ROLE OF AUTOANTIBODIES IN SYSTEMIC SCLEROSIS: RELEVANCE IN CLINICAL PRACTICE

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Disclosures: None

Pittsburgh Scleroderma Center

- 4000+ patients enrolled 1972-present; 5+ visits per patient; 20,000+ patient years of follow-up
- All clinical and laboratory data in MEDLOG, a time-oriented database
- Serum samples (10,000+), DNA samples (1,000+), autopsies (150+)

How do SSc-associated autoantibodies help managing physicians and both clinical and laboratory investigators?

- aid in diagnosis because relatively specific for SSc
- contribute to patient subsetting
- assist in predicting the natural history of disease (skin and other organ system involvement) and survival
- inform the design of future clinical and laboratory studies and clinical trials

CLINICAL CLASSIFICATION OF SYSTEMIC SCLEROSIS (SSc)

a) diffuse cutaneous (dc) SSc (35%): widespread and rapidly progressive skin thickening (proximal to elbows, knees) at any time during the illness; early internal organ involvement (lung, heart, kidney)

b) limited cutaneous (lc) SSc (55%): restricted and non-progressive skin thickening (distal extremities only); late internal organ involvement (pulmonary arterial hypertension)

c) overlap (10%): dc or lc skin thickening with features of another connective tissue disease, e.g. PM/DM, SLE, RA

MODIFIED RODNAN SKIN SCORE

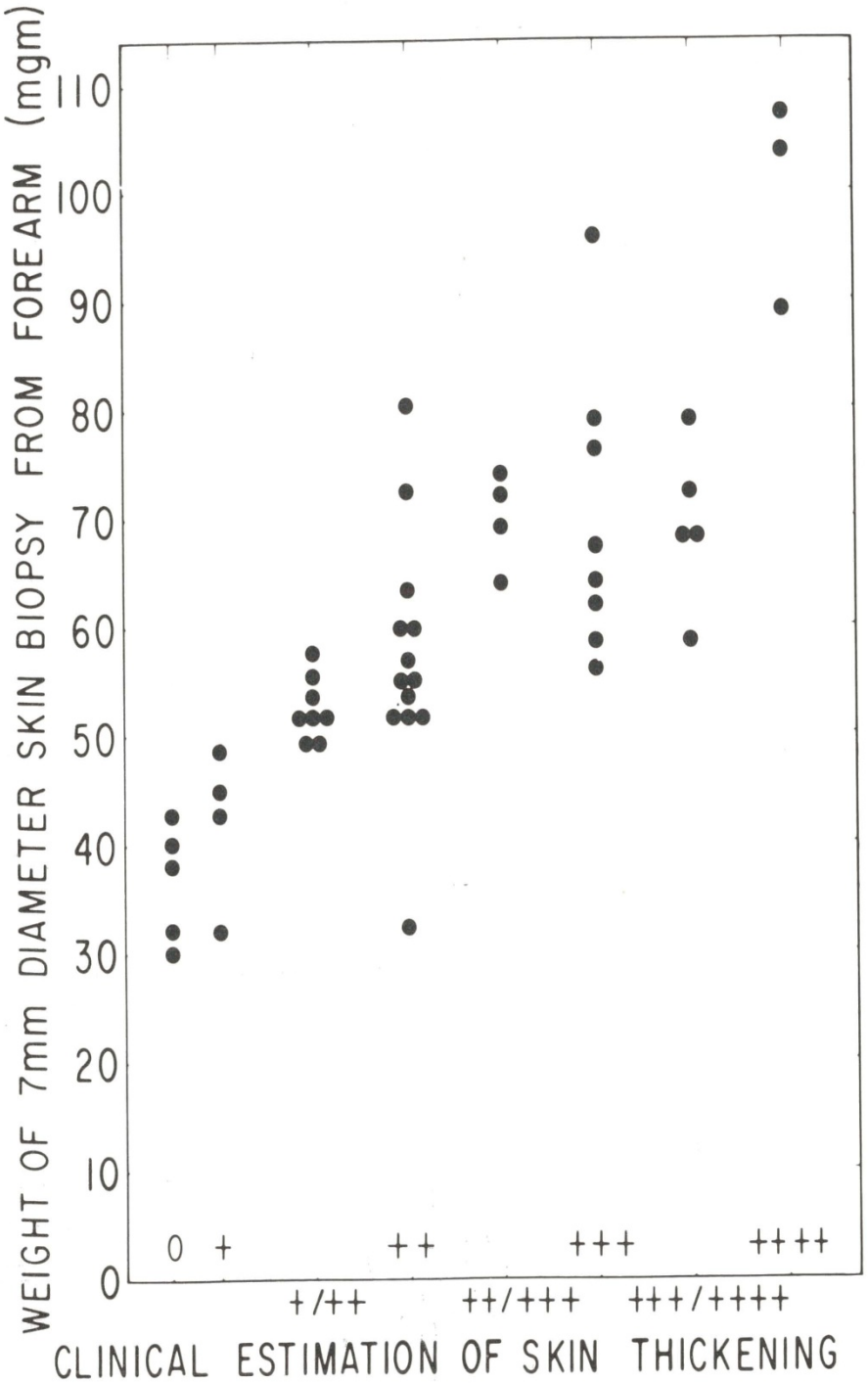
	RIGHT	LEFT
FINGERS	0 1 2 **3**	0 1 2 **3**
HANDS	0 1 **2** 3	0 1 **2** 3
FOREARMS	0 1 **2** 3	0 **1** 2 3
UPPER ARMS	0 **1** 2 3	0 **1** 2 3

FACE	0 **1** 2 3
CHEST	0 1 **2** 3
ABDOMEN	0 **1** 2 3

THIGHS	0 **1** 2 3	0 **1** 2 3
LEGS	**0** 1 2 3	**0** 1 2 3
FEET	0 **1** 2 3	**0** 1 2 3

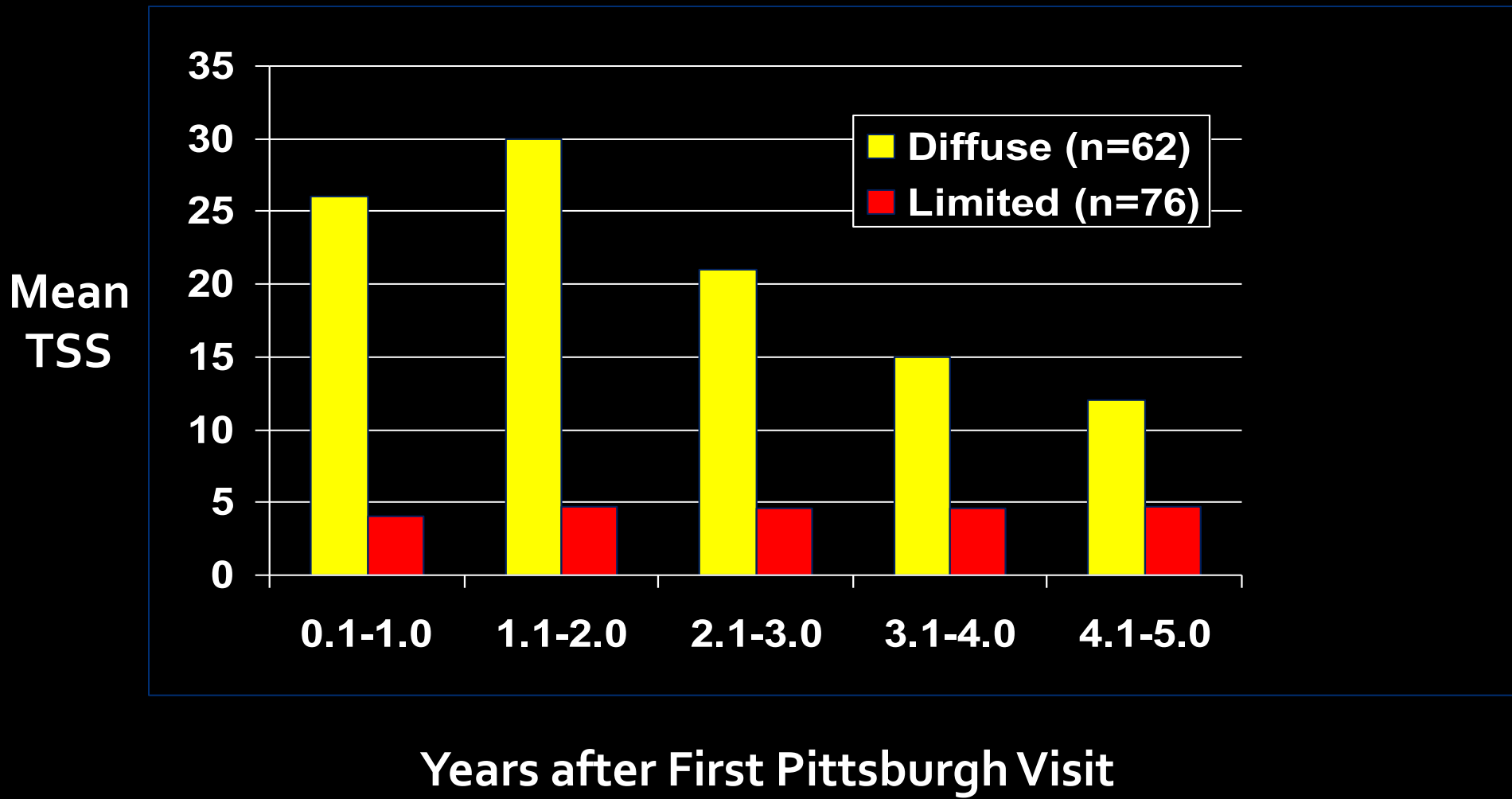
Total Skin Score (TSS) 22

Clements et al. J Rheumatol 1995; 22:1281-5

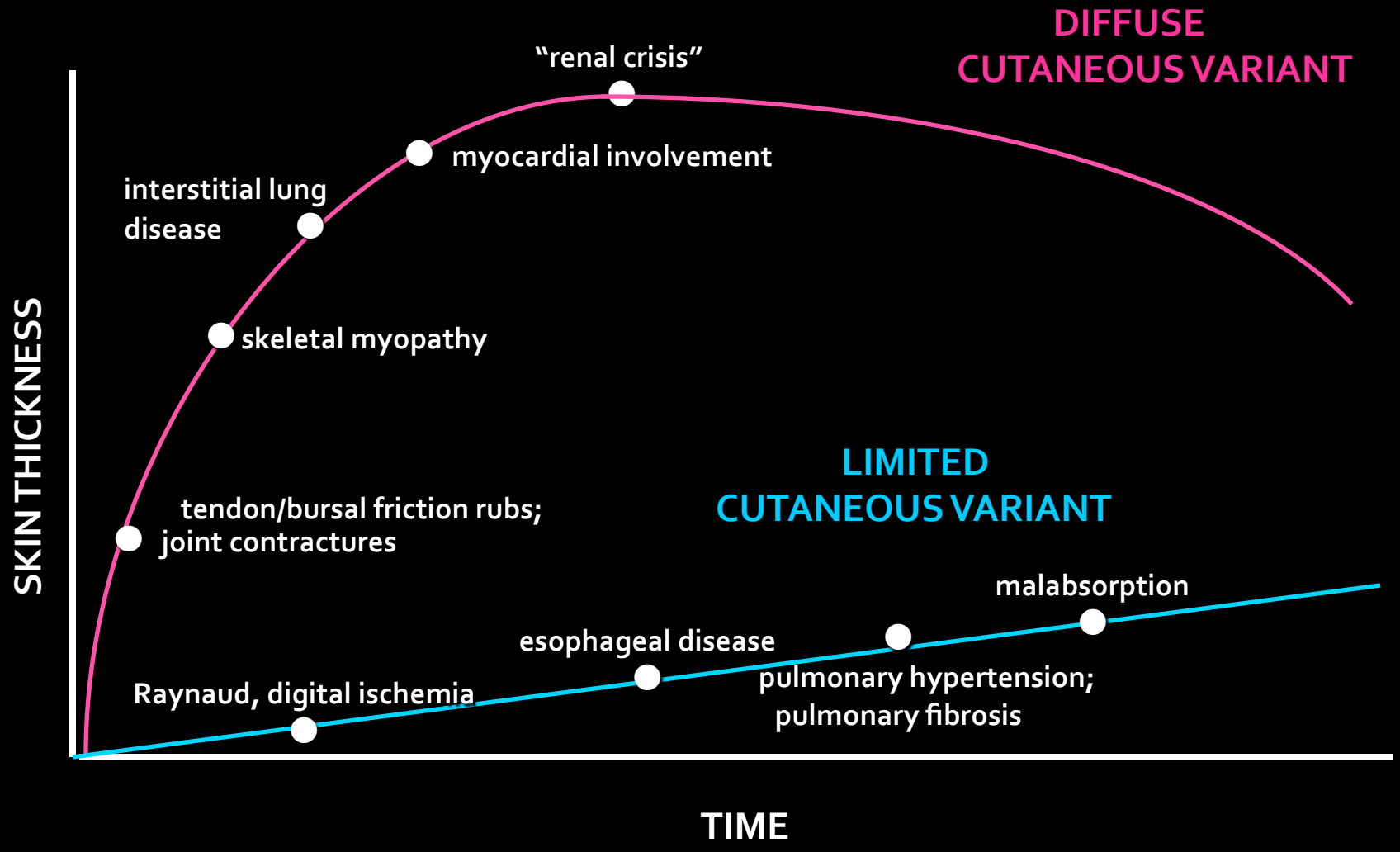


Rodnan et al.
Arthritis Rheum 1979; 22:130-40.

TOTAL SKIN SCORE (TSS) DURING FOLLOWUP



USUAL TIMING OF PROBLEMS IN PATIENTS WITH SYSTEMIC SCLEROSIS



SSc-ASSOCIATED AUTOANTIBODY DETECTION METHODS

1. anti-centromere: indirect immunofluorescence
2. anti-topoisomerase I, -U1RNP, -PM-Scl: double immunodiffusion
3. anti-Th/To, -RNA polymerase III, -U3RNP, -Ku, -U11/U12RNP: immunoprecipitation

MULTIPLEX BEAD METHODS FOR DETECTING SSc AUTOANTIBODIES

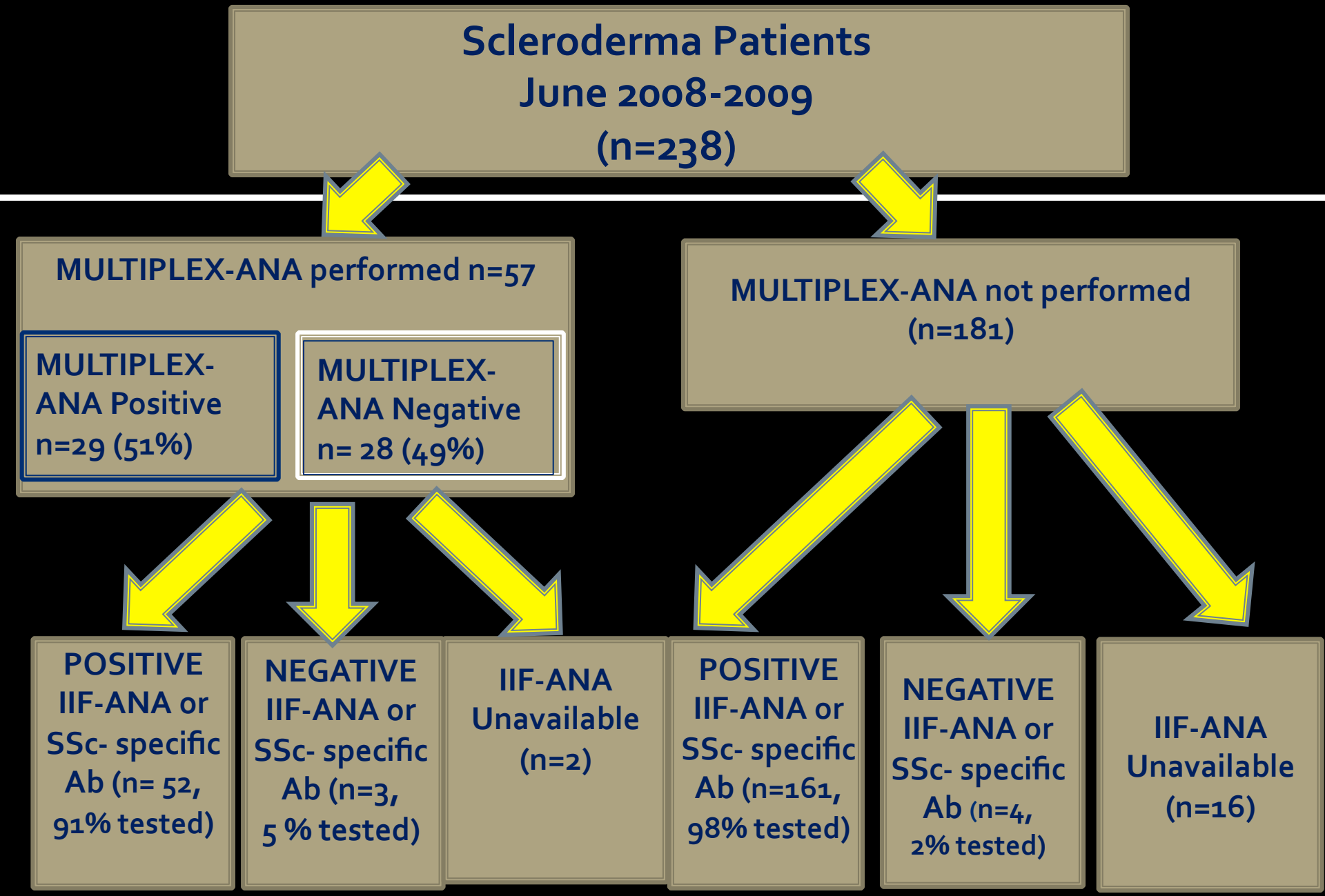
1. ANA testing by indirect immunofluorescence (IIF) may detect antibodies to 100-150 distinct nuclear antigens.

2. ANA testing by mutiplex bead methods depends on
   a. the number of antigens coated onto beads.
   b. the ability of the antigens to react with serum antibodies.

3. Retrospective study of 238 SSc patients comparing IIF ANA and multiplex bead ANA results by 2 commercial laboratories

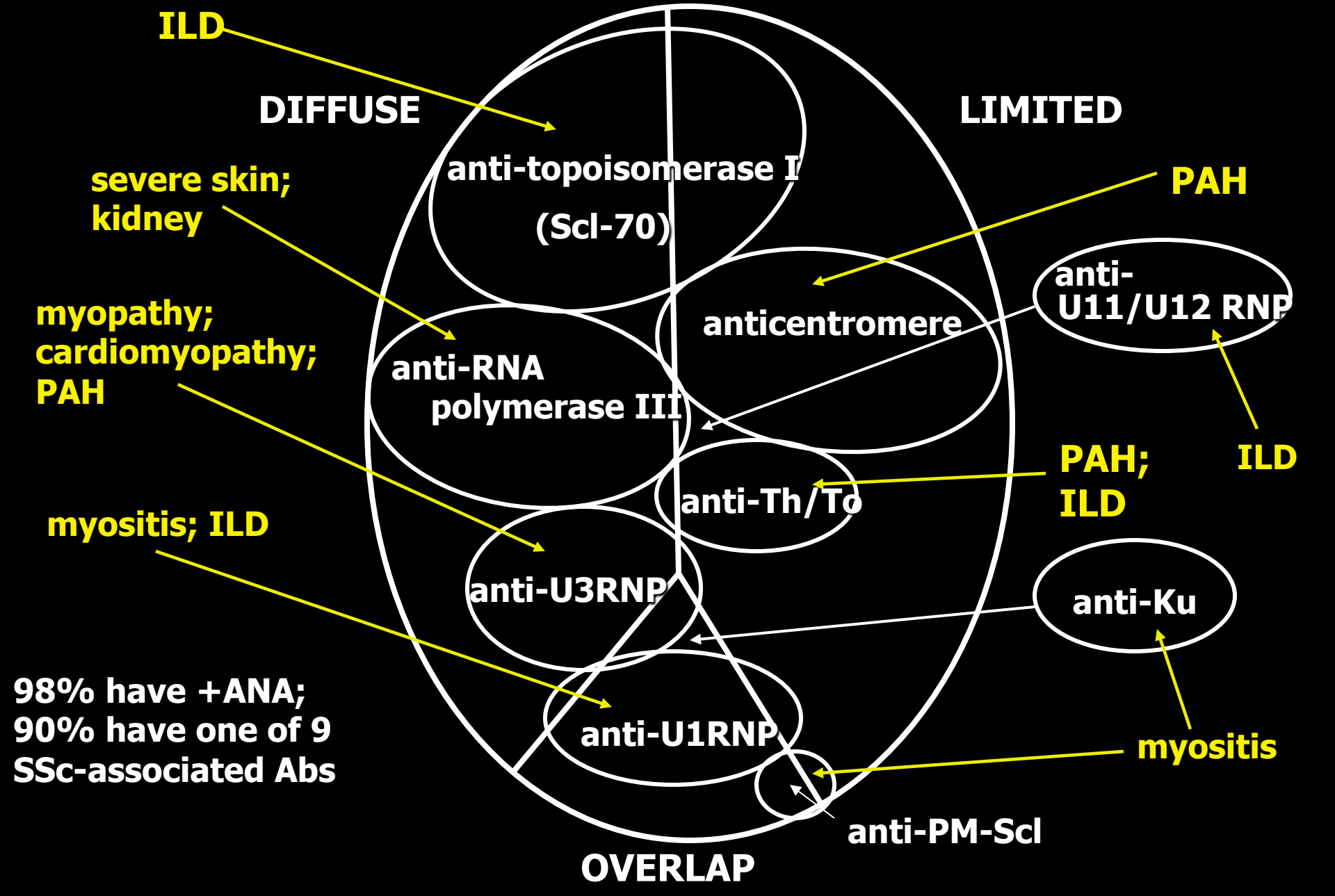
Conclusion: Multiplex bead ANA testing fails to identify 50% of SSc patients, particularly those with anti-RNA polymerase III and antibodies with nucleolar IIF ANA staining.

Shanmugam et al., Clin Rheumatol 2011; 30:1363-8.



Shanmugam et al., Clin Rheumatol 2011; 30:1365.

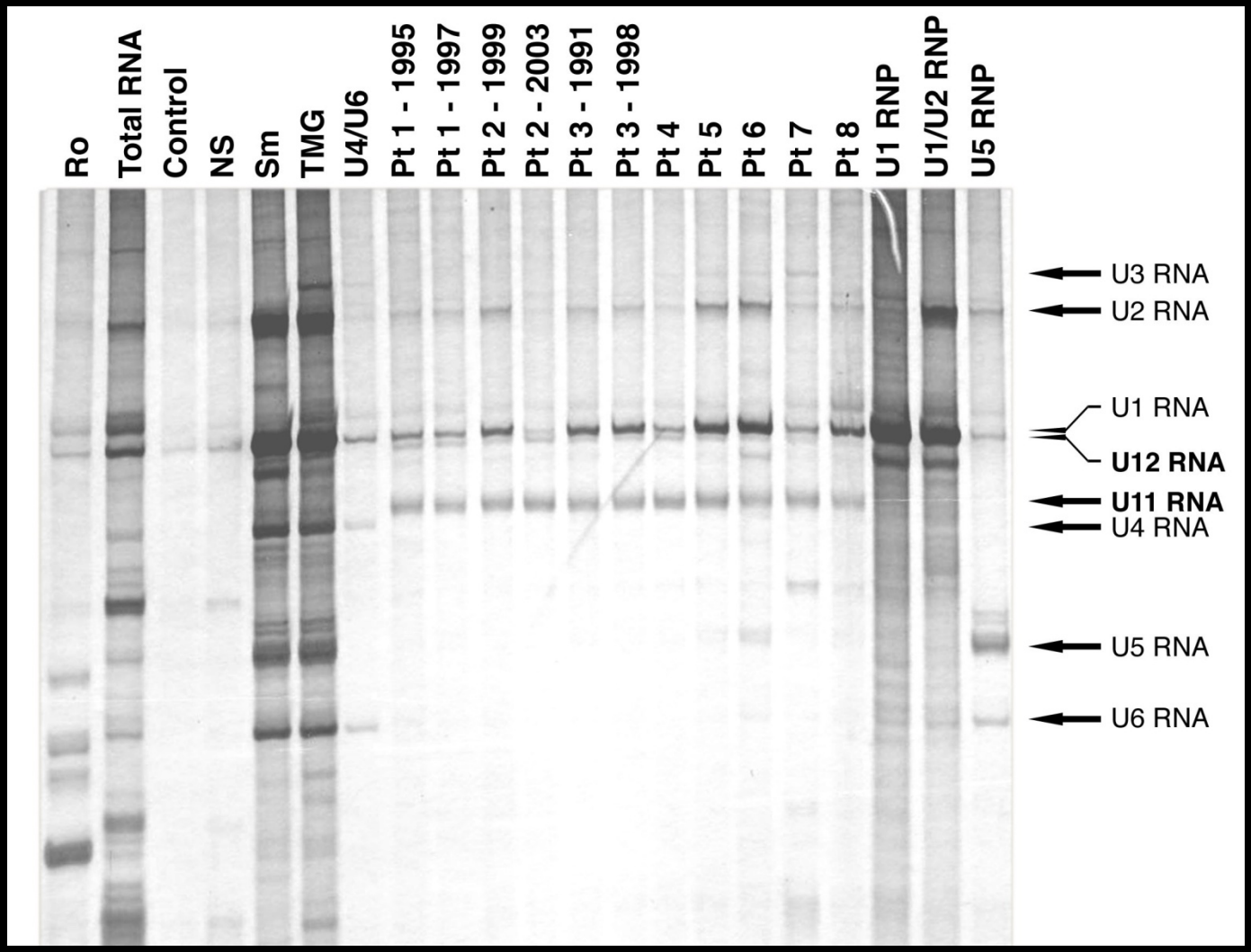
CLINICAL-SEROLOGIC CLASSIFICATION OF SYSTEMIC SCLEROSIS



Anti-U11/U12RNP Antibody

- directed against components of the minor spliceosomal complex
- specificity confirmed by immunoprecipitation, RT-PCR and southern blotting of snRNAs
- 33 SSc patients
- frequency: 3% of new SSc patients over 2 years
- specificity: not found in other CTDs
- dc = lc
- 23/33 (70%) ILD; often severe, rapidly progressive

Fertig et al., Arthritis Rheum 2009; 61: 958-65.

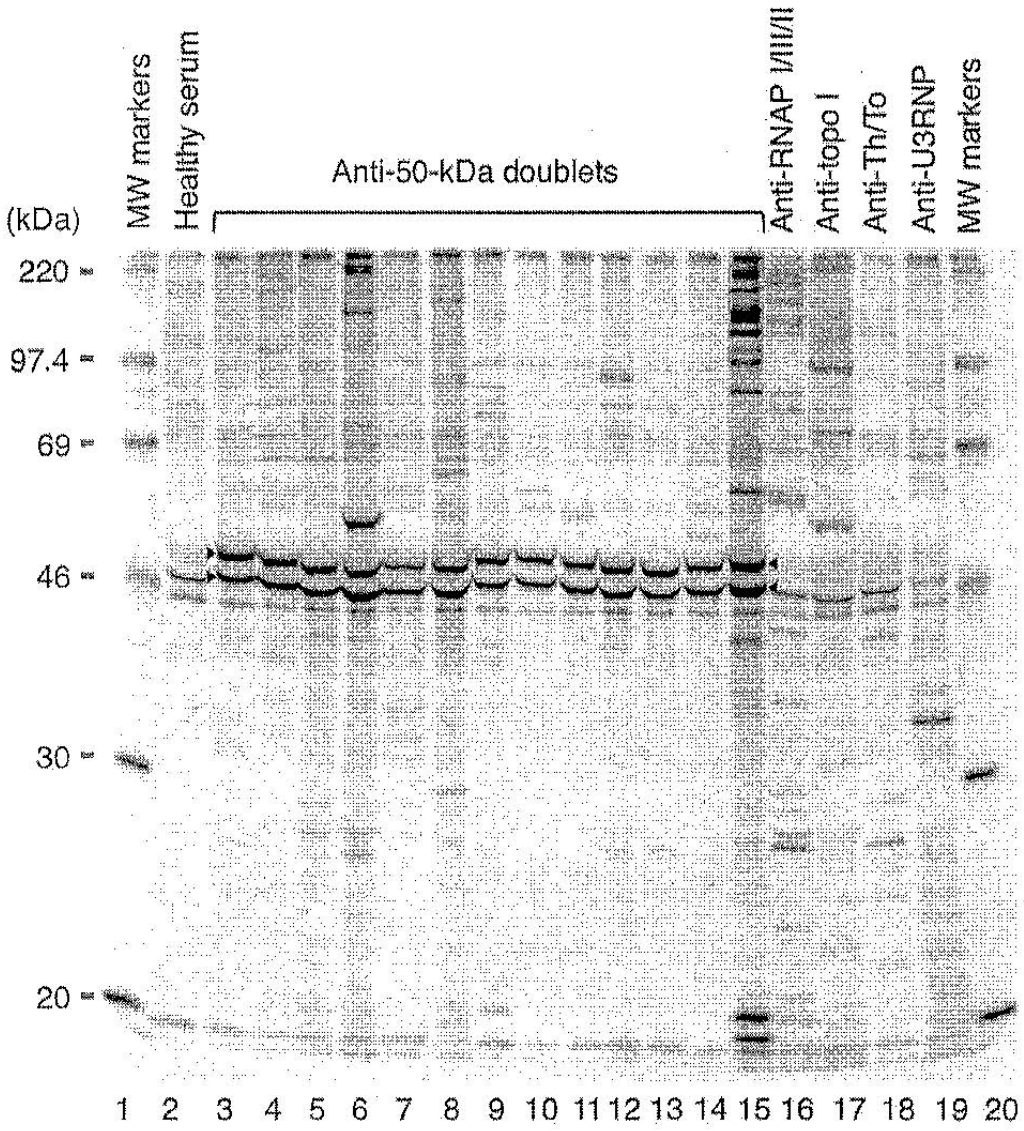


Fertig et al., Arthritis Rheum 2009; 61:962.

Anti-RuvBL1,2 Antibody in SSc

- Directed against antigen couplet including the nuclear proteins RuvBL1 (pontin) and RuvBL2 (reptin); both proteins involved in many cellular processes
- Doublet with MW ≈ 50kDa
- Antibodies detected by protein immunoprecipitation
- 10 Japanese and 27 Pittsburgh patients positive (n=37)
- Relative frequency ≈ 1%
- Specific for SSc (not found in other CTD patients)
- Associations: male sex (41%); dcSSc (68%); myositis overlap (57%), PM>DM

Kaji et al., Arthritis Care & Research 2014; 66: 575-584


(kDa)
MW markers
Healthy serum
Anti-50-kDa doublets
Anti-RNAP I/II/III
Anti-topo I
Anti-Th/To
Anti-U3RNP
MW markers
220
97.4
69
46
30
20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

REFERENCES FOR CLINICAL, LABORATORY AND DISEASE COURSE ASSOCIATIONS OF SSc-ASSOCIATED SERUM ANTIBODIES

1. centromere: Steen et al., Arthritis Rheum 1984: 27:125-131.
2. topo I: Perera et al., Arthritis Rheum 2007; 56: 2740-2746.; Steen et. al. Arthritis Rheum 1988; 31: 196-203.
3. RNA polymerase II: Kuwana et al., Arthritis Rheum 2005; 52:2425-2432.
4. U1RNP: Williams et al., Arthritis Rheum 2005; 52:S590
5. PM-Scl: Koschik et al., Clin Exp Rheumatol 2012; 30(Suppl 71): S12-6.
6. Ku:Williams et al., Arthritis Rheum 2005; 52:S590
7. Th/To: Mitri et al., Arthritis Rheum 2003; 48: 203-209.
8. U3RNP: Aggarwal et al., Arthritis Rheum 2009; 60:1112-8
9. U11/U12 KNP: Fertig et al., Arthritis Rheum 2009; 61:958-65.
10. RuvBL1,2: Kaji et al., Arthritis Care Res 2014 66: 575-584.

AVAILABILITY OF CLIA-CERTIFIED TESTING FOR 10 SSC-ASSOCIATED SERUM AUTOANITBODIES

- centromere: on routine ANA by IIF (reliable); also by ELISA
- topo I: by ELISA (beware that many low level positives are "false positives")
- RNA polymerase III: by ELISA (good assay)
- U1RNP: by ELISA (good assay but some low level positives probably "false positives")
- PM-Scl: by immunodiffusion or ELISA (good assays)
- Ku: Th/To; U3RNP: immunoprecipation (IP) from RDL, possibly other companies; results sometimes different than Pittsburgh IP results; attempting to get Targoff lab (CLIA-certified, Oklahoma Medical Research Foundation) to offer these as part of a "Scleroderma Panel"
- U11/u12 RNP; RuvBL1,2: not commercially available yet

DRw11 ALLELE FREQUENCIES

HLA Type	Anti-Topo I (n=26)	Population Controls (n=67)	p-value	RR
DRB1-1101	3 (12%)	7 (10%)	NS	1.1
DRB1-1102	0	1 (<1%)	NS	
DRB1-1103	2 (8%)	0	NS	
DRB1-1104	17 (65%)	5 (8%)	0.001	23.4

RR = relative risk

Morel et al., Hum Immunol 1994; 40:101-10.

Antibody Profiles in Different SSc Populations

Antibody	Japanese	North American Caucasian	North American African-American	French
Topo I (Scl 70)	**27%**	**20%**	11%	**35%**
RNA polymerase III	5%	**24%**	11%	4%
U3RNP	4%	4%	**37%**	2%
U1RNP	**35%**	14%	**26%**	9%
PM-Scl	0%	2%	0%	6%
Th/To	3%	5%	0%	1%
Centromere	16%	**21%**	11%	18%
Total	90%	90%	96%	75%

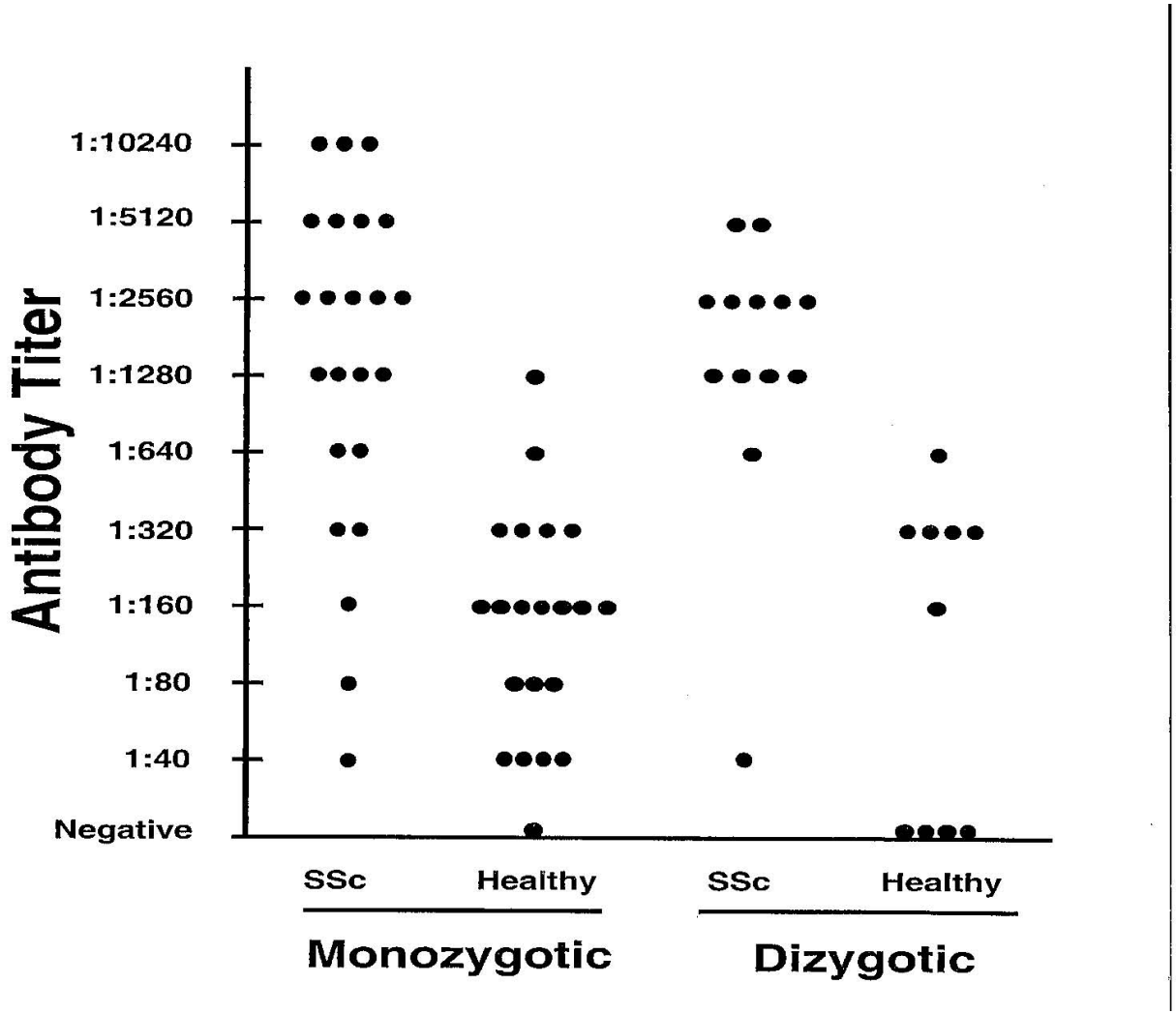
All Ab studies done at University of Pittsburgh

Kuwana et. al., Arthritis Rheum 1994; 37:902-906
Meyer et al., J Rheumatol 2007; 34: 104-109

SSc in Twins

- national recruitment: 42 twin pairs
- monozygotic (MZ) = 24; dizygotic (DZ) = 18
- concordance MZ = 1 (4%); DZ = 1 (6%)
- published concordance rates
  - RA: MZ 12-21%; DZ 0-4%
  - SLE: MZ 10-69%; DZ 0-2%
- Healthy twins often had positive ANA but not SSc-associated autoantibodies.

Feghali-Bostwick et al. Arthritis Rheum 2003; 48: 1956-63.



Feghali-Bostwick et al., Arthritis Rheum 2003; 48:1956-63.

SSc- SPECIFIC SERUM AUTOANTIBODIES DO COEXIST (Pittsburgh, 1972-2009)

(1) Anti-Ku	11/30 (37%)
(2) Anti-U3RNP	24/116 (21%)
(3) Anti-U1RNP	16/142 (11%)
(4) Anti-Th/To	10/182 (5%)
(5) Anti-topoisomerase I	21/576 (4%)
(6) Anti-centromere	19/627 (3%)
(7) Anti-U11/U12RNP	1/37 (3%)
(8) Anti-RNA polymerase III	12/610 (2%)
(9) Anti-PM-Scl	1/87 (1%)

Are the 9 SSc-associated antibodies specific for SSc?

1. centromere: some (5-10%) Raynaud disease (likely pre-SSc); occasional Sjogren syndrome; occasional SLE
2. topo I: specific but beware false positives by ELISA
3. Th/To: 5% Raynaud disease
4. PM-Scl: 30% PM or DM; 5% Raynaud disease
5. Ku: some SLE or PM/DM
6. U1RNP: many SLE, some PM/DM only
7. all others: almost 100% specific

* Lack of long-term follow up is a limitation.

Is antibody titer important?

1. Kuwana et al., Arthritis Rheum 2000; 43: 1074-84.
   - 6/26 SSc patients followed 20+ years lost anti-topo I reactivity.
   - These 6 patients had better skin and pulmonary outcomes and survival.

2. Hu et al. Arthritis Rheum 2003; 48: 1363-73.
   - 59 dcSSc patients studied
   - Anti-topo I IgG levels by ELISA correlated with skin score and physician-judged disease activity.

Correlation of serum anti-topo I antibody with disease activity in SSc

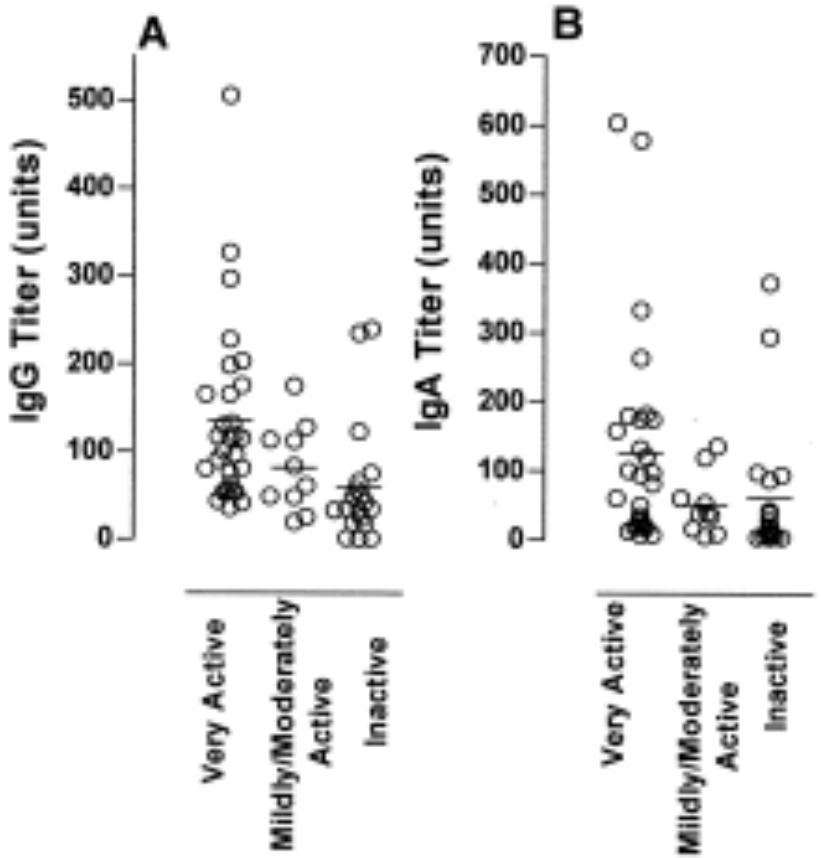


Figure 4. Very active vs. inactive. Mean IgG $p<0.01$ and IgA $p<0.05$.

Hu et al. Arthritis Rheum 2003; 48: 1368

Correlation of serum anti-topo I antibody levels with disease severity and activity in systemic sclerosis

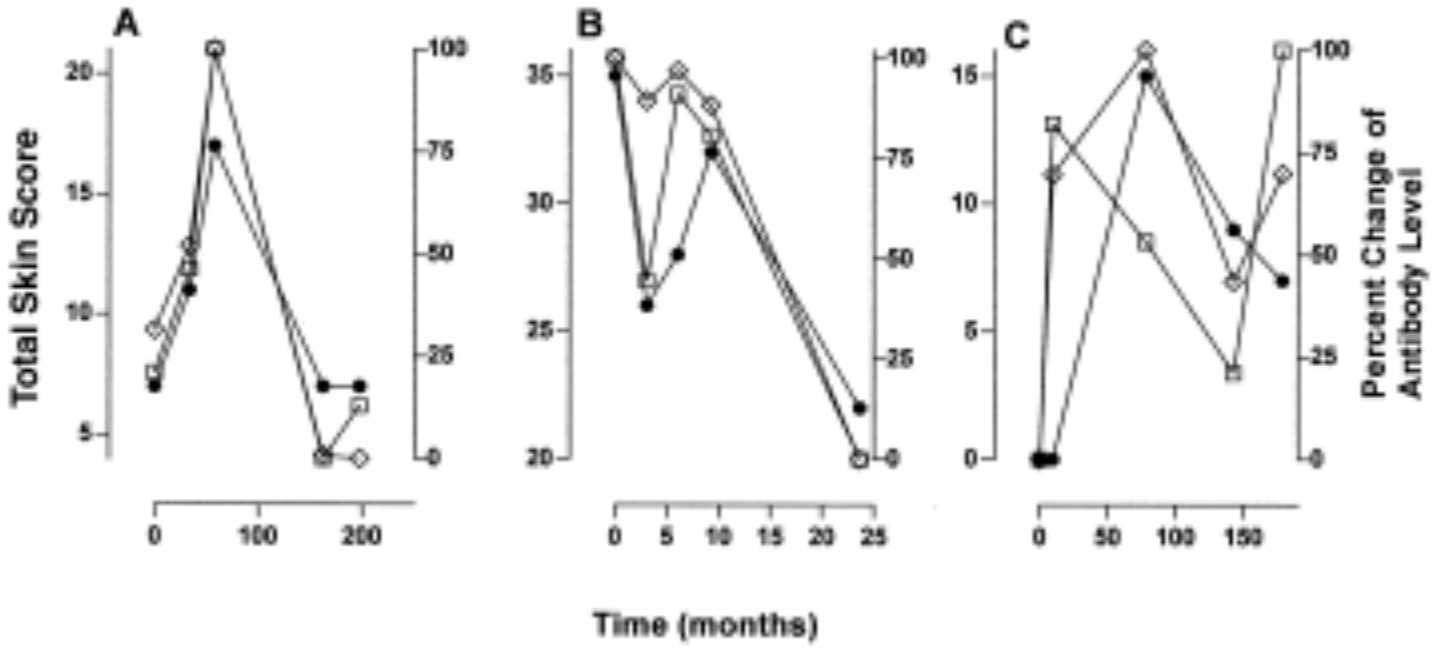


Figure 5. • TSS
☐ IgG; anti-topo I
◊ IgA anti-topo I

Hu et al. Arthritis Rheum 2003; 48: 1369

SURVIVAL IN SSc ACCORDING TO SERUM AUTOANTIBODY (Pittsburgh, 1972-2009)

Cumulative Survival Rate *(CSR)‡

Antibody (number of patients)	5 years	10 years	Decrement
PM-Scl (n=86)	100%	91%	9%
U1RNP (n=126)	91%	83%	8%
Centromere (n=608)	86%	76%	10%
Topo I (n=555)	83%	65%	18%
Ku (n=19)	83%	55%	28%
RNA polymerase III (n=598)	81%	72%	9%
Th/To (n=172)	77%	66%	11%
U11/U12 RNP (n=36)	76%	56%	20%
U3RNP (n=92)	76%	60%	16%

* from first physician diagnosis of SSc; not age/or sex-adjusted
‡Mantel-Haenszel method

ASSOCIATIONS OF GAVE IN SSc

Variable	GAVE (n=65)	SSc Controls *(n=195)	p-value
Female	51 (79%)	162 (83%)	NS
Caucasian	64 (99%)	172 (88%)	0.02
Age at Onset (mean years ± SD)	50.5 ± 16.3	43.6 ± 13.9	0.003
Diffuse Skin Changes	44 (68%)	84 (43%)	0.0007
Renal Crisis	11 (17%)	10 (5%)	0.006
Small Bowel Affected	14 (22%)	18 (9%)	0.01
Antibodies: RNA pol III	38 (59%)	36 (22%)	<0.0001
Topo I	1 (2%)	27 (14%)	0.011
Centromere	10 (15%)	41 (21%)	NS
Medication Use: PPI	51 (78%)	101 (52%)	0.0003
H2	2 (3%)	21 (11%)	NS
OC	3/51 (6%)	6/162 (4%)	NS
HR	15/51 (29%)	21/162 (13%)	0.0117

*Controls = next 3 consecutive new patients, 1983-2009

PPI = proton pump inhibitor; H-2 = H2 blocking drug; OC = oral contraceptive; HR = hormone replacement

Burgess et al. Poster Presentation at Digestive Disease Week, Chicago, IL, May 2011

SCLERODERMA RENAL CRISIS* OUTCOMES BY DECADE (Pittsburgh, 1980-2009)

Decade	Death (D)†	Permanent Dialysis (PD) †	Temporary Dialysis (TD) †	No Dialysis (ND)
1980-89	31	5	7	11
1990-99	13	9	22	13
200-09	8	1	1	12

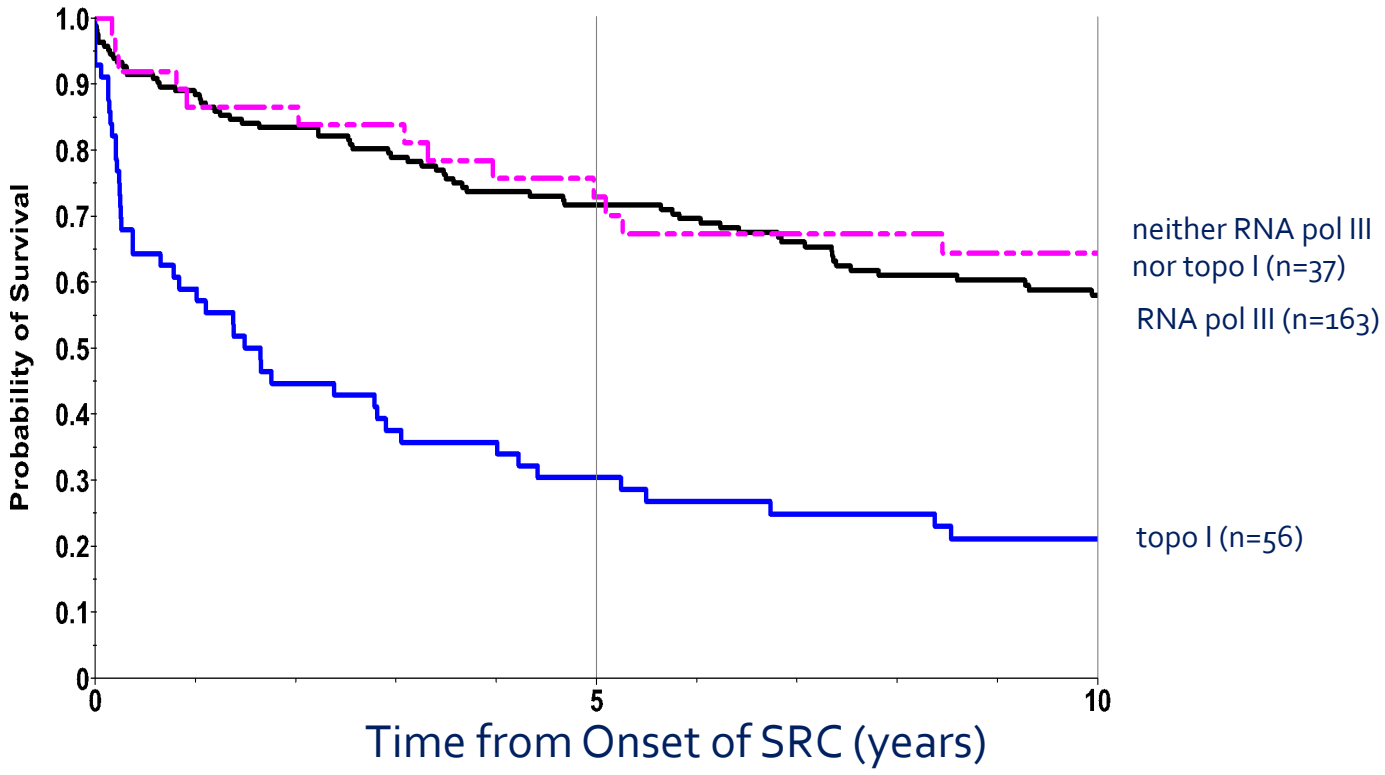
* SRC at or after first Pittsburgh visit; outcome at 3 years after onset of SRC
† D+PD vs. TD+ND; $p<0.08$

SCLERODERMA RENAL CRISIS OUTCOMES BY SERUM AUTOANTIBODY (Pittsburgh, 1980-2009)

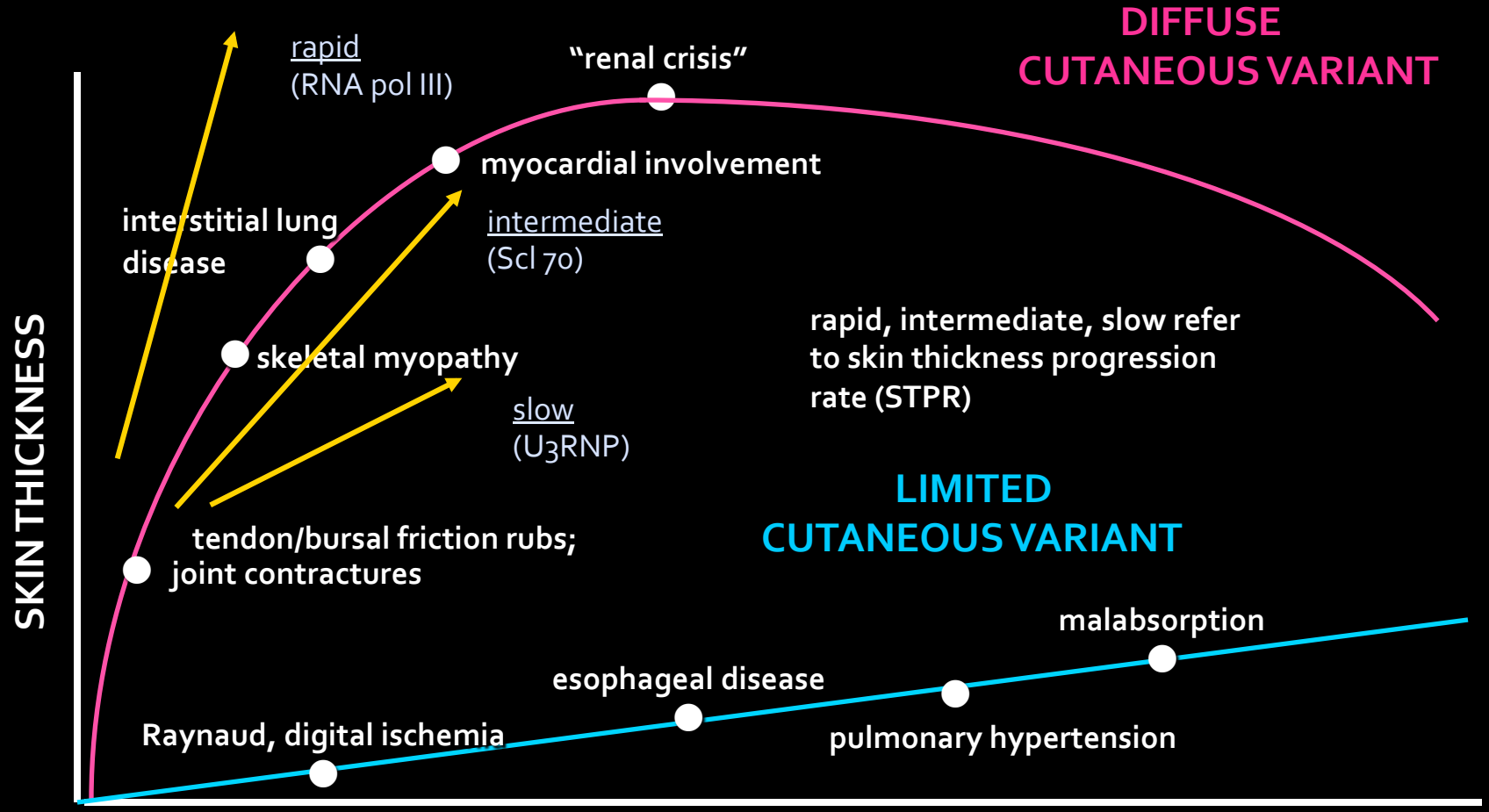
Antibody	D†	PD†	TD	ND
anti-topo I	22 (65%)	2 (6%)	4 (12%)	6 (18%)
anti-RNA pol III	15 (29%)	7 (13%)	9 (17%)	21 (40%)

† D + PD vs. TD + ND, p=0.01

SURVIVAL OF 256 SSc PATIENTS WITH SCLERODERMA RENAL CRISIS (SRC) BY AUTOANTIBODY (Pittsburgh, 1980-present)



USUAL TIMING OF PROBLEMS IN PATIENTS WITH SYSTEMIC SCLEROSIS



Domsic et al., Ann Rheum Dis 2011; 70: 104-109.

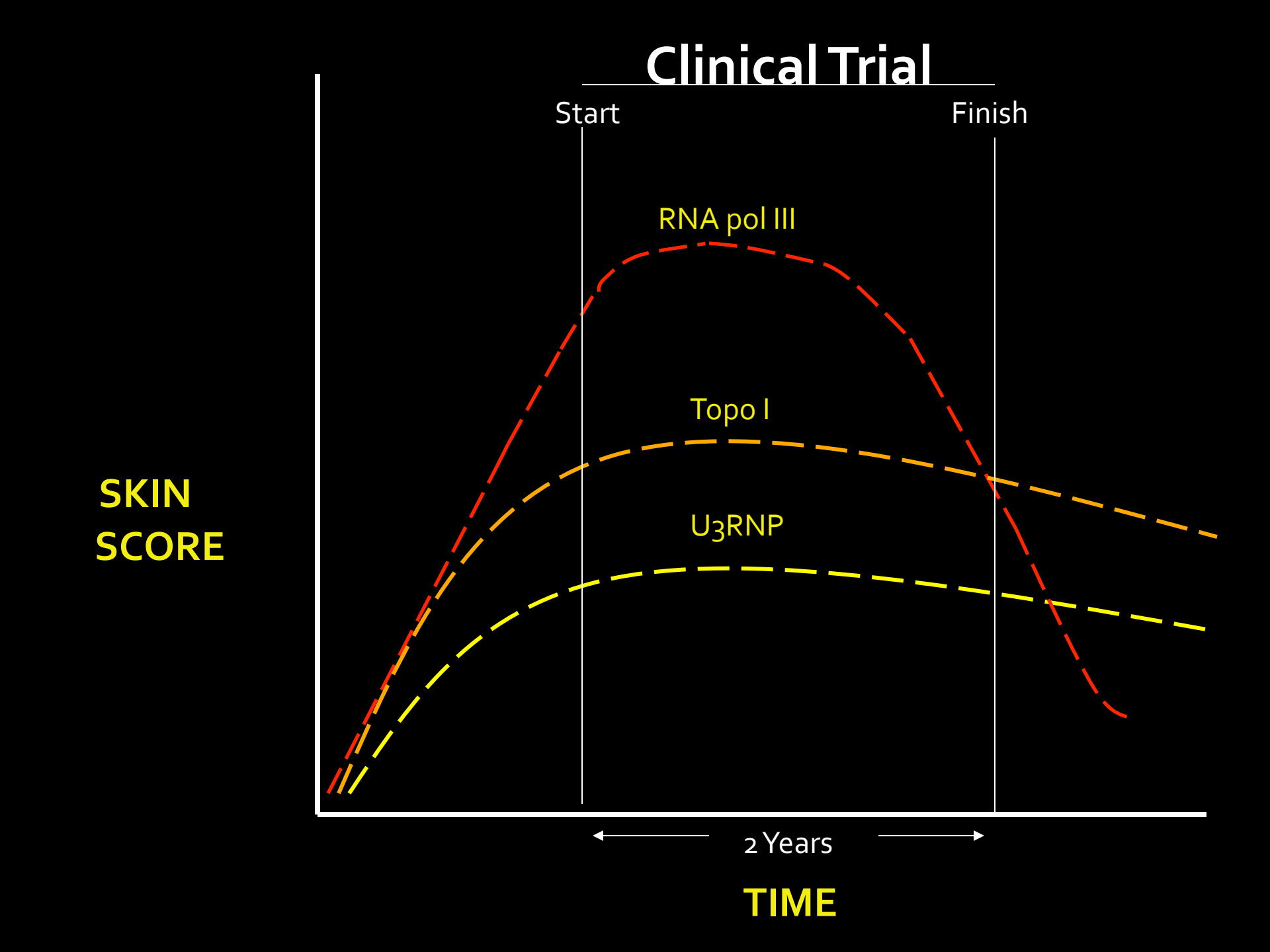
Table 2 Multivariate analysis of first visit variables predictive of mortality at 2 years

	OR	95% CI	p Value
Age >55 years	2.54	1.69 to 3.80	0.0001
Cardiac involvement	2.27	1.37 to 3.74	0.006
Tendon friction rubs	2.13	1.39 to 3.26	0.0005
Rapid STPR	1.74	1.15 to 2.63	0.01
Gastrointestinal involvement	1.83	1.21 to 2.74	0.004
Male gender	1.60	1.03 to 2.49	0.04
Anti-RNA polymerase III antibody	0.61	0.40 to 0.94	0.02

STPR, skin thickness progression rate.

Paradox: anti-RNA pol III protective: associated with rapid STPR but also with better 2 year survival

Domsic et al., Ann Rheum Dis 2011; 70:104-9.


Clinical Trial
Start
Finish
RNA pol III
Topo I
U3RNP
SKIN SCORE
2 Years
TIME

RECOMMENDATIONS FOR FUTURE CLINICAL TRIALS IN EARLY dcSSc

1) Early means very early; duration < 18 months from first SSc symptom

2) Consider stratifying on the basis of:
   a) skin thickness progression rate (STPR)
   b) serum autoantibody: anti-RNA polymerase III; anti-Scl 70; other

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