Scleroderma
Scleroderma

- Sclero (hard) Derma (skin) – Greek
- Autoimmune disease unknown aetiology
- Collagen deposition
- Localised or systemic
- Hallmark autoantibodies
Clinical Classification of Scleroderma
Two sub-set model based on the extent of skin involvement

- **Limited cutaneous systemic sclerosis (LcSSc)**
  - Lengthy history of Raynauds (years)
  - Skin thickening confined to extremities and perioral region

- **Diffuse cutaneous systemic sclerosis (DcSSc)**
  - Raynauds, skin changes, systemic features develop simultaneously
  - Skin thickening proximal to elbows/knees and involves trunk

- **Overlap syndromes**
  - Polymyositis, SLE
Skin Involvement in SSc
Systemic Sclerosis (SSc)

- Peak onset: 30-50
- F:M ratio: 3:1
- Limited (LcSSc) 70%
- Diffuse (DcSSc) 30%
Prevalence of SSc in UK

- 120-140/million
- UK population: 7-8,000
- Royal Free: 12-1500 patients
Scleroderma citrinum
Vascular Involvement
Gastrointestinal involvement

- Oesophageal dysfunction
- Restricted blood supply
- Damage nerves controlling muscle function
- Difficulty swallowing
- 90% of SSc patients
- Small & Large bowel can be affected
Lung Involvement

Pulmonary fibrosis – interstitial lung disease

Fibrosis of lung tissue – loss of elasticity

Pulmonary arterial hypertension
Renal Involvement

- Vasospasm
- High blood pressure
- Failure of homeostatic Mechanisms
- Loss of renal blood supply
- Kidney failure
- Fatal pre-ACE inhibitors
Clinical classification vs Antibody profile

- **LcSSc**
  - Lung fibrosis
  - PAH

- **DcSSc**
  - Renal
  - Muscle
  - Liver
  - Joints
Autoantibodies in Systemic Sclerosis

- Distinct ANA patterns on Hep-2 cells that correlate strongly with presence of specific autoantibodies
- Antibodies occur in non-overlapping populations
Anti-nuclear antibodies in SSc

- >95% of SSc patients are ANA positive

- >85% have a defined ANA specificity

- ~80% have an antibody specific for SSc
Antibody frequency in SSc

<p>| | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>ACA</td>
<td>29%</td>
</tr>
<tr>
<td>ATA</td>
<td>17%</td>
</tr>
<tr>
<td>Total</td>
<td>46%</td>
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</table>
Anti-centromere antibody

- Characteristic pattern
- Insoluble antigen
- Not detected by CIE or Immunoprecipitation
Anti-centromere antibody

- CENP-B – major centromere antigen
- Antibodies associated with LcSSc
- Little internal organ involvement
- PAH major cause of mortality
- Age of onset 50ish
ACA/AMA overlap*

- LcSSc – PBC
- 5% PBC
- 5% LcSSc
- PBC slower to develop
- Less frequent Tx
Anti-Scl-70

- Variable nucleolar stain
- Readily soluble
- Detected as an anti-ENA
- Dense nucleoplasmic speckling almost homogeneous
Anti-Scl-70 – ATA*

- Extent of nucleolar stain is substrate dependent
- Chromatin of dividing cells is strongly stained
- NOR staining
Anti-Scl-70

- DNA topoisomerase I (ATA)
- Recombinant antigen available in EIA
- DcSSc > LcSSc
- Pulmonary fibrosis
- Age of onset 40
Anti-RNA polymerase (I/III) – a third major SSc specific antibody

- ACA 29%
- ATA 17%
- ARA 13%
- total 59%
Anti-RNA polymerase I/III Pattern 1*

- Nucleoplasmic speckled pattern
- Additional bright dots
- Negative nucleolar staining
Anti-RNA polymerase I/III Pattern 2*

- Variable punctate nucleolar stain
- Speckled nucleoplasmic stain
Immunoprecipitation by RNA polymerase I/III antibodies

- Radiolabelled cell extract (35-S Met)
- Protein A sepharose Coupled antibody
ARA by Immunoprecipitation

RNA pol I 190kDa
RNA pol III 155kDa, 138kDa
RNA pol I 120kDa

RNA pol II sub-units 220 & 145 kDa
ELISA development from a recombinant peptide

Kuwana et al. Arthritis Rheum 2002
Kuwana et al. Arthritis Rheum 2005
Anti-RNA polymerase (ARA)

- RNA pol III major antigenic epitope
- Recombinant available
- Immunoprecipitation assay
- DcSSc
- Scleroderma renal crisis (SRC)
- Age of onset 50ish
Scleroderma Renal Crisis

- Who gets it?
  - 16% of all dcSSc; 2% of lcSSc
  - 66% within first year of disease
  - 22% present with SRC as presentation of SSc
  - Association with bad skin disease
  - 60% ARA positive
  - Very poor prognosis prior to ACE inhibitors

- H Penn et al, 2007, QJM 100 485-494
Scleroderma Renal Crisis
ARA by EIA, IIF and IP

Anti-RNA polymerase U/ml

ARA by EIA, IIF and IP
Anti-PM-Scl

- Nucleolar and diffuse nucleoplasmic stain
- Detected as an anti-ENA by CIE, EIA, blot, ippt
Autoantibodies as prognostic indicators

Anti-PM-Scl vs Anti-aats Clinical features and outcome

<table>
<thead>
<tr>
<th>Feature</th>
<th>PM-Scl (n32)</th>
<th>aats (n29)</th>
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<tbody>
<tr>
<td>Raynauds</td>
<td>100%</td>
<td>93%</td>
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<tr>
<td>Arthritis</td>
<td>97</td>
<td>90</td>
</tr>
<tr>
<td>Myositis</td>
<td>88</td>
<td>83</td>
</tr>
<tr>
<td>Lung fibrosis</td>
<td>78</td>
<td>79</td>
</tr>
<tr>
<td>Sicca</td>
<td>34</td>
<td>59</td>
</tr>
<tr>
<td>Telangiectasia</td>
<td>62</td>
<td>31</td>
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<tr>
<td>Dysphagia</td>
<td>47</td>
<td>24</td>
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<tr>
<td>Scleroderma</td>
<td>97</td>
<td></td>
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<tr>
<td>Sclerodactyly</td>
<td></td>
<td>72</td>
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<tr>
<td>Clubbing</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Well</td>
<td>66</td>
<td>48</td>
</tr>
<tr>
<td>Major disability</td>
<td>9</td>
<td>35</td>
</tr>
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</table>
Anti-fibrillarin (U3-RNP)*

- Characteristic staining pattern
- Clumpy nucleolar
- No anti-ENA
- Confirmation by immunoprecipitation
- 34 kD Fibrillarin
- U3-RNA
AFA: Clinical Associations

- DcSSc – in afro-caribbean
- Extensive internal organ involvement
- Pulmonary hypertension
- Myositis
- Renal
- Young onset (<40)
Anti-Th-RNP (Th/To)

- Nucleolar and discrete nucleoplasmic speckles
- Not detected by gel
- Precipitation (CIE)
Anti-Th-RNP

- 40kD protein antigen associated with 7-2 RNA
- Function is RNA processing
- Antibodies associated with LcSSc (have been described in IPF patients)
- Little internal organ involvement
- Age of onset 40ish
Anti-nRNP

- Coarse nucleoplasmic staining
- Not SSc specific
- Readily soluble detected as an anti-ENA
Anti-nRNP

- 68kD, A & C proteins of U1-RNP complex
- Function is mRNA splicing
- Overlap syndromes – SLE like
- Joints and muscles
- Age of onset 36
### Autoantibodies in SSc

167 consecutive patients

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Frequency (n)</th>
<th>%</th>
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<tbody>
<tr>
<td>Centromere</td>
<td>42</td>
<td>25</td>
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<tr>
<td>Centromere + AMA</td>
<td>7</td>
<td>4</td>
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<tr>
<td>Scl-70 (4 with nRNP)</td>
<td>29</td>
<td>17</td>
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<tr>
<td>RNA polymerase</td>
<td>22</td>
<td>13</td>
</tr>
<tr>
<td>PM-Scl</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>nRNP (1 with RoLa)</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>U3-RNP (1 with Ro)</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Th-RNP</td>
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<td>4</td>
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<tr>
<td></td>
<td><strong>147</strong></td>
<td><strong>87</strong></td>
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</tbody>
</table>
Other antibodies in SSc

- Anti-Ku
- Anti-Jo-1
- Anti-SRP
- Anti-Ro
### Clinical Subgroups associated with SSc autoantibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Freq.</th>
<th>Age of onset</th>
<th>Subset</th>
<th>Organ</th>
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<tbody>
<tr>
<td>ACA</td>
<td>29</td>
<td>51</td>
<td>LcSSc</td>
<td>Lung - IPH</td>
</tr>
<tr>
<td>ARA</td>
<td>13</td>
<td>48</td>
<td>DcSSc</td>
<td>Lung - PF</td>
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<tr>
<td>ATA</td>
<td>17</td>
<td>41</td>
<td>Dc&gt;LcSSc</td>
<td>Lung - PF</td>
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<tr>
<td>Th-RNP</td>
<td>4</td>
<td>41</td>
<td>LcSSc</td>
<td>Lung - IPH</td>
</tr>
<tr>
<td>PM-Scl</td>
<td>8</td>
<td>41</td>
<td>SSC/myositis</td>
<td>Muscle</td>
</tr>
<tr>
<td>AFA (U3-RNP)</td>
<td>7</td>
<td>37</td>
<td>Dc&gt;LcSSc</td>
<td>Lung - IPH</td>
</tr>
<tr>
<td>n-RNP</td>
<td>8</td>
<td>36</td>
<td>LcSSc/SLE</td>
<td>Joint</td>
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Conclusions

- Distinctive Immunofluorescence patterns can be identified in sera from SSc patients
- The antibodies form mutually exclusive sub-populations
- Correlate with disease phenotype
- Useful prognostic and diagnostic markers
Acknowledgements

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  Royal Free Hospital

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  Royal Free Hospital