Autoantibodies in Diagnosis and Follow up of Rheumatic Diseases Study Group

Clinically relevant autoantibodies in myositis with interstitial lung disease and cancer-associated myositis

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Collaborational Research
- Medical and Biological Laboratory
Clinical manifestation of PM/DM

Heliotrope rash
V-neck sign
Gottron's sign

From Mimori T ed. PM/DM knowledge book, 2015
Marker Autoantibodies in IIM

- **Myositis-specific antibodies (MSAs)**
  - anti–aminoacyl–tRNA synthetases including anti–Jo-1
  - anti–MDA5 (CADM–140)
  - anti–Mi–2
  - anti–TIF–1γ/α (p155/140)
  - anti–NXP–2 (MJ)
  - anti–SAE
  - anti–SRP (signal recognition particle)
  - anti–HMGCR
  - anti–cN1A

- **Myositis-associated antibodies (MAAs)**
  - anti–U1–RNP
  - anti–Ku
  - anti–PM–Scl
Myositis 
and 
Interstitial Lung Disease
Interstitial lung disease (ILD) associated with polymyositis and dermatomyositis

- Frequency in PM/DM is ~50% (21~78% in recent reports)
- Most important life prognostic factor in PM/DM
- Frequency of ILD is equivalent in PM and DM. However, DM–ILD has severer disease coarse, poorer responsiveness on treatment, and poorer prognosis than PM–ILD.
**Aminoacyl-tRNA Synthetases (ARS)**

- **Amino acid**
- **P-adenosine**
- **tRNA**
- **3′-OH**
- **Aminoacyl-tRNA**
- **ATP**

The cycle involves the conversion of an amino acid into an aminoacyl-tRNA using ATP and P-adenosine in the presence of ARS enzymes.
## Spectrum of Anti-Synthetase Antibodies

<table>
<thead>
<tr>
<th>Autoantibodies</th>
<th>Target Antigens</th>
<th>% in Myositis</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-Jo-1</td>
<td>Histidyl-tRNA synthetase</td>
<td>15–20%</td>
</tr>
<tr>
<td>anti-PL-7</td>
<td>Threonyl-tRNA synthetase</td>
<td>5%</td>
</tr>
<tr>
<td>anti-PL-12</td>
<td>Alanyl-tRNA synthetase</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>anti-EJ</td>
<td>Glycyl-tRNA synthetase</td>
<td>5–10%</td>
</tr>
<tr>
<td>anti-OJ</td>
<td>Isoleucyl-tRNA synthetase</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>anti-KS</td>
<td>Asparaginyl-tRNA synthetase</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>anti-Zo</td>
<td>Phenylalanyl-tRNA synthetase</td>
<td>Case Report</td>
</tr>
<tr>
<td>anti-Ha</td>
<td>Tyrocyl-tRNA synthetase</td>
<td>Case Report</td>
</tr>
</tbody>
</table>
**Anti-Synthetase Syndrome**

Patients who have autoantibodies to any aminoacyl-tRNA synthetases reveal the common clinical symptoms:

- Polymyositis (PM) > Dermatomyositis (DM)
- Interstitial lung disease (ILD) with shrinking lung
- Mechanic’s hand

Others: Polyarthritis/arthritis, Raynaud’s phenomenon, Fever
Response to Treatment and Clinical Course of ILD

A. Response to the initial glucocorticoid therapy of ILD

- Anti-ARS(+) (8/17): 47% (p<0.05)
- Anti-ARS(-): 2/15 (13%)

Anti-ARS(+) ILD responds to GC, but shows recurrent course.

B. Clinical course of ILD and anti-syntetase antibodies

- Anti-ARS(+):
  - Single rush: 5
  - Static or very slow: 8
  - Recurrent: 6
  - Rush and fatal: 1

- Anti-ARS(-):
  - Single rush: 7
  - Static or very slow: 14

(Yoshifuji H, Autoimmunity 2006)
**Chest CT of PM-associated ILD (anti-PL-7-positive case)**

- Basilar GGO
- Reticular opacity
- Traction bronchiectasis

Before treatment (Sept. 2008)  

After treatment (Sept. 2009)

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**Treatment:** high-dose glucocorticoid + calcineurin inhibitor (cyclosporin or tacrolimus)

→ Intravenous cyclophosphamide

(Watanabe K. et al: Resp Med 2011)
“Amyopathic” Dermatomyositis

- Typical skin rash (heliotrope rash or Gottron’s sign) but no evidence of myopathy and normal CK

Amyopathic Dermatomyositis

Hypomyopathic Dermatomyositis

Clinically Amyopathic Dermatomyositis (Sontheimer)

- Associated with intractable RP–ILD in Asia

- Anti-CADM-140 (Sato S. A&R 2005)
The target antigen was identified as MDA5 (IFIH1), a cytoplasmic receptor of viral RNAs.

At first visit
(Random or lower consolidation/ GGO)

After one month
(Diffuse GGO)

**Treatment recommendation:**
Intensive immunosuppressive therapy combined with
high-dose glucocorticoids
+ calcineurin inhibitors
+ intravenous cyclophosphamide pulse
Life prognosis of patients with anti-MDA5-positive CADM/DM-ILD

Patients who received intensive regimen of combined immunosuppressive therapy in early phase (n=14)

Patients who received conventional step up therapy (Historical control, n=14)

Log rank test: p=0.038

(Nakashima R, ACR2011 (Chicago) Abstract #225)
Autoantibodies and ILD in PM/DM

- Chronic ILD with shrinking lung in PM/DM (NSIP, in part UIP or OP)

  Positive anti-synthetase: Response to treatment can be expected, although frequent recurrence

- Acute/subacute ILD in clinically amyopathic DM (CADM) (DAD)

  Positive anti-MDA5: Intractable and poor prognosis
Myositis and Malignancy
### Population-based cohort studies of the association of malignancy with DM/PM

<table>
<thead>
<tr>
<th>Study</th>
<th>Frequency of malignancy</th>
<th>SIR (95% CI)</th>
<th>Frequency of malignancy</th>
<th>SIR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buchbinder et al. 1)</td>
<td>36/85 (42%)</td>
<td>6.2 (3.9-10)</td>
<td>58/321 (18%)</td>
<td>2.0 (1.4-2.7)</td>
</tr>
<tr>
<td>Airio et al. 2)</td>
<td>19/71 (27%)</td>
<td>6.5 (3.9-10)</td>
<td>12/175 (7%)</td>
<td>1.0 (0.5-1.8)</td>
</tr>
<tr>
<td>Stockton et al. 3)</td>
<td>77/286 (27%)</td>
<td>7.7 (5.7-10.1)</td>
<td>71/419 (17%)</td>
<td>2.1 (1.5-2.9)</td>
</tr>
<tr>
<td>Chow et al. 4)</td>
<td>31/203 (15%)</td>
<td>3.8 (2.6-5.4)</td>
<td>26/336 (8%)</td>
<td>1.7 (1.1-2.4)</td>
</tr>
<tr>
<td>Sigurgeirsson et al. 5)</td>
<td>59/392 (15%)</td>
<td>2.4 (1.6-3.6),(male); 3.4 (2.4-4.7),(female)</td>
<td>37/396 (9%)</td>
<td>1.8 (1.1-2.7),(male); 1.7 (1.0-2.5),(female)</td>
</tr>
<tr>
<td>Chen et al. 6)</td>
<td>95/1012 (9%)</td>
<td>5.1 (5.01-5.22)</td>
<td>33/643 (5%)</td>
<td>2.15 (2.08-2.2)</td>
</tr>
</tbody>
</table>

**SIR:** standardized incidence ratio

Risk factors for underlying malignancy in myositis

Increased Risk

Host factor
• Old age (> 45 yrs)

Symptoms
• Cutaneous involvement
• Distal muscle involvement
• Dysphagia
• Refractory to treatment

Laboratory markers
• Elevated CRP/ESR
• Low C4 level
• Anti-p155/140 antibody

Decreased Risk

Host factor
• Young age

Symptoms
• Raynaud’s phenomenon
• Interstitial lung disease
• Arthritis/arthralgia

Laboratory marker
• High titer of antinuclear antibody

Chen YJ et al. British Journal of Dermatology 2001
Zahr ZA et al. Curr Rheumatol Rep 2011
Anti-p155/140 antibody
（Targoff IN: A&R 2006, Kaji K: Rheumatology 2007）

- TIF-1γ (p155) and α (p140) are the autoantigens
- DM-specific autoantibody (20-30% in DM)
- Detected both in adult and juvenile DM
- Association with malignant tumors is speculated
- Skin lesions and dysphagia are intensive
- ANA in indirect fluorescence shows weakly positive
The role of TIF-1 as a transcription coactivator

TIF-1: Transcriptional Intermediary Factor-1

- **TIF-1γ**: 155kDa (TRIM33)
- **TIF-1α**: 140kDa (TRIM24)

Modified from the pamphlet of MBL Co. Ltd.
## Anti-p155 (TIF-1γ) antibody and cancer-associated myositis in DM patients

<table>
<thead>
<tr>
<th>Authors</th>
<th>N</th>
<th>CAM (Ab+)</th>
<th>No CAM (Ab+)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targoff 2006</td>
<td>45</td>
<td>6(6)</td>
<td>39(8)</td>
<td>100</td>
<td>79</td>
<td>42.9</td>
<td>100</td>
</tr>
<tr>
<td>Gunawardena 2008</td>
<td>20</td>
<td>3(3)</td>
<td>17(3)</td>
<td>100</td>
<td>82</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Kaji 2007</td>
<td>52</td>
<td>10(5)</td>
<td>42(2)</td>
<td>50</td>
<td>95</td>
<td>71.4</td>
<td>88.9</td>
</tr>
<tr>
<td>Chinoy 2007</td>
<td>103</td>
<td>15(8)</td>
<td>88(11)</td>
<td>53</td>
<td>87</td>
<td>42.1</td>
<td>91.6</td>
</tr>
<tr>
<td>Fujikawa 2009</td>
<td>30</td>
<td>5(5)</td>
<td>25(0)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Trallero-Araguas 2010</td>
<td>65</td>
<td>14(10)</td>
<td>51(5)</td>
<td>71</td>
<td>92</td>
<td>66.7</td>
<td>92</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>315</strong></td>
<td><strong>53(37)</strong></td>
<td><strong>262(29)</strong></td>
<td><strong>70</strong></td>
<td><strong>89</strong></td>
<td><strong>56</strong></td>
<td><strong>93.5</strong></td>
</tr>
</tbody>
</table>

(Selva-O’Callaghan et al. Curr Opin Rheumatol 2010) with modification
No cancer association is found in patients under 40 year-old.

75% of Ab-positive patients upper 45 y.o. are associated with malignancy.

Temporal relationships of cancer detection with myositis in 75 patients with cancer-associated myositis

Anti-NXP-2 (MJ) antibody

(Gunawardena H: A&R 2009, Espada G: J Rheumatol 2009)

**Antigen:** NXP (nuclear matrix protein)-2

- 140kDa protein is immunoprecipitated
- One of AML/CBF/PEBP2/runt domain transcription factor family
- Act as a transcription factor especially in tissue-specific transcription regulation

**Clinical significance of antibody**

- 20–25% in JDM, 1.5%–17% in adult DM (rare in PM)
- Rare in Japanese (only 1.6%)
- Associated with subcutaneous calcinosis in JDM
- Association with malignancy has been reported
  - Japanese cohort 50% (4/8 cases) (Ichimura Y. Ann Rheum Dis 2012)
  - European cohorts 0–50%

155 kDa → 140 kDa

Lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Malignancy in myositis

• The screening of MSAs is useful not only for diagnosis and prognosis, but also to predict the complication of malignancy.
• For patients with anti-TIF1-γ, intensive surveillance of malignancy is strongly recommended at the diagnosis of myositis. However, younger patients with anti-TIF-1γ less than 40s are rarely affected by malignancy.
• Detection of cancer is usually done within 3 months before or after DM is diagnosed. Stage of malignancies is usually high when cancer is diagnosed.
• Types of malignancies are not related to cancer-associated myositis.
Methods for detecting myositis-specific autoantibodies (Immunoprecipitation (IP))

RNA-IP

Protein-IP ($^{35}$S-Met)
Methods for detecting myositis-specific autoantibodies (Routine tests)

- **Line blot assay** (EUROIMMUN, Germany)
  - Autoimmune Inflammatory Myopathies 16 Ag (EUROLINE) including Jo-1, PL-7, PL-12, EJ, OJ, SRP, MDA5, NXP-2, TIF-1γ, Mi-2α/β, SAE1, Ku, Ro52, PM-Scl100/75

- **ELISA** (MBL Co. Ltd., Japan)
  - MESACUP™ anti-ARS (Jo-1, PL-7, PL-12, EJ and KS), MESACUP™ anti-MDA5, anti-TIF-1γ and anti-Mi-2
## Association Between Autoantibodies and Subsets of Idiopathic Inflammatory Myopathies

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Autoantibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM</td>
<td>anti–synthetase*, anti–SRP</td>
</tr>
<tr>
<td>DM</td>
<td>anti–synthetase*, anti–Mi–2</td>
</tr>
<tr>
<td>(C)ADM</td>
<td>anti–MDA5*</td>
</tr>
<tr>
<td>CAM</td>
<td>anti–TIF–1γ, anti–NXP–2</td>
</tr>
<tr>
<td>JDM</td>
<td>anti–Mi–2, anti–NXP–2</td>
</tr>
<tr>
<td>Overlap myositis</td>
<td>anti–U1RNP, anti–Ku (Japanese), anti–PM–Scl (Caucasian)</td>
</tr>
<tr>
<td>IMNM</td>
<td>anti–SRP, anti–HMGCR</td>
</tr>
<tr>
<td>IBM</td>
<td>anti–cN1A</td>
</tr>
</tbody>
</table>

*anti-synthetase and anti-MDA5 are associated with interstitial pneumonia*
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