Autoantibodies in scleroderma

Dr Sarah Sasson
SydPATH Registrar
28th July 2014

Introduction to Systemic sclerosis (SSc)

- SSc is an idiopathic systemic autoimmune disease characterised by severe and often progressive cutaneous and visceral fibrosis, fibroproliferative vasculopathies and pronounced cellular and humeral immunity abnormalities.
- Incidence 1: 4000 (USA)
- F>M
- Higher incidence in African Americans
- Clinically SSc can be divided into:
  - Limited
  - Diffuse
  - Sine cutaneous
Autoantibodies in SSc

- Autoantibodies that target a variety of intra- and extra-cellular proteins are a widely acknowledged hallmark of SSc
- 95% of pts have detected autoantibodies at diagnosis.
- It is unclear if these autoantibodies play a pathogenic role or are an epiphenomenon of disease
- Autoantibodies in SSc may:
  - Predict subtype
  - Predict severity

SSc and ANA

- 85-99% of patients with SSc have a positive ANA
- With the exception of CENP-B it is difficult to identify most SSc-related ANA by IIF
- Therefore it is recommended an additional tests e.g. ELISA, LIA, ALBIA or IP are used for confirmation.
### Autoantibodies in SSc

- Value is high Specificity and positive likelihood ratio

Table 1. Diagnostic and prognostic associations of autoantibodies

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Technique</th>
<th>Predictive of</th>
<th>Compared with</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Pos LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACA</td>
<td>IF</td>
<td>SSc</td>
<td>Healthy individuals</td>
<td>44</td>
<td>99.9</td>
<td>327</td>
</tr>
<tr>
<td>ACA</td>
<td>IF</td>
<td>SSc</td>
<td>Other CTDs</td>
<td>31</td>
<td>91</td>
<td>12.9</td>
</tr>
<tr>
<td>ACA</td>
<td>IF</td>
<td>lScs</td>
<td>Healthy individuals</td>
<td>44</td>
<td>93</td>
<td>6.1</td>
</tr>
<tr>
<td>ACA</td>
<td>IF</td>
<td>Ps. Arthritis</td>
<td>Healthy individuals</td>
<td>12</td>
<td>71</td>
<td>0.47</td>
</tr>
<tr>
<td>ACA</td>
<td>IF</td>
<td>SSc</td>
<td>Healthy individuals</td>
<td>24</td>
<td>90</td>
<td>2.3</td>
</tr>
<tr>
<td>Anti-Scl 70</td>
<td>ID</td>
<td>SSc</td>
<td>Healthy individuals</td>
<td>20</td>
<td>103</td>
<td>&gt;25</td>
</tr>
<tr>
<td>Anti-Scl 70</td>
<td>ID</td>
<td>SSc</td>
<td>Other CTDs</td>
<td>26</td>
<td>99.5</td>
<td>22</td>
</tr>
<tr>
<td>Anti-Scl 70</td>
<td>ID</td>
<td>SSc</td>
<td>Healthy individuals</td>
<td>28</td>
<td>99</td>
<td>10</td>
</tr>
<tr>
<td>Anti-Scl 70</td>
<td>ID</td>
<td>SSc</td>
<td>Healthy individuals</td>
<td>41</td>
<td>90.4</td>
<td>6.8</td>
</tr>
<tr>
<td>Anti-Scl 70</td>
<td>ID</td>
<td>SSc</td>
<td>Other CTDs</td>
<td>40</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>Anti-Scl 70</td>
<td>ELSA</td>
<td>SSc</td>
<td>Healthy individuals</td>
<td>43</td>
<td>100</td>
<td>&gt;55</td>
</tr>
<tr>
<td>Anti-Scl 70</td>
<td>ELSA</td>
<td>SSc</td>
<td>Other CTD</td>
<td>43</td>
<td>90</td>
<td>4.3</td>
</tr>
<tr>
<td>Anti-Scl 70</td>
<td>ID</td>
<td>Ps. Arthritis</td>
<td>Healthy individuals</td>
<td>37</td>
<td>92</td>
<td>3.0</td>
</tr>
<tr>
<td>Anti-Scl 70</td>
<td>ID</td>
<td>SSc</td>
<td>Healthy individuals</td>
<td>45</td>
<td>81</td>
<td>2.3</td>
</tr>
<tr>
<td>Anti-Scl 70</td>
<td>ID</td>
<td>SSc</td>
<td>Healthy individuals</td>
<td>43</td>
<td>83</td>
<td>2.5</td>
</tr>
<tr>
<td>Anti-Scl 70</td>
<td>ID</td>
<td>SSc</td>
<td>Healthy individuals</td>
<td>12</td>
<td>97</td>
<td>4.0</td>
</tr>
<tr>
<td>Anti-Scl 70</td>
<td>ID</td>
<td>SSc</td>
<td>Healthy individuals</td>
<td>58</td>
<td>94</td>
<td>6.0</td>
</tr>
<tr>
<td>PM-Scl</td>
<td>ID</td>
<td>SSc</td>
<td>Healthy individuals</td>
<td>50</td>
<td>98</td>
<td>31</td>
</tr>
</tbody>
</table>

ACA, anticytoplasm antibody; AFA, antifilament antibody; ELSA, enzyme-linked immunosorbent assay; IF, indirect immunofluorescence; ID, immunodiffusion; ID, immuneidetation; ELSA, enzyme-linked immunosorbent assay; IF, immuneidetation; SSc, systemic sclerosis; lScs, limited cutaneous systemic sclerosis; dSSc, diffuse cutaneous systemic sclerosis; CTD, connective tissue disease; RP, Raynaud phenomenon; Pos LR, positive likelihood ratio.

Published with permission [10].
Autoantibodies relatively specific for SSc

**Centromere (CENP)**
- Detected on IIF on Hep 2 cells and/or ELISA
- 6 types CENP A-F
- CENP-B most common, reactive with nearly all CENP+ sera
- 20-40% of SSc pts are CENP+
- Majority have limited cutaneous/CREST syndrome and associated with a higher risk for calcinosis and ischemic digital loss in pts with SSc.
- ACA+ in a patient with Raynaud’s increased the risk of developing SSc
- Lower frequency of pulmonary fibrosis
- Associated with greater lag time from detection to clinical symptoms, less MSK and cardiac involvement, higher risk HTN and GIT complaints compared with ATA+ pts.

**Topoisomerase I (ATA)/ Scl-70**
- Anti-Scl-70 was initially described as a 70kD target of autoantibodies in SSc.
- Subsequent work has found the autoantigen is in fact topoisomerase I 100kD and Scl-70 is a breakdown product of this. Hence it is more correct to use Topoisomerase-I.
- Detected in 37% of SSc
- Most associated with diffuse disease
- ATA+ in a patient with Raynaud’s increased the risk of developing SSc
- Associated with poorer prognosis, increased mortality, pulmonary fibrosis, musculoskeletal involvement and proteinuria
- ATA+ pts develop Raynaud’s younger than negative counterparts and were younger at age of first digital ulcer
- May be a biomarker of disease activity
- May have a higher rate of malignancies
- Unclear how an autoantibody directed at an intracellular antigen can cause damage associated with SSc

---

Cepeda and Reveille Curr Opin Rheumatology 2004
## Autoantibodies relatively specific for SSc

### Topoisomerase I (ATA)/ Scl-70

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Prevalence in SSc</th>
<th>HLA associations</th>
<th>Clinical and serologic associations</th>
<th>Progress</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Scl-70</td>
<td>9 to 20%</td>
<td>HLA-DRB1*1101,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HLA-DRB1*1104</td>
<td>dSSc</td>
<td>Increased mortality rate</td>
<td>? Relation with cancer, renal arteriopathy, controvers</td>
</tr>
</tbody>
</table>
Autoantibodies relatively specific for SSc

- **Th/To**
  - Directed against subunits of ribonuclease mitochondrial RNA processing and ribonuclease P complexes
  - Typically produce “dotty” nucleolar pattern on IIF on Hep-2
  - Prevalence of 2-5% of SSc and rarely associated with other disorders
  - Clinically associated with limited cutaneous SSc, less vascular and GIT disease

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Prevalence in SSc</th>
<th>HLA associations</th>
<th>Clinical and serologic associations</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Th/To</td>
<td>2 to 5%</td>
<td>HLA-DPB1*11</td>
<td>Milder skin and systemic involvement More severe pulmonary fibrosis</td>
<td></td>
</tr>
</tbody>
</table>

*Cepeda and Reveille Curr Opin Rheumatology 2004*

Autoantibodies relatively specific for SSc

- **Fibrillarin/U3 ribonucleoprotein**
  - Autoantibodies to nuclear fibrillarin is considered specific for SSc but only present in 7% of patients
  - May be associated with muscle involvement, more severe disease and worse prognosis in African-American patients

- **U11/ U12RNP**
  - Antigen is component of the spliceosome
  - Highly specific (99%) but low prevalence 3%
  - Associated with severe lung fibrosis

- **B23/nucleophosmin**
  - Nucleolar protein associated with cell proliferation
  - 80% associate with anti-fibrilian Abs
  - Also found in HCC, SLE and RA
Patterns of autoantibodies detected in SSc

To discuss:

Patterns of autoantibodies detected in SSc

Mehra et al Autoimm Rev 2013
Some patients with SSc do not have autoantibodies to common targets of SSc, but rather autoantibodies more commonly associated with other autoimmune diseases.

Other patients have no detectable autoantibodies.

Mehra et al Autoimm Rev 2013
Autoantigen localization and autoantibody frequency in SSc

4 major patterns of autoantibodies specific for SSc

- **Anti-CENP-B**: Characteristic speckled pattern in interphase
- **Anti-topoisomerase I**: Interphase Nucleolar staining
  - Few nuclear specks
- **Anti-RNA polymerase III**: Dark halo around staining
  - Fluorescent dots associated with nucleolar organisers
  - Diffuse granular/speckled nuclear staining
- **Anti-Th/To**: Few nuclear specks
Geographical variations in prevalence of major SSc autoantibodies

Table 2
Geotrophic variations in the frequency of the major systemic sclerosis autoantibodies to nuclear autoantigens in 4672 patients

<table>
<thead>
<tr>
<th>Country</th>
<th>Patients</th>
<th>Anti-centromere N</th>
<th>Anti-centromere %</th>
<th>Anti-Th/to N</th>
<th>Anti-Th/to %</th>
<th>Anti-topoisomerase I N</th>
<th>Anti-topoisomerase I %</th>
<th>Anti-RNA polymerase III N</th>
<th>Anti-RNA polymerase III %</th>
</tr>
</thead>
<tbody>
<tr>
<td>French</td>
<td>259</td>
<td>42</td>
<td>14.3</td>
<td>21</td>
<td>11.7</td>
<td>35</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Canada</td>
<td>1432</td>
<td>20</td>
<td>62</td>
<td>33</td>
<td>23</td>
<td>92</td>
<td>2</td>
<td>59</td>
<td>23</td>
</tr>
<tr>
<td>USA</td>
<td>1432</td>
<td>5</td>
<td>44</td>
<td>2</td>
<td>14</td>
<td>6</td>
<td>1</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>England</td>
<td>735</td>
<td>25</td>
<td>42</td>
<td>31</td>
<td>42</td>
<td>17</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Sweden</td>
<td>276</td>
<td>22</td>
<td>82</td>
<td>11</td>
<td>42</td>
<td>7</td>
<td>1</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Denmark</td>
<td>230</td>
<td>34</td>
<td>18</td>
<td>15</td>
<td>14</td>
<td>4</td>
<td>1</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>France</td>
<td>127</td>
<td>18</td>
<td>14</td>
<td>1</td>
<td>35</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Italy</td>
<td>1012</td>
<td>36</td>
<td>39</td>
<td>2</td>
<td>39</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Spain</td>
<td>72</td>
<td>41</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Greece</td>
<td>254</td>
<td>11</td>
<td>39</td>
<td>2</td>
<td>39</td>
<td>1</td>
<td>3</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Japan</td>
<td>275</td>
<td>16</td>
<td>28</td>
<td>2</td>
<td>28</td>
<td>5</td>
<td>3</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mean</td>
<td>26.1</td>
<td>3</td>
<td>28.6</td>
<td>2</td>
<td>28.6</td>
<td>10.3</td>
<td>3</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Diagnostic value of autoantibodies in SSc

- Both anti-centromere and anti-topoisomerase-I Abs have very high specificity, PPV and LR for SSc
- There is limited data on the performance of Anti-Th/To and RNPI-III antibodies

Koenig et al Autoimm Rev 2008
Clinical Spectrum of SSc

- Cutaneous vs. visceral involvement
- Rate of disease progression
- Limited vs. diffuse

Limited SSc (CREST)
  - Raynaud’s phenomenon first manifestation
  - Slow progression of skin involvement which remains at distal extremities
  - Slow disease course
  - Late occurrence of pulmonary arterial hypertensions (PAH)

Diffuse (SSc)
  - Short duration or absence of Raynaud’s phenomenon
  - Early and extensive skin involvement
  - Severe visceral involvement: pulmonary fibrosis, renal crises, cardiomyopathy
  - Poorer survival

Autoantibodies as biomarkers for SSc phenotypes

- Limited scleroderma is associated with:
  - Anti CENP-B
  - Anti-Th/To
- Diffuse SSc is associated with:
  - antiRNAPIII
  - Anti-topoisomerase I in certain populations

<table>
<thead>
<tr>
<th>Anti-centromere</th>
<th>Peripheral vascular disease, isolated pulmonary arterial hypertension, primary biliary cirrhosis, limited and intermediate skin involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Th/To</td>
<td>Isolated pulmonary arterial hypertension, pulmonary fibrosis, limited skin involvement</td>
</tr>
<tr>
<td>Anti-topoisomerase I</td>
<td>Pulmonary fibrosis, peripheral vascular disease, severe heart disease, renal crisis, cancer, limited, intermediate or diffuse skin involvement</td>
</tr>
<tr>
<td>Anti-ENA</td>
<td>Renal crisis</td>
</tr>
<tr>
<td>Anti-polymyosin III</td>
<td>Diffuse skin involvement</td>
</tr>
</tbody>
</table>

Koenig et al Autoimm Rev 2008
Is SSc an autoantibody mediated disease?

• Compelling evidence that any of the SSc ANAs cause disease manifestations is lacking.
• Newer autoantibodies have stronger experimental evidence for a causative role in tissue damage:
   Anti-endothelial cell antibodies
   Anti-fibrillin-1
   Anti-MMP1 and -3
   Anti PDGFR

<table>
<thead>
<tr>
<th>Autoantibodies</th>
<th>Specific for SSc</th>
<th>Prevalence in SSc</th>
<th>Pathogenic role in SSc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-endothelial cell antibodies (AECAs)</td>
<td>No</td>
<td>&gt;60%</td>
<td>Induce endothelial cell apoptosis via caspase 9 pathway and induce TGFβ1 expression in apoptotic endothelial cells; activate normal fibroblasts into pro-fibrotic phenotype via causing release of autocrine TGFβ in ECM</td>
</tr>
<tr>
<td>Antibody 1 (FIB1)</td>
<td>Yes</td>
<td>&gt;50%</td>
<td>Promote deposition of extracellular matrix and other fibrotic ECM components</td>
</tr>
<tr>
<td>Anti-collagenous autoantibodies MMP1 and -3</td>
<td>Yes</td>
<td>High percentage</td>
<td>Promote degradation of excessive collagen and other fibrotic ECM components</td>
</tr>
<tr>
<td>Anti-smooth muscle growth factor receptor (PDGF) antibodies</td>
<td>Yes</td>
<td>100%</td>
<td>Stimulate normal fibroblasts via HIF-1α/ERK1/2 pathways and generate reactive oxygen species cascades to induce collagen I production, and convert resting fibroblasts into activated myofibroblasts</td>
</tr>
</tbody>
</table>

Discussion

• SSc is an idiopathic systemic autoimmune disease characterised by severe and often progressive cutaneous and visceral fibrosis, fibroproliferative vasculopathies and pronounced cellular and humoral immunity abnormalities.
• 95% of patients have an autoantibody at diagnosis
• It is unclear of autoantibodies in SSc are pathogenic or an epiphenomenon
• Autoantibodies in SSc may predict:
   Subtype
   Severity
• Autoantibodies have a greater negative predictive value than positive predictive value
Discussion

• The 4 major autoantibodies in SSc are:
  ➢ CENP-B- associated with CREST syndrome; lower risk of pulmonary fibrosis
  ➢ Scl-70/Topoisomerase-1- associated with diffuse disease, worse prognosis and higher mortality
  ➢ RNAPI-III- Often co-exist; Increased risk of renal crisis, HTN and MSK involvement
  ➢ Th/To- Associated with limited cutaneous disease

• Apart from CENP-B autoantibodies in SSc often co-exist in a range of patterns. Some patients may also have autoantibodies more classically associated with other autoimmune diseases, or none at all.

• The prevalence of these autoantibodies display geographical variance.

• Newer autoantibodies in SSc are being described with more pathogenic features.

Thank you

• Questions?