema (4 vs 0) Calcinosis (2 vs 0) PH (5 vs 1)	-

ACA, anticentromere antibodies; *FVC*, forced vital capacity; *ILD*, interstitial lung disease; *IP*, immunoprecipitation; *IPF*, idiopathic pulmonary fibrosis; *PH*, pulmonary hypertension; *SRC*, scleroderma renal crisis; *TSS*, total skin score

cancer patients but not in 210 alimentary tract cancer patients [77]. In nonmalignant diseases, anti-NPM was not detected in 187 chronic hepatitis and liver cirrhosis in one study [77], but others reported 3.3% in liver cirrhosis [75] or 5.4% in chronic hepatitis [76] and 1.7% in healthy controls [76].

Immunoreactivity with NPM in sera from 125 ovarian cancer patients was significantly higher than those with 40 patients with the benign ovarian disease and 40 controls by dot blot [78]; however, the prevalence or individual data were not reported. Lu et al. tested levels of antibodies to 14 currently promising ovarian cancer-related biomarkers including B23 by ELISA, in 151 ovarian cancer patients, 23 borderline ovarian tumors, 55 benign tumors, and 75 healthy controls but found no difference in anti-B23 levels [79].

Dai et al. detected anti-NPM antibodies by WB in 28.2% of prostatic carcinoma (PCa) patients but none

Table 11 Prevalence of anti-PM-Scl antibodies by immunodiffusion or immunoprecipitation

Author, year, country	Methods	SLE	PM/DM	SSc	Others
Reichlin 1984 [57] USA	DID	0/241	12.5%(21/168) SSc-PM 7 SSc-DM 2 DM 7, PM 5	0% (0/40) SSc PMR 1	RA 0/35 SjS 0/40 NHC 0/75
Hirakata 1992 [30] Japan	IP DID	0/126	0/52 PM(+)OL 0/39 PM(−)OL 0/15	0% (0/113)	
Marguerie 1992 [68] UK	CIE IP	0/1689	*N* = 256 27 PM/DM-SSc OL	*N* = 879 27 PM/DM-SSc, 4SSc	
Oddis 1992 [60] USA	DID IP		4.7% (5/106) PM/DM-SSc OL 24.4% (10/41)	1.7% (6/359) Other OL 2/47 (1 SSc-RA, 1 DM-RA)	
Kuwana 1994 [127] Japan	IP DID			0% (0/275)	
Hausmanowa-Petrusewicz 1998 [66] Poland	DID IP		23.8% (20/84) PM 0/19 DM 0/21 SclMy 90.5% (19/21) Unclassified 1/13		
Bunn 1998 [17] UK	CIE IP			5.4% (40/735)	
Troyanov 2005 Canada [152]	IP		100 PM 9 DM 19 PM/DM OL 7.4% (5/68) Cancer-associated 0/4		
Mierau 2011 [43] Germany	DID			4.9% (42/863)	
Krzyszak 2011 [133] USA	IP			4.8% (5/105) Caucasian 5/75 Af Am 0/23	
Koschic 2012 [153] USA	DID			3.1% (75/2425)	
Kaji 2014 [154] Japan	IP			0% (0/588)	
Muro 2015 [61] Japan	IP	0/123	2.3% (3/133) 2 DM 1 CADM	0.9% (2/223)	SjS 0/88 OL 0/17 UCTD 25% (4/16)

Af Am, African American; *CADM*, clinically amyopathic dermatomyositis; *CIE*, counter immunoelectrophoresis; *DID*, double immunodiffusion; *DM*, dermatomyositis; *IP*, immunoprecipitation; *OL*, overlap syndrome; *PM*, polymyositis; *SclMy*, scleromyositis; *UCTD*, undifferentiated connective tissue disease

in benign prostatic hypertrophy (BPH) or NHC [80]. By ELISA using recombinant protein, anti-NPM levels were higher in PCa patients than BPH or NHC. The ROC curve analysis showed a high diagnostic value for PCa to differentiate BPH or NHC. A total of 97.1% of early-stage PCa patients were identified correctly, while 69.2% of BPH patients with elevated prostate-specific antigen (PSA) levels were anti-NPM negative. Anti-NPM levels increased significantly after surgery in patients with early-stage PCa.

Pulford et al. reported autoantibodies to ALK (anaplastic lymphoma kinase)-NPM fusion protein in all 11 ALK-positive ALCL (anaplastic large cell lymphoma) but not in 13 controls (5 healthy, 5 cancer, 3 ALK-negative ALCL) [81]. Damm-Welk et al. detected anti-ALK antibodies in 13 of 21 ALK-positive non-small cell lung cancer (NSCLC) and 13 of 22 ALK translocation positive but NPM-ALK-negative lymphoma patients and one ALK-positive rhabdomyosarcoma patient but not in 20 healthy adults [82]. These sera reacted with ALK-fusion proteins but also with full-length ALK-transfected COS cells, suggesting that antibodies recognize the ALK portion of the fusion protein. Consistent with this study, Knorr et al. showed antibodies

Table 12 Prevalence of autoantibodies to nucleophosmin (NPM)

Authors, year country, methods	Subjects	Prevalence	Association	
Rheumatic and immune-mediated diseases				
Li 1989 [69] Australia ELISA, WB	164 ANoA (120 RD, 44 SSc)	7/164	-	5/7 were SLE or SLE variants
Ulanet 2003 [70] USA IP-WB, ELISA	92 SSc	10.9% positive	Low %FVC PH (60% vs 24%)	-
Lartigue 2005 [71] France ELISA, WB	82 SLE	28.0%	Associated with anti-CL (65.2% vs 28.8%)	5.8% (6/103) HC by ELISA
Wesierska-Gadek 1992 [72] Finland, WB	32 allogeneic BMT (19 extensive GVHD, 3 limit c-GVHD, 10 no c-GVHD)	Ext GVHD 10/19 Lim c-GVHD 0/3 No c-GVHD 0/10	-	HC 0/48 Autologous BMT 0/8 ANoA + SSc 1/10
Malignancy				
Brankin1998 [73] USA ELISA	100 breast cancer 50 recurred, 50 no recurrence	Levels associated with recurrence	-	-
Mojtahedi 2011 [74] Iran 2DWB	9 HER2-BC, 9 HER2 + BC, 9 controls	4/9, 4/9, 1/9	-	-
Imai 1992 [77] Japan WB	433 cancer (184 HCC, 210 alimentary tract ca, 187 hepatitis/cirrhosis, 37 lung ca)	1/184 HCC 1/37 lung ca 1 dysgerminoma	-	-
Liu 2015 [75] USA ELISA	HCC76 Liver cirrhosis 30 Chronic hepatitis 30 SLE 43 NHC 89	22.4% 3.3% 0 0 1.1%	-	-
Wang 2017 [76] China ELISA	AFP(−)HCC 56 AFP(+)HCC 86 CLD 168 NHC 59	21.4% 10.5% 5.4% 1.7%	-	-
Taylor 2009 [78] USA DB	125 OvCa, 40 benign ovarian disease, 40 healthy	OvCa patients had higher levels of anti-NPM	-	-
Lu 2011 [79] USA ELISA	151 OvCa, 23 borderline ov.disease, 55 benign tumors, 75 healthy	Anti-NPM levels were not higher in OvCa	-	-
Dai 2016 [80] USA WB	PCa 39 BPH 21 NHC 30	28.2% 0% 0%	-	-

AFP, alpha fetoprotein; *BC*, breast cancer; *BMT*, bone marrow transplant; *BPH*, benign prostatic hypertrophy; *ca*, cancer; *cGVHD*, chronic GVHD; *CL*, cardiolipin; *CLD*, chronic liver disease; *DB*, dot blot; *ext GVHD*, extensive GVHD; *FVC*, forced vital capacity; *GVHD*, graft versus host disease; *HCC*, hepatocellular carcinoma; *NHC*, normal healthy control; *NPM*, nucleophosmin; *ov*, ovarian; *OvCa*, ovarian cancer; *PCa*, prostatic carcinoma; *RD*, rheumatic diseases

from patients with NPM-ALK-positive ALCL patients recognized epitopes on ALK-portion of NPM-ALK by overlapping peptide microarray analysis [83].

In summary, anti-NPM antibodies were reported in rheumatic diseases including SLE and SSc, GVHD, and various types of malignancies including breast cancer, HCC, ovarian cancer, and prostatic cancer. Systematic studies are lacking and clinical association of anti-NPM antibodies does not appear to be established or consistent. Generally, studies by WB tend to show a lower prevalence of anti-NPM antibodies than studies using ELISA. In the majority of studies, whether anti-NPM-positive patients by ELISA or WB showed ANoA by immunofluorescence was not described.

Nucleolin/C23

Nucleolar phosphoprotein originally described as C23 was characterized as a ~ 110 kD protein nucleolin that plays roles in ribosome biogenesis and intranuclear transport of pre-ribosomal particles. Autoantibodies to nucleolin/C23 were reported in SLE and other diseases by WB using serum dilution of 1:10 (IgM) or 1:20 (IgG) [84]. IgM antibodies were positive in 64% (27/42) and IgG in 17% (7/42) adult SLE. The reactivity was mainly IgM and was not specific for SLE. The prevalence of IgM or IgG antibodies were as follows: childhood SLE (15/15), SjS (17/24), SSc (7/16), PM (2/10), RA (1 /20), or polymyalgia rheumatica (PMR) (1 /20), patients with acute hepatitis A (16/20) or infectious mononucleosis (4/20), and none in 10 normal subjects.

In a study on ANA in chronic GVHD after bone marrow transplant (Table 13), 95% of 19 cGVHD with SSc had ANA and 58% had ANoA [67]. Anti-nucleolin antibodies were detected in three of them but not in 18 cGVHD without SSc by WB though ANA and ANoA were positive in 58% and 22%, respectively, in these subsets.

Anti-nucleolin antibodies were also reported in kidney or heart transplant patients [85]. The prevalence appears to be high in patients with the irreversible rejection of kidney transplants and heart allografts with transplant-related coronary disease.

In a mouse study, anti-nucleolin antibodies were found by WB in sera from MRL/*lpr* mice (100%, 10/10) and NZB/W F1 mice (80%, 8/10) but not BALB/c mice (0/10) [86]. Among the 150 kD, 110 kD (nucleolin), 75 kD, and 55 kD proteins often targeted by autoantibodies in these mice, anti-nucleolin antibodies were produced first in all 10 MRL/*lpr* mice, whereas it was first in 7/10 NZB/W F1 mice.

The clinical association and significance of anti-nucleolin antibodies are not well understood as studies are very limited and no systematic analysis in various diseases is available. Moreover, the relationship between the results by different immunoassays is not known, and thus no gold standard for anti-nucleolin detection is accepted.

Nucleolar RNA Helicase II/Gu

Autoantibodies to nucleolar RNA helicase II (RHA II)/Gu was originally described using a serum "Gu" from a patient with gastric antral vascular ectasia (GAVE, watermelon stomach) that has high titer ANoA [87], and it was shown that Gu antigen is identical to RHA II.

In the following study, anti-RHA II/Gu was screened by WB using the recombinant protein in 108 sera with ANoA from 3408 sera tested [88]. Anti-RHA II/Gu antibodies were found in 11 (10%) of 108 ANoA-positive sera, including 3 of 46 patients with SSc (7%), 3 of 17 patients with SLE (18%), 4 of 9 patients with UCTD (44%), and 1 healthy relative of a SSc patient. It was not found in 11 ANoA-positive SjS, 11 PM/DM, 5 primary antiphospholipid syndromes (APS), and 3 RA. None of the anti-Gu-positive patients had symptoms suggestive of gastric antral vascular ectasia (GAVE). Two other sera positive for ANoA from patients with GAVE were negative for RHA II/Gu [89].

Table 13 Prevalence of anti-nucleolin antibodies in patients with transplant and GVHD

	Subjects	*N*	Anti-nucleolar abs (%)	Anti-nucleolin abs (%)
Wesierska-Gadek 1992 [72] **Austria** **WB**	Allogeneic BMT Extensive cGVHD	19	63.2% (12/19)	31.6% (6/19)
	Limited cGVHD	3	0	0
	No cGVHD	10	0	0
	Autologous BMT	8	0	0
	SSc	10	10% (1/10)	0
	Controls	48	0	0
Bell 1996 [67] **Germany** **WB**	Chronic GVHD after bone marrow transplant (with SSc)	19	58%	15.8%
	cGVHD without SSc	18	22%	0%
Qin 2011 [155] **USA** **ELISA**	normal	49		2.0%
	Waiting for kidney transplant	66		9.1%
	Irreversible rejection of kidney transplant	51		25.5%
	Heart allografts recipient	129		17.1%
	Heart allografts with TCAD	89		43.8%

BMT, bone marrow transplant; *cGVHD*, chronic graft versus host disease; *TCAD*, transplant-related coronary artery disease; *WB*, western blot

Anti-RHA II/Gu antibodies occur in low frequencies in patients with connective tissue diseases (CTDs) who have ANoA, but they are not specific for SSc or GAVE.

In conclusion, anti-RNA helicase II/Gu antibodies were reported in ANoA-positive SSc, SLE, and UCTD, but their clinical associations will need to be evaluated in the future.

No55

No55 was identified using serum from a female patient with interstitial cystitis from Finland [23]. The antibody was named based on its nucleolar staining in immunofluorescence and ~ 55 to 58 kD doublet proteins recognized in WB. The anti-No55 staining pattern was unique in demonstrating uniform staining throughout the interphase nucleolus, chromosomal staining in mitotic cells, and no apparent staining of coiled bodies.

In a study on patients with prostate cancer, No55 was identified as a protein recognized by 7 out of the 47 sera (14.9%) but not by 20 healthy male controls [90]. Six out of 45 patients with local disease and one of two patients with metastatic disease were positive for anti-No55.

In conclusion, anti-No55 antibodies are reported only in a case of interstitial cystitis and 7 cases of prostate cancer.

Nop52

Nop52 was identified using a human serum from a bone marrow-transplanted male patient as a 52-kD nucleolar autoantigen colocalized with nucleolar proteins involved in the late processing step such as hPop1 and B236. cDNA cloning and sequence revealed that it was identical to NNP1 [91]. A study using a pool of 30 sera including a patient from neuroblastoma identified an autoantigen NNP3 as a novel amino-terminal variant of NNP1 [92]. Antibodies to NNP3 were detected in 1/30 healthy volunteers (titer 1:100), 1/10 neuroblastoma, and 1/10 non-neuroectodermal malignancies (1/2 Hodgkin's disease) (titer 1:100 and 1:1000) by a plaque assay.

Clumpy Nucleolar (AC-9): Anti-U3RNP/Fibrillarin Antibodies

Autoantibodies to the U3RNA-protein complex were originally described using sera from ANoA-positive SSc patients [24]. A 34-kD protein fibrillarin associated with the U3RNP complex was identified as a target antigen [93]. A monoclonal antibody that recognized a 34-kD nucleolar antigen was established from a lupus-prone New Zealand black × New Zealand white (NZB/NZW) F1 mouse, and the identity of the antigen with fibrillarin was confirmed [94]. Specific induction of ANoA by subcutaneous injection of mercuric chloride in susceptible strains of mice with H-2 s MHC was reported. The target nucleolar antigen was identified as fibrillarin and established as a model of chemical induce autoimmunity [95, 96]. U3 small nucleolar RNP (snoRNP) is a box C/D snoRNPs and contains fibrillarin and several other proteins including Nop58, Nop56, 15.5 K, U3-55 k, Mpp10, Imp3, and Imp4 [97]. The structure of U3 snoRNPs appears quite complex and autoantibodies to components other than fibrillarin have not been fully studied. For clinical purposes, autoantibodies to U3 snoRNP in SSc are called anti-fibrillarin, anti-U3RNP, or anti-fibrillarin/U3RNP.

Anti-U3RNP/fibrillarin antibodies are considered a serological marker of SSc associated with dcSSc. While it is likely to be true, the studies examining the prevalence of anti-U3RNP by IP in various SARDs are limited [98] (Table 2). Anti-U3RNP was detected in 5.8% of SSc but none in SLE, PM/DM, or UCTD, and the only positive non-SSc patients has primary Raynaud's phenomenon [98]. In a study from Japan, anti-U3RNP was found in 7.1% of SSc, and only one SLE patient was positive in other SARDs [30]. There was a study testing anti-U3RNP by ELISA and western blot (WB); however, the antibodies reported in the article appeared to be very different than conventional anti-U3RNP antibodies [99].

Anti-U3RNP antibodies in SSc patients were tested by standard IP assay in many studies. The majority of studies reported the prevalence of 2–8% in the analysis in different countries. In SSc, association with dsSSc based on the higher prevalence of anti-U3RNP in dcSSc than lcSSc has been shown in most studies (Table 14). There were some other studies that detected anti-U3RNP by WB or other methods or in selected patients [35, 51, 64, 100]. One study using IF and WB reported a case of anti-U3RNP in malignancy [77] (Table 15).

Discussing the prevalence of anti-U3RNP antibodies in SSc as a whole may be confusing and misleading because the prevalence appears to be quite different depending on race/ethnicity (Table 16). In all studies, the high prevalence of anti-U3RNP antibodies in African Americans is shown (15.8–43.5%), while it is low (0–7.1%) in Caucasians and Asians. In one study in Afro-Caribbean patients, they also showed a high prevalence (22.2%), while Latin appears to be in the middle (6.1–12.5%). Furthermore, the association of anti-U3RNP with lcSSc vs dcSSc subset also appears to be depending on race/ethnicity (Table 17) [15, 98, 101]. In general, the association of anti-U3RNP with dcSSc has been shown in most studies; however, this association is limited to African Americans, and it is not seen in Caucasians or other races in one study [98]. In contrast, another study showed the same clinical association was only in Caucasians and Latin, and it was not seen in African Americans (Table 17).

Table 14 Prevalence of anti-U3RNP/fibrillarin antibodies identified by IP in SSc patients

Author, year, country	SSc	lcSSc	dcSSc	SSc overlap	
Kipnis 1990 [31] USA	7.1% (8/112) SSc-related disease	9.7% (3/31) CREST	5.3% (2/38)	6.7% (1/15)	RP 7.1% (2/28)
Okano 1992 [98] USA	5.8% (24/416)	4.0% (7/174)	8.9% (17/191)		Non-SSc RD 244 (SLE 118, primary Raynaud's 81, PM/DM 29, UCTD 13, SLE-PM/DM OL 3 0.4% (1/244, primary RP)
Hirakata 1992 [30] Japan	7.1% (8/113)				PM/DM 0/52 SLE 0.8% (1/126) Overlap PM + 0/39 Overlap PM − 0/15
Kuwana 1994 [127] Japan	3.6% (10/275)	4 cases	5 cases	1 case	
Harvey 1997 [128] UK, USA, Russia	6.9% (4/58)	2.9% (1/35)	13.0% (3/23)	-	
Jacobsen, 1998 [50] Denmark	3.5% (8/230)	-	-	-	
Falkner 2000 [102] USA	8.2% (24/292)	-	-	-	
Tormey 2001 [101] UK	4.1% (42/1026)	16 cases	26 cases	(3 cases PM/SSc OL)	2 UCTD, 1 Raynaud's 13 males, 29 females 16 (38%) lcSSc 26 (62%) dcSSc
Reveille 2001 [18] USA	8.1% (28/345)	-	-	-	-
Jacobsen 2001 [129] Denmark	4.6% (8/174)	-	-	-	-
Gunduz 2001 [51] USA	4.7% (11/232)	-	-	-	SRC + PH 2/11 SRC only 2/23 PH only 2/15
Steen 2005 [49] USA	3.8% (55/1432)	-	35 cases	-	-
Meyer 2007 [131] France, USA	France 1.6% (2/127) USA 2.4% (6/247)	-	-	-	-
Hamaguchi 2008 [48] Japan	2.5% (5/203)	0.9% (1/112)	4.4% (4/91)	-	-
Ceribelli 2010 [132] Italy	0% (0/213)	-	-	-	Only ACA, topo I, RNAP III negative sera were tested
Krzyszak 2011 [133] USA	8.6% (9/105)	-	-	-	Caucasian Am 2.7% (2/75) African Am 30.4% (7/23)
Van Praet 2011 [134] Bonroy 2012 [135] Belgium	0% (0/145)	0% (0/84)	0% (0/20)	-	-
Nandiwada 2016 [15] USA	7.9 + % (78 + /990) 3Ab neg ANoA + 160	3.3% (17/508)	12.7% (61/482)	-	-
Nandiwada 2016 [15] USA	Af Am 22.8 + % (52 + /228)	20.5 + % (15 + /73)	24.5 + % (37 + /151)	-	ns
Nandiwada 2016 [15] USA	White 1.8 + % (10 + /555)	0% (0/342)	8.9 + % (10 + /210)	-	$P < 0.0001$
Nandiwada 2016 [15] USA	Hisp 6.1 + % (12 + /196)	2.5 + % (2 + /81)	8.9 + % (10 + /112)	-	ns

3Ab neg, anti-topoisomerase, centromere, and RNA polymerase III antibodies negative; *ACA*, anticentromere antibodies; *ns*, not significant; *PH*, pulmonary hypertension; *RD*, rheumatic diseases; *RP*, Raynaud's phenomenon; *SRC*, scleroderma renal crisis

Table 15 Additional studies reporting anti-U3RNP/fibrillarin prevalence based on detection methods

Author, year	Subjects	Methods	Prevalence	Note
Reimer1988 [64] USA	646 SSc 53 ANoA	WB (IP)	59.5% (22/37) ANoA (+)SSc	Described as "negative by IP"
Arnett 1996 [100] USA	335 SSc	IP of rec Fib	8.1 + % (27 + /335)	-
Poormoghim 2000 [35] USA	ssSSc 0/16 lcSSc 5/136	IP	-	-
Gunduz 2001 [51] USA	SRC + PH 2/11 SRC only 2/23 PH only 2/15	IP	-	-
Malignancy				
Imai 1992 [77] USA	HCC 184, CH/LC 187, GIC 210, LC 37, OC 2, NC 229	IF WB	Cancer 0.17% (1/620) ANoA	HCC 1/184 HCC ANoA 1/12

CH, chronic hepatitis; *GIC*, gastrointestinal cancer; *HCC*, hepatocellular carcinoma; *IP*, immunoprecipitation; *LC*, liver cirrhosis; *NC*, normal control; *OC*, ovarian cancer; *Rec Fib*, recombinant fibrillarin; *SRC*, scleroderma renal crisis; *ssSSc*, systemic sclerosis sine scleroderma; *WB*, western blot

Tormey reported a high prevalence of anti-U3RNP antibodies in Afro-Caribbean (22.2%) compared with Caucasian (3.4%) patients [101]. All 8 Afro-Caribbean patients, 2 Oriental and an Asian patient had dcSSc, whereas only 47% of Caucasians with anti-U3RNP had dcSSc, indicating the association of anti-U3RNP with dcSSc is stronger in Afro-Caribbean and Asian ethnicity. The distinctive clinical association of anti-U3RNP antibodies in different races will need to be carefully evaluated in future studies.

The association of anti-U3RNP antibodies with clinical features is summarized in Table 18. It is not straightforward to describe clinical association within SSc because the

Table 16 Prevalence of anti-U3RNP antibodies by IP and race

	White	African American	Latin	Afro-Caribbeans	Asian
Okano 1992 [98] USA	4.2% (14/332)	43.5% (10/23)	-	-	-
Hirakata 1992 [30] Japan					7.1% (8/113)
Kuwana 1994 Japan	-	-	-	-	3.6% (10/275)
Arnett 1996 [100] USA (IP rec)	4.8% (11/227)	15.8% (12/76)	12.5% (4/32)	-	-
Jacobsen 1998 [50] Denmark	3.5% (8/230)	-	-	-	-
Reveille 2001 [18] USA	2.1% (4/191)	22.1% (17/77)	9.1% (7/77)	-	-
Tormey 2001 [101] UK	3.4% (31/900)	-	-	22.2% (8/36)	-
Hamaguchi 2008 [48] Japan	-	-	-	-	2.5% (5/203)
Ceribelli 2010 [132] Italy	0 + % (0 + /213)*	-	-	-	-
Krzyszak 2011 [133] USA	2.7% (2/75)	30.4% (7/23)	0% (0/5)	-	-
Van Praet 2011 [134] Bonroy 2012 [135] Belgium	0% (0/145)	-	-	-	-
Nandiwada 2016 [15] USA	1.8% (10/555)	22.8% (52/228)	6.1% (12/196)	-	-

*Only sera that were negative for anticentromere, topoisomerase I, and RNA polymerase III negative were tested

Table 17 Prevalence of anti-U3RNP antibodies in SSc patients by race, sex, and disease classification

Race, sex	SSc all	lcSSc	dcSSc	*P*
Okano [98] **1990, USA**				
All Black	43.5% (10/23)	14.3% (1/7)	56.3% (9/16)	$P=0.089$
Black woman	43.8% (7/16)	0% (0/5)	63.6% (7/11)	$P=0.034$
Black men	42.9% (3/7)	50% (1/2)	40% (2/5)	
All White	4.2% (14/332)	3.7% (6/162)	4.7% (8/170)	
White women	4.4% (12/274)	3.6% (5/139)	5.2% (7/135)	
White men	3.4% (2/58)	4.3% (1/23)	2.9% (1/35)	
Other races	0% (0/10)	0% (0/5)	0% (0/5)	
Total	6.6% (24/365)	4.0% (7/174)	8.9% (17/191)	$P=0.089$
Nandiwada [15] **2016, USA**				
African American	22.8 + % (52 + /228)	20.5 + % (15 + /73)	24.5 + % (37 + /151)	
White	1.8 + % (10 + /555)	0% (0/342)	8.9 + % (10 + /210)	$P<0.0001$
Hispanic	6.1 + % (12 + /196)	2.5 + % (2 + /81)	8.9 + % (10 + /112)	$P=0.077$

method of comparison varies, and also for possible differences between different races, but common features associated with anti-U3RNP include a high prevalence in African Americans, in male patients, with muscle inflammation, calcinosis, esophageal, and small bowel involvement, cardiac involvement, severe ILD, and PH [46, 49, 98, 100, 102].

Table 18 Clinical association of anti-U3RNP antibodies detected by IP

	Prevalence in SSc lcSSc vs dcSSc	Increased in anti-U3RNP	Reduced/low in anti-U3RNP
SSc			
Reimer 1988 [64] USA	*N* = 22 vs RNAP I, PM-Scl, without ANoA	Male Initial symptom RP	Onset age Arthralgia/arthritis
Okano 1992 [98] USA	5.8% (24/416) 4.0% vs 8.9% *N* = 24	Digital pitting scars/ulcers Hyper/hypopigmentation Calcinosis Muscle inflammation Small bowel involvement Primary PAH	-
Kuwana 1994 [127] Japan	3.6% (10/275) *N* = 10	-	Arthritis Pulmonary interstitial fibrosis
Jacobsen 1998 [50] Denmark	3.5% (8/230) *N* = 8	calcinosis esophageal	-
Falkner 2000 [102] USA	8.2% (24/292) *N* = 24	esophageal involvement small bowel involvement PH, Heart	Lung fibrosis Kidney
Steen 2005 [49] USA	*N* = 55	Male, African American muscle inflammation Severe GI Severe lung fibrosis Isolated PH Severe heart disease	-
Arnett 1996 [100] USA (IP of rec Fib)	*N* = 27	Male dcSSc Pulmonary fibrosis Cardiac, renal, gut, muscle	-
Subset of SSc			
Gunduz 2001 [51] USA	SRC + PHT 2/11 SRC only 2/23 PHT only 2/15	-	-

dcSSc, diffuse cutaneous SSc; *GI*, gastrointestinal involvement; *PAH*, pulmonary arterial hypertension; *PH*, pulmonary hypertension; *RNAP*, RNA polymerase; *RP*, Raynaud's phenomenon

The reduced prevalence of arthritis or kidney involvement is shown in some studies and also reduced the prevalence of pulmonary fibrosis [46, 102].

Punctate Nucleolar (AC-10): Anti-NOR-90 and -RNA Polymerase I Antibodies

NOR90/Human Upstream Binding Factor, hUBF

Antibodies to NOR90 were originally described in 6 patients with rheumatic diseases based on immunofluorescence staining of the nucleolar organizing region (NOR) (Fig. 1) and reactivity with doublet proteins of ~ 90 kD in WB [103] and IP (Fig. 4). cDNA clones for NOR-90 were identified as hUBF [31]. From a cohort of rheumatic diseases, the original study reported four of 6 patients with anti-NOR90 were SSc [103]; however, another study showed seven out of 9 patients had SjS including primary and secondary SjS with

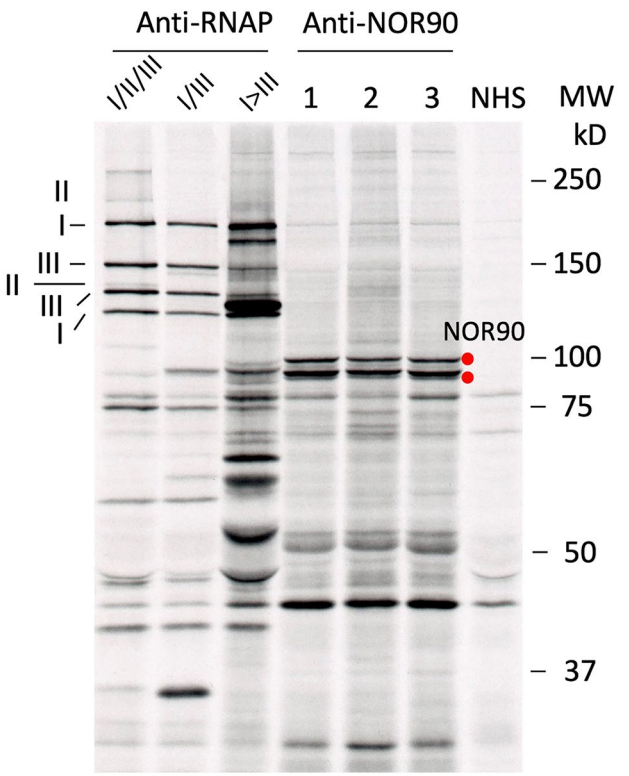

Fig. 4 Immunoprecipitation using sera with anti-RNA polymerases or -NOR-90 antibodies. ^{35}S-methionine-labeled K562 cell extract was immunoprecipitated by anti-RNA polymerases or -NOR-90 human autoimmune sera and protein components were analyzed by 8% SDS-PAGE and autoradiography. The two largest subunits of RNA polymerase I, II, and III are indicated on the left. Lane I/II/III is a serum with antibodies to all 3 RNA polymerases, whereas lane I/III is a serum with both anti-RNAPI and RNAP III. Lane I > III is a serum with predominant anti-RNAP I compared with anti-RNAP III. NOR-90 proteins are shown with red dots

RA [104]. Anti-NOR90 is not a common specificity, and the number of cases in each report is small; nevertheless, anti-NOR90 has been detected in various rheumatic diseases including SLE, RA, SjS, UCTD, and arthritis (Table 19). Despite the identification of high anti-NOR90 titers, 1:320–1:12,800, no specific association with a particular disease or clinical features has been confirmed yet. The detection of anti-NOR90 in malignancy was also reported, however, at low frequency [105–108]. Anti-NOR-90 tested by LIA has been reported (Table 20); however, technical limitations on sensitivity and specificity of the assay should be noted because this assay has not been fully validated compared with other immunoassays.

ASE-1

ASE-1 was described as a nucleolar autoantigen similar to NOR-90 immunofluorescence pattern and intracellular localization, with similar mobility in SDS-PAGE. ASE-1 was identified as a 55 kD protein based on its sequence; however, it migrates ~ 90 kD close to NOR-90 [109].

In one study, anti-ASE-1 was evaluated by immunofluorescence, WB, and IP of in vitro transcription and translation (TnT) product, compared with anti-NOR90 reactivity [108]. Four of 23 sera were anti-ASE-1 positive, 6 were anti-ASE-1, and NOR-90 positive, 10 were only anti-NOR-90 positive. Thus, in addition to similar localization and mobility, these two specificities also coexist quite often. Clinical features were available in 7 patients with anti-ASE-1 who showed 3 slowly progressive SSc with Raynaud's phenomenon (one also had lung cancer), 3 patients with malignancy (2 lung cancer, 1 melanoma), and 2 had seronegative arthritis.

Another study reported ASE-1 as an autoantigen in SLE. Anti-ASE-1 was tested in 92 SLE, 35 RA, 50 SSc, and 100 controls by WB using 3 recombinant proteins [110]. The reactivity with each recombinant protein was 42–55% with SLE sera, while it was 11–31% in RA, 7–14% in SSc, and 15–21% in the unaffected community controls. Reactivity to all 3 proteins was seen in 25% of SLE, 8.5% of RA, 6% of SSc, and 1% of control, and in SLE, it was associated with serositis.

Anti-RNA Polymerase I Antibodies

It has been suggested that nucleolar staining pattern by IIF is often seen in SSc; however, the fine specificity was not described until the late 1980s [64, 111]. One of the reasons for the delay in characterizing nucleolar SSc antigen may be because none of the SSc-specific ANoA make precipitin lines in DID that have been used to detect and define autoantibody specificities until the 1980s. The first description of antibodies to RNA polymerases (RNAP) was anti-RNA polymerase I antibodies as one of the specificities for the ANoA in SSc

Table 19 Prevalence of anti-NOR90 antibodies

Rheumatic diseases and others					
Author, year	**Country**	**Subjects**	**Methods**	**Prevalence**	**Note**
Rheumatic diseases and others					
Rodriguez-Sanchez 1987 [103]	Spain	RD 254 NOR staining 10	IF, WB cell (90 kD doublet?)	RD 2.4% (6/254) NOR 6/10 (IF titer, 1:640–10,000)	4/6 SSc 2/6 unknown
Kipnis 1990 [31]	USA	RD 112 (SSc 38, CREST 31, SSc-OL 15, Raynaud's 28)	IF, IP	RD 0% (0/112)	0/38 SSc 0/31 CREST 0/15 OL, 0/28 RP
Imai 1994 [104]	USA, Canada, Italy	Unknown (cancer, RD)	IP, IF	13 anti-NOR-90 7/8 F IF titer high (7/8 1:1600–12,800)	8/13 had clinical info SLE 2, SSc 1, RA 1, UCTD 1 arthritis 1, Malignancy 2, (4/8 RP+)
Dick 1995 [105]	Germany	RD 26,631(SSc 108)	IF, WB cell	RD 0.03% (9/26631) IF titer 8/9 1:320–2560 9/9 F	RA 2, SLE 1, SLE susp 1, SSc 1, UCTD 1, OA 1, Alv proteinosis 1, SSc 1/108
Fritzler 1995 [106, 156]	Canada, Brazil	RD children 238 (JIA 28%, IIM 14%, SLE 12%, OL/MCTD 6%, SSc 5%, SjS 4%, other 31%)	IF, WB, IP	RD 0.8% (2/238) IF titer 1:640–1280 2/2 F	RP 1 SLE(RP+) 1
Fujii 1996 [107]	Japan	ANoA 91 (SLE 21, SSc 21, RA 14, SjS 13, UCTD 10, vasculitis 4, ITP 3, OA 2, Hashimoto 2, PMR 1)	IF WB recombinant	ANoA 9.9% (9/91) 7/9 F	SS 7/9(pSS 2, RA 3, RA + SSc 1), SSc 2
Whitehead 1998 [108]	Canada, USA	Unknown	IF, WB, TnT IP,	4 anti-NOR-90, all also had anti-ASE-1 4/4 F	SSc 2 (1 with melanoma), seronegative arthritis 2
Dagher 2002 [157]	Canada	Unknown	IF, WB	1 F	Limited SSc 1
Satoh 2012 [8]	USA	4754 general population 670 ANA pos-tested by IP	IP	0.02% (1/4754) 0.15% (1/670)	
Malignancy					
Imai 1992 [77]	USA	HCC 184, CH/LC 187, GIC 210, LC 37, OC 2, NC 229	IF WB	Cancer 0.17% (1/620) ANoA 6% (16/17) F	HCC 1/184 HCC ANoA 1/12
Zhang 2002 [158]	China	HCC 137, LD 77, NC 30	IF, WB, TnT IP	1 F IF titer 1:5120	HCC 1/137 HCC ANoA 1/13

Alv, alveolar; *CH*, chronic hepatitis; *HCC*, hepatocellular carcinoma; *IF*, immunofluorescence; *IIM*, idiopathic inflammatory myopathies; *LC*, liver cirrhosis; *LD*, liver disease; *GIC*, gastrointestinal cancer; *LC*, *JIA*, juvenile idiopathic arthritis; lung cancer; *NC*, normal controls; *OA*, osteoarthritis; *OC*, ovarian cancer; *NC*, normal control; *RD*, rheumatic diseases; *TnT*, in vitro transcription and translation product

[111]. There were a series of earlier studies reporting anti-RNA polymerase I antibodies by a solid-phase radioimmunoassay (RIA) in the majority of patients with SLE, MCTD, and RA, and lupus-prone MRL/*lpr* mice [112–115]. However, these data are quite different from the data in all studies after 1987 by radioimmunoprecipitation and ELISA, which show high specificity for SSc [49, 116–118]. Thus, earlier studies by solid-phase RIA appear to be detecting antibodies different than anti-RNAP I antibodies that coexist with anti-RNAP III antibodies and are specific for SSc as we currently detect.

RNA polymerase I localizes to nucleoli with punctate pattern (AC-10), whereas RNA polymerase III and II distribute in nuclei showing a coarse speckled pattern (AC-5) based on the study using monoclonal antibodies (mAbs) [119].

Table 20 Prevalence of anti-NOR-90 by line immunoassay

Author, year, country	SSc	lcSSc	dcSSc	Others	HC
Low 2012 [159] China	1.5% (1/68)	-	-	SLE 2% (1/49)	1.4% (1/73, OA/HC)
Villalta 2012 [139] Italy	4.8% (10/210)	6.2% (9/146)	1.6% (1/64)	ANoA 17.9% (10/56)	-
Mehra 2013 [140] Australia	2.8% (15/528)	-	-	-	-
Wielosz 2014 [142] Poland	6.9% (6/87)	9.6% (5/52)	2.9% (1/35)	-	-
Chang 2015 [143] New Zealand	1.7% (1/60)	0% (0/41)	6.7% (1/15)	SSc-OL 0% (0/4)	-
Patterson 2015 [144] Australia	3.8% (19/505)	-	-	-	-
Liaskos 2017 [145] Greece	6.1% (8/131)	15% (6/40)	2.4% (2/82)	-	-
Marou 2017 [146] Greece	6.0% (5/84)	-	-	-	-
Liaskos 2018 [147] Greece	4.4% (7/158)	6.3% (6/95)	1.6% (1/63)	-	-
Liu, 2019 [148] China	2.19% (7/320)	-	-	Non-SSc CTD 2.0% (2/100)	3.3% (1/30)
Gauderon 2020[14] Switzerland	ANoA (≥ 1:320) or SSc susp 0.3% (1/386)	-	-	-	-

CTD, connective tissue disease; *HC*, healthy controls; *OA*, osteoarthritis; *OL*, overlap syndrome

Predominant nucleolar staining by uncommon human sera that show IP of strong RNAP I with weak RNAP III [120] is consistent with the data by mAbs. Commercially available kits for anti-RNA polymerases are only for the detection of anti-RNAP III antibodies [118]. Thus, positive patients are expected to have a nuclear large/coarse speckled pattern (AC-5). Since virtually all anti-RNAP III sera also contain anti-RNAP I, nucleolar staining may be reported; however, reporting punctate nucleolar staining with a background of a coarse speckled pattern may require careful interpretation and experience [121].

Anti-RNA polymerase III immunoassay has become a part of standard screening tests when patients are suspected or diagnosed as SSc [49, 118]. Virtually, all anti-RNAP III sera also have anti-RNAP I, and the screening test is only for RNAP III. Commercial immunoassay for anti-RNAP I is not available. There are no studies separating the clinical significance of anti-RNAP I from that of anti-RNAP III because they coexist in virtually all cases. Thus, a well-established association of anti-RNAP III with SSc, mainly dcSSc often complicated with renal crisis, will be applied for anti-RNAP I.

Many sera immunoprecipitate RNAP III and RNAP I at comparable intensity; however, some sera predominantly immunoprecipitate anti-RNAP I with very weak RNAP III [120, 122]. Some of these patients may show atypical clinical presentation. These sera tend to be negative in anti-RNAP III ELISA, though anti-RNAP III ELISA can be positive up to 80–95% of anti-RNAP III IP positive sera in general [118].

Summary of Clinical Association

The association of each ANoA pattern, specific autoantibodies, and clinical diagnosis are summarized (Fig. 5). The association of anti-Th/To (AC-8), -PM-Scl (AC-8), -fibrillarin(U3RNP) (AC-9), and -RNAP I (AC-10) with SSc or SSc-OL is well established. Anti-Th/To and -PM-Scl are associated with lcSSc, while anti-fibrillarin and RNAP I are associated with dcSSc. Many anti-Th/To-positive patients do not represent typical SSc and may be considered ssSSc or UCTD or show only certain features related to SSc including ILD, PH, or Raynaud's phenomenon. Limited studies reported anti-RNA helicase II/Gu (AC-8), -NOR90/hUBF, and ASE-1 mainly in patients with SARDs but not necessarily SSc. Several specificities with the AC-8 pattern, including anti-nucleolin/C23, nucleophosmin/B23, No55, and Nop-52/NNP1, have been reported in SARDs, malignancies, GVHD, and other diseases and clinical significance remains to be established.

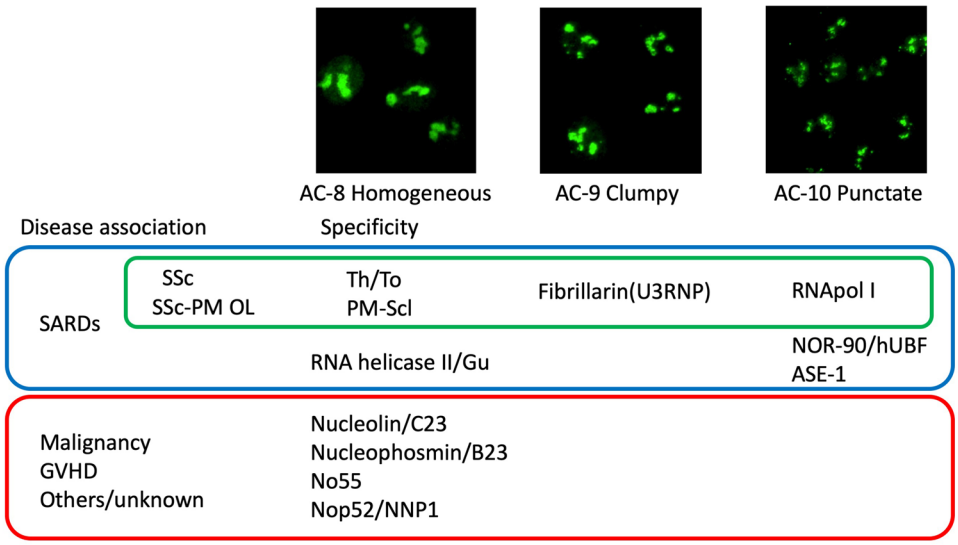

Fig. 5 AC patterns, specific autoantibodies, and clinical diagnosis. Association of each ANoA pattern, specific autoantibodies, and clinical diagnosis is summarized

Conclusions

ANoA by immunofluorescence has a long history of over 60 years. Many target antigens of ANoA have been identified and characterized; however, there are still many ANoA-positive sera with unknown specificity. Among the characterized specific ANoA, many articles have been published on anti-Th/To, -PM-Scl, and -U3RNP/fibrillarin, and their clinical association with SSc or SSc overlap syndrome is well established. However, the clinical relevance of other specific ANoA is not well understood despite the fact that many of these specificities were identified decades ago. One important factor and a difference between these two groups are the roles of immunoassays. For anti-Th/To, PM-Scl, and U3RNP, reliable detection by IP (and DID for anti-PM-Scl) contributed significantly to establishing their clinical significance. IP is considered reliable but not available for most clinicians, whereas currently available immunoassays have limitations in sensitivity and specificity. Future important issues will be the development and validation of widely available immunoassay for these. For the latter group of ANoA with unknown clinical relevance, the establishment of widely available assay and systematic screening would help future clinical uses of these specificities.

Acknowledgements This work was supported by JSPS KAKENHI (Grants-in-Aid for Scientific Research) grant number 19K08617 (for MS).

Declarations

Ethics Approval The study was conducted in accordance with the Declaration of Helsinki after approval by the institutional review board of University of Occupational and Environmental Health, Japan.

Consent to Participate Written informed consent was obtained from all individual participants included in the study.

Conflict of Interest The authors declare no competing interests.

References

1. Beck JS (1961) Variations in the morphological patterns of "autoimmune" nuclear fluorescence. Lancet 1:1203–1205
2. Fennell RH Jr, Rodnan GP, Vazquez JJ (1962) Variability of tissue-localizing properties of serum from patients with different disease states. Lab Invest 2:24–31
3. Beck JS, Anderson JR, Mc EA, Rowell NR (1962) Antinucleolar antibodies. Lancet 2:575–577
4. Chan EK, Damoiseaux J, Carballo OG, Conrad K, de Melo CW, Francescantonio PL et al (2015) Report of the first international consensus on standardized nomenclature of antinuclear antibody HEp-2 cell patterns 2014–2015. Front Immunol 6:412
5. Andrade LEC, Klotz W, Herold M, Conrad K, Rönnelid Y, Fritzler MJ, von Mühlen CA, Satoh M, Damoiseaux J, de Melo Cruvinel W, Chan EKL (2018) On behalf of the Executive Committee of ICAP. International Consensus on Antinuclear Antibody Patterns: definition of the AC-29 pattern associated with antibodies to DNA topoisomerase I. Clin Chem Lab Med 56:17835–1788
6. Herold M, Klotz W, Andrade LEC, Conrad K, Damoiseaux J, Fritzler MJ, von Muhlen, Satoh M, Chan EKL (2018) And the other members of the Executive Committee of ICAP. International Consensus on Antinuclear Antibody Patterns on defining negative results and recommendation in reporting unidentified patterns. Clin Chem Lab Med 56:1799–1802
7. Damoiseaux J, Andrade LEC, Carballo OG, Conrad K, Francescantonio PLC, Fritzler MJ et al (2019) Clinical relevance of HEp-2 indirect immunofluorescent patterns: the International Consensus on ANA Patterns (ICAP) perspective. Ann Rheum Dis 78:879–889
8. Satoh M, Chan EK, Ho LA, Rose KM, Parks CG, Cohn RD et al (2012) Prevalence and sociodemographic correlates of

antinuclear antibodies in the United States. Arthritis Rheum 64:2319–2327
9. Dinse GE, Parks CG, Weinberg CR, Co CA, Wilkerson J, Zeldin DC et al (2020) Increasing prevalence of antinuclear antibodies in the United States. Arthritis Rheumatol 72:1026–1035
10. Terao C, Ohmura K, Yamada R, Kawaguchi T, Shimizu M, Tabara Y et al (2014) Association between antinuclear antibodies and the HLA class II locus and heterogeneous characteristics of staining patterns: the Nagahama study. Arthritis Rheumatol 66:3395–3403
11. Roberts-Thomson PJ, Nikoloutsopoulos T, Cox S, Walker JG, Gordon TP (2003) Antinuclear antibody testing in a regional immunopathology laboratory. Immunol Cell Biol 81:409–412
12. Sener AG, Afsar I, Demirci M (2014) Evaluation of antinuclear antibodies by indirect immunofluorescence and line immunoassay methods': four years' data from Turkey. APMIS 122:1167–1170
13. Mengeloglu Z, Tas T, Kocoglu E, Aktas G, Karabork S (2014) Determination of anti-nuclear antibody pattern distribution and clinical relationship. Pak J Med Sci 30:380–383
14. Gauderon A, Roux-Lombard P, Spoerl D (2020) Antinuclear antibodies with a homogeneous and speckled immunofluorescence pattern are associated with lack of cancer while those with a nucleolar pattern with the presence of cancer. Front Med (Lausanne) 7:165
15. Nandiwada SL, Peterson LK, Mayes MD, Jaskowski TD, Malmberg E, Assassi S et al (2016) Ethnic differences in autoantibody diversity and hierarchy: more clues from a US cohort of patients with systemic sclerosis. J Rheumatol 43:1816–1824
16. Ferri C, Bernini L, Cecchetti R, Latorraca A, Marotta G, Pasero G et al (1991) Cutaneous and serologic subsets of systemic sclerosis. J Rheumatol 18:1826–1832
17. Bunn CC, Denton CP, Shi-Wen X, Knight C, Black CM (1998) Anti-RNA polymerases and other autoantibody specificities in systemic sclerosis. Br J Rheumatol 37:15–20
18. Reveille JD, Fischbach M, McNearney T, Friedman AW, Aguilar MB, Lisse J et al (2001) Systemic sclerosis in 3 US ethnic groups: a comparison of clinical, sociodemographic, serologic, and immunogenetic determinants. Semin Arthritis Rheum 30:332–346
19. Hesselstrand R, Scheja A, Shen GQ, Wiik A, Akesson A (2003) The association of antinuclear antibodies with organ involvement and survival in systemic sclerosis. Rheumatology (Oxford) 42:534–540
20. Sulli A, Ruaro B, Smith V, Pizzorni C, Zampogna G, Gallo M et al (2013) Progression of nailfold microvascular damage and antinuclear antibody pattern in systemic sclerosis. J Rheumatol 40:634–639
21. Sharmin S, Ahmed S, Abu Saleh A, Rahman F, Choudhury MR, Hassan MM (2014) Association of immunofluorescence pattern of antinuclear antibody with specific autoantibodies in the Bangladeshi population. Bangladesh Med Res Counc Bull 40:74–78
22. Nishimura S, Nishiya K, Hisakawa N, Chikazawa H, Ookubo S, Nakatani K et al (1996) Positivity for antinuclear antibody in patients with advanced rheumatoid arthritis. Acta Med Okayama 50:261–265
23. Ochs RL, Stein TW Jr, Peebles CL, Gittes RF, Tan EM (1994) Autoantibodies in interstitial cystitis. J Urol 151:587–592
24. Reddy R, Tan EM, Henning D, Nohga K, Busch H (1983) Detection of a nucleolar 7–2 ribonucleoprotein and a cytoplasmic 8–2 ribonucleoprotein with autoantibodies from patients with scleroderma. J Biol Chem 258:1383–1386
25. Hashimoto C, Steitz JA (1983) Sequential association of nucleolar 7–2 RNA with two different autoantigens. J Biol Chem 258:1379–1382
26. Mahler M, Fritzler MJ, Satoh M (2015) Autoantibodies to the mitochondrial RNA processing (MRP) complex also known as Th/To autoantigen. Autoimmun Rev 14:254–257
27. Van Eenennaam H, Vogelzangs JH, Lugtenberg D, Van Den Hoogen FH, Van Venrooij WJ, Pruijn GJ (2002) Identity of the RNase MRP- and RNase P-associated Th/To autoantigen. Arthritis Rheum 46:3266–3272
28. Okano Y, Medsger TA Jr (1990) Autoantibody to Th ribonucleoprotein (nucleolar 7–2 RNA protein particle) in patients with systemic sclerosis. Arthritis Rheum 33:1822–1828
29. Kuwana M, Kimura K, Hirakata M, Kawakami Y, Ikeda Y (2002) Differences in autoantibody response to Th/To between systemic sclerosis and other autoimmune diseases. Ann Rheum Dis 61:842–846
30. Hirakata M, Mimori T, Akizuki M, Craft J, Hardin JA, Homma M (1992) Autoantibodies to small nuclear and cytoplasmic ribonucleoproteins in Japanese patients with inflammatory muscle disease. Arthritis Rheum 35:449–456
31. Kipnis RJ, Craft J, Hardin JA (1990) The analysis of antinuclear and antinucleolar autoantibodies of scleroderma by radioimmunoprecipitation assays. Arthritis Rheum 33:1431–1437
32. Verheijen R, Wiik A, De Jong BA, Hoier-Madsen M, Ullman S, Halberg P et al (1994) Screening for autoantibodies to the nucleolar U3- and Th(7–2) ribonucleoproteins in patients' sera using antisense riboprobes. J Immunol Methods 169:173–182
33. Van Eenennaam H, Vogelzangs JH, Bisschops L, Te Boome LC, Seelig HP, Renz M et al (2002) Autoantibodies against small nucleolar ribonucleoprotein complexes and their clinical associations. Clin Exp Immunol 130:532–540
34. Mitri GM, Lucas M, Fertig N, Steen VD, Medsger TA Jr (2003) A comparison between anti-Th/To- and anticentromere antibody-positive systemic sclerosis patients with limited cutaneous involvement. Arthritis Rheum 48:203–209
35. Poormoghim H, Lucas M, Fertig N, Medsger TA Jr (2000) Systemic sclerosis sine scleroderma: demographic, clinical, and serologic features and survival in forty-eight patients. Arthritis Rheum 43:444–451
36. Fischer A, Meehan RT, Feghali-Bostwick CA, West SG, Brown KK (2006) Unique characteristics of systemic sclerosis sine scleroderma-associated interstitial lung disease. Chest 130:976–981
37. Fischer A, Pfalzgraf FJ, Feghali-Bostwick CA, Wright TM, Curran-Everett D, West SG et al (2006) Anti-th/to-positivity in a cohort of patients with idiopathic pulmonary fibrosis. J Rheumatol 33:1600–1605
38. Mahler M, Satoh M, Hudson M, Baron M, Chan JY, Chan EK et al (2014) Autoantibodies to the Rpp25 component of the Th/To complex are the most common antibodies in patients with systemic sclerosis without antibodies detectable by widely available commercial tests. J Rheumatol 41:1334–1343
39. Mahler M, Gascon C, Patel S, Ceribelli A, Fritzler MJ, Swart A et al (2013) Rpp25 is a major target of autoantibodies to the Th/To complex as measured by a novel chemiluminescent assay. Arthritis Res Ther 15:R50
40. Mahler M, Fritzler MJ (2014) Antinuclear antibodies in children. J Rheumatol 41:1260–1262
41. Markusse IM, Meijs J, de Boer B, Bakker JA, Schippers HPC, Schouffoer AA et al (2017) Predicting cardiopulmonary involvement in patients with systemic sclerosis: complementary value of nailfold videocapillaroscopy patterns and disease-specific autoantibodies. Rheumatology (Oxford) 56:1081–1088
42. Koenig M, Bentow C, Satoh M, Fritzler MJ, Senecal JL, Mahler M (2019) Autoantibodies to a novel Rpp38 (Th/To) derived B-cell epitope are specific for systemic sclerosis and associate with a distinct clinical phenotype. Rheumatology (Oxford) 58:1784–1793

43. Mierau R, Moinzadeh P, Riemekasten G, Melchers I, Meurer M, Reichenberger F et al (2011) 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 13:R172
44. Muller R, Benyamine A, Bertin D, Harle JR, Kaplanski G, Mazodier K et al (2020) Characteristics of systemic sclerosis patients with positive anti-Th/To antibodies: about 6 patients and literature review. Rev Med Interne 41:440–445
45. Falkner D, Wilson J, Medsger TA Jr, Morel PA (1998) HLA and clinical associations in systemic sclerosis patients with anti-Th/To antibodies. Arthritis Rheum 41:74–80
46. Kuwana M, Kaburaki J, Okano Y, Tojo T, Homma M (1994) Clinical and prognostic associations based on serum antinuclear antibodies in Japanese patients with systemic sclerosis. Arthritis Rheum 37:75–83
47. Harvey G, Black C, Maddison P, McHugh N (1997) Characterization of antinucleolar antibody reactivity in patients with systemic sclerosis and their relatives. J Rheumatol 24:477–484
48. Hamaguchi Y, Hasegawa M, Fujimoto M, Matsushita T, Komura K, Kaji K et al (2008) The clinical relevance of serum antinuclear antibodies in Japanese patients with systemic sclerosis. Br J Dermatol 158:487–495
49. Steen VD (2005) Autoantibodies in systemic sclerosis. Semin Arthritis Rheum 35:35–42
50. Jacobsen S, Halberg P, Ullman S, Van Venrooij WJ, Hoier-Madsen M, Wiik A et al (1998) Clinical features and serum antinuclear antibodies in 230 Danish patients with systemic sclerosis. Br J Rheumatol 37:39–45
51. Gunduz OH, Fertig N, Lucas M, Medsger TA Jr (2001) Systemic sclerosis with renal crisis and pulmonary hypertension: a report of eleven cases. Arthritis Rheum 44:1663–1666
52. Ceribelli A, Satoh M, Chan EK (2012) A new immunoprecipitation-real time quantitative PCR assay for anti-Th/To and anti-U3RNP antibody detection in systemic sclerosis. Arthritis Res Ther 14:R128
53. Hardin JA, Rahn DR, Shen C, Lerner MR, Wolin SL, Rosa MD et al (1982) Antibodies from patients with connective tissue diseases bind specific subsets of cellular RNA-protein particles. J Clin Invest 70:141–147
54. Yamane K, Ihn H, Kubo M, Kuwana M, Asano Y, Yazawa N et al (2001) Antibodies to Th/To ribonucleoprotein in patients with localized scleroderma. Rheumatology (Oxford) 40:683–686
55. Koenig M, Joyal F, Fritzler MJ, Roussin A, Abrahamowicz M, Boire G et al (2008) Autoantibodies and microvascular damage are independent predictive factors for the progression of Raynaud's phenomenon to systemic sclerosis: a twenty-year prospective study of 586 patients, with validation of proposed criteria for early systemic sclerosis. Arthritis Rheum 58:3902–3912
56. Wolfe JF, Adelstein E, Sharp GC (1977) Antinuclear antibody with distinct specificity for polymyositis. J Clin Invest 59:176–178
57. Reichlin M, Maddison PJ, Targoff I, Bunch T, Arnett F, Sharp G et al (1984) Antibodies to a nuclear/nucleolar antigen in patients with polymyositis overlap syndromes. J Clin Immunol 4:40–44
58. Reimer G, Scheer U, Peters JM, Tan EM (1986) Immunolocalization and partial characterization of a nucleolar autoantigen (PM-Scl) associated with polymyositis/scleroderma overlap syndromes. J Immunol 137:3802–3808
59. Mahler M, Raijmakers R (2007) Novel aspects of autoantibodies to the PM/Scl complex: clinical, genetic and diagnostic insights. Autoimmun Rev 6:432–437
60. Oddis CV, Okano Y, Rudert WA, Trucco M, Duquesnoy RJ, Medsger TA Jr (1992) Serum autoantibody to the nucleolar antigen PM-Scl. Clinical and immunogenetic associations Arthritis Rheum 35:1211–1217
61. Muro Y, Hosono Y, Sugiura K, Ogawa Y, Mimori T, Akiyama M (2015) Anti-PM/Scl antibodies are found in Japanese patients with various systemic autoimmune conditions besides myositis and scleroderma. Arthritis Res Ther 17:57
62. Kohara A, Yanaba K, Muro Y, Ito H, Nakagawa H, Noda K et al (2017) Anti-PM/Scl antibody-positive dermatomyositis in a Japanese patient: a case report and review of the literature. Int J Rheum Dis 20:2186–2189
63. Nishida T, Nakano K, Satoh M, Fukuyo S, Akashi K, Tanaka Y (2021) A Japanese patient with anti-PM/Scl and centromere antibody-positive scleroderma-amyopathic dermatomyositis overlap syndrome who developed renal crisis. Mod Rheumatol Case Rep
64. Reimer G, Steen VD, Penning CA, Medsger TA Jr, Tan EM (1988) Correlates between autoantibodies to nucleolar antigens and clinical features in patients with systemic sclerosis (scleroderma). Arthritis Rheum 31:525–532
65. Jablonska S, Blaszczyk M (1999) Scleroderma overlap syndromes. Adv Exp Med Biol 455:85–92
66. Hausmanowa-Petrusewicz I, Kowalska-Oledzka E, Miller FW, Jarzabek-Chorzelska M, Targoff IN, Blaszczyk-Kostanecka M et al (1997) Clinical, serologic, and immunogenetic features in Polish patients with idiopathic inflammatory myopathies. Arthritis Rheum 40:1257–1266
67. Bell SA, Faust H, Mittermuller J, Kolb HJ, Meurer M (1996) Specificity of antinuclear antibodies in scleroderma-like chronic graft-versus-host disease: clinical correlation and histocompatibility locus antigen association. Br J Dermatol 134:848–854
68. Marguerie C, Bunn CC, Copier J, Bernstein RM, Gilroy JM, Black CM et al (1992) The clinical and immunogenetic features of patients with autoantibodies to the nucleolar antigen PM-Scl. Medicine (Baltimore) 71:327–336
69. Li XZ, McNeilage LJ, Whittingham S (1989) Autoantibodies to the major nucleolar phosphoprotein B23 define a novel subset of patients with anticardiolipin antibodies. Arthritis Rheum 32:1165–1169
70. Ulanet DB, Wigley FM, Gelber AC, Rosen A (2003) Autoantibodies against B23, a nucleolar phosphoprotein, occur in scleroderma and are associated with pulmonary hypertension. Arthritis Rheum 49:85–92
71. Lartigue A, Drouot L, Jouen F, Charlionet R, Tron F, Gilbert D (2005) Association between anti-nucleophosmin and anti-cardiolipin antibodies in (NZW x BXSB)F1 mice and human systemic lupus erythematosus. Arthritis Res Ther 7:R1394–R1403
72. Wesierska-Gadek J, Penner E, Hitchman E, Kier P, Sauermann G (1992) Nucleolar proteins B23 and C23 as target antigens in chronic graft-versus-host disease. Blood 79:1081–1086
73. Brankin B, Skaar TC, Brotzman M, Trock B, Clarke R (1998) Autoantibodies to the nuclear phosphoprotein nucleophosmin in breast cancer patients. Cancer Epidemiol Biomarkers Prev 7:1109–1115
74. Mojtahedi Z, Safaei A, Yousefi Z, Ghaderi A (2011) Immunoproteomics of HER2-positive and HER2-negative breast cancer patients with positive lymph nodes. OMICS 15:409–418
75. Liu M, Varela-Ramirez A, Li J, Dai L, Aguilera RJ, Zhang JY (2015) Humoral autoimmune response to nucleophosmin in the immunodiagnosis of hepatocellular carcinoma. Oncol Rep 33:2245–2252
76. Wang T, Liu M, Zheng SJ, Bian DD, Zhang JY, Yao J et al (2017) Tumor-associated autoantibodies are useful biomarkers in immunodiagnosis of alpha-fetoprotein-negative hepatocellular carcinoma. World J Gastroenterol 23:3496–3504
77. Imai H, Ochs RL, Kiyosawa K, Furuta S, Nakamura RM, Tan EM (1992) Nucleolar antigens and autoantibodies in hepatocellular carcinoma and other malignancies. Am J Pathol 140:859–870

78. Taylor DD, Gercel-Taylor C, Parker LP (2009) Patient-derived tumor-reactive antibodies as diagnostic markers for ovarian cancer. Gynecol Oncol 115:112–120
79. Lu D, Kuhn E, Bristow RE, Giuntoli RL 2nd, Kjaer SK, Shih Ie M et al (2011) Comparison of candidate serologic markers for type I and type II ovarian cancer. Gynecol Oncol 122:560–566
80. Dai L, Li J, Xing M, Sanchez TW, Casiano CA, Zhang JY (2016) Using serological proteome analysis to identify serum anti-nucleophosmin 1 autoantibody as a potential biomarker in European-American and African-American patients with prostate cancer. Prostate 76:1375–1386
81. Pulford K, Falini B, Banham AH, Codrington D, Roberton H, Hatton C et al (2000) Immune response to the ALK oncogenic tyrosine kinase in patients with anaplastic large-cell lymphoma. Blood 96:1605–1607
82. Damm-Welk C, Siddiqi F, Fischer M, Hero B, Narayanan V, Camidge DR et al (2016) Anti-ALK Antibodies in patients with ALK-positive malignancies not expressing NPM-ALK. J Cancer 7:1383–1387
83. Knorr F, Weber S, Singh VK, Pulford K, Reiter A, Woessmann W et al (2018) Epitope mapping of anti-ALK antibodies in children with anaplastic large cell lymphoma. Clin Immunol 195:77–81
84. Minota S, Jarjour WN, Roubey RA, Mimura T, Winfield JB (1990) Reactivity of autoantibodies and DNA/anti-DNA complexes with a novel 110-kilodalton phosphoprotein in systemic lupus erythematosus and other diseases. J Immunol 144:1263–1269
85. Yoshikawa H, Komatsu W, Hayano T, Miura Y, Homma K, Izumikawa K et al (2011) Splicing factor 2-associated protein p32 participates in ribosome biogenesis by regulating the binding of Nop52 and fibrillarin to preribosome particles. Mol Cell Proteomics 10:M110 006148
86. Hirata D, Iwamoto M, Yoshio T, Okazaki H, Masuyama J, Mimori A et al (2000) Nucleolin as the earliest target molecule of autoantibodies produced in MRL/lpr lupus-prone mice. Clin Immunol 97:50–58
87. Valdez BC, Henning D, Busch RK, Woods K, Flores-Rozas H, Hurwitz J et al (1996) A nucleolar RNA helicase recognized by autoimmune antibodies from a patient with watermelon stomach disease. Nucleic Acids Res 24:1220–1224
88. Arnett FC, Reveille JD, Valdez BC (1997) Autoantibodies to a nucleolar RNA helicase protein in patients with connective tissue diseases. Arthritis Rheum 40:1487–1492
89. Garcia MC, Zhou J, Henning D, Arnett FC, Valdez BC (2000) Unique epitopes in RNA helicase II/Gu protein recognized by serum from a watermelon stomach patient. Mol Immunol 37:351–359
90. Fossa A, Siebert R, Aasheim HC, Maelandsmo GM, Berner A, Fossa SD et al (2000) Identification of nucleolar protein No55 as a tumour-associated autoantigen in patients with prostate cancer. Br J Cancer 83:743–749
91. Jansen E, Meulemans SM, Orlans IC, Van de Ven WJ (1997) The NNP-1 gene (D21S2056E), which encodes a novel nuclear protein, maps in close proximity to the cystatin B gene within the EPM1 and APECED critical region on 21q22.3. Genomics 42:336–41
92. Behrends U, Jandl T, Golbeck A, Lechner B, Muller-Weihrich S, Schmid I et al (2002) Novel products of the HUD, HUC, NNP-1 and alpha-internexin genes identified by autologous antibody screening of a pediatric neuroblastoma library. Int J Cancer 100:669–677
93. Lischwe MA, Ochs RL, Reddy R, Cook RG, Yeoman LC, Tan EM et al (1985) Purification and partial characterization of a nucleolar scleroderma antigen (Mr = 34,000; pI, 8.5) rich in NG,NG-dimethylarginine. J Biol Chem 260:14304–10
94. Reimer G, Pollard KM, Penning CA, Ochs RL, Lischwe MA, Busch H et al (1987) Monoclonal autoantibody from a (New Zealand black x New Zealand white)F1 mouse and some human scleroderma sera target an Mr 34,000 nucleolar protein of the U3 RNP particle. Arthritis Rheum 30:793–800
95. Hultman P, Enestrom S (1988) Mercury induced antinuclear antibodies in mice: characterization and correlation with renal immune complex deposits. Clin Exp Immunol 71:269–274
96. Hultman P, Enestrom S, Pollard KM, Tan EM (1989) Anti-fibrillarin autoantibodies in mercury-treated mice. Clin Exp Immunol 78:470–477
97. Welting TJ, Raijmakers R, Pruijn GJ (2003) Autoantigenicity of nucleolar complexes. Autoimmun Rev 2:313–321
98. Okano Y, Steen VD, Medsger TA Jr (1992) Autoantibody to U3 nucleolar ribonucleoprotein (fibrillarin) in patients with systemic sclerosis. Arthritis Rheum 35:95–100
99. Kasturi KN, Hatakeyama A, Spiera H, Bona CA (1995) Antifibrillarin autoantibodies present in systemic sclerosis and other connective tissue diseases interact with similar epitopes. J Exp Med 181:1027–1036
100. Arnett FC, Reveille JD, Goldstein R, Pollard KM, Leaird K, Smith EA et al (1996) Autoantibodies to fibrillarin in systemic sclerosis (scleroderma). An immunogenetic, serologic, and clinical analysis. Arthritis Rheum 39:1151–60
101. Tormey VJ, Bunn CC, Denton CP, Black CM (2001) Anti-fibrillarin antibodies in systemic sclerosis Rheumatology (Oxford) 40:1157–1162
102. Falkner D, Wilson J, Fertig N, Clawson K, Medsger TA Jr, Morel PA (2000) Studies of HLA-DR and DQ alleles in systemic sclerosis patients with autoantibodies to RNA polymerases and U3-RNP (fibrillarin). J Rheumatol 27:1196–1202
103. Rodriguez-Sanchez JL, Gelpi C, Juarez C, Hardin JA (1987) Anti-NOR 90. A new autoantibody in scleroderma that recognizes a 90-kDa component of the nucleolus-organizing region of chromatin. J Immunol 139:2579–84
104. Imai H, Fritzler MJ, Neri R, Bombardieri S, Tan EM, Chan EK (1994) Immunocytochemical characterization of human NOR-90 (upstream binding factor) and associated antigens reactive with autoimmune sera. Two MR forms of NOR-90/hUBF autoantigens. Mol Biol Rep 19:115–24
105. Dick T, Mierau R, Sternfeld R, Weiner EM, Genth E (1995) Clinical relevance and HLA association of autoantibodies against the nucleolus organizer region (NOR-90). J Rheumatol 22:67–72
106. Fritzler MJ, von Muhlen CA, Toffoli SM, Staub HL, Laxer RM (1995) Autoantibodies to the nucleolar organizer antigen NOR-90 in children with systemic rheumatic diseases. J Rheumatol 22:521–524
107. Fujii T, Mimori T, Akizuki M (1996) Detection of autoantibodies to nucleolar transcription factor NOR 90/hUBF in sera of patients with rheumatic diseases, by recombinant autoantigen-based assays. Arthritis Rheum 39:1313–1318
108. Whitehead CM, Fritzler MJ, Rattner JB (1998) The relationship of ASE-1 and NOR-90 in autoimmune sera. J Rheumatol 25:2126–2130
109. Whitehead CM, Winkfein RJ, Fritzler MJ, Rattner JB (1997) ASE-1: a novel protein of the fibrillar centres of the nucleolus and nucleolus organizer region of mitotic chromosomes. Chromosoma 106:493–502
110. Edworthy S, Fritzler M, Whitehead C, Martin L, Rattner JB (2000) ASE-1: an autoantigen in systemic lupus erythematosus. Lupus 9:681–687
111. Reimer G, Rose KM, Scheer U, Tan EM (1987) Autoantibody to RNA polymerase I in scleroderma sera. J Clin Invest 79:65–72
112. Stetler DA, Rose KM, Wenger ME, Berlin CM, Jacob ST (1982) Antibodies to distinct polypeptides of RNA polymerase I in sera

from patients with rheumatic autoimmune disease. Proc Natl Acad Sci U S A 79:7499–7503
113. Stetler DA, Jacob ST (1984) Phosphorylation of RNA polymerase I augments its interaction with autoantibodies of systemic lupus erythematosus patients. J Biol Chem 259:13629–13632
114. Stetler DA, Sipes DE, Jacob ST (1985) Anti-RNA polymerase I antibodies in sera of MRL lpr/lpr and MRL +/+ autoimmune mice. Correlation of antibody production with delayed onset of lupus-like disease in MRL +/+ mice. J Exp Med 162:1760–70
115. Stetler DA, Cavallo T (1987) Anti-RNA polymerase I antibodies: potential role in the induction and progression of murine lupus nephritis. J Immunol 138:2119–2123
116. Kuwana M, Kaburaki J, Mimori T, Tojo T, Homma M (1993) Autoantibody reactive with three classes of RNA polymerases in sera from patients with systemic sclerosis. J Clin Invest 91:1399–1404
117. Okano Y, Steen VD, Medsger TAJ (1993) Autoantibody reactive with RNA polymerase III in systemic sclerosis. Ann Intern Med 119:1005–1013
118. Kuwana M, Okano Y, Pandey JP, Silver RM, Fertig N, Medsger TA Jr (2005) Enzyme-linked immunosorbent assay for detection of anti-RNA polymerase III antibody: analytical accuracy and clinical associations in systemic sclerosis. Arthritis Rheum 52:2425–2432
119. Jones E, Kimura H, Vigneron M, Wang Z, Roeder RG, Cook PR (2000) Isolation and characterization of monoclonal antibodies directed against subunits of human RNA polymerases I, II, and III. Exp Cell Res 254:163–172
120. Ceribelli A, Krzyszczak ME, Li Y, Ross SJ, Chan JY, Chan EK et al (2011) Atypical clinical presentation of a subset of patients with anti-RNA polymerase III–non-scleroderma cases associated with dominant RNA polymerase I reactivity and nucleolar staining. Arthritis Res Ther 13:R119
121. Yamasaki Y, Honkanen-Scott M, Hernandez L, Ikeda K, Barker T, Bubb MR et al (2006) Nucleolar staining cannot be used as a screening test for the scleroderma marker anti-RNA polymerase I/III antibodies. Arthritis Rheum 54:3051–3056
122. Satoh M, Vazquez-Del Mercado M, Krzyszczak ME, Li Y, Ceribelli A, Burlingame RW et al (2012) Coexistence of anti-RNA polymerase III and anti-U1RNP antibodies in patients with systemic lupus erythematosus: two cases without features of scleroderma. Lupus 21:68–74
123. Terao C, Ohmura K, Yamada R, Kawaguchi T, Shimizu M, Tabara Y et al (2014) Association between antinuclear antibodies and the HLA class II locus and heterogeneous characteristics of staining patterns: the Nagahama study. Arthritis Rheumatol 66:3395–3403
124. Vermeersch P, Bossuyt X (2013) Prevalence and clinical significance of rare antinuclear antibody patterns. Autoimmun Rev 12:998–1003
125. Daschakraborty S, Aggarwal A, Aggarwal R (2012) Non-organ-specific autoantibodies in Indian patients with chronic liver disease. Indian J Gastroenterol 31:237–242
126. Wu Y, Cai B, Tang J, Bai Y, Wang L (2011) Tacrolimus may induce the production of nucleolar anti-nuclear antibody in liver transplant patients. J Gastrointestin Liver Dis 20:267–270
127. Kuwana M, Kaburaki J, Okano Y, Tojo T, Homma M (1994) Clinical and prognostic associations based on serum antinuclear antibodies in Japanese patients with systemic sclerosis. Arthritis Rheum 37:75–83
128. Harvey G, Black C, Maddison P, McHugh N (1997) Characterization of antinucleolar antibody reactivity in patients with systemic sclerosis and their relatives. J Rheumatol 24:477–484
129. Jacobsen S, Ullman S, Shen GQ, Wiik A, Halberg P (2001) Influence of clinical features, serum antinuclear antibodies, and lung function on survival of patients with systemic sclerosis. J Rheumatol 28:2454–2459
130. Reveille JD (2003) Ethnicity and race and systemic sclerosis: how it affects susceptibility, severity, antibody genetics, and clinical manifestations. Curr Rheumatol Rep 5:160–167
131. Meyer OC, Fertig N, Lucas M, Somogyi N, Medsger TA Jr (2007) Disease subsets, antinuclear antibody profile, and clinical features in 127 French and 247 US adult patients with systemic sclerosis. J Rheumatol 34:104–109
132. Ceribelli A, Cavazzana I, Franceschini F, Airo P, Tincani A, Cattaneo R et al (2010) Anti-Th/To are common antinucleolar autoantibodies in Italian patients with scleroderma. J Rheumatol 37:2071–2075
133. Krzyszczak ME, Li Y, Ross SJ, Ceribelli A, Chan EK, Bubb MR et al (2011) Gender and ethnicity differences in the prevalence of scleroderma-related autoantibodies. Clin Rheumatol 30:1333–1339
134. Van Praet JT, Van Steendam K, Smith V, De Bruyne G, Mimori T, Bonroy C et al (2011) Specific anti-nuclear antibodies in systemic sclerosis patients with and without skin involvement: an extended methodological approach. Rheumatology (Oxford) 50:1302–1309
135. Bonroy C, Van Praet J, Smith V, Van Steendam K, Mimori T, Deschepper E et al (2012) Optimization and diagnostic performance of a single multiparameter lineblot in the serological workup of systemic sclerosis. J Immunol Methods 379:53–60
136. Mahler M, Satoh M, Hudson M, Baron M, Chan JY, Chan EK et al (2014) Autoantibodies to the Rpp25 component of the Th/To complex are the most common antibodies in patients with systemic sclerosis without antibodies detectable by widely available commercial tests. J Rheumatol 41:1334–1343
137. Rodriguez-Reyna TS, Hinojosa-Azaola A, Martinez-Reyes C, Nunez-Alvarez CA, Torrico-Lavayen R, Garcia-Hernandez JL et al (2011) Distinctive autoantibody profile in Mexican Mestizo systemic sclerosis patients. Autoimmunity 44:576–584
138. Graf SW, Hakendorf P, Lester S, Patterson K, Walker JG, Smith MD et al (2012) South Australian Scleroderma Register: autoantibodies as predictive biomarkers of phenotype and outcome. Int J Rheum Dis 15:102–109
139. Villalta D, Imbastaro T, Di Giovanni S, Lauriti C, Gabini M, Turi MC et al (2012) Diagnostic accuracy and predictive value of extended autoantibody profile in systemic sclerosis. Autoimmun Rev 12:114–120
140. Mehra S, Walker J, Patterson K, Fritzler MJ (2013) Autoantibodies in systemic sclerosis. Autoimmun Rev 12:340–354
141. Poormoghim H, Moghadam AS, Moradi-Lakeh M, Jafarzadeh M, Asadifar B, Ghelman M et al (2013) Systemic sclerosis: demographic, clinical and serological features in 100 Iranian patients. Rheumatol Int 33:1943–1950
142. Wielosz E, Dryglewska M, Majdan M (2014) Serological profile of patients with systemic sclerosis. Postepy Hig Med Dosw (Online) 68:987–991
143. Chang WS, Schollum J, White DH, Solanki KK (2015) A cross-sectional study of autoantibody profiles in the Waikato systemic sclerosis cohort. New Zealand Clin Rheumatol 34:1921–1927
144. Patterson KA, Roberts-Thomson PJ, Lester S, Tan JA, Hakendorf P, Rischmueller M et al (2015) Interpretation of an extended autoantibody profile in a well-characterized Australian systemic sclerosis (Scleroderma) cohort using principal components analysis. Arthritis Rheumatol 67:3234–3244
145. Liaskos C, Marou E, Simopoulou T, Barmakoudi M, Efthymiou G, Scheper T et al (2017) Disease-related autoantibody profile in patients with systemic sclerosis. Autoimmunity 50:414–421
146. Marou E, Liaskos C, Efthymiou G, Dardiotis E, Daponte A, Scheper T et al (2017) Increased immunoreactivity against

human cytomegalovirus UL83 in systemic sclerosis. Clin Exp Rheumatol 35(Suppl 106):31–34
147. Liaskos C, Marou E, Simopoulou T, Gkoutzourelas A, Barmakoudi M, Efthymiou G et al (2018) Multiparametric autoantibody profiling of patients with systemic sclerosis in Greece. Mediterr J Rheumatol 29:120–126
148. Liu C, Hou Y, Yang Y, Xu D, Li L, Li J et al (2019) Evaluation of a commercial immunoassay for autoantibodies in Chinese Han systemic sclerosis population. Clin Chim Acta 491:121–125
149. Mendes C, Viana VST, Pasoto SG, Leon EP, Bonfa E, Sampaio-Barros PD (2020) Clinical and laboratory features of African-Brazilian patients with systemic sclerosis. Clin Rheumatol 39:9–17
150. Tahiat A, Allam I, Abdessemed A, Mellal Y, Nebbab R, Ladjouze-Rezig A et al (2020) Autoantibody profile in a cohort of Algerian patients with systemic sclerosis. Ann Biol Clin (Paris) 78:126–133
151. Steen V, Medsger TA Jr (2003) Predictors of isolated pulmonary hypertension in patients with systemic sclerosis and limited cutaneous involvement. Arthritis Rheum 48:516–522
152. Troyanov Y, Targoff IN, Tremblay JL, Goulet JR, Raymond Y, Senecal JL (2005) Novel classification of idiopathic inflammatory myopathies based on overlap syndrome features and autoantibodies: analysis of 100 French Canadian patients. Medicine (Baltimore) 84:231–249
153. Koschik RW 2nd, Fertig N, Lucas MR, Domsic RT, Medsger TA Jr (2012) Anti-PM-Scl antibody in patients with systemic sclerosis. Clin Exp Rheumatol 30:S12–S16
154. Kaji K, Fertig N, Medsger TA Jr, Satoh T, Hoshino K, Hamaguchi Y et al (2014) Autoantibodies to RuvBL1 and RuvBL2: a novel systemic sclerosis-related antibody associated with diffuse cutaneous and skeletal muscle involvement. Arthritis Care Res (Hoboken) 66:575–584
155. Qin Z, Lavingia B, Zou Y, Stastny P (2011) Antibodies against nucleolin in recipients of organ transplants. Transplantation 92:829–835
156. Fritzler MJ, von Muhlen CA, Toffoli SM, Staub HL, Laxer RM (1995) Autoantibodies to the nucleolar organizer antigen NOR-90 in children with systemic rheumatic diseases. J Rheumatol 22:521–524
157. Dagher JH, Scheer U, Voit R, Grummt I, Lonzetti L, Raymond Y et al (2002) 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 29:1543–1547
158. Zhang JY, Wang X, Peng XX, Chan EK (2002) Autoantibody responses in Chinese hepatocellular carcinoma. J Clin Immunol 22:98–105
159. Low AH, Wong S, Thumboo J, Ng SC, Lim JY, Ng X et al (2012) Evaluation of a new multi-parallel line immunoassay for systemic sclerosis-associated antibodies in an Asian population. Rheumatology (Oxford) 51:1465–1470