Antibody testing in peripheral neuropathies and myopathies

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Outline

• Anti-ganglioside and anti-glycolipid antibodies
• Antibodies in nodopathies and paranodopathies
• MAG antibody
• Antibodies related to dermatomyositis
• Antibodies related to antisynthetase syndrome
• Antibodies related to necrotizing myopathy
• Antibodies related to IBM
Anatomy of nodes/paranodes

With permission from:
Stathopoulos P et al Nat Rev Neurol 2015
Ganglioside and glycolipid antibodies: structure of antigens

- Letter M, D, T, Q = number of Sialic acid (NeuAc) molecules attached: 1, 2, 3, 4 respectively
- Letter a or b:
## Syndromes associated with antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM1 IgG</td>
<td>AMAN (~50%), AMSAN</td>
</tr>
<tr>
<td>GM2 IgG (or IgM)</td>
<td>AIDP or AMAN after CMV infection</td>
</tr>
<tr>
<td>GD1a IgG</td>
<td>AMAN (~25%), AMSAN</td>
</tr>
<tr>
<td>GD1b IgG</td>
<td>Acute sensory ataxic neuropathy</td>
</tr>
<tr>
<td>GT1a IgG</td>
<td>PCB variant, MFS, acute bulbar palsy</td>
</tr>
<tr>
<td>GQ1b IgG</td>
<td>MFS, BBE, acute bulbar palsy, acute ophthalmoplegia</td>
</tr>
<tr>
<td>GalNAc- GD1a IgG</td>
<td>AMAN, AMSAN</td>
</tr>
<tr>
<td>GM1 IgM</td>
<td>Multifocal Motor Neuropathy (MMN)</td>
</tr>
<tr>
<td>GalNAc-GD1a IgM</td>
<td>MMN (few cases)</td>
</tr>
<tr>
<td>GD1b IgM</td>
<td>CANADA, CANOMAD</td>
</tr>
</tbody>
</table>

**Notes:**

1. IgG antibodies—usually acute immune neuropathies, IgM—chronic
2. Antibodies typically NOT detected in classic AIDP or CIDP

Abbreviations: AMAN= acute motor axonal neuropathy, AMSAN= acute motor/sensory axonal neuropathy, CANADA= chronic ataxic neuropathy with disialosyl antibodies, CANOMAD= chronic ataxic neuropathy, ophthalmoplegia, M-protein, agglutination, disialosyl antibodies, PCB= pharyngocervicobrachial, MFS= Miller Fisher syndrome, BBE=Bickerstaff brainstem encephalitis
Pathogenic role of ganglioside antibodies-focus on GM1 and GQ1b

- GM1-antibody positive AMAN/AMSAN often preceded by C. jejuni diarrhea
- Antibodies proven to be pathogenic by passive transfer experiments, active immunization, and pathology models
- Can cause either rapidly reversible conduction failure (excellent prognosis, recovery in 3-4 weeks) OR complement-mediated axonal degeneration (more poor prognosis)
- GQ1b antigens enriched in extraocular muscles (specifically their neuromuscular junction) and cerebellar molecular layer
Pathogenesis of GM1-AMAN

Ganglioside/glycolipid antibodies: detection methods

- Specificity and sensitivity vary greatly by detection method and by lab
- Most commonly used technique is ELISA
- Target antigen must highly purified
- **False positives can occur, especially in low titers**
- **Some blood samples may contain polyspecific antibodies, especially if obtained while on intravenous immunoglobulin treatment**—avoid it
- Other techniques: Western blotting, IHC, thin layer chromatography (TLC)
- TLC assays useful when lipids difficult to purify because they are present in very small amount in tissues (SGPG), but cumbersome to perform, variable results, difficult quantification
- Emerging role of glycolipid antibody complexes (eg LM-1)

Antibodies against paranodal proteins: Neurofascin-155

- Discovered in 2012¹
- CIDP phenotype with subacute onset, sensory ataxia, distal weakness and severe tremor²
- Combined central and peripheral demyelination syndrome (CCPD, aka MS+CIDP)³
- Importantly: Poor response to IVIG, partial response to steroids in ~50%
- IgG4 antibody, Rituximab may work best²
- Likely pathogenic- distinct nerve biopsy features with paranodal axo-glial detachment, lack of findings seen in typical CIDP⁴ (segmental demyelination, inflammation, onion bulbs)
- IgM NF-155 antibody⁵- 5 patients recently described, 4 CIDP, 1 GBS, similar phenotype to IgG-NF155, more neuropathic pain
- IgM NF-155- nerve biopsy did not show paranodal pathology, treatment response to IVIG in four patients

Antibodies against paranodal proteins: contactin-1 and contactin-associated protein 1 (CASPR1)

• Contactin-1: Described by two groups\textsuperscript{1,2}
• Phenotype: subacute onset, either motor predominant, or sensory ataxia
• Poor response to IVIG, 73\% respond to steroids, good response to Rituximab
• CASPR1: 2 patients (1 GBS, 1 CIDP)
• In GBS, antibody was IgG3, in CIDP, IgG4
• Quadriparesis, sensory symptoms
• Prominent pain was a common feature- binding of patient’s sera to TRPV1 dorsal ganglia neurons\textsuperscript{3}
• CIDP patient responded poorly to IVIG and well to Rituximab

Paranode pathology- antibody negative CIDP (left) vs contactin-1 positive CIDP (right)-

With permission from Koike H et al. JNNP 2017 Jun;88(6):465-473
• 5 patients with IgG reactivity against the nodes of Ranvier (four IgG4, one IgG3)¹
• Severe phenotype associated with conduction block or decreased distal motor amplitude
  4/5 with subacute sensory ataxia
• **Concomitant features: Nephrotic syndrome (2), retroperitoneal fibrosis (1)**
• IVIG and/or corticosteroids effective in 3, Rituximab in 1
• 7% of patients with idiopathic neuropathy tested + for neurofascin antibodies in recent study²
• Unclear significance of + antibodies in idiopathic neuropathy or transient IgM responses in patients with GBS

Neurofascin antibodies: detection methods

- ELISA
- Western Blot
- Cell based assays/flow cytometry
- IHC in rat sural nerve
- Rate of detection: 4-18%\(^1,2,3,4\)
- Optimal method still debated, more research needed

1. Ng JK et al. Neurology 2012; 79: pp. 2241-2248

Images from Kadoya M et al. (with permission).
MAG/SGPG neuropathy

- Associated with IgM monoclonal gammopathy most times
- Typical phenotype: Slowly progressive, distal sensorimotor neuropathy
- Sensory loss and ataxia, tremor (~30-40%), distal weakness
- Electrophysiology: Distally accentuated demyelination (DADS), low terminal latency index (TLI) <0.25
- Antibodies are pathogenic: IgM deposition in myelin sheaths (top image), myelin wide-spacing (bottom image) animal model
- MAG is present in CNS>PNS, but in most cases, antibodies react against the ganglioside SGPG too, which is unique to PNS
- Method of detection: ELISA (most sensitive), Western Blot (far more specific, all + ELISAs should be confirmed)

From neuromuscular.wustl.edu (top image), Hand Clin Neurol 2015 (bottom)
MAG/SGPG neuropathy: recent updates

• **Phenotype more complicated**- some cases with acute or chronic sensorimotor polyradiculoneuropathies, (17%) or even asymmetric neuropathies (3%)\(^1\)

• Response to steroids or IVIG generally poor, PLEX does not remove IgM antibodies well

• **Rituximab treatment**: 2 RCTs, none met primary endpoint (INCAT leg disability score-trial 1, INCAT sensory score-trial 2), but some meaningful secondary endpoints met in both (time 10 minute walk, functional abilities, etc)\(^2,3\)

• **Rituximab helps about 30-50% of patients**, more likely if subacute course, muscle weakness (CIDP-like)\(^4\), less likely if older age, demyelinating pattern

• **15% of patients with paradoxical worsening** after starting Rituximab, can be reversed with IVIG\(^5\)

• Other options: fludarabine, chlorambucil, cyclophosphamide, other B-cell depleting agents, HNK-1 targeted therapy\(^6\)

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When do I order antibodies for neuropathy, and what

<table>
<thead>
<tr>
<th>Clinical syndrome and Electrophysiology</th>
<th>Antibody ordered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute neuropathy/GBS- whether “demyelinating” or “axonal” by initial EMG</td>
<td>GM1, GD1a, GD1b, GT1a, GQ1b, GalNac-GD1a, ?asialoGM1, LM1, *neurofascin-155</td>
</tr>
<tr>
<td>Miller Fisher syndrome</td>
<td>GQ1b, GT1a</td>
</tr>
<tr>
<td>Chronic purely motor syndrome, axonal/demyelinating, suspicious for MMN</td>
<td>GM1, GalNac-GD1a, GM1/GalC complex</td>
</tr>
<tr>
<td>Chronic purely motor syndrome, axonal, strong suspicion of ALS</td>
<td>None</td>
</tr>
<tr>
<td>Sensory ganglionopathy, axonal</td>
<td>IgG GD1b, FGFR3, ?TSHDS, paraneoplastic (Dr Li’s talk)</td>
</tr>
<tr>
<td>CIDP (not DADS)</td>
<td>Contactin-1, Neurofascin-140, 155, and 186, MAG only if IgM monoclonal present</td>
</tr>
<tr>
<td>DADS</td>
<td>MAG, SGPG</td>
</tr>
<tr>
<td>Axonal sensorimotor length dependent neuropathy</td>
<td>None</td>
</tr>
</tbody>
</table>
Antibodies in myopathy

- Myositis-associated (ANA, RNP, Ku) vs Myositis-specific
- Will discuss 4 groups:
  - anti synthetase antibodies
  - antibodies specific to dermatomyositis
  - antibodies related to immune mediated necrotizing myopathy (IMNM)
  - NT5C1-alpha IgG
Antisynthetase antibodies

- Jo1, PL-7, PL-12, OJ, EJ, Ks, Zo (all antigens are amino-acylo tRNA synthetases)
- Jo-1 most well studied
- Antisynthetase syndrome:
  - Weakness due to myositis, myalgia
  - Interstitial lung disease (present in variable percentages, with some antibodies close to 100%) - can flare independently from myositis and cause great morbidity and mortality
- Non-erosive arthritis
- Raynaud syndrome
- Characteristic skin rashes (mechanical hands, shawl sign, usually not typical Gottron’s papules or heliotrope)
- **Characteristic muscle biopsy** - perimysial fragmentation and thickening, necrotic/regenerating fibers adjacent to perimysium, marked histiocytic inflammation (acid, alkaline phosphatase, CD68), intranuclear actin aggregates seen on electron microscopy
Antisynthetase syndrome
## Antibodies related to dermatomyositis

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen function</th>
<th>Epidemiology</th>
<th>Skin features</th>
<th>Juvenile DM</th>
<th>Cancer</th>
<th>ILD</th>
<th>Amyopathic</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mi-2</td>
<td>Nuclear RNA helicase (SNF2)</td>
<td>Hispanic s, young</td>
<td>Cuticle overgrowth, shawl and V sign</td>
<td>+</td>
<td>No</td>
<td>No</td>
<td>-</td>
<td>Excellent, monophasic</td>
</tr>
<tr>
<td>TIF1-γ(p155/140)</td>
<td>Nuclear transcription factor</td>
<td>Bimodal (juvenile +&gt;45)</td>
<td>Typical DM</td>
<td>+++ (20-30%)</td>
<td>+++ (adults)</td>
<td>No</td>
<td>+</td>
<td>Variable, worse with Ca</td>
</tr>
<tr>
<td>NXP-2</td>
<td>Cytoplasm, MORC3</td>
<td>Bimodal</td>
<td>Calcinosis, edema, typical DM</td>
<td>++ (&gt;20%)</td>
<td>++ (adults)</td>
<td>Rare</td>
<td>-</td>
<td>Variable</td>
</tr>
</tbody>
</table>
# Antibodies related to dermatomyositis

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<th>Amyopathic</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-5</td>
<td>Cytoplasm, RNA helicase, IFN induced</td>
<td>Japan/China</td>
<td>Palmar papules, nailfold ulcers</td>
<td>-</td>
<td>Rare</td>
<td>+++ (severe)</td>
<td>+++</td>
<td>Poor</td>
</tr>
<tr>
<td>SAE(SUMO)</td>
<td>Cytoplasm</td>
<td>Typical DM</td>
<td>-</td>
<td>No</td>
<td>No</td>
<td>++ (at onset)</td>
<td>Good</td>
<td></td>
</tr>
</tbody>
</table>
The many skin manifestations of DM/AS
The many skin manifestations of DM/AS
Antibodies related to immune necrotizing myopathy (IMNM)

- **IMNM** is an immune myopathy with extensive myofiber necrosis but minimal to primary inflammation on biopsy
- **Signal recognition particle (SRP) IgG**
  - Two epitopes (44 and 72 kDA)
  - Very severe rapidly progressive myopathy, very high CK levels (>5,000-10,000), often treatment resistant (corticosteroids+IVIG+ Rituximab or MTX ), myocarditis and respiratory muscle involvement
  - If not treated early, fibrosis and irreversible weakness can occur within 6 months or less
- **HMG-CoA reductase antibody (HMGCR) IgG**
  - Associated with previous statin exposure only in 2/3 cases¹
  - **Cases without statin exposure- younger, more aggressive disease, and paraneoplastic**²
  - Careful with some low positive ELISA titers- can be false

Antibody related to IBM: NT5C1-alpha IgG

- Sensitivity 35-70% (so negative does not exclude disease)$^{1,2}$
- Specificity high (~90%) but can have false positives, especially in SLE/Sjogren/MCTD$^3$
- Differences between NT5+ and NT5- IBM patients still to be determined
- Possible that NT5+ associated with more severe course/mortality, dysphagia, facial weakness, lower FVC, less vacuoles and more mitochondrial abnormalities on biopsy$^{4,5}$
- Does not replace the need for muscle biopsy to diagnose IBM

Antibodies: New studies (Pm-Scl)

- Proximal arm > proximal leg weakness, head drop
- ILD ultimately 60%
- Mechanic hands, Raynaud, sclerodactyly
- Biopsy: perivascular inflammation
Antibodies: New studies (U1-RNP)

Muscular and extramuscular features of myositis patients with anti-U1-RNP autoantibodies

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Abstract
Objective
To define the clinical phenotype of patients with myositis with anti-U1-ribonucleoprotein (RNP) autoantibodies.

Methods
In this longitudinal cohort study, the prevalence and severity of clinical features at disease onset and during follow-up in patients with anti-U1-RNP-positive myositis were compared to those with dermatomyositis (DM), immune-mediated necrotizing myopathy (IMNM), and the antisynthetase syndrome (AS).

Results
Twenty anti-U1-RNP-positive patients, 178 patients with DM, 133 patients with IMNM, and 132 patients with AS were included. Anti-U1-RNP-positive patients were younger (~37 years) and more likely to be black (60%) than patients with AS, DM, or IMNM. Muscle weakness was presenting feature in 15% of anti-U1-RNP-positive patients; 80% eventually developed weakness. Four of 7 anti-U1-RNP-positive patients had necrotizing muscle biopsies. Arthritis occurred in 60% of anti-U1-RNP-positive patients; this was increased compared to DM (18%) or IMNM (6%) (all p < 0.01). DM-specific skin features developed in 60% of anti-U1-RNP-positive patients. Interstitial lung disease (ILD) occurred in 43% of anti-U1-RNP-positive patients; fewer patients with DM (13%) and IMNM (6%) and more patients with AS (80%) developed ILD (all p < 0.01). Glomerulonephritis and pericarditis occurred in 25% and 40% of anti-U1-RNP-positive patients, respectively, but rarely in the other groups; these features occurred only in those with concomitant anti-Ro52 autoantibodies. No anti-U1-RNP patient had cancer-associated myositis or died during the study period.

Conclusions
Patients with anti-U1-RNP myositis typically present with proximal weakness and necrotizing muscle biopsies. Arthritis, dermatitis, and ILD are the most common extramuscular clinical features. Pericarditis and glomerulonephritis are uniquely found in patients with anti-U1-RNP-positive myositis.

- Biopsies usually show necrotizing myopathy
- 45% ILD
- Pericarditis and glomerulonephritis distinguishing features
A nice overview of antibodies in myopathy
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Neuropathy antibodies-more controversial topics: NS6S

Pestronk A et al JNNP 2010

Nobile Orazio E et al JNNP 2014
Neuropathy antibodies-more controversial topics: NS6S

- Pathogenicity never convincingly demonstrated (unlike GM1)
- Many false positives in sensory neuropathies (per authors)
- Few false positives in ALS (personal observation, unpublished)
- Results not reproduced by another group- sensitivity in detection of MMN did not improve with addition of NS6S antibody testing
Neuropathy antibodies-more controversial topics:

**TSHDS**

- **TSHDS IgM** antibodies reported by the Washington University group
- Painful sensory>motor axonal neuropathies, non-length dependent (hands>feet), IgM M-protein common\(^1\)
- Pathogenicity never convincingly documented and **no information about treatment response**
- 6/58 cases nerve biopsy- 5 with capillary pathology and C5b9 complement deposition\(^1\).
- Thickened capillaries is completely non-specific
- C5b9 deposition in endoneurial capillaries not specific for immune disease- can be seen with diabetic neuropathy
- No biopsy showed abnormal IgM staining of capillaries or myelin sheaths unlike other antibodies associated with IgM-paraproteinemic neuropathy (MAG)
- May be meaningful to order this antibody with (large or small fiber) non-length dependent sensory neuropathies, but not routinely in length dependent distal sensory neuropathies

Neuropathy antibodies-more controversial topics:

**FGFR3**

- Described in 2014 by a French group\(^1\)
- 16/106 patients with idiopathic neuropathy, +sera, 1/211 controls
- 90% of antibody positive patients fulfilled criteria for **sensory ganglionopathy** (large or small fiber), trigeminal nerve involvement common
- Many had coexistent autoimmune disorders
- **No information about treatment response**
- Not meaningful to order this antibody in length-dependent axonal sensorimotor neuropathies, demyelinating neuropathies, motor neuropathies, or phenotypes not fitting with the original description.

1. Antoine JC et al JNNP 2014
Biggest problem with FGFR3 antibodies

• 5 patients with positive antibodies in variable titers recently reported\(^1\)
• Subsequent re-testing 6 months later- undetectable in two, significant drop in one
• Variable presentations- from length dependent axonal sensorimotor neuropathy to small fiber neuropathy, to demyelinating neuropathy with proximal weakness
• Comorbidities present: diabetes, sarcoidosis, polycythemia
• Major changes in antibody titers between two measurements despite no immunotherapy indicates variability and inconsistency of ELISA results