DETECTION AND CLINICAL RELEVANCE OF THE FINE SPECIFICITY OF ANTINUCLEOLAR AUTOANTIBODIES FOR THE DIAGNOSIS OF SYSTEMIC SCLEROSIS

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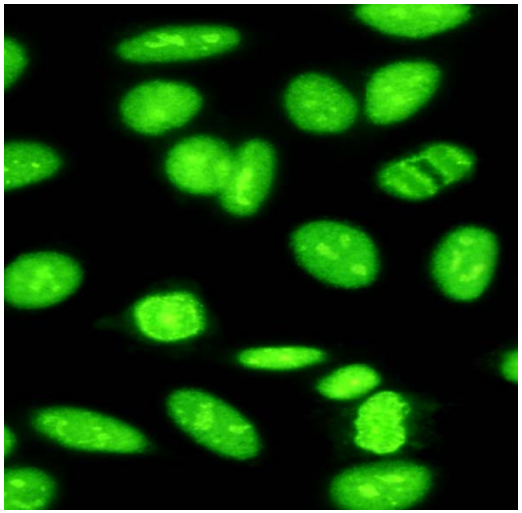
Anti-nuclear antibodies (ANA) in systemic sclerosis (SSc)

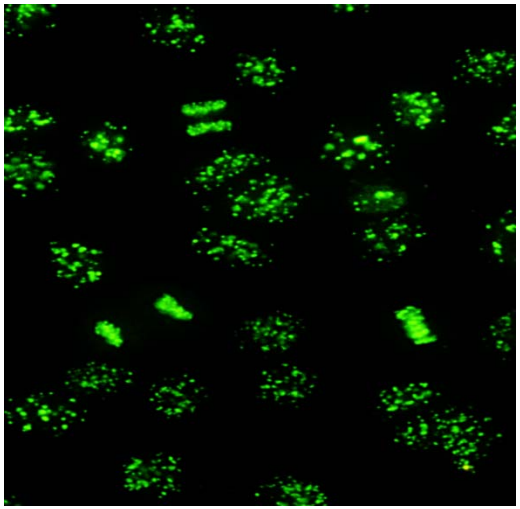
>90%

Staining patterns characteristic for SSc (IIF on HEp-2 cells)



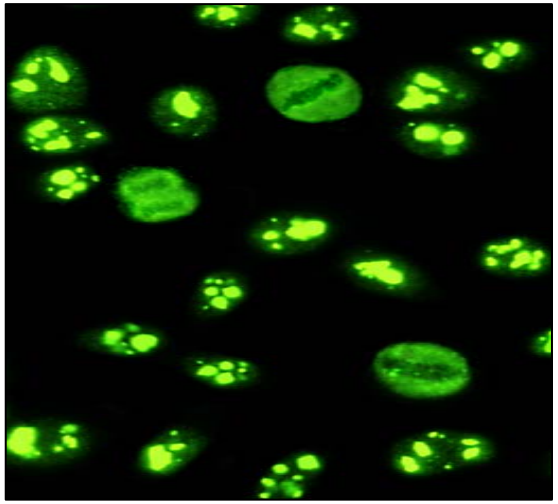
Homogeneous + nucleolar (anti-topo I)

15-30%



anti-centromere (ACA)

20-35%



anti-nucleolar (ANoA)

15-40%

ANTINUCLEOLAR AUTONTIBODIES (ANoA): HISTORICAL NOTES

ANoA were first identified in 1962 by Beck JS et al. *Lancet*

Association with systemic sclerosis (SSc) **(15-40%)** has been known since at least 1970 (*Ritchie RF, NEJM 1970*)

…… **but ANoAs by IIF are not specific for SSc**

Many nucleolar enzymes and proteins can be targeted by ANoAs:

-1977 *(Wolfe et al)* PM-Scl

-1983 *(Reddy R et al)* Th/To

-1985 *(Lischwe MA et al)* U3-RNP (fibrillarin)

-1987 *(Reimer G et al)* RNAP I

-1987 *(Chan EK et al)* NOR 90 (anti-hUBF)

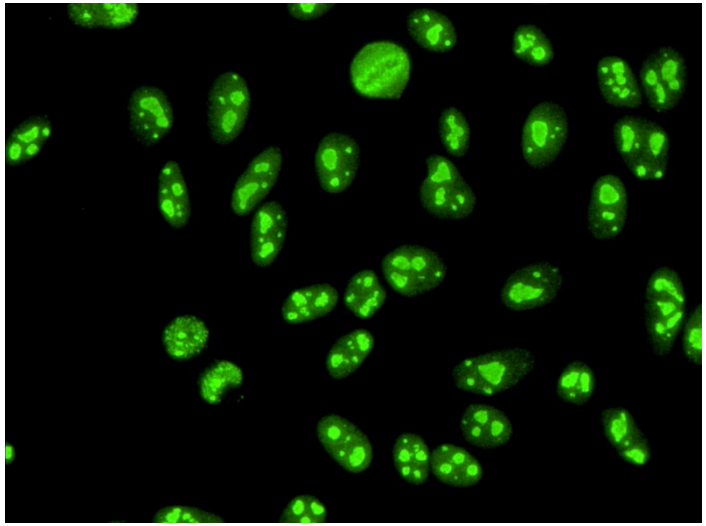
-1993 *(Okano Y et al, Hirakata M et al)* RNAP III and II (not always show an ANoA pattern on IIF)

Other ANoAs: anti-B23 (nucleophosmin), other anti-snoRNPs

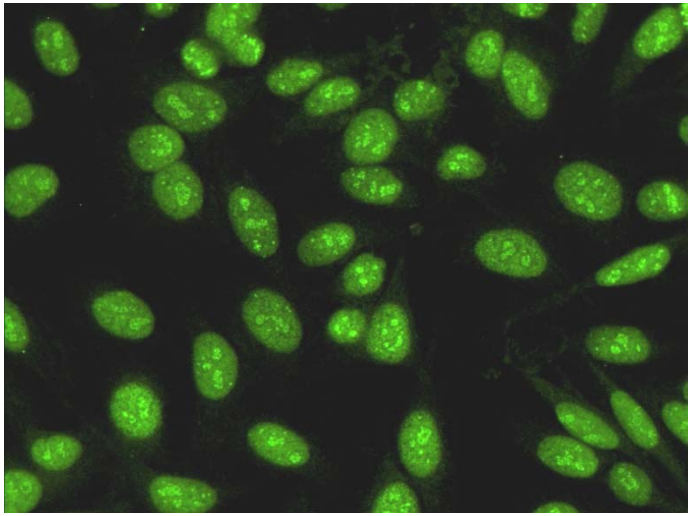
Autoantibody	Prevalence in SSc	Clinical associations	Prognosis
Anti-U3-RNP/AFA	~4% Caucasians 16-22% African descent (HLA-DQB1 alleles)	*dcSSC Myositis, PAH Renal, cardiac invol.?	Younger patients with greater internal organ involvement
Anti-RNAP I/III	4-25% (HLA-DQB1 alleles)	*dcSSc PAH, righ heart failure, Renal crisis	Increased mortality
Anti-PM-Scl	1-6% (HLA-DRB1; DQA1; DQB1 alleles)	*lcSSc Pulmonary fibrosis Digital ulceration *PM/SSc overlap **(~50%)**	Benign/chronic corse with better response to steroid
Anti-Th/To	1-7% (HLA-DRB1 alleles)	*lcSSc Pulmonary fibrosis PAH?	Worse prognosis

KT Ho & JD Reveille. Arthritis Res Ther 2002 (modified)

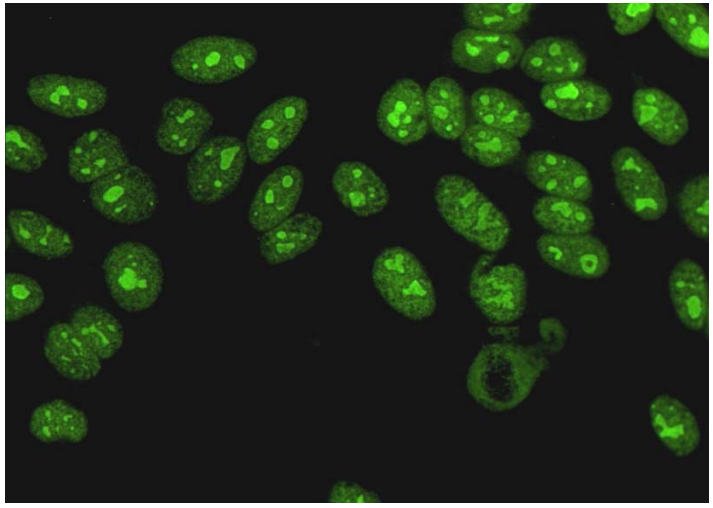
ANoA patterns



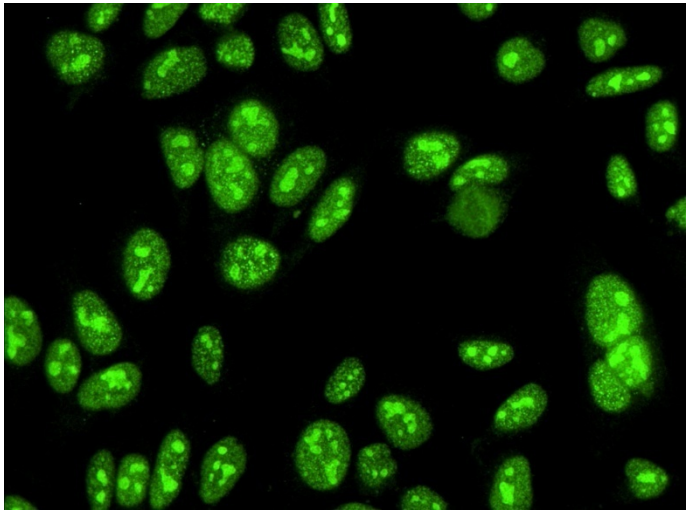
Clumpy (AFA)



Speckled (RNAP III)

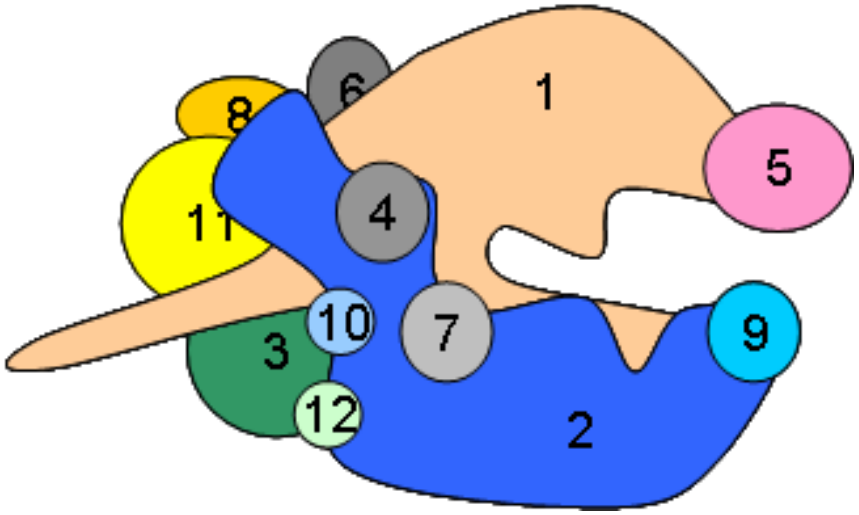


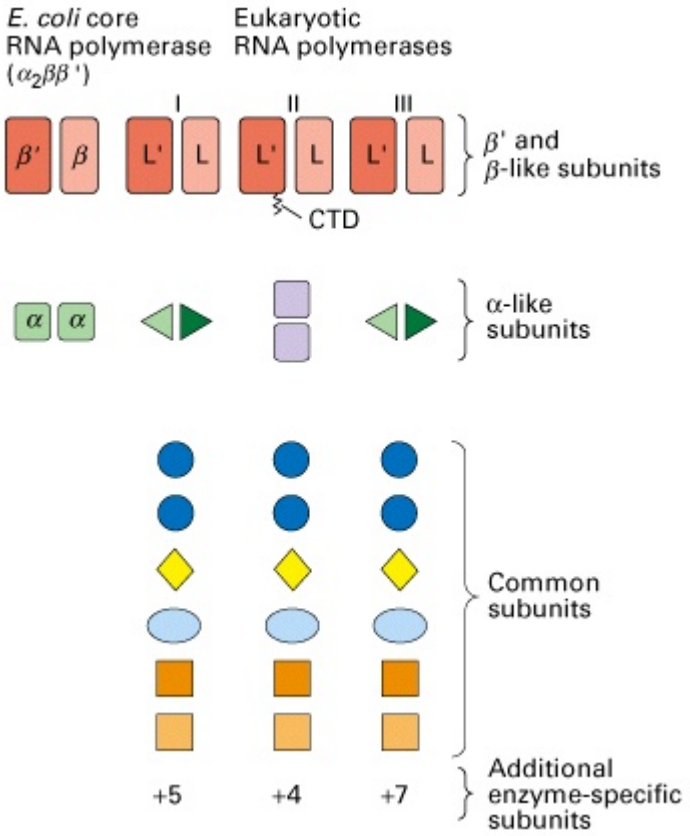
Homogeneous (PM-Scl)



Homogeneous (Th/To)

RNA polymerases





Kuwana M et al. Arthritis Rheum 2002

Immunodominant epitope of RNAP III

(aa residues 891-1020 on RPC 155 the largest subunit of RNAP III)

ARTHRITIS & RHEUMATISM
Vol. 52, No. 8, August 2005, pp 2425–2432
DOI 10.1002/art.21232
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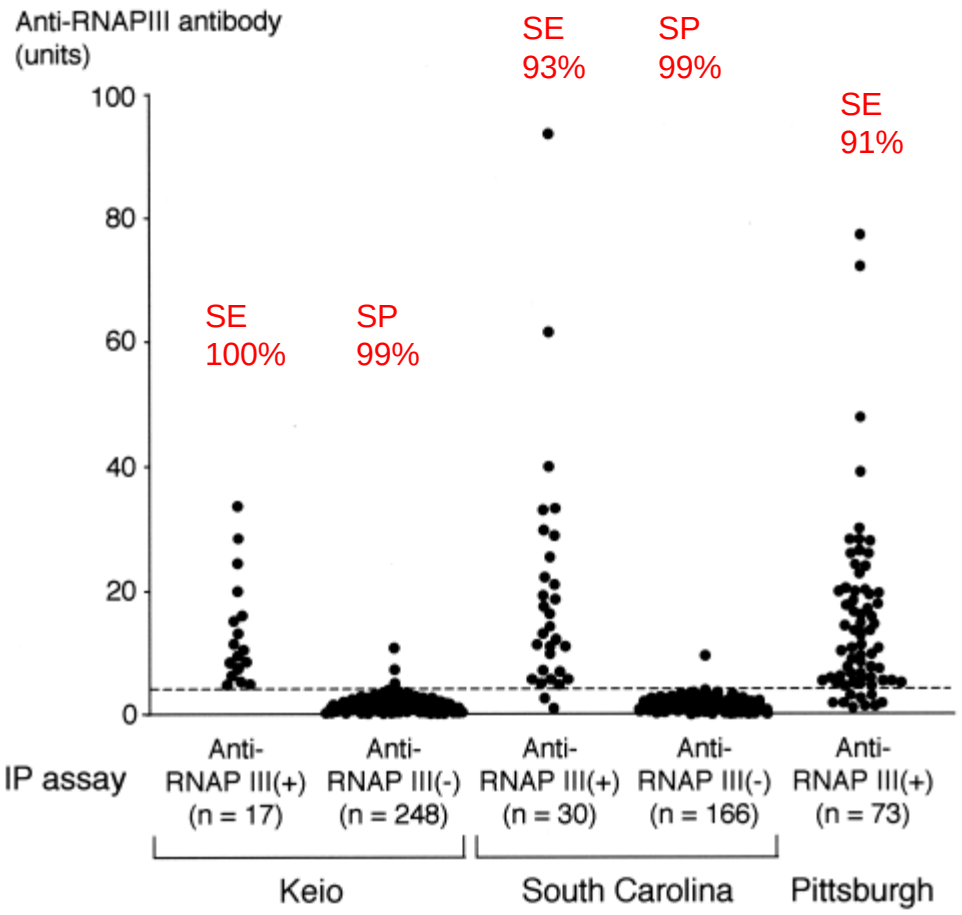
Enzyme-Linked Immunosorbent Assay for Detection of Anti–RNA Polymerase III Antibody

Analytical Accuracy and Clinical Associations in Systemic Sclerosis

Masataka Kuwana,[1] Yutaka Okano,[2] Janardan P. Pandey,[3] Richard M. Silver,[3] Noreen Fertig,[4] and Thomas A. Medsger, Jr.[4]

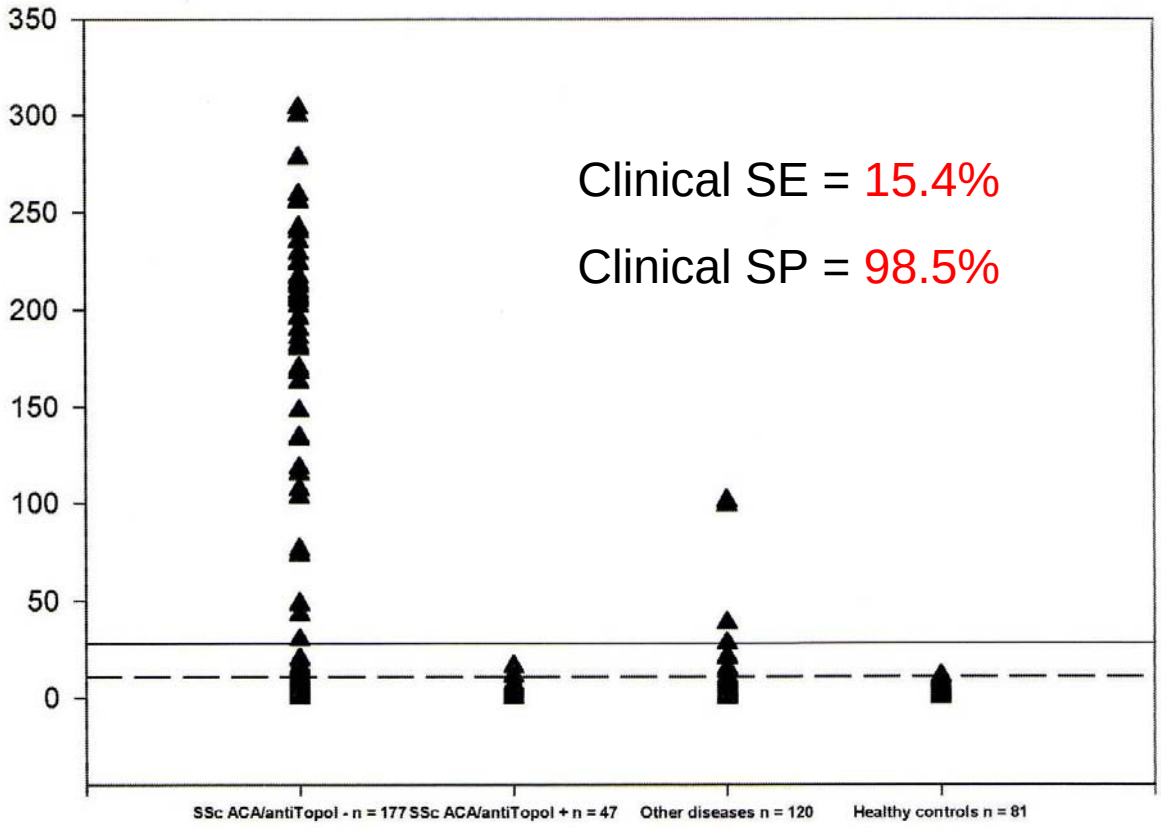


Clinical SE 17%

Clinical SP 98%

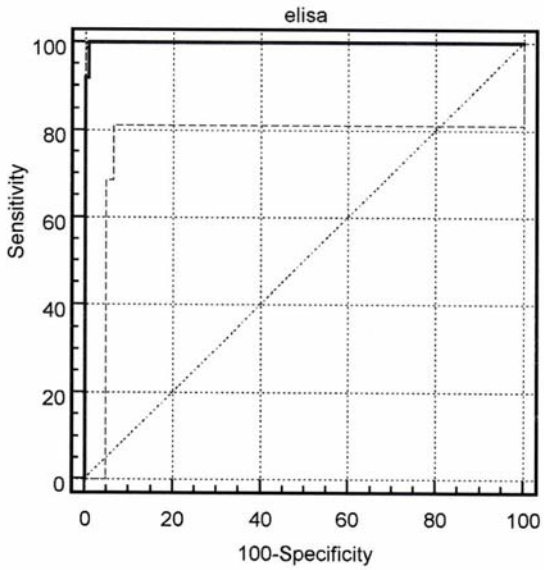
Validation of a new immunoenzymatic method to detect antibodies to RNA polymerase III in systemic sclerosis

V. Codullo, G. Morozzi, A. Bardoni, R. Salvini, G. Deleonardi, O. De Pità, V. Riccieri, A. Ruffatti, A. Tincani, R. Tozzoli, G. Valesini, C. Montecucco; Forum Interdisciplinare per la Ricerca sulle Malattie Autoimmuni (F.I.R.M.A.) study group.



Clinical SE = 15.4%

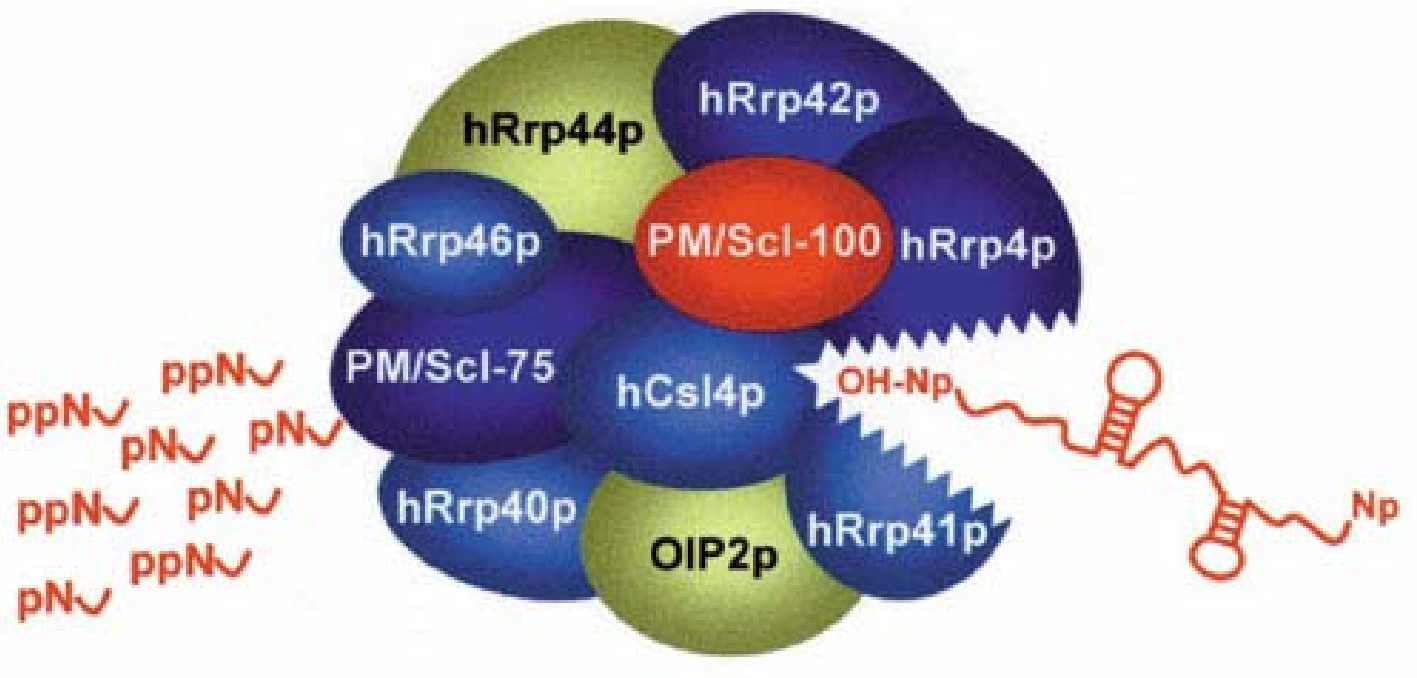
Clinical SP = 98.5%



Clin Exp Rheumatol 2007

PM/Scl

autoantigen: human counterpart of yeast exosome



PM/Scl Ab reactivity:

- **PM/Scl 100**
- **PM/Scl 75**
- **Other proteins = low percentage**

Immunodominant epitope PM/Scl 100 aa 231-245

Synthetic peptide (PM1-alpha)

Brouwer R. et al. Arthritis Res 2000

Contents lists available at ScienceDirect

Autoimmunity Reviews

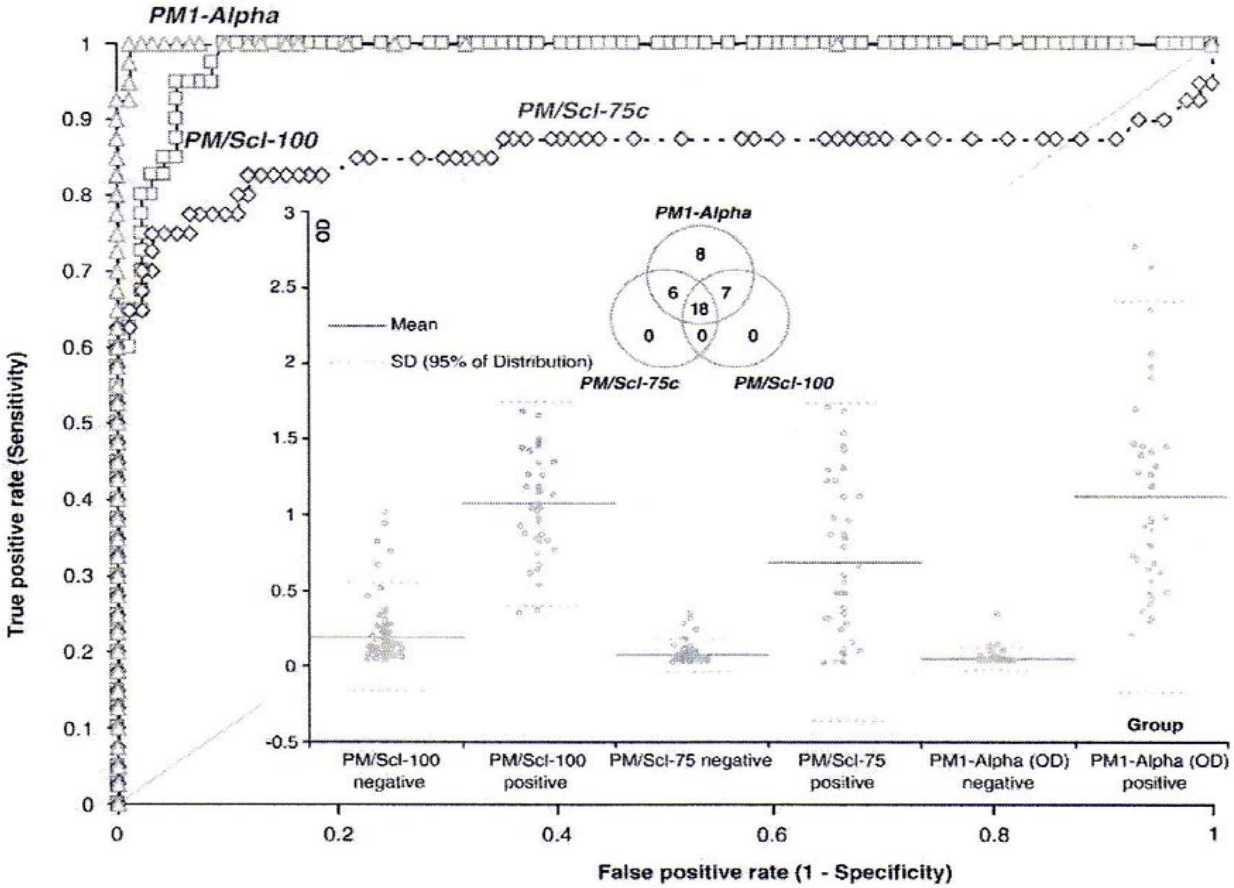
journal homepage: www.elsevier.com/locate/autrev

Review

PM1-Alpha ELISA: The assay of choice for the detection of anti-PM/Scl autoantibodies?

Michael Mahler [a,*], Marvin J. Fritzler [b]

[a] Dr. Fooke Laboratorien GmbH, Neuss, Germany
[b] Faculty of Medicine, University of Calgary, Canada

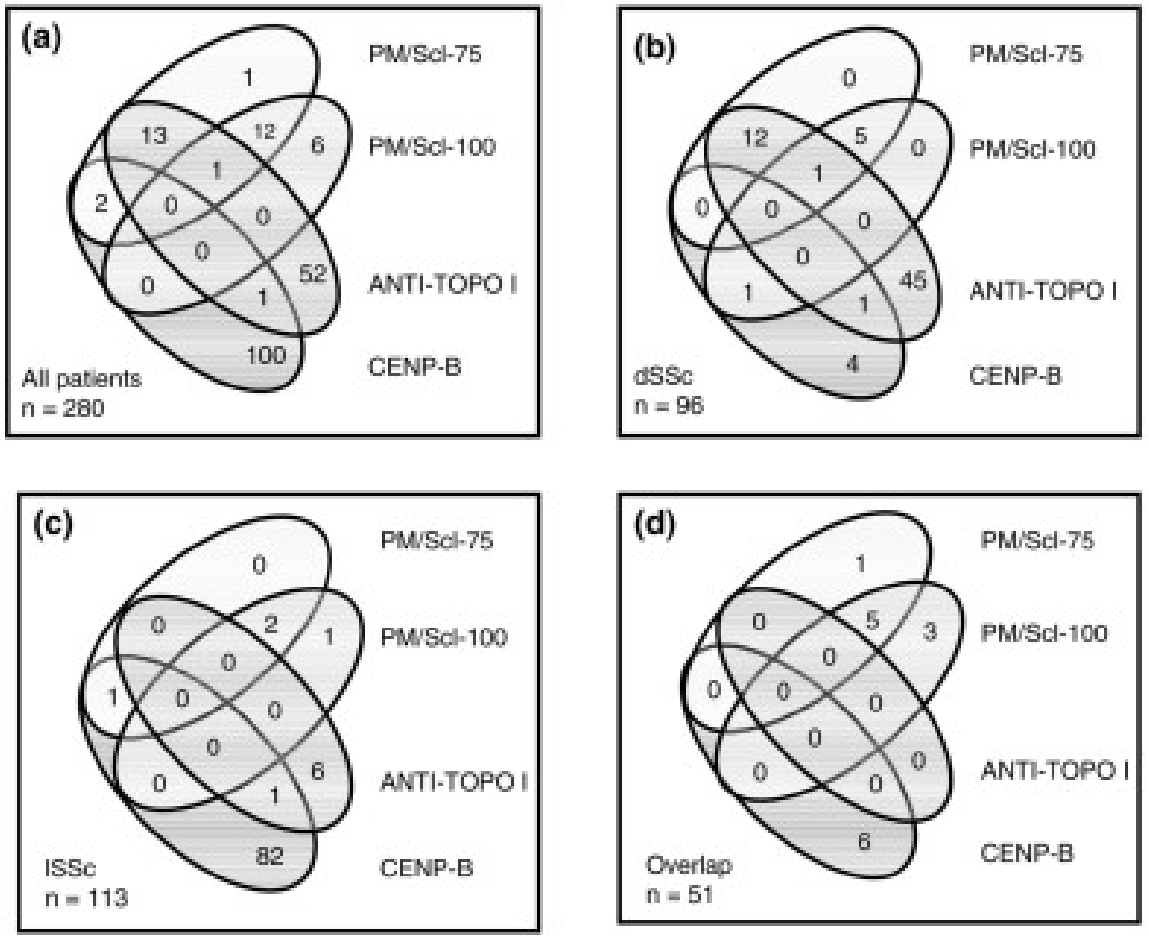


Research article

Arthritis Res Ther 2009

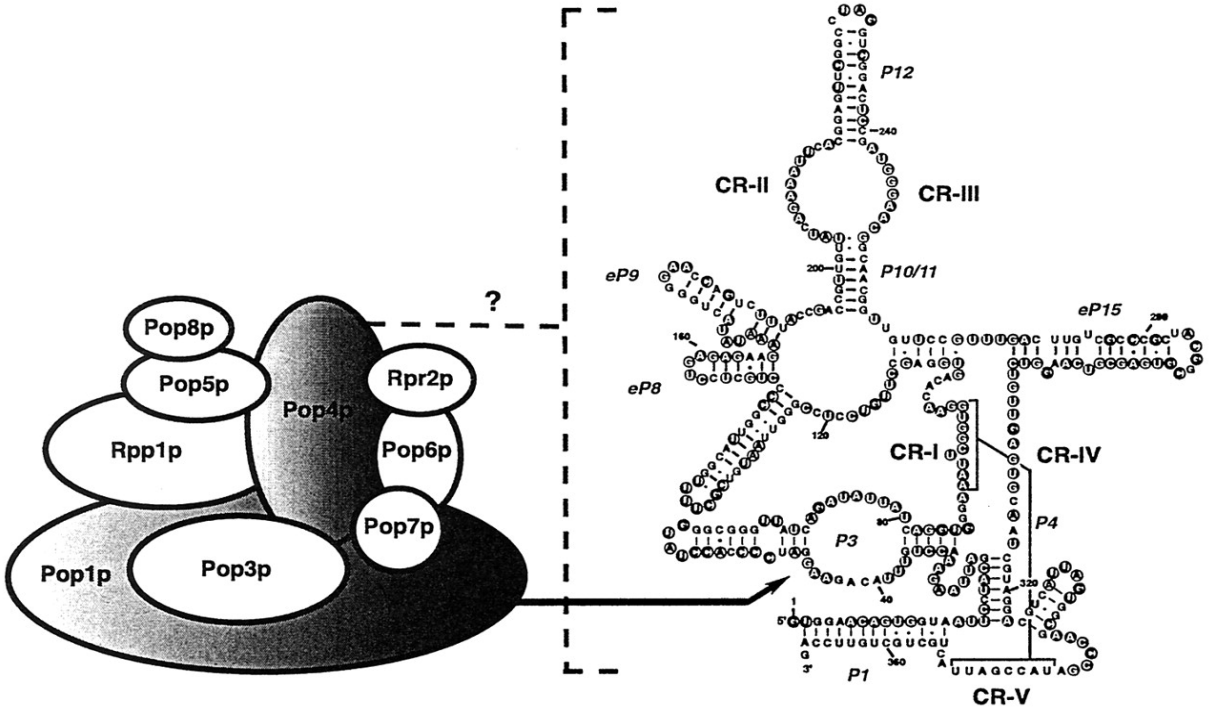
Antibodies against PM/Scl-75 and PM/Scl-100 are independent markers for different subsets of systemic sclerosis patients

Katharina Hanke[1], Claudia S Brückner[1], Cornelia Dähnrich[2], Dörte Huscher[3], Lars Komorowski[2], Wolfgang Meyer[2], Anthonia Janssen[2], Marina Backhaus[1], Mike Becker[1], Angela Kill[1], Karl Egerer[1], Gerd R Burmester[1], Falk Hiepe[1], Wolfgang Schlumberger[2] and Gabriela Riemekasten[1]



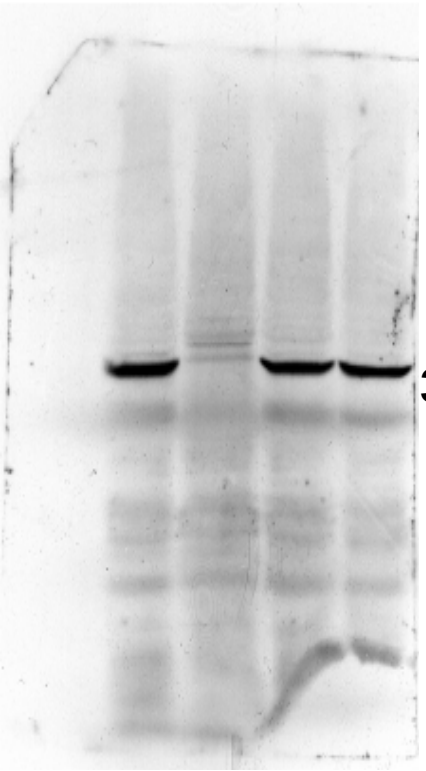
Th/To

Ribonuclease MRP and ribonuclease P complexes



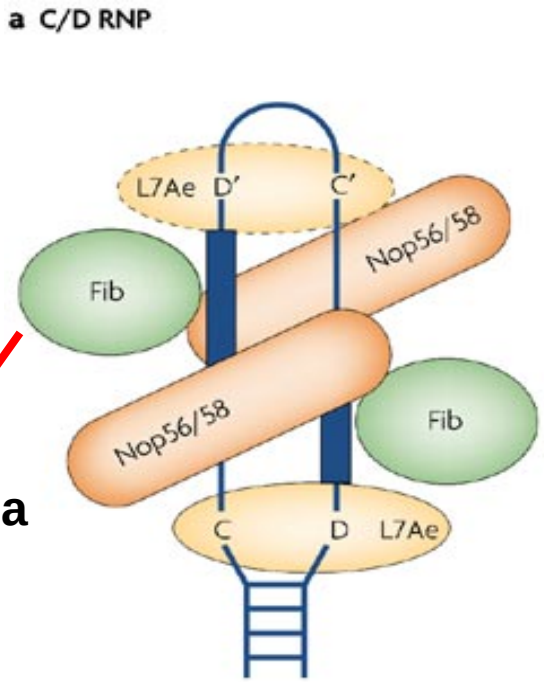
Houser-Scott F. et.al. PNAS 2002;99:2684-2689

snoRNP macromolecular complexes

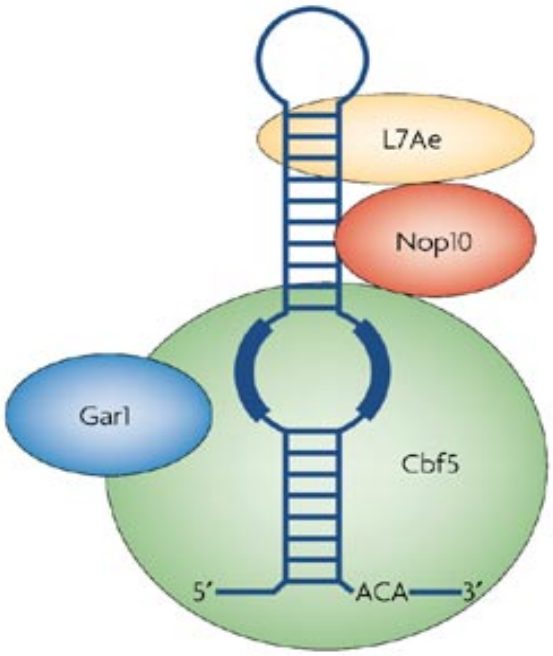


34 kDa

U3snoRNP/fibrillarin



Box H/ACA snoRNP



C/D RNPs

Archaea	Human
Fibrillarin	Fibrillarin
L7Ae	15.5K/NHPX
Nop56/58	NOP56
	NOP58

H/ACA RNPs

Archaea	Human
Cbf5	Dyskerin
L7Ae	NHP2
Gar1	GAR1
Nop10	NOP10

Nature Reviews | Molecular Cell Biology

PHADIA ELISA

Autoantibody	Autoantigen
anti-fibrillarin (AFA)	Full-lenght recombinant human protein (Baculovirus)
anti-RNAP III	200 aa-long fragment of RPC subunit of RNA pol III (Baculovirus)
anti-PM-Scl 100	100 kDa full-lenght recombinant human protein (E. coli)

Patients

50 SSc Topo I and ACA negative [37/50 (74%) ANoA positive]

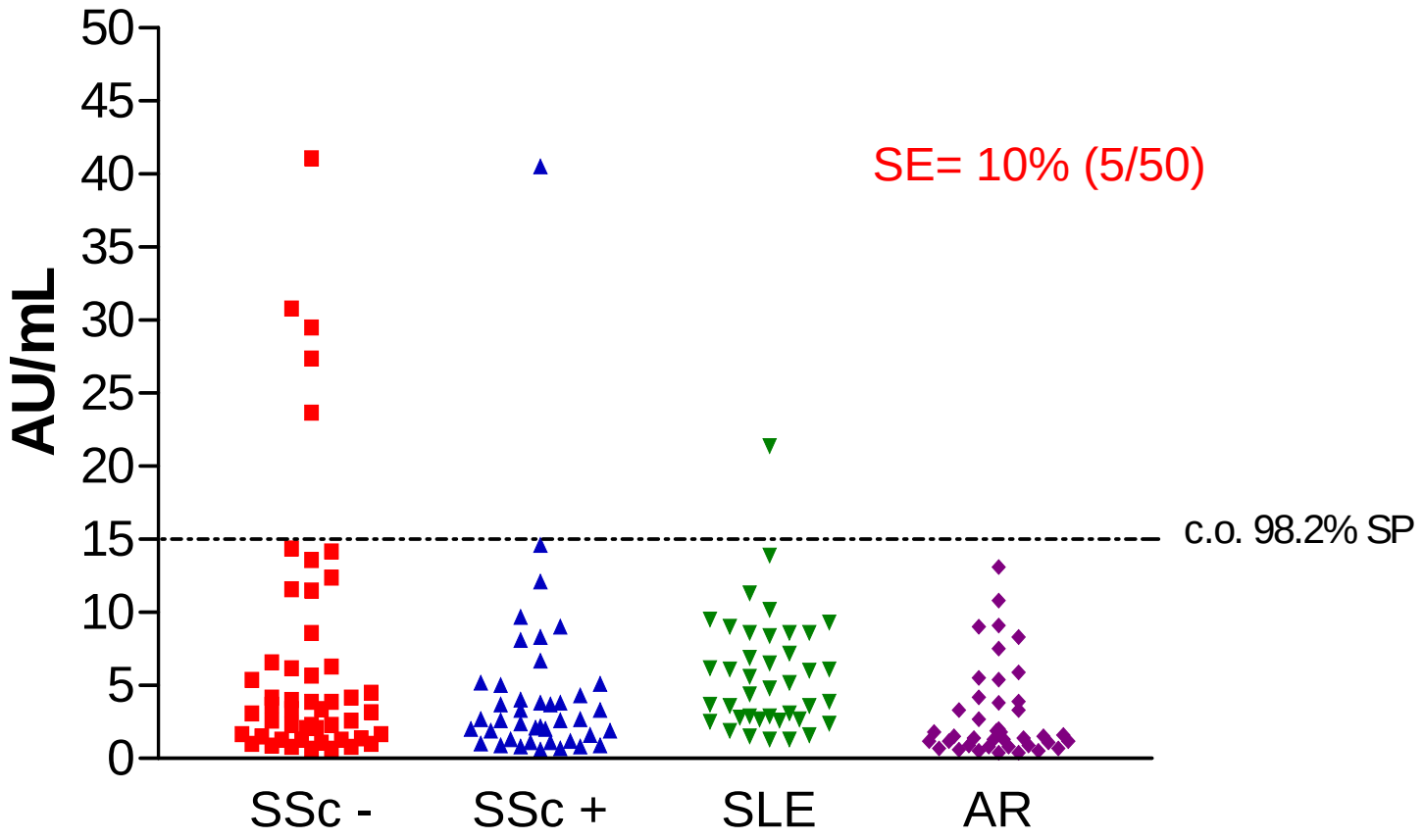
Controls

42 SSc (32 ACA Pos; 10 Topo I pos)

40 Systemic lupus erythematosus (SLE)

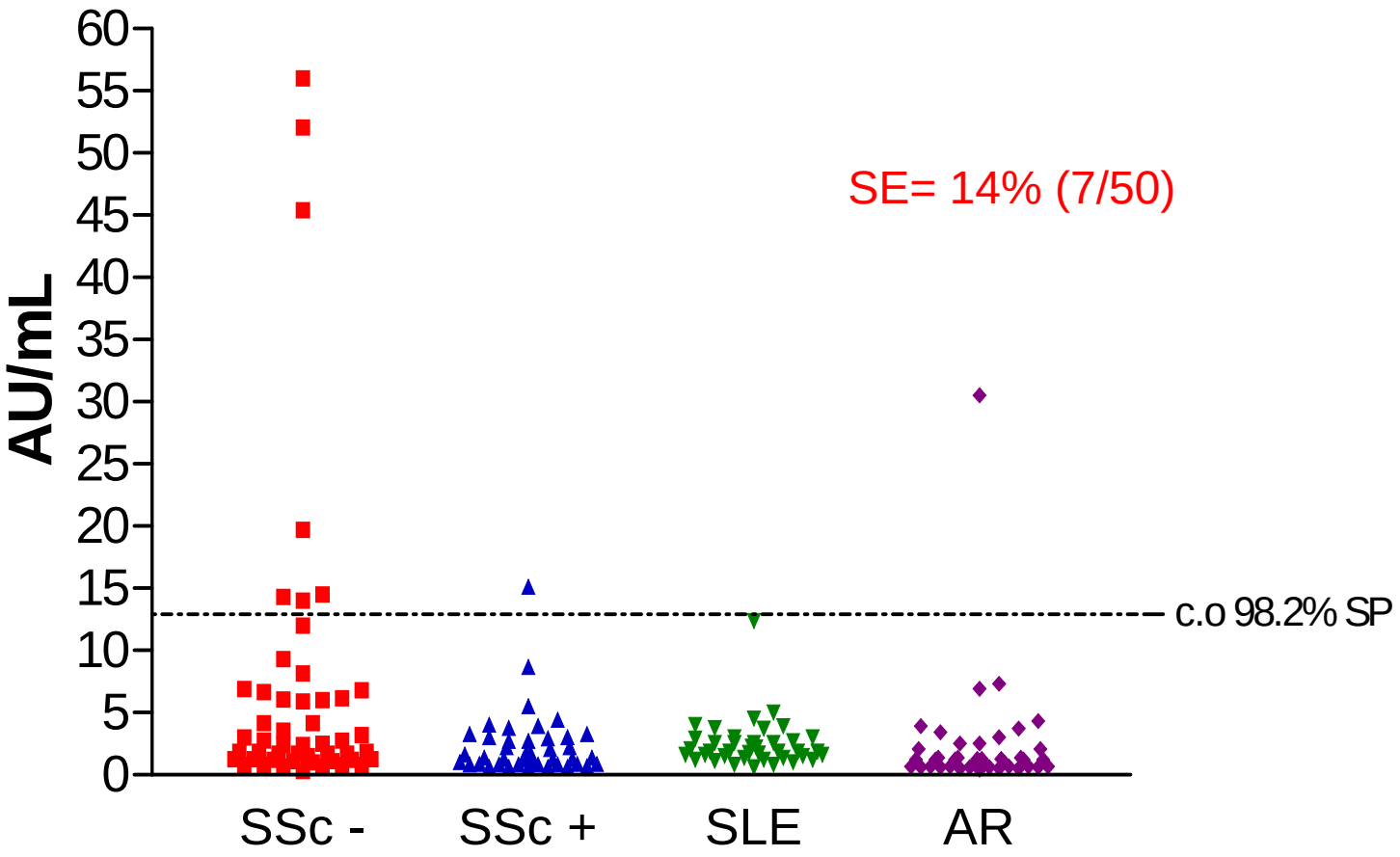
40 Rheumatoid arthritis AR)

anti-fibrillarin

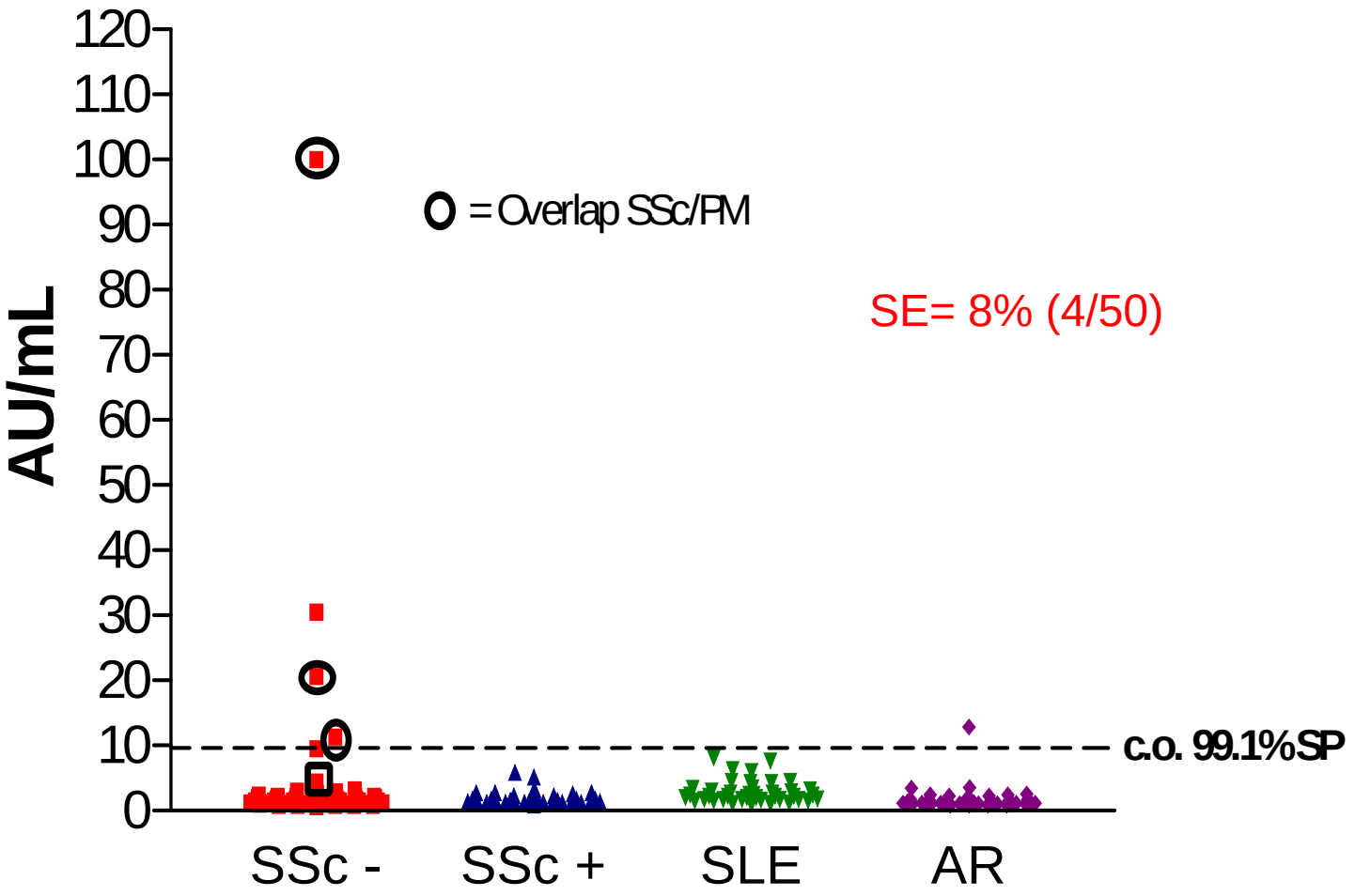


Villalta D et al. Clin Chim Acta 2010;441:710-13

anti-RNA polymerase III



anti-PM-Scl

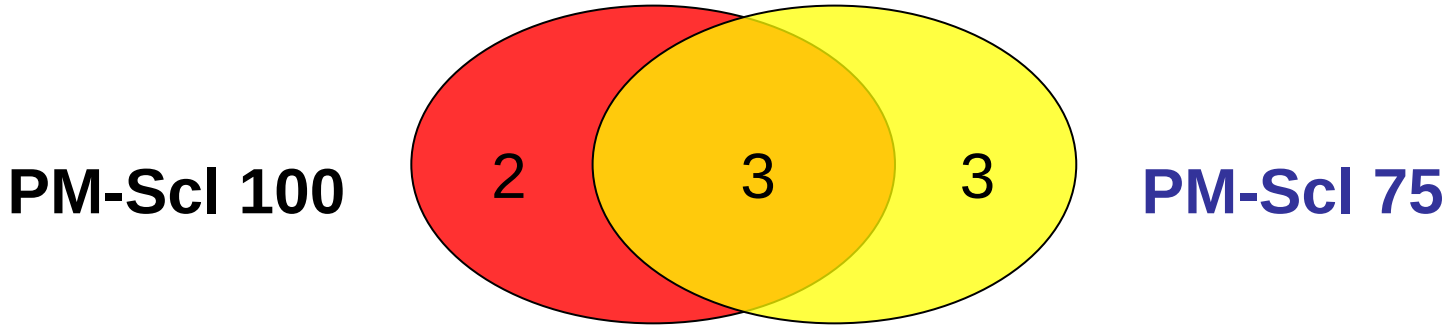

120
110
100
90
80
70
60
50
40
30
20
10
0
AU/mL
O = Overlap SSc/PM
SE= 8% (4/50)
c.o. 99.1%SP
SSc -
SSc +
SLE
AR

Clinical characteristics and fluorescence pattern on HEp-2 cells of sera positive for AFA, RNAP III and PM-Scl

Antibody	No. positive sera	IIF pattern	Organ involvement/clinical features						
			Skin	Lung	PAH	Heart	Kidney	GI	Myositis
AFA	5	5 Nucleolar	4 dcSSc 1 lcSSc	4	1	-	-	3	-
anti-RNAP III	7	4 Speckled 1 Nucleolar 2 Nucleolar + Speckled	6 dcSSc 1 lcSSc	4	-	2	-	4	-
anti-PM-Scl	4	3 Nucleolar 1 Nucleolar + Speckled	3 PM/Scl 1 dcSSc	2	-	-	-	1	3

	ELISA (Phadia)	LIA Multiparametric (Euroimmun)
RNAP III	7/50 (r. Baculo)	8/50 (r E.coli)
PM-Scl (100)	4/50 (r. E.coli)	5/50 (r. Baculo)
AFA	5/50 (r.Baculo)	1/50 (r.E.coli)

	LIA (overall)	LIA (ANoA +)
RNAP III	**8/50 (16%)**	**5/37**
PM-Scl (100)	**5/50 (10%)**	**5/37 (3 PM-Scl 75 +)**
AFA	**1/50 (2%)**	**1/37**
Th/To	**2/50 (4%)**	**2/37**
NOR-90	**3/50 (6%)**	**3/37**
PM-Scl (75)	**7/50 (3 PM-Scl 100 +) (14%)**	**6/37 (3 PM-Scl 100 +)**
Ku	**4/50 (8%)**	**0/37**
PDGFR	**1/50 (2%)**	**0/37**
Total SSc -	**28/50 (56%)**	**19/37 (51.3%)**



Conclusions

- The detection of ANoAs SSc-associated is useful for the diagnosis and disease subtyping of SSc
- The recent introduction of ELISA ad LIA assays for ANoA specificity detection allows their detection in routine clinical practice
- With the increasing number of diagnostic assays and platforms to test ANoA specificities, more diligent attention needs to be given to standardizing the autoantigens used in the assays and the various platforms (ELISA, LIA, etc.) in which they are employed
- Highly characterized autoantigens or synthetic peptides autoantigens seem to be associated with high performances immunoassays and may facilitate the standardization process
- The availability of international reference sera could be an important step towards standardization (IUIS/WHO/AF/CDC ANA#11 for PM-Scl 100)