Autoantibody Testing in the Diagnosis of Autoimmune Neurological Disorders

Lisa K. Peterson, Ph.D., D(ABMLI)
Assistant Professor, University of Utah School of Medicine
Medical Director, Immunology
Learning Objectives:

• Understand the role of autoantibody testing in diagnosis and management of autoimmune neurologic disorders

• Compare and contrast methods used to detect the relevant autoantibodies

• Describe different strategies for autoantibody testing
What are autoimmune neurologic disorders?

- Disorders of the nervous system caused by an aberrant immune response
- Identified by autoantibody marker detected in serum or cerebrospinal fluid (CSF)
- Antigen-specific
- Paraneoplastic or idiopathic
- Presentation:
  - Subacute onset of symptoms
  - Can affect any part of the nervous system
  - Often multifocal
  - Fluctuating disease course
- Risk factors:
  - Coexisting autoimmune disease  
    (type 1 diabetes mellitus, thyroid disease)
  - Family history of autoimmune disease
  - Cancer history
  - Smoking history

http://www.aruplab.com/topics/PNS
Symptoms of Autoimmune Neurologic Disorders

- Fever
- Headache
- Pain
- Seizures
- Cognitive impairment (confusion, memory issues, attention deficit, dementia)
- Psychosis, agitation (hallucinations, delusions, paranoia)
- Loss of consciousness
- Speech, hearing and language dysfunction
- Loss of sensation or paralysis in certain areas of the face or body
- Muscle weakness
- Movement disorders (myoclonus, tremor, dyskinesia)
- Dysautonomia (hypoventilation, tachycardia, hypertension, hyperthermia)
- Optic neuropathy/retinopathy

www.encephalitis.info/support/information/practical-resources-on-encephalitis/effects-of-encephalitis/
Differential Diagnosis

- Viral, bacterial and other (protozoan e.g. toxoplasmosis)
- Brain tumors
- Stroke
- Drug reactions
- Metabolic disturbances
- Psychiatric disorders
- Neurodegenerative disorders
Evaluation of Autoimmune Neurologic Disorders

Linnoila and Pittock. Semin Neurol. 382-396
Neuronal Autoantibodies

- Autoantibodies defined by cellular location of target antigens
  - Intracellular
    - Nuclear
    - Cytoplasmic
    - Enzymes transcription factors
    - RNA-binding proteins
  - Plasma membrane or secreted protein
    - Neurotransmitter receptors
    - Ion channels
    - Ion channel–complex components
    - Water channels

- Significance
  - Diagnostic
  - Prognostic
  - Determine treatment and management strategies

https://www.aacc.org/publications/cln/articles/2012/march/autoantibody-markers
Autoimmune Neurologic Disorders

- Antibody-associated disorders of the nervous system
- Diverse group of syndromes
- Currently can be broadly divided into 2 categories based on cellular location:
  - Autoimmune disorders associated with antibodies to intracellular neuronal antigens (cytosolic or nuclear)
    - Classic paraneoplastic neurological syndrome (PNS), very rare
  - Autoimmune disorders associated with antibodies to neuronal cell-surface or synaptic receptors, common
    - Autoimmune encephalitis
    - Autoimmune gastrointestinal dysmotility
    - Autoimmune epilepsy
    - Autoimmune dysautonomia
    - Autoimmune dementia
    - Autoimmune neuronopathy
    - Autoimmune Neuromuscular Junction (NMJ) disorders
Classic Paraneoplastic Neurological Syndromes (PNSs)

- Associated with remote effects of tumors; occur in less than 1% of all cancers
- Characterized by the presence of onconeural antibodies, highly specific markers of underlying malignancy
- Antibodies target tumor antigens that are normally expressed only in neurons
- Antibodies may be beneficial by keeping the tumor in check, but can cause severe neuronal damage when they gain access to the nervous system
- First antibodies identified using brain tissue sections
  - Intracellular proteins
  - Poor prognosis - irreversible neuronal killing
- Monophasic, limited clinical response, and affect older adults
- Symptoms often precede tumor detection, alert search for tumor or recurrence
- Not typically responsive to immunotherapy, but improvement is seen upon removal of tumor
Classic Paraneoplastic Neurological Syndromes (PNSs)

- **Syndromes of the central nervous system (CNS)**
  - Paraneoplastic encephalomyelitis (PEM)
  - Limbic encephalomyelitis (LE)
  - Paraneoplastic cerebellar degeneration (PCD)
  - Opsoclonus-myoclonus (OM)

- **Syndromes of peripheral nervous syndromes (PNS)**
  - Paraneoplastic sensory neuronopathy (PSN)
  - Chronic gastrointestinal pseudo-obstruction

- **Syndromes of the neuromuscular junction and muscles**
  - Myasthenia gravis (MG)
  - Lambert-Eaton myasthenic syndrome (LEMS)
  - Acquired neuromyotonia

http://www.aruplab.com/topics/PNS
# Classic Antibody-associated Paraneoplastic Neurological Syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Antibody</th>
<th>Common Cancer Associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEM including cortical, limbic, brainstem encephalitis, PCD, myelitis, PSN, autonomic dysfunction</td>
<td>Anti-Hu</td>
<td>SCLC</td>
</tr>
<tr>
<td>PCD</td>
<td>Anti-Yo</td>
<td>Gynecological, breast</td>
</tr>
<tr>
<td>PCD, brainstem encephalitis, opsoclonus-myoclonus</td>
<td>Anti-Ri</td>
<td>Gynecological, breast, SCLC</td>
</tr>
<tr>
<td>PEM, PCD, chorea, peripheral neuropathy</td>
<td>Anti-CV2/CRMP5</td>
<td>SCLC, thymoma</td>
</tr>
<tr>
<td>Limbic, hypothalamic, brainstem encephalitis (infrequently PCD)</td>
<td>Anti-Ma</td>
<td>Germ-cell tumors of testis</td>
</tr>
<tr>
<td>Cancer-associated retinopathy</td>
<td>Anti-recoverin</td>
<td>SCLC</td>
</tr>
<tr>
<td>PCD</td>
<td>Anti-Tr</td>
<td>Hodgkin lymphoma</td>
</tr>
</tbody>
</table>

Abbreviations: PEM, paraneoplastic encephalomyelitis; PCD, paraneoplastic cerebellar degeneration; PSN, paraneoplastic sensory neuronopathy; CRMP5, collapsin response mediator protein 5.
### NUCLEAR AND CYTOPLASMIC SPECIFICITIES

<table>
<thead>
<tr>
<th>ANTIBODY</th>
<th>ONCOLOGICAL ASSOCIATION</th>
<th>APPROX. FREQUENCY OF CANCER</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANNA-1</td>
<td>Small-cell lung carcinoma, neuroblastoma, thymoma</td>
<td>90%</td>
</tr>
<tr>
<td>ANNA-2</td>
<td>Small-cell lung carcinoma, breast adenocarcinoma</td>
<td>90%</td>
</tr>
<tr>
<td>ANNA-3</td>
<td>Aerodigestive carcinoma</td>
<td>90%</td>
</tr>
<tr>
<td>AGNA-1 (SOX1)</td>
<td></td>
<td>90%</td>
</tr>
<tr>
<td>PCA-2</td>
<td>Small-cell lung carcinoma</td>
<td>90%</td>
</tr>
<tr>
<td>PCA-Tr</td>
<td>Hodgkin lymphoma</td>
<td>90%</td>
</tr>
<tr>
<td>CRMP-5</td>
<td>Small-cell lung carcinoma, thymoma, thyroid, or renal</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td>carcinoma</td>
<td></td>
</tr>
<tr>
<td>Amphiphysin</td>
<td>Small-cell lung carcinoma, breast adenocarcinoma</td>
<td>90%</td>
</tr>
<tr>
<td>GAD65</td>
<td>Occasionally (e.g., thymoma)</td>
<td>&lt; 10%</td>
</tr>
</tbody>
</table>

### PLASMA MEMBRANE SPECIFICITIES

<table>
<thead>
<tr>
<th>ANTIBODY</th>
<th>ONCOLOGICAL ASSOCIATION</th>
<th>APPROX. FREQUENCY OF CANCER</th>
</tr>
</thead>
<tbody>
<tr>
<td>VGKC-complex*</td>
<td>Small-cell lung carcinoma, thymoma, adenocarcinoma of breast, prostate</td>
<td>&lt; 15%</td>
</tr>
<tr>
<td>(Kv1 potassium channel)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NMDA receptor</td>
<td>Teratoma (ovarian or extra-ovarian)</td>
<td>50%</td>
</tr>
<tr>
<td>AMPA receptor</td>
<td>Thymoma, lung and breast carcinoma</td>
<td>70%</td>
</tr>
<tr>
<td>GABA-B receptor</td>
<td>Small-cell lung carcinoma, other neuroendocrine neoplasm</td>
<td>70%</td>
</tr>
<tr>
<td>P/Q and N-type</td>
<td>Lung, breast or gynecologic carcinoma</td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td>calcium channel</td>
<td></td>
</tr>
<tr>
<td>Muscle AChR</td>
<td>Thymoma, lung, breast, gynecologic, or prostate carcinoma</td>
<td>&lt; 15%</td>
</tr>
<tr>
<td>Neuronal ganglionic AChR</td>
<td>Miscellaneous carcinomas, thymoma</td>
<td>&lt; 15%</td>
</tr>
</tbody>
</table>

**Abbreviations:** AGNA, anti-glia/neuronal nuclear antibody; ANNA, antisynaptic nuclear antibody; PCA, Purkinje cell cytoplasmic antibody; CRMP-5, collapsin response-mediator protein-5; GAD65, glutamic acid decarboxylase-65; VGKC, voltage-gated potassium channel; NMDA, N-methyl D-aspartate; AMPA, \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid; GABA, gamma-aminobutyric acid; AChR, acetylcholine receptor.
Encephalitis

- Acute inflammation of the brain
  - Infection invading the brain (infectious)
  - Immune attack
    - Post-infection
    - Autoimmune
- Variable symptoms and rate of development which reflect the specific areas of the brain affected by inflammation
- Onset associated with ‘flu-like illness or headache
- Alteration in level of consciousness is usually serious
  - May range from mild confusion or drowsiness, to loss of consciousness and coma
- Other symptoms include a high temperature, seizures, aversion to bright lights, inability to speak or control movement, sensory changes, neck stiffness, or uncharacteristic behavior
- Some individuals may also experience hallucinations and vivid nightmares during the acute period of the encephalitis
- Differential diagnosis includes infectious, metabolic and toxic causes of encephalitis, but it is essential that an autoimmune etiology is considered early in the differential diagnosis due to the potential benefit of immunotherapy and the potential to trigger the search for cancer.

Autoimmune Encephalitis

- Autoantibodies in serum and/or CSF
  - Some cases are paraneoplastic
- Diverse clinical presentation
- Improves with immunotherapy
- Limbic encephalitis
  - Confusional state with loss of orientation (delirium), and usually occurs with 1 or more signs of cognitive decline (generally memory problems), seizures, altered mood and personality, and sleep disorders
- NMDA receptor encephalitis
  - Affects more regions of the brain than the limbic system and therefore is not classified as a limbic encephalitis. However, it is often discussed in association with the limbic encephalitis disorders.
  - Progressive illness that typically starts with psychosis, memory deficits, seizures and verbal deficits developing into a state of unresponsiveness with catatonic features.

http://aaemra.blogspot.com/2015/02/a-whole-herd-of-zebras.html
Autoantibodies associated with autoimmune encephalitis

Since 2007 (~1 per year)

- NMDA
- VGKC complex (LGI1, CASPR2, others?)
- AMPA
- GABA-B
- GABA-A
- DPPX
- GlyR
- mGluR5
- IgLON5
Disorders of the Neuromuscular Junction (NMJ)

- Specialized synapse with a complex structural and functional organization
- Types of disease
  - Myasthenia gravis, the most common NMJ disorder
    - Muscle weakness that can vary in type and severity, ptosis, diplopia, unstable or waddling gait, a change in facial expression, difficulty swallowing, shortness of breath, dysarthria
    - Most patients have antibodies to the muscle acetylcholine receptor (AChR)
    - ~10% have AChR antibodies that are only identified by novel methods
    - ~5% muscle-specific kinase (MUSK) antibody positive, ocular disease
  - Lambert Eaton Myasthenic syndrome (LEMS), less common
    - Proximal muscle weakness, depressed tendon reflexes, posttetanic potentiation, and autonomic changes
    - Initial presentation can be similar to that of myasthenia gravis (MG)
    - Presynaptic failure to release enough packets of ACh
    - Antibodies to the presynaptic voltage-gated calcium channels (VGCC)
  - Neuromyotonia, Morvan syndrome, faciobrachial dystonic seizures, others
    - Muscular hyperactivity, muscle cramps, stiffness, myotonia-like symptoms, associated walking difficulties, hyperhidrosis, myokymia, fasciculations, fatigue, exercise intolerance, myoclonic jerks and other related symptoms
    - Antibodies to the voltage-gated potassium channels (VGKC) complex (targets include LGI1, CASPR2, and other undefined antigens)
  - Autonomic neuropathy
    - Dysautonomia and GI dysmotility
    - Ganglionic AChR antibodies
  - Neuromyelitis optica
    - Main symptoms are loss of vision and spinal cord function
    - Most patients have antibodies to the aquaporin 4 receptor (AQP4)
- Usually responsive to immunotherapies
  - Variable correlation with cancers

Role of autoantibodies in the pathogenesis of autoimmune neurologic diseases

- Antibodies are markers of disease, only a few have been shown to be pathogenic.
- Antibody does not predict how the disease presents but can predict what type of malignancy you should go hunting for.
- PNS: Expression of neuronal proteins by a cancer breaks immune tolerance to proteins normally expressed in the nervous system.
- It is unclear what the trigger is for antibody production in patients in whom cancer is never detected. Infection?

Role of autoantibodies in the pathogenesis of autoimmune neurologic diseases (continued)

- Antigens in different cellular locations may be associated with different types of autoimmune pathogenesis
- Many of antigens associated with autoimmune neurologic disorders are expressed in many different regions of the CNS which can explain the diversity of symptoms and clinical syndromes associated with a single antibody
- However, it remains to be determined why in some cases specific regions of the nervous system are targeted despite the antibody being widely expressed (isoform, conformation, exposure/availability)

Diagnostic criteria for paraneoplastic neurological syndromes (PNS)

Table 4  Diagnostic criteria for paraneoplastic neurological syndromes (PNS)

**Definite PNS**
1. A classical syndrome and cancer that develops within five years of the diagnosis of the neurological disorder.
2. A non-classical syndrome that resolves or significantly improves after cancer treatment without concomitant immunotherapy, provided that the syndrome is not susceptible to spontaneous remission.
3. A non-classical syndrome with onconeural antibodies (well characterised or not) and cancer that develops within five years of the diagnosis of the neurological disorder.
4. A neurological syndrome (classical or not) with well characterised onconeural antibodies (anti-Hu, Yo, CV2, Ri, Ma2, or amphiphysin), and no cancer.

**Possible PNS**
1. A classical syndrome, no onconeural antibodies, no cancer but at high risk to have an underlying tumour.
2. A neurological syndrome (classical or not) with partially characterised onconeural antibodies and no cancer.
3. A non-classical syndrome, no onconeural antibodies, and cancer present within two years of diagnosis.

Diagram:
- **Neurological syndrome**
  - Classical
    - Tumour present
    - Onconeural antibodies absent or present
    - High risk for cancer
    - Definite
  - Tumour absent
    - Onconeural antibodies absent
    - Definite
  - Tumour absent
    - Onconeural antibodies present
    - Partially characterised onconeural antibodies
    - Possible
  - Tumour present
    - Onconeural antibodies absent
    - Improvement after cancer therapy or onconeural antibodies present
    - Definite

Graus et al. J Neurol Neurosurg Psych 2004;75:1135-40
Diagnostic criteria for definite autoimmune limbic encephalitis

Diagnosis can be made when all four* of the following criteria have been met:

1. **Subacute onset** (rapid progression of less than 3 months) of working memory deficits, seizures, or psychiatric symptoms suggesting involvement of the limbic system

2. **Bilateral brain abnormalities** on T2-weighted fluid-attenuated inversion recovery MRI highly restricted to the medial temporal lobes†

3. At least one of the following:
   1. CSF pleocytosis (white blood cell count of more than five cells per mm³)
   2. EEG with epileptic or slow-wave activity involving the temporal lobes

4. **Reasonable exclusion** of alternative causes

*If one of the first three criteria is not met, a diagnosis of definite limbic encephalitis can be made only with the detection of antibodies against cell-surface, synaptic, or onconeural proteins. †¹⁸ Fluorodeoxyglucose (¹⁸F-FDG) PET can be used to fulfil this criterion.

Diagnosis of anti-NMDA receptor encephalitis

Probable anti-NMDA receptor encephalitis

1. **Rapid onset** (less than 3 months) of **at least four** of the six following major groups of symptoms:
   - Abnormal (psychiatric) behavior or cognitive dysfunction
   - Speech dysfunction (pressured speech, verbal reduction, mutism)
   - Seizures
   - Movement disorder, dyskinesias, or rigidity/abnormal postures
   - Decreased level of consciousness
   - Autonomic dysfunction or central hypoventilation

2. **At least one** of the following **laboratory study** results:
   - Abnormal EEG (focal or diffuse slow or disorganized activity)
   - CSF with pleocytosis or oligoclonal bands

3. **Reasonable exclusion** of other disorders

Definite anti-NMDA receptor encephalitis

Presence of **one or more** of the six major group of symptoms and **IgG anti-GluN1 antibodies** after **reasonable exclusion** of other disorders

Criteria for autoantibody-negative but probable autoimmune encephalitis

Diagnosis can be made when all four of the following criteria have been met:

1. **Rapid progression** (less than 3 months) of working memory deficits (short-term memory loss), altered mental status, or psychiatric symptoms

2. **Exclusion of well defined syndromes** of autoimmune encephalitis (eg, typical limbic encephalitis, Bickerstaff’s brainstem encephalitis, acute disseminated encephalomyelitis)

3. **Absence of well characterized autoantibodies** in serum and CSF, and at least two of the following criteria:
   - MRI abnormalities suggestive of autoimmune encephalitis*
   - CSF pleocytosis, CSF-specific oligoclonal bands or elevated CSF IgG index, or both*
   - Brain biopsy showing inflammatory infiltrates and excluding other disorders (eg, tumour)

4. **Reasonable exclusion** of alternative causes

Importance of autoantibodies in the diagnosis of autoimmune neurologic diseases

- Autoantibodies should be included in the differential diagnosis early in the evaluation.
- Detection of neural autoantibodies can aid in confirming a diagnosis of autoimmune neurologic disease.
- Lack of detection of a neural autoantibody does not eliminate the possibility of autoimmune neurologic disease.
- Tests for detecting neural autoantibodies have complexities that must be considered.
- Results must be interpreted within the clinical context, since taking them as conclusive evidence of autoimmune encephalitis could be a mistake.

https://www.autoimmuneencephalitis.net/diagnosis
Detection of neuronal autoantibodies in the clinical laboratory

Waters et al. Handbook of Clinical Neurology, Vol. 133, Chapter 9, pgs.147-163
## Autoantibodies and Methods for Their Detection in the Clinical Laboratory

<table>
<thead>
<tr>
<th>Category</th>
<th>Specific antibodies</th>
<th>Detection methods*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IHC/IFA*</td>
</tr>
<tr>
<td>Intracellular antigens</td>
<td>ANNA-1 (Hu)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>ANNA-2 (RI)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>ANNA-3</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>CRMP-5 (CV2)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>Ma/Ta</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>AGNA-1 (Sox-1)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>PCCA-1 (Yo)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>PCCA-2</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>PCCA-Tr (DNER)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>GAD65</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>Amphiphysin</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>Recoverin</td>
<td>x</td>
</tr>
<tr>
<td>Neuronal surface antigens</td>
<td>NMDAR</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>LG1</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>CASPR2</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>AMPAR</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>GABA_B_R</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>mGlurR</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>Gyr</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>STR</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>AQP4</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>PQ-VGCC</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>N-VGCC</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>AchRIBIN</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>gAChR</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>VGKC</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>DPPX</td>
<td>x</td>
</tr>
</tbody>
</table>

Tissue-based Indirect Immunofluorescence or Immunohistochemistry

Advantages
- Antigens are in their native form
- Can screen for many auto-antibodies at the same time
- Can discover new autoantibodies

Disadvantages
- Requires significant training to become proficient
- Several antibodies can yield the same staining pattern, must be confirmed using another assay
- Difficult to identify multiple coexisting antibodies
- Some antibodies are very rare so it is difficult to validate and to maintain competency
- Subjective
- Time consuming
- Lacks standardization
- Requires a second method to confirm specific autoantibody

Waters et al. Handbook of Clinical Neurology, Vol. 133, Chapter 9, pgs.147-163
Western Blot or Line Blot Testing

**Advantages**
- Can screen for and identify multiple antibodies at the same time
- Increased sensitivity and specificity compared to IFA
- Less subjective than IFA
- Higher throughput, can be automated

**Disadvantages**
- Antigens are not in their native form (false negatives)
- Can be difficult to obtain rare positive samples for validation and as controls (manufacturer controls often contain a single antibody)
- Clinical relevance of WB or IB positive but IFA negative results is questionable
ELISAs

**Advantages**
- Increased sensitivity and specificity compared to IFA
- Less subjective than IFA
- Higher throughput, can be automated

**Disadvantages**
- Antigens are not in their native form (false negatives)
- False positives due to nonspecific binding (plate, heterophile antibodies, etc.)

Waters et al. Handbook of Clinical Neurology, Vol. 133, Chapter 9, pgs.147-163
Cell-Based Assays

**Advantages**
- Antigens are in their native form
- Less subjective than IFA
- Requires less training for proficiency
- Very sensitive and specific method for detecting antibodies against many of the cell surface targets

**Disadvantages**
- Can only be used to detect antibodies against the transfected antigen
- Can’t identify new autoantibodies

*Preferred method for detecting antibodies to cell surface receptors

Adapted from Waters et al. Handbook of Clinical Neurology, Vol. 133, Chapter 9, pgs.147-163 and www.euroimmun.com
Radio- or Fluorescent Immunoprecipitation Assays

**Advantages**
- Antigens are in their native form
- Increased sensitivity compared to IFA, WB, LB, ELISA
- Less subjective than IFA

*Preferred method for detecting antibodies to synaptic receptors*

**Disadvantages**
- Radioactivity
- May identify multiple autoantibodies due to immunoprecipitation of a protein complex, which may have to be confirmed using an additional assay (eg. VGKC complex ➔ LGI1 and CASPR2)

Waters et al. Handbook of Clinical Neurology, Vol. 133, Chapter 9, pgs.147-163
Primary Cell Culture-Based IFA

**Advantages**
- Antigens are in their native form
- Can screen for many antibodies at the same time
- Can discover new autoantibodies

**Disadvantages**
- Labor intensive, time consuming
- Requires significant training for proficiency
- Several antibodies can yield the same pattern
- Difficult to identify coexisting antibodies
- Rare antibodies are difficult to validate and to maintain competency
- Subjective
- Primarily performed on a research basis

Waters et al. Handbook of Clinical Neurology, Vol. 133, Chapter 9, pgs.147-163
Comparison Between Sample Types for Autoantibody Detection

<table>
<thead>
<tr>
<th>Serum</th>
<th>CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantages</strong></td>
<td><strong>Advantages</strong></td>
</tr>
<tr>
<td>• Less invasive, more suitable for monitoring response to tx</td>
<td>• Less nonspecific binding, fewer false positives</td>
</tr>
<tr>
<td>• Antibodies present at higher titers</td>
<td>• Can be more sensitive and specific than serum for neuronal cell antibodies</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td><strong>Disadvantages</strong></td>
</tr>
<tr>
<td>• Nonspecific binding can cause false positives</td>
<td>• More invasive</td>
</tr>
<tr>
<td>• Some antibodies are produced intrathecally, so serum can be negative (false negative)</td>
<td>• Antibodies present at lower titer than in serum or not at all, which can cause false negatives</td>
</tr>
</tbody>
</table>
Current challenges for detection of autoantibodies associated with neurologic disease

- Testing for some autoantibodies is proprietary/patented and only available at select labs
- Some autoantibodies are very rare making it difficult to acquire positive samples to validate and properly control assays (in addition the manufacturer’s do not provide positive controls)
- Overlap of symptoms associated with multiple autoantibodies makes determining sensitivity of antibody tests difficult since the diseases are defined by the presence of the antibody
- Detection based on patterns of staining on cerebellum and hippocampus sections requires significant training and proficiency to accurately identify specific autoantibodies
- The number of autoantibodies associated with autoimmune neurologic diseases is continuing to increase
- Exponential growth in the number of samples tested (mainly in order to exclude an immunotherapy responsive cause) is associated with some equivocal or clinically irrelevant positive test results
Testing strategies for detecting autoantibodies in autoimmune neurologic diseases:

- Comprehensive
- Targeted
- Single autoantibody

Antibody Evaluation of Paraneoplastic Neurologic Syndrome and Related Diseases

Acquired neuromuscular junction disorders**

Motor weakness preferentially affecting proximal, bulbar, or extraocular muscles; autonomic symptoms such as dry mouth, constipation, impotence, and bladder urgency. Muscle twitching or myokymia, cramps, muscle stiffness, and in chronic cases, muscle hypertrophy.

AChR\text{BnR}, STMR, gAChR, VGCC (P/Q- and N-types), VGKC\text{4}

AChR positive

STM positive

AChR modulating

STM titre

VGKC positive

LG11 and CASPR2

Paraneoplastic neurologic syndrome

Encephalitis, encephalomyelitis, cerebellar degeneration, Stiff-person syndrome, myoclonus/opsoclonus, neuronopathy, neuropathy

Hippocampus (neuronal/glial surface and synaptic proteins)\text{1}

NMDAR, VGKC (LG1, CASPR2), AMPAR, GABA(B/R), NMO

Cerebellum (intracellular and surface proteins)\text{2}

Hu, Yo, Ri, Ma/Ta, CRMP5, PCCA-Tr, GAD65, Amphiphysin, Sox-1

Tebo et al., Clin Chim Acta. 2016;459:162-9
Distribution of antibody-positivity in patients evaluated using a 15 autoantibody paraneoplastic panel

![Pie chart showing distribution of antibody-positivity](image)

<table>
<thead>
<tr>
<th># of Antibodies</th>
<th># of patients (n=78,889)</th>
<th># of patients (n=1,589)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None detected</td>
<td>69,701 (88.4%)</td>
<td>1,363 (85.8%)</td>
</tr>
<tr>
<td>Single</td>
<td>7,592 (9.6%)</td>
<td>173 (10.9%)</td>
</tr>
<tr>
<td>Two</td>
<td>1,319 (1.7%)</td>
<td>33 (2.1%)</td>
</tr>
<tr>
<td>Three</td>
<td>213 (0.3%)</td>
<td>13 (0.8%)</td>
</tr>
<tr>
<td>Four</td>
<td>52 (0.1%)</td>
<td>6 (0.4%)</td>
</tr>
<tr>
<td>Five</td>
<td>9 (0.01%)</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>Six</td>
<td>1 (0.001%)</td>
<td>0</td>
</tr>
</tbody>
</table>


Majority of autoantibodies identified target neuromuscular antigens (both individual and co-existing)

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Number positive (%)</th>
<th>First</th>
<th>Number positive (%)</th>
<th>Second</th>
<th>Number positive (%)</th>
<th>Third</th>
<th>Number positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Striational</td>
<td>3,483 (4.42)</td>
<td>mACHR</td>
<td>684 (20)</td>
<td>VGKC</td>
<td>192 (6)</td>
<td>gACHR</td>
<td>174 (5)</td>
</tr>
<tr>
<td>VGKC</td>
<td>2,194 (2.78)</td>
<td>Str</td>
<td>192 (9)</td>
<td>mACHR</td>
<td>98 (4)</td>
<td>gACHR</td>
<td>90 (4)</td>
</tr>
<tr>
<td>gACHR</td>
<td>1,696 (2.15)</td>
<td>Str</td>
<td>174 (10)</td>
<td>mACHR</td>
<td>146 (9)</td>
<td>VGKC</td>
<td>90 (5)</td>
</tr>
<tr>
<td>mACHR</td>
<td>1,370 (1.74)</td>
<td>Str</td>
<td>684 (50)</td>
<td>gACHR</td>
<td>146 (11)</td>
<td>VGKC</td>
<td>98 (7)</td>
</tr>
<tr>
<td>VGCC_N</td>
<td>889 (1.13)</td>
<td>VGCC_P/Q</td>
<td>233 (26)</td>
<td>VGKC</td>
<td>74 (8)</td>
<td>Str</td>
<td>64 (7)</td>
</tr>
<tr>
<td>VGCC_P/Q</td>
<td>863 (1.09)</td>
<td>VGCC_N</td>
<td>233 (27)</td>
<td>VGKC</td>
<td>85 (10)</td>
<td>Str</td>
<td>62 (8)</td>
</tr>
<tr>
<td>ANNA-1</td>
<td>252 (0.32)</td>
<td>CRMP5</td>
<td>28 (11)</td>
<td>VGCC_P/Q</td>
<td>25 (10)</td>
<td>VGCC_N</td>
<td>18 (7)</td>
</tr>
<tr>
<td>CRMP5</td>
<td>156 (0.20)</td>
<td>Str</td>
<td>30 (19)</td>
<td>ANNA-1</td>
<td>28 (18)</td>
<td>mACHR</td>
<td>24 (15)</td>
</tr>
<tr>
<td>PCA-1</td>
<td>82 (0.10)</td>
<td>Str</td>
<td>7 (9)</td>
<td>VGKC, gACHR</td>
<td>3 (4)</td>
<td>VGCC_N</td>
<td>2 (2)</td>
</tr>
<tr>
<td>SOX1</td>
<td>39 (0.05)</td>
<td>VGCC_P/Q</td>
<td>13 (33)</td>
<td>VGCC_N</td>
<td>10 (26)</td>
<td>ANNA-1</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Amphiphysin</td>
<td>39 (0.05)</td>
<td>ANNA-1, VGCC_P/Q</td>
<td>9 (23)</td>
<td>VGCC_N</td>
<td>4 (10)</td>
<td>Str, gACHR, CRMP5</td>
<td>2 (5)</td>
</tr>
<tr>
<td>PCA-2</td>
<td>24 (0.03)</td>
<td>CRMP5</td>
<td>6 (25)</td>
<td>ANNA-1</td>
<td>5 (21)</td>
<td>VGCC_N</td>
<td>4 (17)</td>
</tr>
<tr>
<td>ANNA-2</td>
<td>20 (0.03)</td>
<td>VGCC_P/Q, VGCC_N</td>
<td>2 (10)</td>
<td>gACHR, Str, amphiphysin</td>
<td>1 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCA-Tr</td>
<td>8 (0.01)</td>
<td>mACHR, Str</td>
<td>1 (13)</td>
<td>ANNA-1</td>
<td>1 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANNA-3</td>
<td>7 (0.01)</td>
<td>ANNA-1</td>
<td>1 (14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Majority of autoantibodies identified target neuromuscular junction receptors

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Number positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VGKC</td>
<td>67 (4.23)</td>
</tr>
<tr>
<td>STR</td>
<td>66 (4.16)</td>
</tr>
<tr>
<td>PQ-VGCC</td>
<td>38 (2.42)</td>
</tr>
<tr>
<td>gAChR</td>
<td>32 (2.06)</td>
</tr>
<tr>
<td>AChR-BIN</td>
<td>30 (1.89)</td>
</tr>
<tr>
<td>N-VGCC</td>
<td>20 (1.26)</td>
</tr>
<tr>
<td>ANNA-1 (Hu)</td>
<td>7 (0.44)</td>
</tr>
<tr>
<td>PCCA-1 (Yo)</td>
<td>5 (0.31)</td>
</tr>
<tr>
<td>CRMP-5 (CV2)</td>
<td>5 (0.31)</td>
</tr>
<tr>
<td>Amphiphysin</td>
<td>3 (0.19)</td>
</tr>
<tr>
<td>ANNA-2 (Ri)</td>
<td>2 (0.13)</td>
</tr>
<tr>
<td>ANNA-3</td>
<td>1 (0.06)</td>
</tr>
<tr>
<td>PCCA-2</td>
<td>1 (0.06)</td>
</tr>
<tr>
<td>AGNA-1</td>
<td>0</td>
</tr>
</tbody>
</table>

Neural antibody clusters can guide search for cancer

Table 2. Clinical and oncological associations of the most common duos and trios autoantibody clusters

<table>
<thead>
<tr>
<th></th>
<th>mMChR and Str</th>
<th>VGCC_{P/Q} and VGCC_{N}</th>
<th>Str and VGK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trio</td>
<td>Duo</td>
<td>gAChR</td>
<td>VCRC or CRMP5</td>
</tr>
<tr>
<td>----------</td>
<td>---------------</td>
<td>--------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Number with histories available</td>
<td>122</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Patients with tumor, n (%)</td>
<td>55 (45)</td>
<td>8 (67)</td>
<td>13 (81)</td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymoma, n (%)</td>
<td>20 (26)</td>
<td>0</td>
<td>11 (85)</td>
</tr>
<tr>
<td>Prostate, n (% of men with cancer)</td>
<td>9 (20)</td>
<td>4 (50)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Lung, n (%)</td>
<td>6 (11)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clinical</td>
<td>MG or LES, n (% of patients)</td>
<td>MG 69 (57)</td>
<td>MG 10 (78)</td>
</tr>
<tr>
<td></td>
<td>Duo</td>
<td>mAChR</td>
<td>VGCC_{P/Q}</td>
</tr>
<tr>
<td>----------</td>
<td>---------------</td>
<td>--------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Number with histories available</td>
<td>53</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Patients with tumor, n (%)</td>
<td>10 (19)</td>
<td>7 (78)</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymoma, n (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prostate, n (% of men with cancer)</td>
<td>4 (44)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lung, n (%)</td>
<td>2 (13)</td>
<td>2 (68)</td>
<td>6 (66)</td>
</tr>
<tr>
<td>Clinical</td>
<td>MG or LES, n (% of patients)</td>
<td>MG 63 (67)</td>
<td>MG 10 (78)</td>
</tr>
</tbody>
</table>

Abbreviations: MG, myasthenia gravis; LES, Lambert–Eaton myasthenic syndrome; NS, not statistically significant.

*P value for comparison between the duo mMChR/Str cluster with the trio clusters in gray.

*P value for comparison between the duo mMChR/Str cluster with either trio cluster in gray, or comparison between trio clusters in gray.

*P value for comparison between the duo cluster VGCC_{P/Q}/VGCC_{N} and the trio cluster VGCC_{P/Q}, VGCC_{N}, and SOX1.

*P value for comparison between the trio cluster mAChR, Str, and VGK and the other clusters in dark gray.

Summary

- Majority of patients tested for autoimmune neurologic disease have a single autoantibody
- Autoantibodies against neuromuscular junction antigens are more common than autoantibodies against intracellular targets
- Autoantibodies that occur in clusters primarily involve those targeting the neuromuscular junctions
- Detection of autoantibody clusters is associated with increased incidence of cancer
- Most antibodies have low positivity rates – initial testing should take these rates of positivity into account (along with age, sex, clinical phenotype and presence of a tumor)
Comparison between strategies for testing for neural autoantibodies

**Comprehensive**

**Advantages**
- Can identify multiple Abs
- Can rule out multiple Abs

**Disadvantages**
- Can take weeks to receive results which can delay tx
- Not all antibodies are relevant for all patients (age, sex, tumor, clinical symptoms)
- Many of the Abs are very rare, not cost effective to test everyone
- Negative result does not rule out autoimmune neurologic disease
- Expensive
- Overlap between comprehensive panels

**Targeted or Single**

**Advantages**
- Faster, tx can be initiated sooner
- More cost-effective
- Clusters of Ab tend to include similar antigens
- Focus on Abs relevant for specific patients (age, sex, tumor, clinical symptoms)

**Disadvantages**
- Negative result does not rule out autoimmune neurologic disease
- Testing for Abs one at a time can delay diagnosis and tx
Summary

• Autoantibodies are markers of autoimmune neurologic disease, only a few have been shown to be pathogenic.
• Detection of specific autoantibodies significantly impacts diagnosis and management of patients.
• Autoantibody does not predict how the disease presents but can predict treatment response and/or what type of malignancy you should go hunting for.
• Failure to detect a neural autoantibody does not rule out autoimmune neurologic disease.
• Problem with testing in this country is that it is very segmented with only some labs able to offer testing for certain autoantibodies due to patents.
• Field is constantly evolving, we are constantly learning more about these diseases and continuing to identify new autoantibodies.
Learning Objectives:

• Understand the role of autoantibody testing in diagnosis and management of autoimmune neurologic disorders

• Compare and contrast methods used to detect the relevant autoantibodies

• Describe different strategies for autoantibody testing
P.A.C.E.®/FL Password:

AND62217

Go to www.aruplab.com/autoantibody and click on the P.A.C.E.®/FL Credit Redemption Link

Credit redemption for this webinar will be available through July 6, 2017

This webinar can be viewed after July 31, 2017 at www.arup.utah.edu where CME/SAM, P.A.C.E.® and Florida continuing education credit will be available.