Antibody mediated conditions in the peripheral nervous system; What to order?

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Learning objectives

• To review and understand some aspects of the more common antibody mediated peripheral neurological conditions
• To know the latest recommendations on laboratory diagnostic algorithms in these conditions
Disclosures

• UBC Neuroimmunology Lab – Medical Director
• Neurocode Labs (UBC sequencing spin off)—Medical Director
• Icellate Medical AB -- CMO
The conditions

- Myasthenia conditions
- Conditions related to antibodies against Gangliosides
- Paraneoplastic conditions in PNS
Myasthenia gravis the disease

- **Antibody-mediated autoimmune disorder of the neuromuscular junction**
  - Specialized synapse between motor neurons and skeletal muscle fibers
  - Autoantibodies targeting proteins at NMJ disrupt neuromuscular transmission
- Characterized by fluctuating skeletal muscle weakness & fatiguability

**Epidemiology**
- Incidence: 1.7-21.3 per million per year
- Prevalence: 15-170 per million
- Both incidence and prevalence increasing, especially among elderly
- Age of onset: bimodal peak in women ages 30-40 & ~70, and unimodal in men ~70
Clinical features of MG

• Weakness worsens with repeated activity, improves with rest
• 3 subtypes: ocular, bulbar, generalized
  
  Ptosis (drooping of upper eyelids)
  Diplopia (double vision)
  Ophthalmoplegia (paralysis of extraocular muscles)
  Dysarthria (difficulty with articulation)
  Dysphagia (difficulty swallowing)
  Dysphonia (eg. hoarseness)
  Facial muscle weakness (eg. chewing)
  Weakness of limb muscles (arms and legs)
  Difficulty with respiration / crisis

• Thymic abnormalities
  • thymic lymphoid hyperplasia, characterized by B cell germinal centers
  • Thymoma, thymic epithelial tumour
Muscle weakness result of impairment in physiology of neuromuscular junction

Koneczny et al, J Anat. 2013
Myasthenia Gravis Antibodies

• Acetylcholine receptor antibodies (RIPA and CBA)
• Muscle specific kinase antibodies
• Cortactin; positive in 24% of dSNMG, and promise mild disease
• Titin; Thymoma in younger patients, 13% of dSNMG
• Ryanodine; Thymoma
• LRP4; overlapping, of questionable use clinically
• Kv1.4 potassium channel Ab in severe MG, cardiac and thymoma

• A. Vincent, Ann NY Acad Sci., 2018 Jan;
Acetylcholine receptor antibodies (AChR Abs)

- **AChR** is main autoantigen in MG
- ~85% of GMG patients have AChR Abs

**AChR Structure**
- Transmembrane ligand-gated ion channel
- 5 subunits arranged around central ion channel
- 2 isotypes (fetal & adult)
- 2 binding sites for ACh
- Main immunogenic region

- AChR-Abs induce **loss of functional AChR at NMJ**
  - Complement-mediated damage of muscle endplate by IgG1 and IgG3 antibodies
  - Antigenic modulation
  - Blocking of ACh binding site

- Loss of AChR results in lower sensitivity to ACh, and **decreased amplitude of endplate potential** to below threshold required for action potential
Acetylcholine receptor antibodies (AchR Ab)

- IgG 1-3
- Complement mediated destruction and antibody mediated internalization
- Radio Immuno Precipitation Assay (RIPA) is the gold standard but...cell based assay (CBA) is even more sensitive and as specific
- CBA is superior in children (antibodies with less affinity)
- Some Ab’s bind one alpha subunit on two neighboring AchR...

- A. Vincent, Ann NY Acad Sci., 2018 Jan
MuSK Ab driven MG – symptoms

• MuSK+ MG a serious disease with a high rate of life-threatening symptoms
• Lack of diurnal symptom fluctuations
• Unresponsiveness to acetylcholinesterase inhibitors (AChE-Is)
• Negative results of electrophysiologic testing (facial or neck muscles)
• Difficulty in differentiating MuSK+ MG from amyotrophic lateral sclerosis
• Cholinergic hyperactivity is a well-known trait of MuSK+ MG.
• Responsible for patients’ frequent intolerance to AChE-Is, muscle cramps, and fasciculation's

• A. Vincent, Ann NY Acad Sci., 2018 Jan
MuSK Ab driven muscle atrophy

- Atrophy of facial, bulbar, and extrinsic ocular muscles
- Weakness, a myopathy pattern on electromyography
- Muscle thinning and fatty replacement on magnetic resonance imaging
- Childhood-onset disease, widespread atrophy involving limb and paraspinal muscles with severe scoliosis
- Muscle wasting evident in the early stages
- Chronic in some patients -- causing disability
- Muscle biopsy studies in MuSK\(^+\) MG patients reported myopathic features and mitochondrial abnormalities

- A. Vincent, Ann NY Acad Sci., 2018 Jan
Muscle-specific kinase antibodies (MuSK-Abs)

- Of 15% AChR-Ab negative patients, variable proportion have antibodies against MuSK.
- Clinically, MuSK-MG patients have more severe disease course, with bulbar symptoms, more likely to be hospitalized for respiratory problems, most often female.

MuSK Structure

- Single domain transmembrane protein
- Large extracellular sequence of 4 IgG-like domains
- Intracellular tyrosine kinase domain
- Upon activation by agrin, MuSK induces clustering of AChR through pathway involving rapsyn.

- MuSK-Abs appear to have different pathogenic mechanism than AChR-Abs
- IgG4; not complement activating, monovalent
- Likely directly interfere with function of MuSK to cluster AChR.
MuSK antibodies

• IgG4 most pathological (non complement activating)
• Act by reducing MuSK phosphorylation
• Agrin is released from the motor nerve, interacts with LRP4
• Prevents the activation of DOK7 pathway
• Prevents clustering of AchR at the NMJ via Rapsyn
• Rituximab often provides sustained clinical improvement/remission
• Drugs that enhance MuSK phosphorylation such as SPH2 phosphatase inhibitor may be useful in patients with severe disease

• A. Evoli, Ann NY Acad Sci., 2018 Jan;1412(1):82-89
Utility of MuSK Ab

• Mutually exclusive to AchR Ab
• Different pathogenesis (blocking vs. destruction/increased recycling)
• Different treatment -- Rituximab, phosphatase inhibitor (not IV-IG, Mestinon)
• 5% of Ocular only patients are positive – predictive
• Symmetrical ophthalmoparesis of lateral gaze
• MuSK+ MG is a severe disease and though an earlier diagnosis associated with a more timely treatment prevents symptom progression to the most severe clinical manifestations
• Most patients who go into complete stable remission receive early and aggressive treatment with steroids
• All Pt. with suspected MG should be tested as a reflex from a negative AchR Ab
  
  • A. Evoli, Ann NY Acad Sci., 2018 Jan;1412(1):82-89
Rituximab in MuSK Ab driven MG

• Evidence from observational studies and meta-analyses favors the use of rituximab in all MG subtypes, particularly in MuSK+ MG. I

• A great majority of patients with MuSK Abs respond to rituximab, improvement is significant and long-lasting, and immunosuppressive medication could be reduced or withdrawn

• Clinical response was associated with a marked reduction of serum MuSK Ab titer

• Measure MuSK Ab levels for recurrence

• A. Evoli, Ann NY Acad Sci., 2018 Jan;1412(1):82-89
Clinical Case Study: Rituximab Treated MuSK-MG

- 44-year-old woman who presented with diplopia
- Positive for anti-MuSK antibodies
- After 1 year, developed weakness of tongue and facial muscles – slurred speech, fatigued chewing, choking episodes; ADL & titer increased
- First treated with PLEX with resolution of symptoms, decrease of ADL
- Upon symptom return, treated with 2 infusions of Rituximab over 2 weeks -> anti-CD20 monoclonal antibody therapy; depletes circulating B cells
- Patient achieved complete remission and negative MuSK-Ab titer for 6mo
IgG-specific cell-based assay detects potentially pathogenic MuSK-Abs in seronegative MG

**ABSTRACT**

**Objective:** To increase the detection of MuSK-Abs using a CBA and test their pathogenicity.

**Methods:** Sera from 69 MuSK-RIA-positive patients with myasthenia gravis (MG) (Definite MuSK-MG), 169 patients negative for MuSK-RIA and AChR-RIA (seronegative MG, SNMG), 35 healthy individuals (healthy controls, HC), and 16 NMDA receptor-Ab-positive (NMDAR-Ab) disease controls were tested for binding to MuSK on a CBA using different secondary antibodies.

**Results:** Initially, in addition to 18% of SNMG sera, 11% of HC and 19% of NMDAR-Ab sera showed positive binding to MuSK-transfected cells; this low specificity was due to anti-IgG (H+L) detection of IgM bound nonspecifically to MuSK. Using an IgG Fc gamma-specific secondary antibody, MuSK-Abs were detected by CBA in 68/69 (99%) of Definite MuSK-MG, 0/35 HC, 0/16 NMDAR-Ab, and 14/169 (8%) of SNMG sera, providing increased sensitivity with high specificity. The RIA-negative, CBA-positive MuSK-IgG sera, but not IgM-MuSK-binding sera, reduced agrin-induced AChR clustering in C2C12 myotubes, qualitatively similar to RIA-positive MuSK-Abs.

**Conclusions:** An IgG-specific MuSK-CBA can reliably detect IgG MuSK-Abs and increase sensitivity. In the MuSK-CBA, IgG specificity is essential. The positive sera demonstrated pathogenic potential in the in vitro AChR-clustering assay, although less effective than Definite MuSK-MG sera, and the patients had less severe clinical disease. Use of IgG-specific secondary antibodies may improve the results of other antibody tests.

**Classification of evidence:** This study provides Class III evidence that an IgG-specific MuSK-CBA identifies patients with MG. *Neurol Neuroimmunol Neuroinflamm 2017;4:e357; doi: 10.1212/NXI.0000000000000357*
Biacore Technology

• Biosensor technology that uses surface plasmon resonance to monitor bio-molecular interactions in real time

• Principle
  • Ligand is immobilized (covalently bound) onto a sensor chip surface
  • The analyte is passed over the surface
  • Binding of analyte monitored by SPR
Optimization of Parameters & Assay Specificity

- Varied immobilization density, serum dilution, sample buffer
- 20 HC, 10 MuSK-Ab negative, and 10 MuSK-Ab positive sera
- Assay cutoff = mean + 3SD of 20 healthy controls

- Optimized assay conditions: high MuSK density (>4500RU), 1/10 serum dilution, HBS-EP-BSA sample buffer
- Assay highly specific for MuSK-Abs in serum
MuSK antibody Biacore assay highly reproducible

- Intra-assay variability – 3 samples assayed 12x

<table>
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<th>Sample</th>
<th>Mean Binding Response (RU)</th>
<th>SD</th>
<th>% CV</th>
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<td>Positive Medium</td>
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<td>Positive High</td>
<td>228.12</td>
<td>18.55</td>
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- Inter-assay variability – 3 samples assayed 3x on 3 days

<table>
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<th>Sample</th>
<th>Mean Binding Response (RU)</th>
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<th>% CV</th>
</tr>
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<tbody>
<tr>
<td>Positive Low</td>
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<td>Positive Medium</td>
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<tr>
<td>Positive High</td>
<td>226.63</td>
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MuSK-Ab Isotype and IgG Subclass Analysis

• Following sample injection, sequential injection of antiserum against human IgM, IgG, IgG1, 2, 3, 4

• Isotype/subclass trends in 19 MuSK-MG patients
  – Predominant IgG subclass was IgG4; all 19 patients expressed continuously over course of disease
  – Followed by IgG1 (13/19 patients)
  – IgG3 at low levels by 13/19, IgG2 expressed only by 1 patient
  – 11/19 patients had IgM antibodies
    • 4 expressed continuously, one patient had high levels until month 60
Peripheral nervous systems (PNS)

• Highly complex structure
• Longest axons in the human body
• Motor, sensory, and autonomic neurons
• Supported by both myelin-forming and non-myelinating Schwann cells
• Most resides within an immunologically privileged site within the blood–nerve barrier (the PNS equivalent of the blood–brain barrier)
• Exception: the dorsal and ventral spinal roots and the sensory and motor nerve terminals that lie in sites more freely exposed to circulating factors

• J. Goodfellow et al. Progress in Molecular Biology and Translational Science, Vol. 156, 2018
Gangliosides

• On the bimolecular lipidic layers of all the cell membranes,

• Part of a large family of glycosphingolipids composed of a molecule of ceramide binds a sialyl oligosaccharide (beta-glycosidic bond) and form clusters (lipid rafts) on the surface of neuronal membranes

• Nomenclature of Svennerholm:

• – G refers to the ganglioside series. – The second letter refers to the number of sialic acid residues (M, D, T, Q, P: mono, di, tri, quarter, penta). – The number (1, 2, 3) refers to the order of migration of the ganglioside on thin-layer chromatography – The lower case letter (a, b, c) refers to isomeric forms

GBS

• Guillain, Barre’, and Strohl in 1916
• A syndrome of acute, flaccid weakness with loss of tendon reflexes, high cerebrospinal fluid (CSF) protein levels and normal CSF inflammatory cell counts
• Homologies in membrane oligosaccharides of microbes and humans favor the hypothesis that the phenomenon known as “molecular mimicry”
• Campylobacter jejuni enteric infections are most commonly associated with the axonal forms of Guillain-Barré syndrome (GBS)
• Lipo-oligosaccharide (LOS) structures existed on C.jeuni that are structurally similar to gangliosides
GBS

- Cytomegalovirus is a frequent virus to be associated with GBS and associates with the AIDP variant
- Mycoplasma pneumoniae infection is associated with neurological complications, including encephalitis and GBS
- Haemophilus influenzae type b is also reported in 1%–9% of cases as a trigger of GBS
- Vaccinations
- Zika virus

- J. Goodfellow et al. Progress in Molecular Biology and Translational Science, Vol. 156, 2018
Guillian Barre syndrome – the acute

1. Acute inflammatory demyelinating polyneuropathy (AIDP), North America and Europe; no autoantibody has been clearly identified in association with AIDP

2. Axonal form, acute motor axonal neuropathy (AMAN), Asia and South America, associated with GD1a, GM1a, GM1b, and GalNAcGD1a antibodies (IgG isotype)

3. Axonal form with sensory involvement, acute motor and sensory axonal neuropathy (AMSAN), Asia and South America, associated with GM1 and GD1a antibodies (IgG isotype)

4. Miller Fisher syndrome, characterized by ophthalmoplegia, ataxia, and areflexia, was associated with GQ1b and GT1a antibodies, IgG isotype

J. Goodfellow et al. Progress in Molecular Biology and Translational Science, Vol. 156, 2018
Pharyngeal-Cervical-Brachial Variant

- Acute oropharyngeal, neck, and shoulder weakness without significant limb weakness
- AntiGT1a or anti-GD1a IgG

- J. Goodfellow et al. Progress in Molecular Biology and Translational Science, Vol. 156, 2018
Multifocal motor neuropathy (MMN)

- With conduction blocks: is a slowly progressive disease, with asymmetrical motor deficit of the limbs, without or with minimum sensory deficit, often with distal involvement of an upper limb
- Antibody reactivity against GM1 ganglioside, of IgM type but polyclonal
- The antibody is present in 30–80% of patients but also in small percentages of patients with other dysimmune neuropathies, or with motor neuron disease
- Anti-GM1 IgM seropositivity is not sufficient to confirm the diagnosis, which
- Three patterns of antibody specificity: GD1b and asialo-GM1, GM1 and GM2, isolated GM1 MMN

- J. Goodfellow et al. Progress in Molecular Biology and Translational Science, Vol. 156, 2018
Chronic Inflammatory Demyelinating Polyradiculoneuropathy -- CIDP

• A relapsing-remitting and less fulminant version of AIDP
• Clinically there is areflexia and variable sensory impairment and motor weakness
• Corticosteroids have also been shown to be effective in treating CIDP but not in AIDP
• Lack of clear association with antiganglioside antibodies

• J. Goodfellow et al. Progress in Molecular Biology and Translational Science, Vol. 156, 2018
Laboratory aspects

• Anti-ganglioside antibody detection keeps being affected by technical difficulties
• Thin layer chromatography (TLC) is the gold standard and reference method
• In-house or commercial ELISAs and immuno-line/-dot blots share suboptimal analytical performance vs TLC
• “GA Generic Assays GmbH” immuno-blot stand out for its analytical performance, the most similar to TLC
• INCAT-ELISA for the determination of anti-GM1 IgM and IgG, and anti-GQ1b IgG is fairly reliable

Combinations of two gangliosides appear to form target epitopes in biological membranes
What tests to use?

• Test patients who manifest clinical phenotypes highly compatible with phenotype-specific anti-ganglioside antibodies and to restrict the testing to anti-GM1 and anti-GQ1b antibodies

• Additional antibodies should be selected when particular conditions are suspected: GM2 IgM in GM1 IgM-negative MMN; GD1b, GD3, GT1b, GQ1b IgM in CANOMAD; GT1a IgG in pharyngeal cervical-brachial variant of GBS

• Consider only high levels of anti-ganglioside antibody positivity, as low levels of positivity have non-specific meaning

Anti-MAG neuropathy

- A chronic, slowly progressive demyelinating neuropathy
- Mainly affecting the sensory nerves manifesting as a coarse postural tremor of the arms
- Progresses over time to a more widespread and disabling sensory ataxia with impaired gait and eventually often motor involvement with distal muscle weakness

Anti MAG antibodies
MAG Ab testing

• Anti-MAG IgM testing is carried out with a commercial ELISA kit (Bühlmann, Schönenbuch, Switzerland)
• Manufacturer suggest 1000 BTU (Buhlmann Titer Unit)
• 1000-10000 BTU is Low Positive with False positives
• Above 10000 BTU High specificity (close to 100%) but loss of some sensitivity (25%)

Who should be tested for MAG Ab

• Testing for anti-MAG antibodies is required in:
  • 1. All patients with both IgM MGUS/Waldenstoms/IgM secreting lymphoma and neuropathy
  • 2. In those with a slowly progressive neuropathy of unknown etiology, even in the absence of detectable IgM paraproteins, as serum monoclonal IgM concentrations can be below the detection limits of the immunoelectrophoretic techniques

CANOMAD

• Anti-MAG antibody seronegative patients with IgM-MGUS

• Autoantibodies against a “disialosyl epitope” common to a series of ganglioside “b isomers” including GD1b, GD3, GT1b, and GQ1b.

• Clinical phenotype of this paraproteinemic neuropathy is summarized in the acronym CANOMAD (Chronic Ataxic Neuropathy, Ophthalmoplegia, IgM paraprotein, cold Agglutinin, Disialosyl antibodies)

Paraneoplastic neuropathies

• 1. A direct (pathogenic) link between the tumor and neuropathy is demonstrated
• 2. Seronegative sensory neuronopathies (SNNs) with cancer
• 3. Neuropathies improved by tumor treatment
• 4. Any neuropathy occurring within 2 years of a cancer

• J-C Antoine, Current Opinion in Neurology. 30(5):513–520, OCT 2017
Paraneoplastic neurologic disorders in small cell lung carcinoma
A prospective study

Paul Gozzard, Mark Woodhall, Caroline Chapman, Anjan Nibber, Patrick Waters, Angela Vincent, Bethan Lang, Paul Maddison

First published June 24, 2015, DOI: https://doi.org/10.1212/WNL.00000000000001721
Most frequent autoantibodies in SCLC

Peripheral Neurological Disorders (PND) were quite prevalent (9.4%),

Most frequent; Lambert-Eaton myasthenic syndrome (3.8%), subacute sensory neuronopathy (1.9%) and limbic encephalitis (1.5%)

Eighty-seven percent of all patients with PNDs had antibodies to SOX2 (62.5%), HuD (41.7%), or P/Q VGCC (50%), irrespective of their syndrome.

Lower frequencies: GABAb receptor [12.5%] and N-type VGCC [20.8%]) or very rarely (GAD65, amphiphysin, Ri, CRMP5, Ma2, Yo, VGKC complex, CASPR2, LGI1, and NMDA receptor [all <5%]

*P. Gozzard, Neurology 2015(3)*
Lambert Eaton Myasthenic syndrome

- Paraneoplastic or primary autoimmune neuromuscular junction disorder characterized by proximal weakness, autonomic dysfunction and ariflexia
- 90-100% antibodies against the P/Q-type voltage-gated calcium channels (motor neuron) on presynaptic nerve terminals or N-type (autonomic)
- SOX1 Ab
- History of cancer up to 6 years later… but NT-LEMS is 50% (different treatment)
- SCLC most common
- Electro physiological: Low CMAP amplitude at rest, a decremental response at low rates of RNS, an incremental response at high-rate stimulation or after brief exercise
- 3,4-diaminopyridine is effective symptomatic treatment of LEMS

- V. Kesner et al. Neurologic Clinics, 2018-05-01, Volume 36, Issue 2, Pages 379-394
Subacute sensory neuropathy

• The (most) frequent paraneoplastic neurological syndrome
• T-cell mediated and targets sensory neurons in dorsal root ganglia
• Onset is subacute or rapidly progressive
• Sensory loss is frequently multifocal or asymmetrical involving the upper limbs
• Anti-Hu Ab (and anti-CV2/CRMP5)

• J-C Antoine, Current Opinion in Neurology. 30(5):513–520, OCT 2017
Summary – auto antibodies in peripheral neurological conditions

• MG: Standard of care; AchR Ab with reflex to MuSK Ab for every patient suspected of MG (even ocular only Pt.). In addition, Cell based high sensitivity AchR for dSNMG

• MG: other Ab’s are more experimental and clinical utility is to be validated further, most interesting are Titin and Cortactin in dSNMG

• Antibodies directed to GM1, GQ1b, and disyalilated gangliosides, and anti-MAG antibodies in acute or chronic motor or sensory-motor neuropathies with or without monoclonal IgM

• VGCC, anti-Hu and (anti-CV2/CRMP5) antibodies allow when they are detected the diagnosis of paraneoplastic neuropathies
Thanks for your attention
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