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
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

<table>
<thead>
<tr>
<th></th>
<th>RIGHT</th>
<th>LEFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>FINGERS</td>
<td>0 1 2 3</td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>HANDS</td>
<td>0 1 2 3</td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>FOREARMS</td>
<td>0 1 2 3</td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>UPPER ARMS</td>
<td>0 1 2 3</td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>FACE</td>
<td>0 1 2 3</td>
<td></td>
</tr>
<tr>
<td>CHEST</td>
<td>0 1 2 3</td>
<td></td>
</tr>
<tr>
<td>ABDOMEN</td>
<td>0 1 2 3</td>
<td></td>
</tr>
<tr>
<td>THIGHS</td>
<td>0 1 2 3</td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>LEGS</td>
<td>0 1 2 3</td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>FEET</td>
<td>0 1 2 3</td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>Total Skin Score</td>
<td>(TSS) 22</td>
<td></td>
</tr>
</tbody>
</table>

Rodnan et al.
TOTAL SKIN SCORE (TSS) DURING FOLLOWUP

Mean TSS

Years after First Pittsburgh Visit

- Diffuse (n=62)
- Limited (n=76)
USUAL TIMING OF PROBLEMS IN PATIENTS WITH SYSTEMIC SCLEROSIS

DIFFUSE CUTANEOUS VARIANT

- "renal crisis"
- myocardial involvement
- interstitial lung disease
- skeletal myopathy
- tendon/bursal friction rubs; joint contractures
- Raynaud, digital ischemia

LIMITED CUTANEOUS VARIANT

- malabsorption
- esophageal disease
- pulmonary hypertension; pulmonary fibrosis
- skin thickness tendency

TIME

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

2. ANA testing by multiplex 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.

Scleroderma Patients
June 2008-2009
(n=238)

MULTIPLEX-ANA performed n=57

MULTIPLEX-ANA Positive n=29 (51%)

MULTIPLEX-ANA Negative n=28 (49%)

MULTIPLEX-ANA not performed (n=181)

POSITIVE IIF-ANA or SSc-specific Ab (n=52, 91% tested)

NEGATIVE IIF-ANA or SSc-specific Ab (n=3, 5% tested)

IIF-ANA Unavailable (n=2)

POSITIVE IIF-ANA or SSc-specific Ab (n=161, 98% tested)

NEGATIVE IIF-ANA or SSc-specific Ab (n=4, 2% tested)

IIF-ANA Unavailable (n=16)
CLINICAL-SEROLOGIC CLASSIFICATION OF SYSTEMIC SCLEROSIS

DIFFUSE
- anti-topoisomerase I (Scl-70)
- anti-RNA polymerase III

LIMITED
- anticentromere
- anti-Th/To
- anti-U3RNP
- anti-U1RNP
- anti-PM-Scl
- anti-Ku

OVERLAP
- anti-U11/U12 RNP

ILD
- myositis; ILD
- myopathy; cardiomyopathy; PAH

severe skin; kidney

98% have +ANA; 90% have one of 9 SSc-associated Abs

PAH; ILD
- myositis

PAH
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

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

4. **U1RNP**: Williams et al., Arthritis Rheum 2005; 52:S590
8. **U3RNP**: Aggarwal et al., Arthritis Rheum 2009; 60:1112-8
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: immunoprecipitation (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

<table>
<thead>
<tr>
<th>HLA Type</th>
<th>Anti-Topo I (n=26)</th>
<th>Population Controls (n=67)</th>
<th>p-value</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1-1101</td>
<td>3 (12%)</td>
<td>7 (10%)</td>
<td>NS</td>
<td>1.1</td>
</tr>
<tr>
<td>DRB1-1102</td>
<td>0</td>
<td>1 (&lt;1%)</td>
<td>NS</td>
<td>1.1</td>
</tr>
<tr>
<td>DRB1-1103</td>
<td>2 (8%)</td>
<td>0</td>
<td>NS</td>
<td>1.1</td>
</tr>
<tr>
<td>DRB1-1104</td>
<td>17 (65%)</td>
<td>5 (8%)</td>
<td>0.001</td>
<td>23.4</td>
</tr>
</tbody>
</table>

RR = relative risk

# Antibody Profiles in Different SSc Populations

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Topo I (Scl 70)</td>
<td>27%</td>
<td>20%</td>
<td>11%</td>
<td>35%</td>
</tr>
<tr>
<td>RNA polymerase III</td>
<td>5%</td>
<td>24%</td>
<td>11%</td>
<td>4%</td>
</tr>
<tr>
<td>U3RNP</td>
<td>4%</td>
<td>4%</td>
<td>37%</td>
<td>2%</td>
</tr>
<tr>
<td>U1RNP</td>
<td>35%</td>
<td>14%</td>
<td>26%</td>
<td>9%</td>
</tr>
<tr>
<td>PM-Scl</td>
<td>0%</td>
<td>2%</td>
<td>0%</td>
<td>6%</td>
</tr>
<tr>
<td>Th/To</td>
<td>3%</td>
<td>5%</td>
<td>0%</td>
<td>1%</td>
</tr>
<tr>
<td>Centromere</td>
<td>16%</td>
<td>21%</td>
<td>11%</td>
<td>18%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>90%</strong></td>
<td><strong>90%</strong></td>
<td><strong>96%</strong></td>
<td><strong>75%</strong></td>
</tr>
</tbody>
</table>

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.

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

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Positive/Total (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Anti-Ku</td>
<td>11/30 (37%)</td>
</tr>
<tr>
<td>(2) Anti-U3RNP</td>
<td>24/116 (21%)</td>
</tr>
<tr>
<td>(3) Anti-U1RNP</td>
<td>16/142 (11%)</td>
</tr>
<tr>
<td>(4) Anti-Th/To</td>
<td>10/182 (5%)</td>
</tr>
<tr>
<td>(5) Anti-topoisomerase I</td>
<td>21/576 (4%)</td>
</tr>
<tr>
<td>(6) Anti-centromere</td>
<td>19/627 (3%)</td>
</tr>
<tr>
<td>(7) Anti-U11/U12RNP</td>
<td>1/37 (3%)</td>
</tr>
<tr>
<td>(8) Anti-RNA polymerase III</td>
<td>12/610 (2%)</td>
</tr>
<tr>
<td>(9) Anti-PM-Scl</td>
<td>1/87 (1%)</td>
</tr>
</tbody>
</table>
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?

   - 6/26 SSc patients followed 20+ years lost anti-topo I reactivity.
   - These 6 patients had better skin and pulmonary outcomes and survival.

   - 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.
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

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

### Cumulative Survival Rate *(CSR)‡*

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

* from first physician diagnosis of SSc; not age/or sex-adjusted
‡Mantel-Haenszel method
## ASSOCIATIONS OF GAVE IN SSc

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

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

PPI = proton pump inhibitor; H-2 = H2 blocking drug; OC = oral contraceptive; HR = hormone replacement
**SCLERODERMA RENAL CRISIS* OUTCOMES BY DECADE (Pittsburgh, 1980-2009)**

<table>
<thead>
<tr>
<th>Decade</th>
<th>Death (D)†</th>
<th>Permanent Dialysis (PD) †</th>
<th>Temporary Dialysis (TD) †</th>
<th>No Dialysis (ND)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1980-89</td>
<td>31</td>
<td>5</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>1990-99</td>
<td>13</td>
<td>9</td>
<td>22</td>
<td>13</td>
</tr>
<tr>
<td>200-09</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>12</td>
</tr>
</tbody>
</table>

* 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)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>D†</th>
<th>PD†</th>
<th>TD</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-topo I</td>
<td>22</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>(65%)</td>
<td>(6%)</td>
<td>(12%)</td>
<td>(18%)</td>
</tr>
<tr>
<td>anti-RNA pol III</td>
<td>15</td>
<td>7</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>(29%)</td>
<td>(13%)</td>
<td>(17%)</td>
<td>(40%)</td>
</tr>
</tbody>
</table>

† D + PD vs. TD + ND, p=0.01
SURVIVAL OF 256 SSc PATIENTS WITH SCLERODERMA RENAL CRISIS (SRC) BY AUTOANTIBODY (Pittsburgh, 1980-present)

neither RNA pol III nor topo I (n=37)
RNA pol III (n=163)
topo I (n=56)
USUAL TIMING OF PROBLEMS IN PATIENTS WITH SYSTEMIC SCLEROSIS

DIFFUSE CUTANEOUS VARIANT

- rapid (RNA pol III)
- interstitial lung disease
- skeletal myopathy
- slow (U3RNP)
- tendon/bursal friction rubs; joint contractures
- “renal crisis”
- myocardial involvement

LIMITED CUTANEOUS VARIANT

- rapid, intermediate, slow refer to skin thickness progression rate (STPR)
- rapid, digital ischemia
- esophageal disease
- pulmonary hypertension
- malabsorption

SKIN THICKNESS

TIME

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

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

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

STPR, skin thickness progression rate.
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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