Autoantibody Testing in the Diagnosis of Autoimmune Neurological Disorders

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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

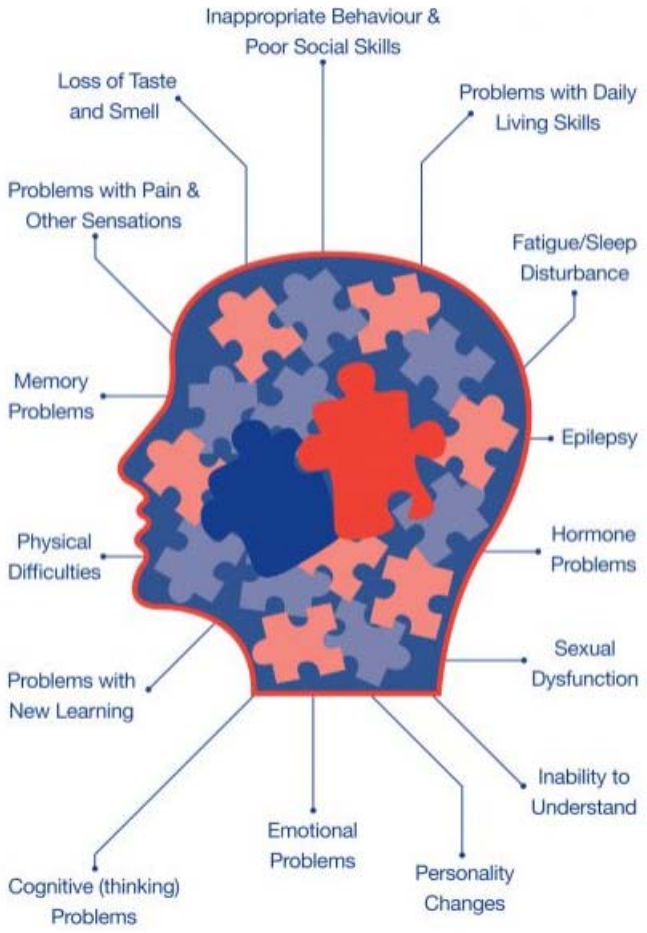
What are autoimmune neurologic disorders?

- 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**

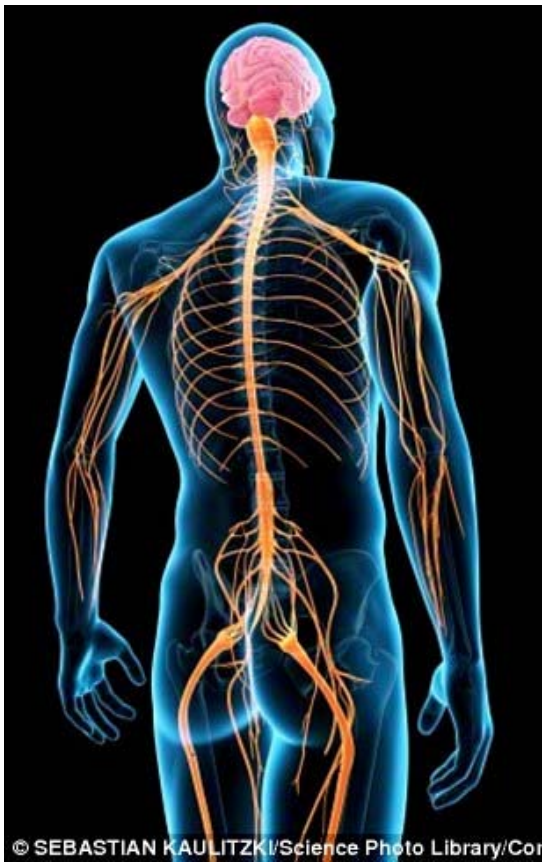


www.encephalitis.info/support/information/practical-resources-on-encephalitis/effects-of-encephalitis/

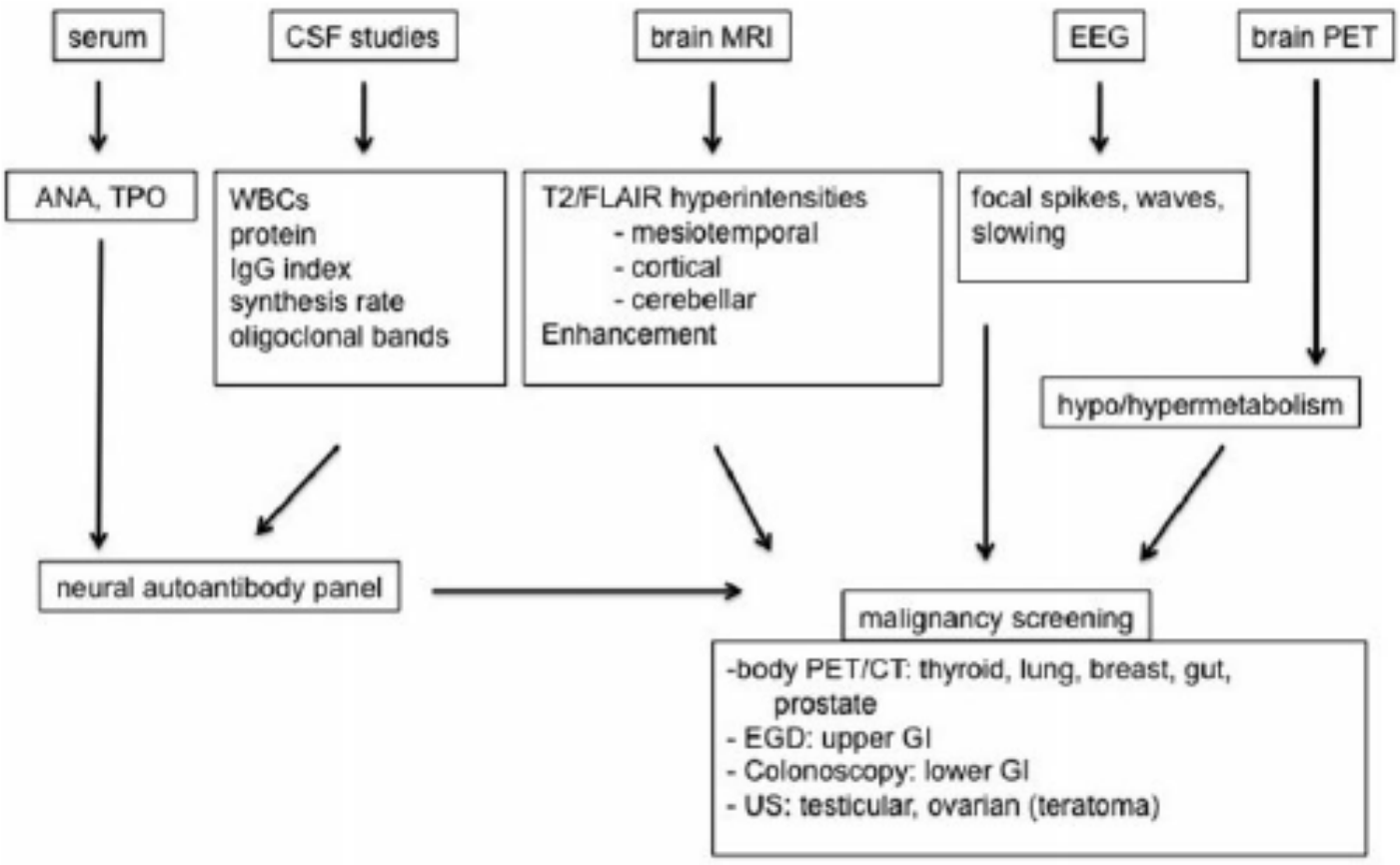
Diagnosis of autoimmune neurologic disease

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

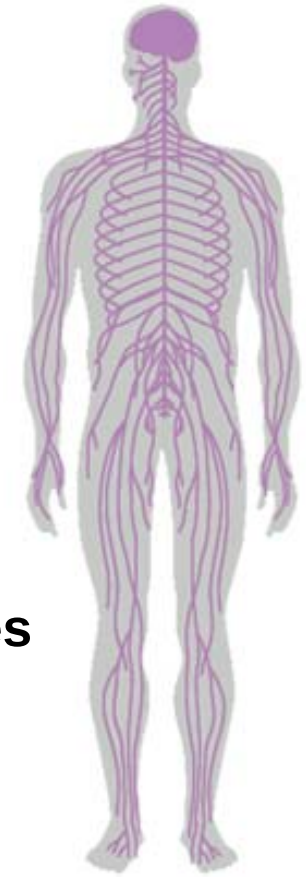
- Antibody-associated disorders of the nervous system
- Diverse group of syndromes
- Currently can be broadly divided into 2 categories based on cellular location:
  - Autoimmune disorders associated with antibodies to intracellular neuronal antigens (cytosolic or nuclear)
    - Classic paraneoplastic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

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

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

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

NUCLEAR AND CYTOPLASMIC SPECIFICITIES

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

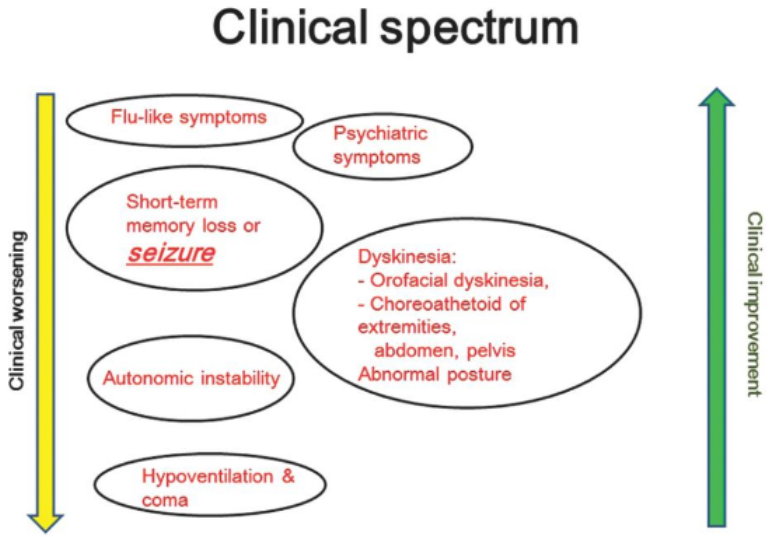
PLASMA MEMBRANE SPECIFICITIES

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

Abbreviations: AGNA, anti-glial/neuronal nuclear antibody; ANNA, antineuronal 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, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic 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.



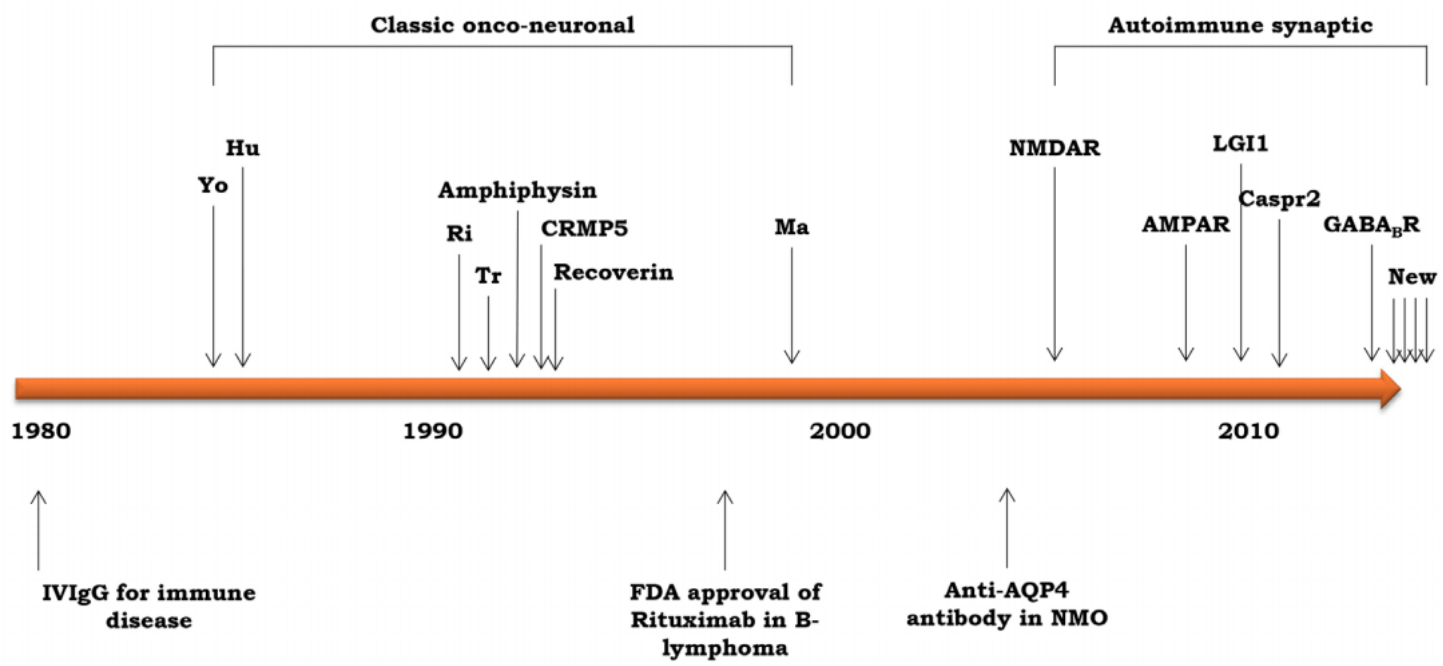
Nawa-apisak et al. 2016. Neuroimmunol Neuroinflamm 3: 79-85

Autoimmune Encephalitis

http://aaemrsa.blogspot.com/2015/02/a-whole-herd-of-zebras-anti-nmda.html

- 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.

Autoantibodies associated with autoimmune encephalitis

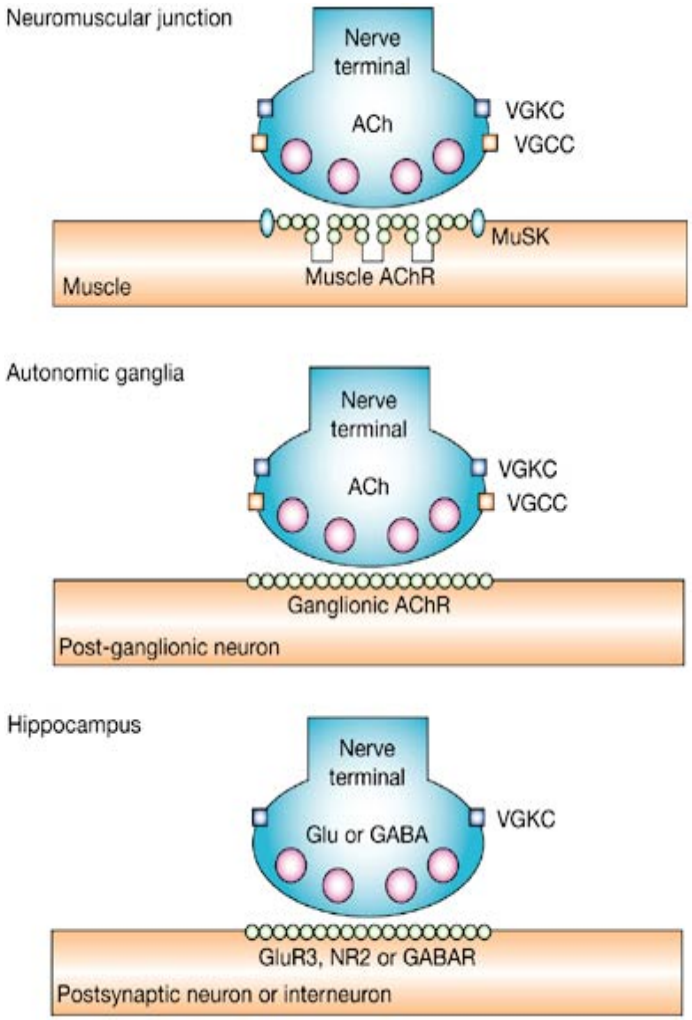


Since 2007 (~1 per year)

- NMDA
- VGKC complex (LGI1, CASPR2, others?)
- AMPA
- GABA-B
- GABA-A
- DPPX
- GlyR
- mGluR5
- IgLON5

Disorders of the Neuromuscular Junction (NMJ)

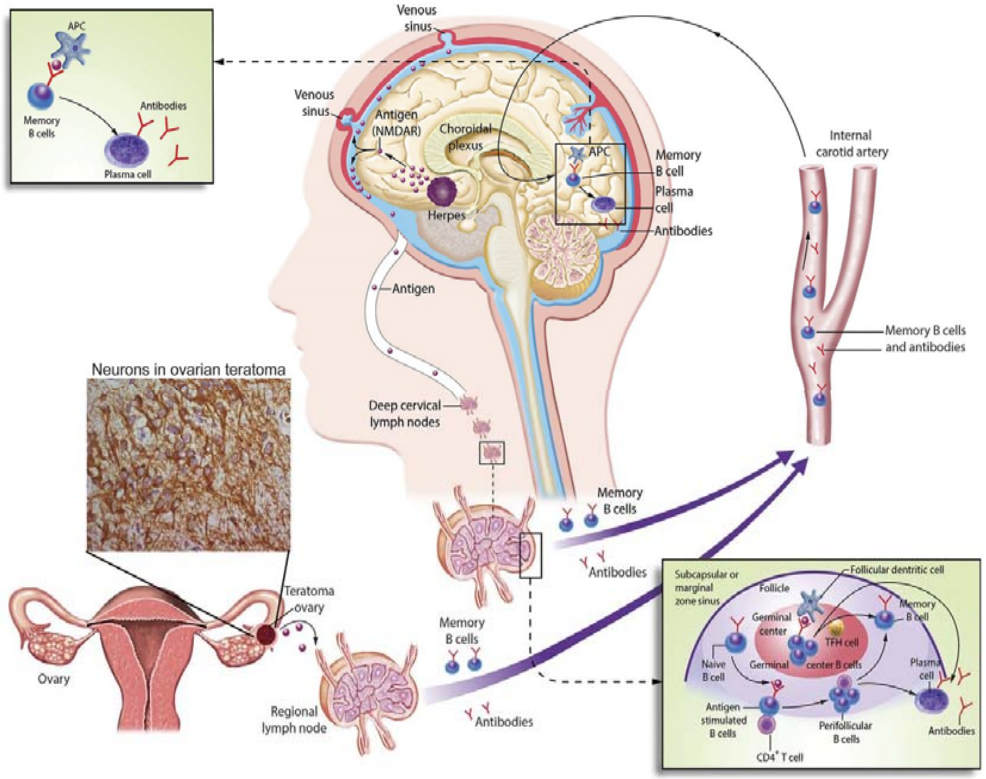
- Specialized synapse with a complex structural and functional organization
- Types of disease
  - Myasthenia gravis, the most common NMJ disorder
    - Muscle weakness that can vary in type and severity, ptosis, diplopia, unstable or waddling gait, a change in facial expression, difficulty swallowing, 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



Auton Neurosci. 2009; 146(1-2): 3–7.

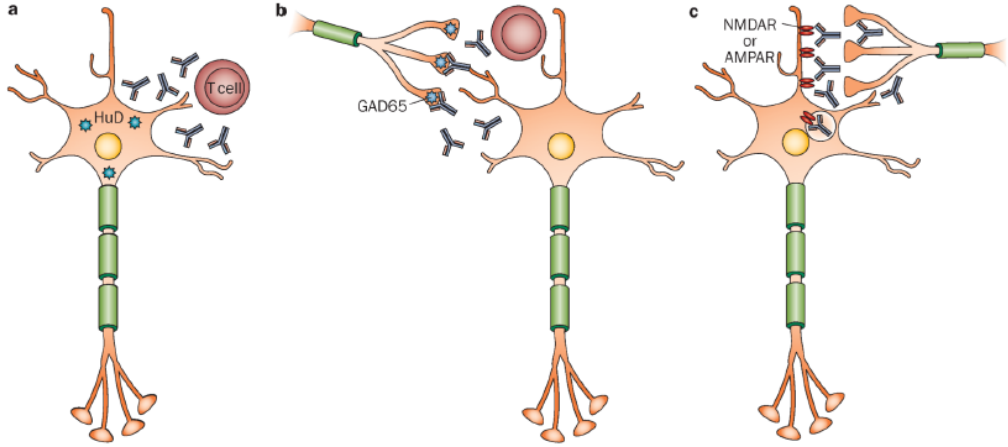
Role of autoantibodies in the pathogenesis of autoimmune neurologic diseases

- 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?



Dalmau et al. Lancet Neurol 2011;10:63–74

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)

Lancaster and Dalmau. 2012. Nat Rev Neurol. 8(7): 380–390

Diagnostic criteria for paraneoplastic neurological syndromes (PNS)

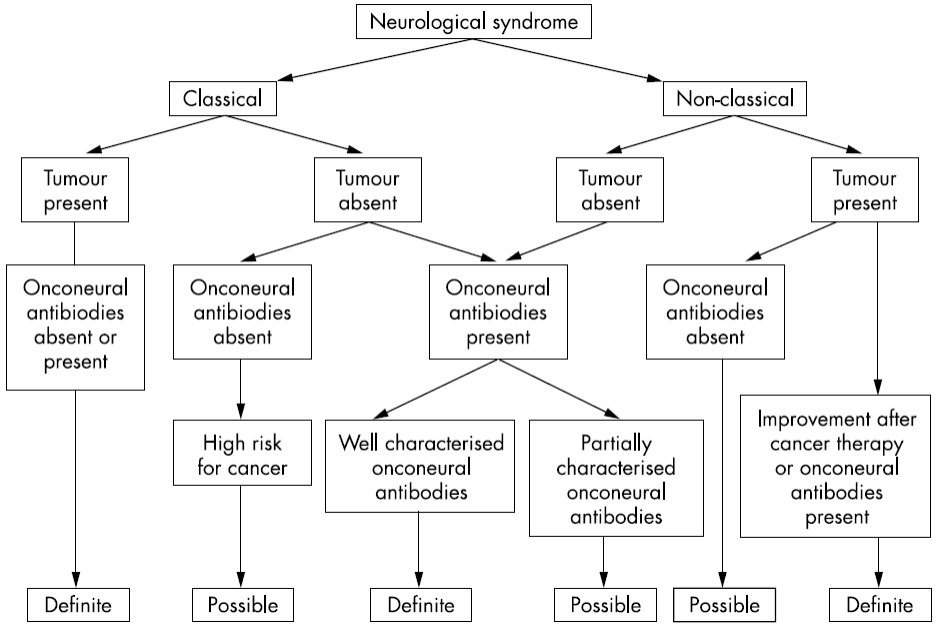
Table 4 Diagnostic criteria for paraneoplastic neurological syndromes (PNS)

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.



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 mm^3)
    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 (^{18}F-FDG) PET can be used to fulfil this criterion.

Graus et al. Lancet Neurol 2016;15:391–404

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 mo**re of the six major group of symptoms and **IgG anti-GluN1 antibodies** after **reasonable exclusion** of other disorders

Graus et al. Lancet Neurol 2016;15:391–404

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

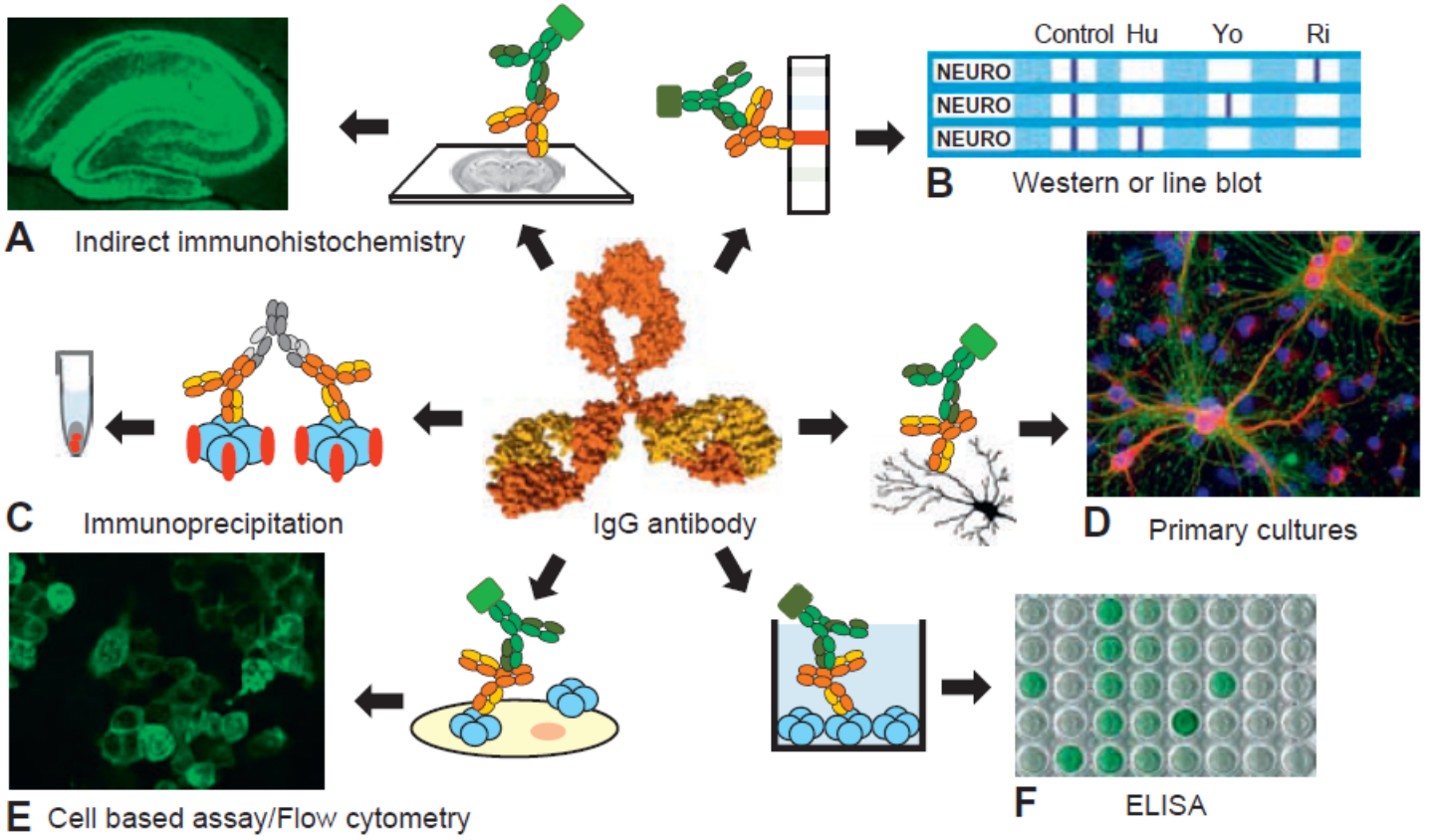
Graus et al. Lancet Neurol 2016;15:391–404

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



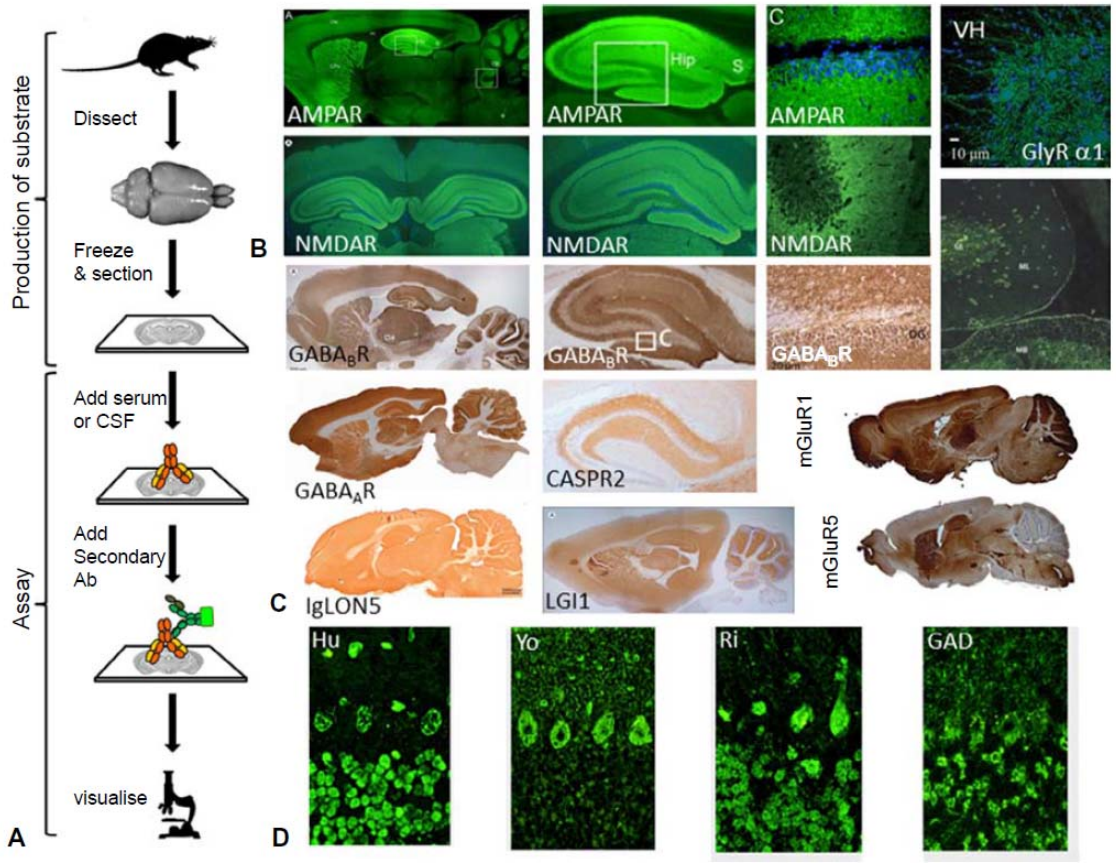
Waters et al. Handbook of Clinical Neurology, Vol. 133, Chapter 9, pgs.147-163

Autoantibodies and Methods for Their Detection in the Clinical Laboratory

Category	Specific antibodies	Detection methods[a]					
		IHC/IFA[a]	WB	LIA	RIA	ELISA	CBA
Intracellular antigens	ANNA-1 (Hu)	x	x	x	–	x	–
	ANNA-2 (Ri)	x	x	x	–	x	–
	ANNA-3	x	–	–	–	–	–
	CRMP-5 (CV2)	x	x	x	–	x	–
	Ma/Ta	x	x	x	–	–	–
	AGNA-1 (Sox-1)	x	x	x	–	x	–
	PCCA-1 (Yo)	x	x	x	–	x	–
	PCCA-2	x	–	–	–	–	–
	PCCA-Tr (DNER)	x	x	x	–	x	–
	GAD65	x	x	x	x	x	–
	Amphiphysin	x	x	x	–	x	–
	Recoverin	x	x	x	–	x	–
Neuronal surface antigens	NMDAR	x	–	–	–	x	x
	LGI1	x	–	–	–	–	x
	CASPR2	x	–	–	–	–	x
	AMPAR	x	–	–	–	–	x
	$GABA_BR$	x	–	–	–	–	x
	mGluR	x	–	–	–	–	x
	GyR	x	–	–	–	–	x
Neuromuscular junction or channel antigen	STR	x	–	–	–	x	–
	AQP4	x	x	x	–	x	x
	PQ-VGCC	–	–	–	x	–	–
	N-VGCC	–	–	–	x	–	–
	AChRBIN	–	–	–	x	–	–
	gAChR	–	–	–	x	–	–
	VGKC	–	–	–	x	–	–
	DPPX	x	–	–	x	–	x

Tebo et al. Clin Chim Acta. 2016;459:162-9

Tissue-based Indirect Immunofluorescence or Immunohistochemistry



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

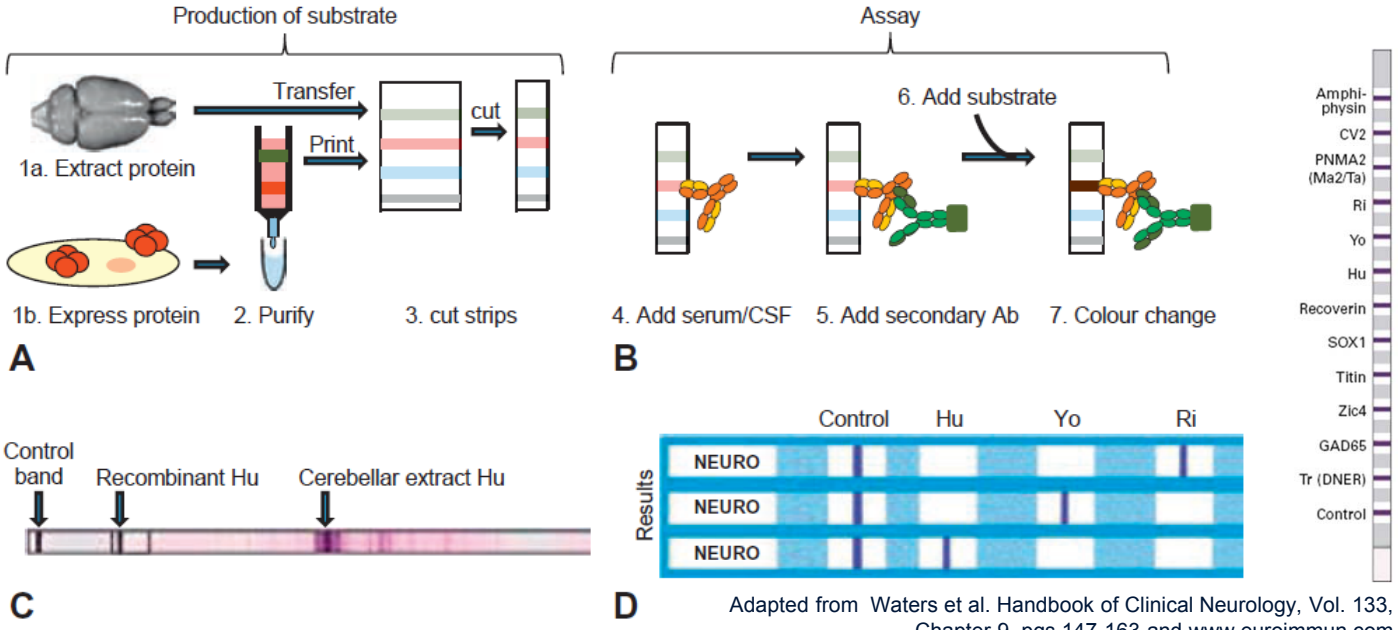
Advantages

- 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

Western Blot or Line Blot Testing



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

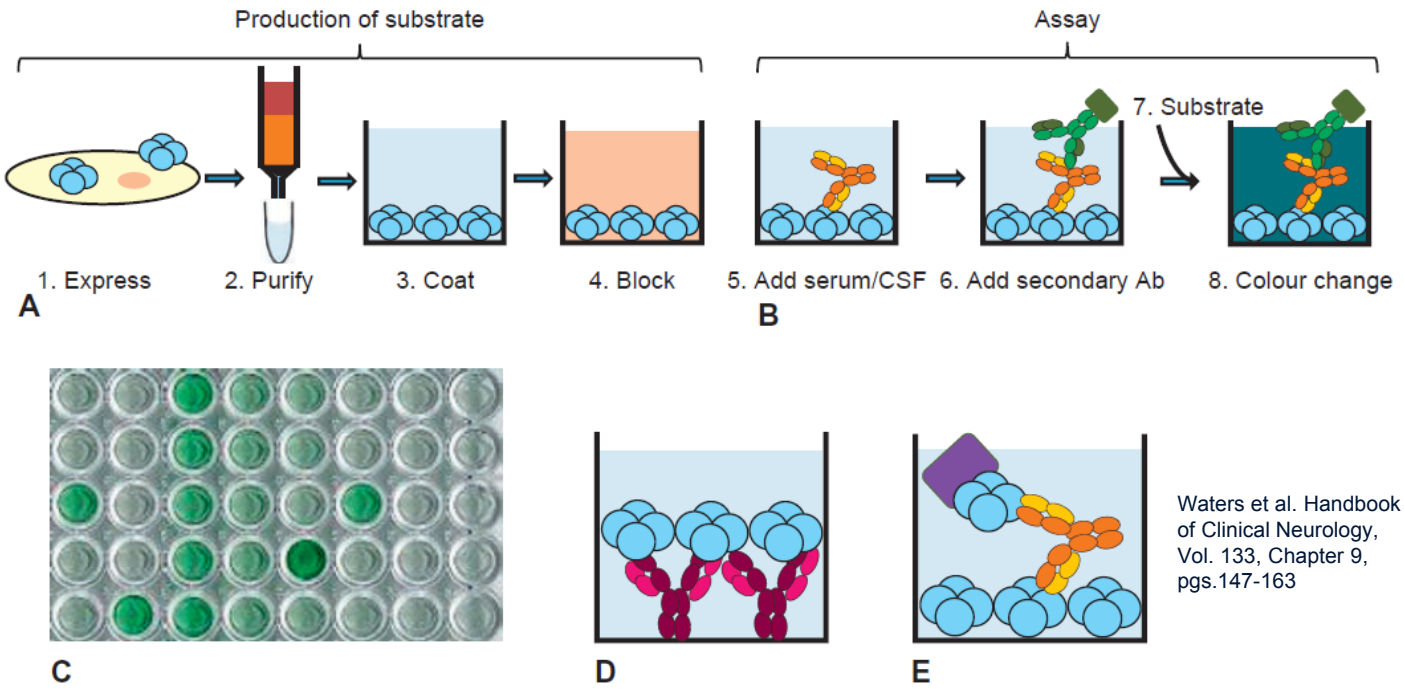
Advantages

- 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



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

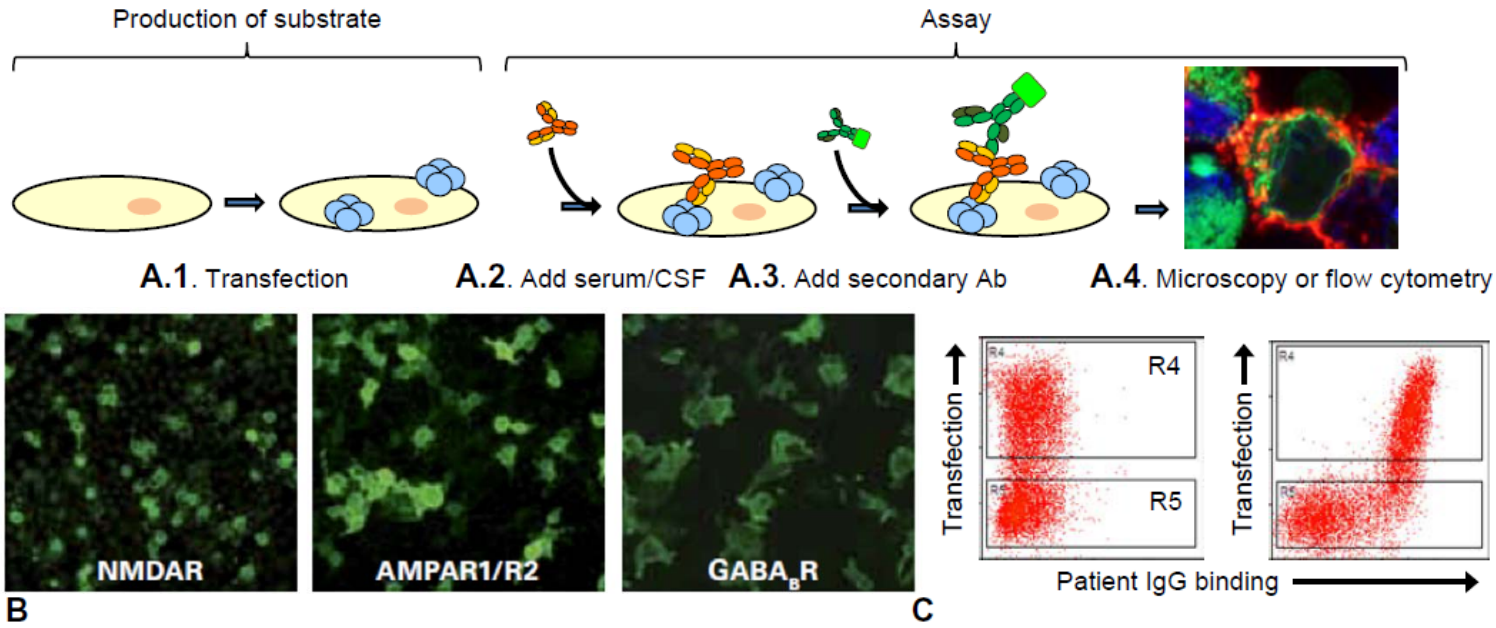
Advantages

- 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.)

Cell-Based Assays



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

Advantages

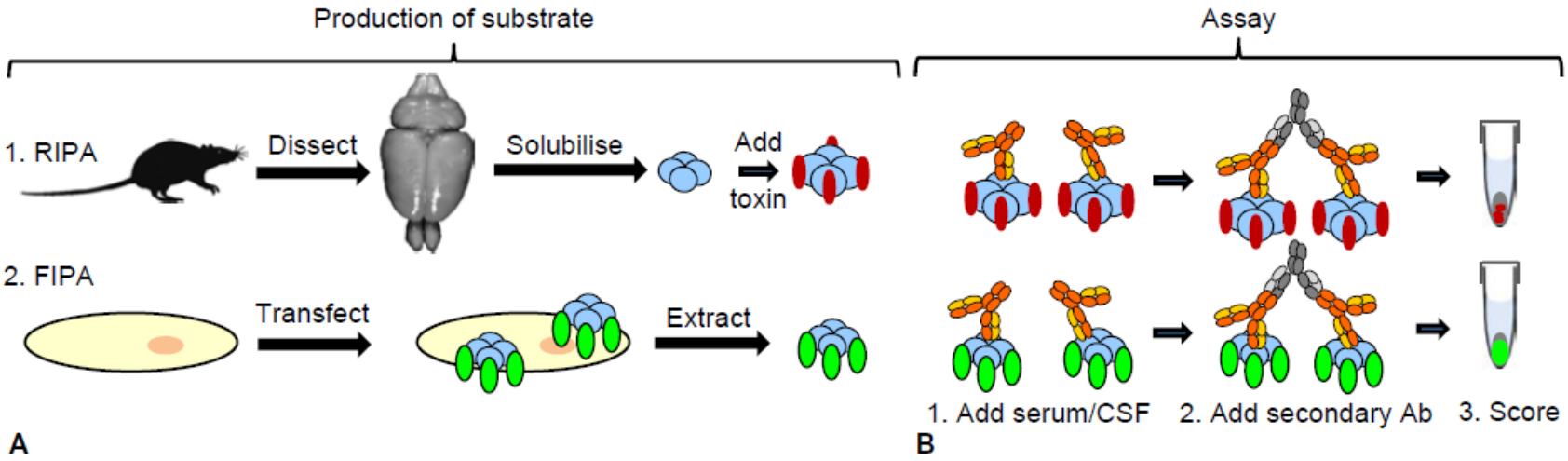
- Antigens are in their native form
- Less subjective than IFA
- Requires less training for proficiency
- Very sensitive and specific method for detecting antibodies against many of the cell surface targets

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

Radio- or Fluorescent Immunoprecipitation Assays



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

Advantages

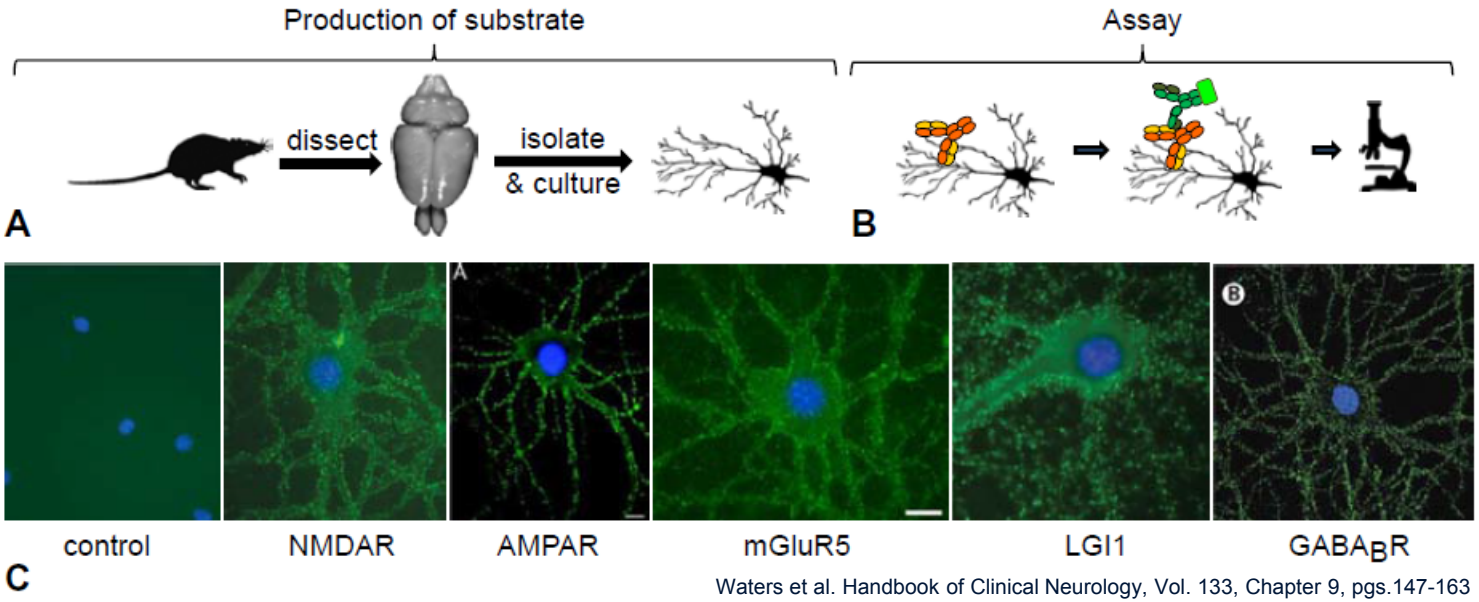
- Antigens are in their native form
- Increased sensitivity compared to IFA, WB, LB, ELISA
- Less subjective than IFA

*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)

Primary Cell Culture-Based IFA



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

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

Comparison Between Sample Types for Autoantibody Detection

Serum

Advantages

- Less invasive, more suitable for monitoring response to tx
- Antibodies present at higher titers

Disadvantages

- Nonspecific binding can cause false positives
- Some antibodies are produced intrathecally, so serum can be negative (false negative)

CSF

Advantages

- Less nonspecific binding, fewer false positives
- Can be more sensitive and specific than serum for neuronal cell antibodies

Disadvantages

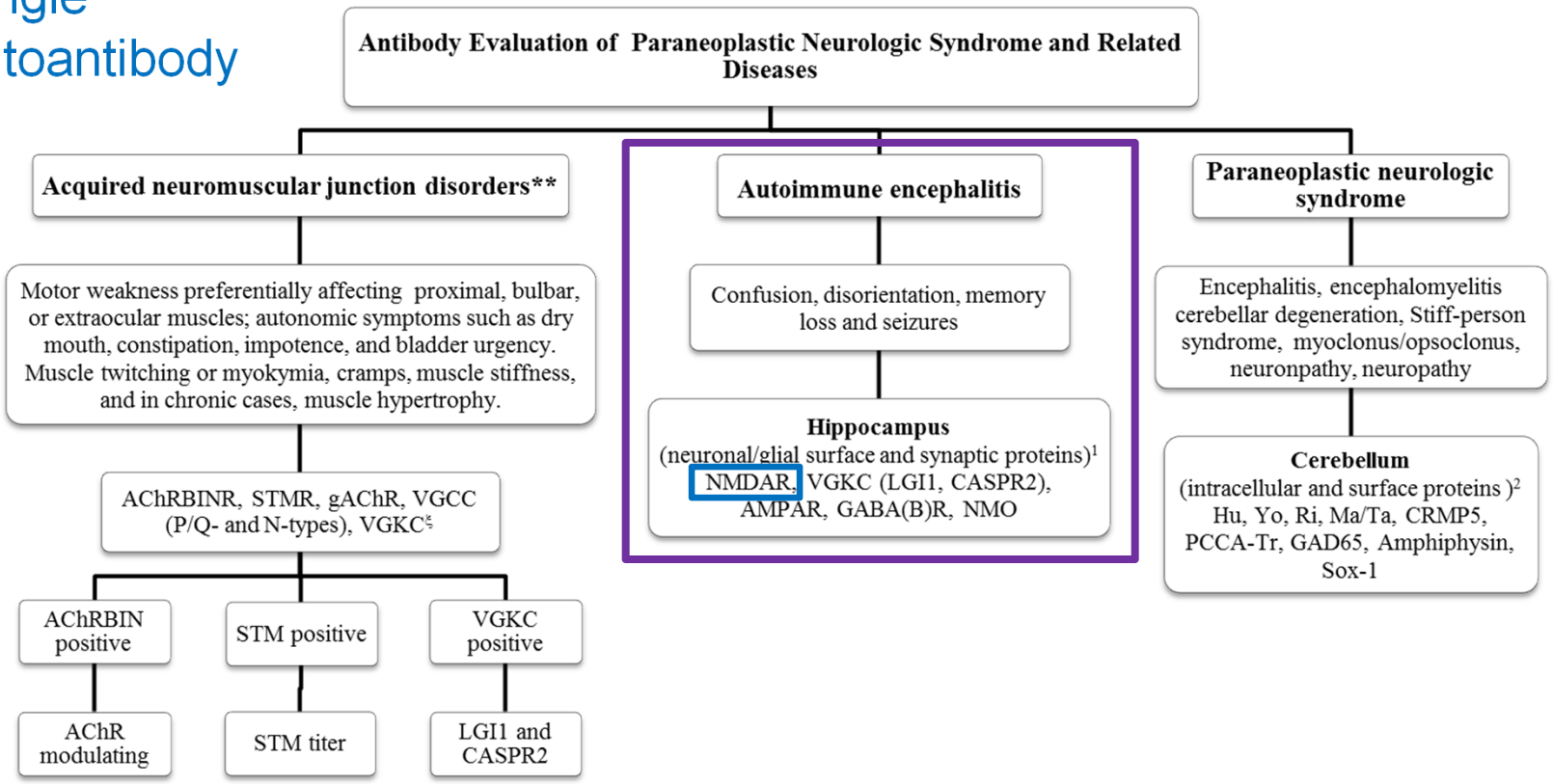
- More invasive
- Antibodies present at lower titer than in serum or not at all, which can cause false negatives

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

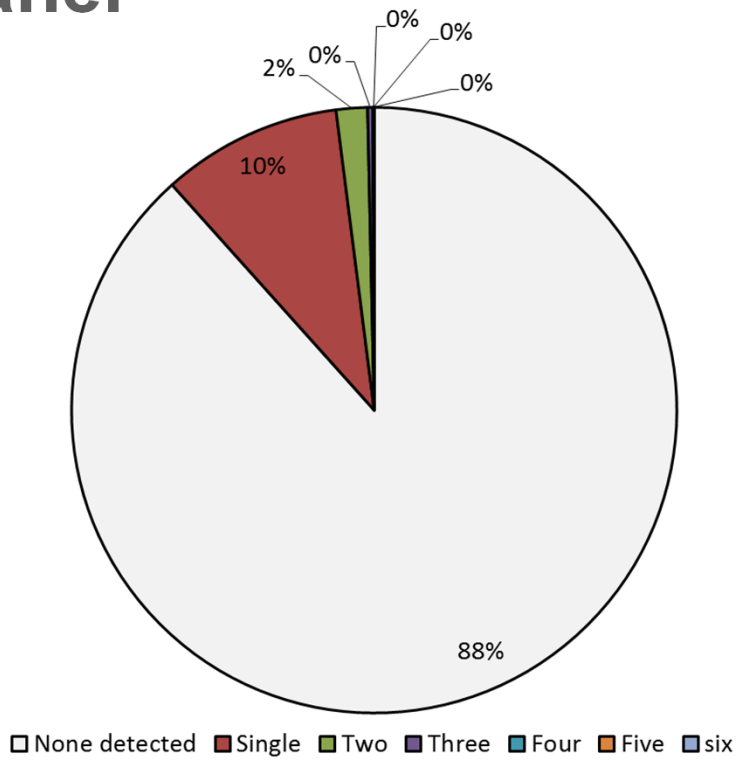
Testing strategies for detecting autoantibodies in autoimmune neurologic diseases:

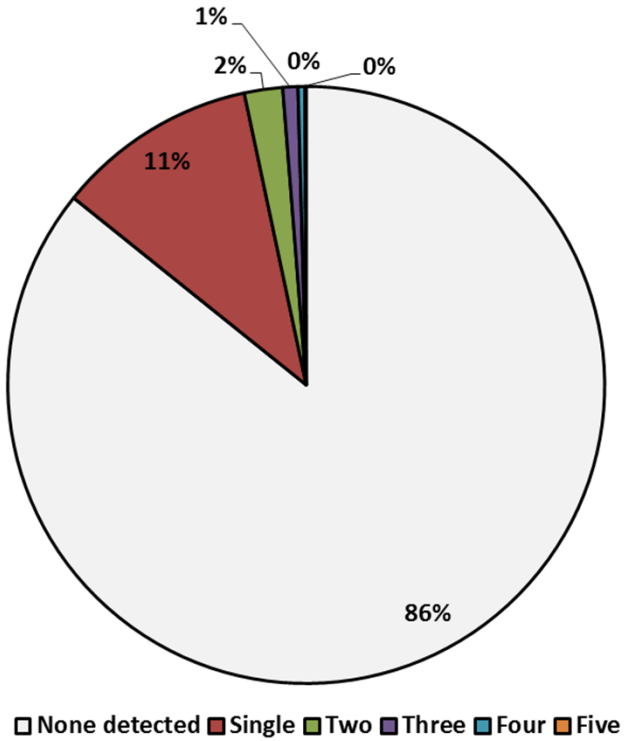
- Comprehensive
- Targeted
- Single autoantibody



Tebo et al., Clin Chim Acta. 2016;459:162-9

Distribution of antibody-positivity in patients evaluated using a 15 autoantibody paraneoplastic panel





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

Horta et al. Clin Cancer Res. 2014;20(14):3862-9

Tebo et al. Clin Chim Acta. 2016;459:162-9

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

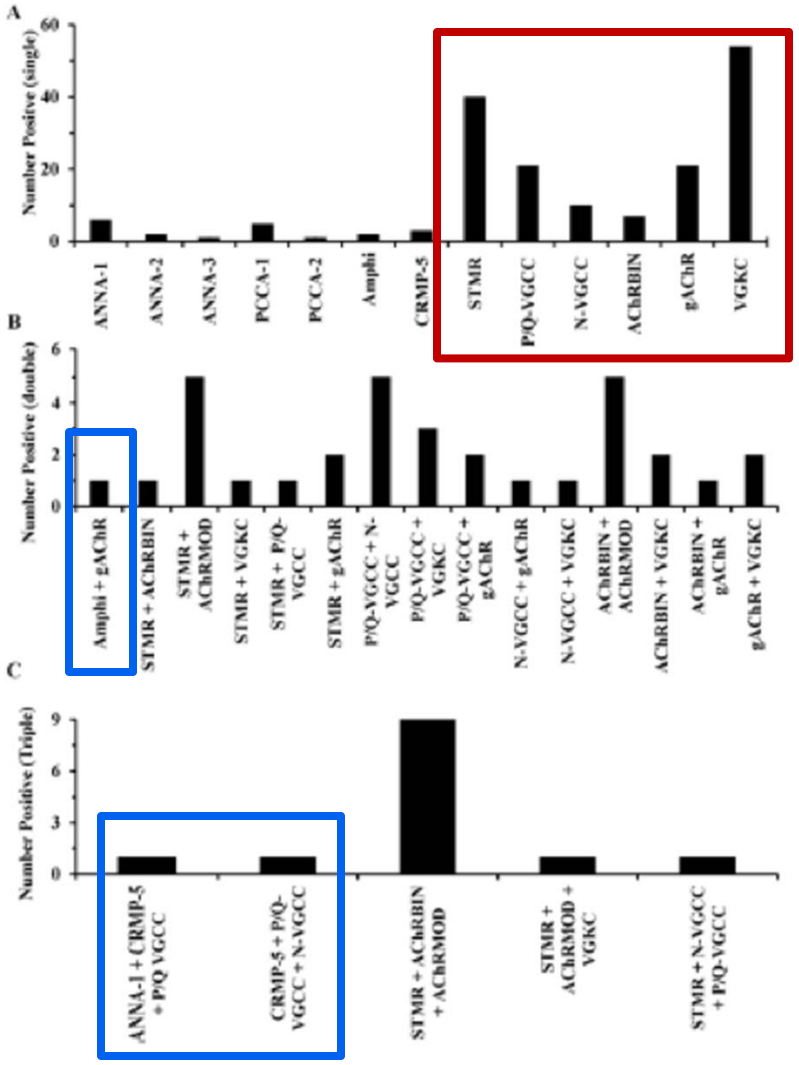
Table 1. Frequency of coexisting autoantibodies among 78,889 sera (15 antibodies tested)

		Coexisting autoantibody by order of frequency					
Autoantibody	**Number positive (%)**	**First**	**Number positive (%)**	**Second**	**Number positive (%)**	**Third**	**Number positive (%)**
Striational	3,483 (4.42)	mAChR	684 (20)	VGKC	192 (6)	gAChR	174 (5)
VGKC	2,194 (2.78)	Str	192 (9)	mAChR	98 (4)	gAChR	90 (4)
gAChR	1,696 (2.15)	Str	174 (10)	mAChR	146 (9)	VGKC	90 (5)
mAChR	1,370 (1.74)	Str	684 (50)	gAChR	146 (11)	VGKC	98 (7)
$VGCC_N$	889 (1.13)	$VGCC_{P/Q}$	233 (26)	VGKC	74 (8)	Str	64 (7)
$VGCC_{P/Q}$	863 (1.09)	$VGCC_N$	233 (27)	VGKC	85 (10)	Str	69 (8)
ANNA-1	252 (0.32)	CRMP5	28 (11)	$VGCC_{P/Q}$	25 (10)	$VGCC_N$	18 (7)
CRMP5	156 (0.20)	Str	30 (19)	ANNA-1	28 (18)	mAChR	24 (15)
PCA-1	82 (0.10)	Str	7 (9)	VGKC, gAChR	3 (4)	$VGCC_N$	2 (2)
SOX1	39 (0.05)	$VGCC_{P/Q}$	13 (33)	$VGCC_N$	10 (26)	ANNA-1	2 (5)
Amphiphysin	39 (0.05)	ANNA-1, $VGCC_{P/Q}$	9 (23)	$VGCC_N$	4 (10)	Str, gAChR, CRMP5	2 (5)
PCA-2	24 (0.03)	CRMP5	6 (25)	ANNA-1	5 (21)	$VGCC_N$, Str	4 (17)
ANNA-2	20 (0.03)	$VGCC_{P/Q}$, $VGCC_N$ ANNA-1	2 (10)	gAChR, Str, amphiphysin	1 (5)		
PCA-Tr	8 (0.01)	mAChR, Str	1 (13)				
ANNA-3	7 (0.01)	ANNA-1	1 (14)				

Horta et al. Clin Cancer Res. 2014;20(14):3862-9

Majority of autoantibodies identified target neuromuscular junction receptors

Autoantibody	Number positive (%)
VGKC	67 (4.23)
STR	66 (4.16)
PQ-VGCC	38 (2.42)
gAChR	32 (2.06)
AChRBIN	30 (1.89)
N-VGCC	20 (1.26)
ANNA-1 (Hu)	7 (0.44)
PCCA-1 (Yo)	5 (0.31)
CRMP-5 (CV2)	5 (0.31)
Amphiphysin	3 (0.19)
ANNA-2 (Ri)	2 (0.13)
ANNA-3	1 (0.06)
PCCA-2	1 (0.06)
AGNA-1	0



Tebo et al. Clin Chim Acta. 2016;459:162-9

Neural antibody clusters can guide search for cancer

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

	mAChR and Str				$VGCC_{P/Q}$ and $VGCC_N$				Str and VGKC			
		Trio				Trio				Trio		
	Duo	gAChR	VGKC or CRMP5	*P*	Duo	VGKC or striational or mAChR	SOX1	*P*	Duo	mAChR	$VGCC_{P/Q}$	*P*
Number with histories available	122	12	16		47	14	10		53	9	5	
Patients with tumor, *n* (%)	55 (45)	8 (67)	13 (81)	0.006[a]	15 (32)	4 (29)	7 (70)	NS	10 (19)	7 (78)	1 (20)	0.021[d]
Tumor type												
Thymoma, *n* (%)	20 (36)	0	11 (85)	<0.004[b]	0	0	0	NS	0	5 (71)	0	0.008[d]
Prostate, *n* (% of men with cancer)	9 (20)	4 (50)	1 (13)	NS	4 (44)	0	0	NS	1 (25)	1 (14)	0	NS
Lung, *n* (%)	6 (11)	0	0	NS	2(13)	2 (68)	6 (86)	0.002[c]	1 (10)	0	0	NS
Clinical												
MG or LES, *n* (% of patients)	MG 69 (57)	MG 10 (78)	MG 11 (69)	NS	LES 3 (6)	LES 2 (14)	LES 3 (30)	NS	0	MG 7 (78)	0	<0.001[d]

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

[a]*P* value for comparison between the duo mAChR/Str cluster with the trio clusters in gray.

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

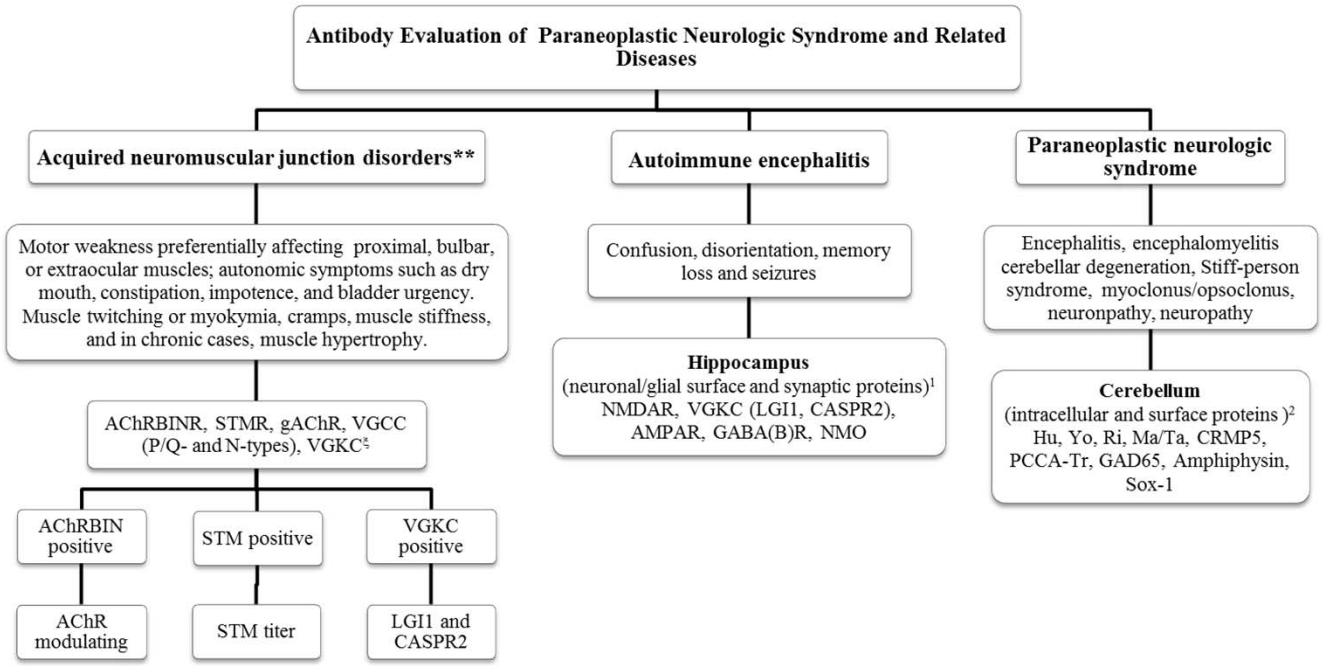
[c]*P* value for comparison between the duo cluster $VGCC_{P/Q}$/$VGCC_N$ and the trio cluster $VGCC_{P/Q}$, $VGCC_N$, and SOX1.

[d]*P* value for comparison between the trio cluster mAChR, Str, and VGKC and the other clusters in dark gray.

Horta et al. Clin Cancer Res. 2014;20(14):3862-9

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)



Tebo et al. Clin Chim Acta. 2016;459:162-9

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

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Department of Pathology