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## A clinical approach to diagnosis of autoimmune encephalitis

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Position Paper

Acute encephalitis is a debilitating disorder that develops as a rapidly progressive encephalopathy (less than 6 weeks) caused by brain inflammation.

The estimated incidence is 5-10 patients per 100000 inhabitants per year.

The most frequent recognized causes of encephalitis are infectious and thus the existing diagnostic criteria and consensus guidelines are focused on an infectious origin.

In the past ten years a growing number of non-infectious, mostly autoimmune, encephalitis have been identified. These newly identified forms of autoimmune encephalitis frequently associate with **antibodies against neuronal cell-surface or synaptic proteins**. Some of them can develop with core symptoms resembling infectious encephalitis, and also with neurological and psychiatric manifestations without fever or CSF pleocytosis.

It is not realistic to include antibody status as part of the early diagnostic criteria of autoimmune encephalitis in view of the fact that:

- antibody testing is not readily accessible in many centers, or it can take weeks to obtain results
- absence of antibodies does not exclude an autoimmune origin

Moreover, response to immunotherapy as part of diagnostic criteria is not practical because **many patients with autoimmune encephalitis do not respond to the most frequently used first line immunotherapies (steroids, IVIg plasma exchange) or the response may take several weeks potentially delaying the diagnosis**.

Clinical facts and reported evidence suggesting that early immunotherapy improves outcome were considered in the development of the current guidelines, in which **conventional neurological examination and standard diagnostic tests prevail in the initial assessment**. This approach should allow the **initiation of preliminary treatment while other studies and antibody tests are processed** and used to refine the diagnosis and treatment.

These guidelines should be applied with caution in children, particularly in they are younger than 5 years.

**Panel 1. Diagnostic criteria for possible autoimmune encephalitis**

All three of the following:

1.- **Subacute onset** (less than 3 months) of **working memory deficits, altered mental status or psychiatric symptoms**.

2.- At least one of the following:

- New focal CNS findings
- Seizures (new onset)
- CSF pleocytosis (more than five cells per mm<sup>3</sup> in white cell count)
- MRI features suggestive of encephalitis

3.- Reasonable exclusion of alternative causes: CNS infection, septic encephalopathy, metabolic encephalopathy, drug toxicity (including seizures by drugs, posterior reversible encephalopathy, serotoninergic syndrome, neuroleptic malignant syndrome, drug withdrawal), cerebrovascular disease, neoplastic disorders, Creutzfeldt-Jakob disease, epileptic disorders, rheumatologic disorders (lupus, sarcoidosis, others), Kleine-Levin, Reye syndrome (in children), mitochondrial diseases, inborn errors metabolism (children)

**Panel 2. Diagnostic criteria for definite autoimmune limbic encephalitis**

All four of the following criteria:

1.- Subacute onset (less than 3 months) of working memory deficits, seizures or psychiatric symptoms.

2.- Bilateral MRI brain abnormalities on medial temporal lobes (PET may be more sensitive)

3.- At least one of the following:

- CSF pleocytosis (more than 5 white cells per mm<sup>3</sup>)
- EEG with epileptic or slow wave activity in temporal lobes

4.- Reasonable exclusion of alternative causes: Lupus, Sjögren's, Kikuchi, Behçet, glioma, herpes, syphilis, Whipple.

If one of the first three criteria is not met diagnosis of definite LE can be made only by the detection of antibodies against cell-surface, synaptic or onconeural proteins.

**Panel 3. Diagnostic criteria for definite acute disseminated encephalomyelitis** (International Pediatric MS Study Group)

All five of the following:

- 1.- A first multifocal clinical CNS event of presumed inflammatory demyelinating cause
- 2.- Encephalopathy that cannot be explained by fever
- 3.- Abnormal brain MRI:
  - Diffuse, poorly demarcated large (1-2 cm) lesions in white matter
  - T1 hypointense lesions in white matter in rare cases
  - Deep gray matter abnormalities (thalamus, basal ganglia) can be present
- 4.- No new clinical or MRI findings after 3 months of symptom onset
- 5.- Reasonable exclusion of alternative causes

**Panel 4. Diagnostic criteria for anti-NMDA receptor encephalitis (anti-NMDAR)**

**Probable anti-NMDAR**

All three of the following:

- 1.- Rapid onset (less 3 months) of at least four of the six following major groups of symptoms:
  - Abnormal (psychiatric) behaviour or cognitive dysfunction
  - Speech dysfunction (pressured speed, verbal reduction, mutism)
  - Movement disorder, dyskinesias, or rigidity/abnormal postures
  - Decreased level of consciousness
  - Autonomic dysfunction or central hypoventilation
- 2.- At least one of the following lab study results:
  - Abnormal EEG (focal or diffuse slow, epileptic activity or extreme delta brush pattern)
  - CSF with pleocytosis or oligoclonal bands
- 3.- Reasonable exclusion of other disorders

Diagnosis can also be made in the presence of three of the above groups of symptoms accompanied by a systemic teratoma

**Definite anti-NMDAR**

Diagnosis can be made in the presence of one or more of the six major groups of symptoms and IgG anti-GluN1 antibodies after reasonable exclusion of other disorders. Antibody testing should include CSF. If only serum is available, confirmatory test should be included (live neurons or tissue immunohistochemistry in addition to cell-based assay)

### **Panel 5. Diagnostic criteria for Bickerstaff's brainstem encephalitis**

Probable: When both of the following criteria have been met:

1.- Subacute onset (less than 4 weeks)

- Decreased levels of consciousness
- Bilateral external ophthalmoplegia
- Ataxia

2.- Reasonable exclusion of alternative causes

Definite: Diagnosis can be made in the presence of positive IgG anti-GQ1b antibodies even if bilateral external ophthalmoplegia is not complete or ataxia cannot be assessed or if recovery has occurred within 12 weeks after onset

### **Panel 6. Diagnostic criteria for Hashimoto's encephalopathy**

All six of the following:

1.- Encephalopathy with seizures, myoclonus, hallucinations or stroke-like episodes

2.- Subclinical or mild overt thyroid disease (usually hypothyroidism)

3.- Brain MRI normal or non-specific abnormalities

4.- Presence of serum thyroid (TPO, TGB) antibodies (no disease-specific cutoff)

5.- Absence of well characterized neuronal antibodies in serum or CSF

6.- Reasonable exclusion of alternative cause

### **Panel 7. Criteria for autoantibody-negative but probable autoimmune encephalitis**

All four of the following:

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 (Bickerstaff's brainstem, typical limbic, acute disseminated encephalomyelitis)

3.- Absence of well characterized autoantibodies in serum and CSF, and at least two of the following:

- MRI abnormalities suggestive of autoimmune encephalitis (excluding some mitochondrial or metabolic causes with symmetrical patterns)
- CSF pleocytosis, CSF-specific oligoclonal bands or elevated CSF IgG index, or both
- Brain biopsy showing inflammatory infiltrates and excluding other disorders (eg. tumor)

4.- Reasonable exclusion of alternative causes

### **Clinical key points**

- A substantial number of patients with autoimmune encephalitis (AE) do not present with a well-defined syndrome but in some cases, demographic information and some comorbidities (diarrhea, ovarian teratoma, faciobrachial dystonic seizures) might initially suggest the underlying disorder (DPPX, NMDAR or LGI1 autoantibodies), although these features are not pathognomonic and might be absent in some patients. In such cases, the diagnosis of definite autoimmune encephalitis depends on the results of autoantibody tests.
- In other patients the clinical syndrome and MRI findings allow for classification as probable or definite AE before the autoantibody status is known (limbic encephalitis, acute disseminated encephalomyelitis).
- In limbic encephalitis (LE) there is a rapid development of confusion, working memory deficit, mood changes and often seizures. The subacute memory loss is a hallmark of the disorder but it can be overlooked easily because of the presence of other symptoms. CSF analysis shows mild to moderate lymphocytic pleocytosis in 60-80% of patients (usually less than 100 white cells per mm<sup>3</sup>) and elevated IgG index or oligoclonal bands in 50%. Among all the subtypes, patients with LGI1 antibodies present with a lower frequency of CSF pleocytosis (41%). In these patients, the absence of inflammatory changes in the CSF might initially suggest a non-inflammatory encephalopathy. MRI often shows increased signal on T2 FLAIR in the medial aspect of the temporal lobes. LE can present with unilateral involvement or with normal MRI and then the diagnosis requires positive antibodies. The onconeural antibodies that more frequently occur with LE are anti-Hu and anti-Ma2. The neuronal cell-surface antibodies more frequently involved are LGI1, GABARb, and AMPAR. Patients with antibodies against GAD (an intracellular antigen) may also develop limbic encephalitis.
- Acute disseminated encephalomyelitis (ADEM) is a monophasic, inflammatory disease of the CNS that mainly occurs in children and adults younger than 40 years. ADEM can be preceded by an acute systemic infection or vaccination. There is a variable encephalopathy along with focal neurological deficits. The CSF usually shows mild pleocytosis ( $\leq 50$  cells/mm<sup>3</sup>). Oligoclonal bands are uncommon (less than 7% of patients). Criteria of ADEM require absence of new clinical and MRI findings 3 months after symptom onset. Transient detection of MOG (myelin oligodendrocyte glycoprotein) antibodies occur in 50% of children with ADEM, but these antibodies are not part of the criteria.
- In Susac's syndrome, a rare autoimmune vasculopathy, there is microvessel involvement of the brain, retina, or inner ear and up to 75% of the patients develop encephalopathy. Typical findings include branch retinal occlusions and MRI abnormalities involving the corpus callosum and periventricular regions.
- Anti-NMDAR encephalitis is frequently recognizable on clinical grounds and is associated with **CSF IgG antibodies against the GluN1 subunit of the NMDA**

**receptor.** In a series of 577 patients by Titulaer et al., **95% of the patients were younger than 45 years**, and 37% younger than 18 years. The ratio **female:male was 4:1**. An underlying ovarian teratoma was identified in **58% of women older than 18 years**. Patients usually develop **abnormal behaviour** (psychosis, delusions, hallucinations, agitation, aggression or catatonia) along with **irritability, insomnia, speech dysfunction, dyskinesias, memory deficits, autonomic instability**, and decrease of the level of **consciousness**. **Seizures** can occur at any time during the disease, but tend to occur earlier in males. **Young children** more frequently present with **abnormal movement or seizures**. When the diagnostic criteria shown in Panel 4 were applied, **80% of the patients fulfilled these criteria within the first month of symptom onset**. The criteria were fulfilled by **75% of patients without teratoma and 90% of patients with teratoma**. Antibody studies should include CSF analysis; a **risk of false-negative or false-positive diagnoses exist if only serum is used**. Findings from three other studies have suggested that serum testing is less consistent, or showed antibodies in patients without anti-NMDAr or immune-mediated disorders.

- Analysis of **CSF for the presence of anti-NMDAr antibodies is mandatory** in patients with **relapsing symptoms after herpes simplex encephalitis**. This complication occurs in 20% of patients with this viral encephalitis, and manifests with new-onset choreoathetosis (in children) or psychiatric symptoms (adults and teenagers), a few weeks (rarely months) after the viral infection.
- **Bickerstaff's encephalitis** is usually preceded by an infectious event, runs a monophasic course, and has a good outcome. In addition to symptoms of brainstem dysfunction (pupillary, facial, bulbar), patients develop generalized limb weakness, which overlaps with features of Guillain-Barré syndrome. CSF pleocytosis occurs in 45% of the patients, and FLAIR brain MRI abnormalities in up to 20%. Anti-GQ1b antibodies are highly specific for this disorder and the related Miller-Fisher syndrome, facilitating the diagnosis in patients with incomplete, atypical symptoms or with associated encephalopathy. The differential diagnosis includes Listeria, enterovirus EV71, paraneoplastic or infectious brainstem encephalitis, CLIPPERS, neurosarcoidosis, and primary CNS lymphoma.

## **Antibody testing**

- Detection of specific autoantibodies establishes a **definite diagnosis of AE**, identifies **immunological subtypes of LE**, and assists in the **differential diagnosis of atypical clinical cases**. Therefore, antibody testing is a **crucial step** in the definite diagnosis of many types of AE.
- Several concepts that apply to classic onconeural or GAD antibodies are not applicable to antibodies against neuronal cell-surface proteins. **Onconeural and GAD antibodies target intracellular proteins** and because these antibodies are present in serum and CSF, and their epitopes are linear, they are detectable with many techniques including ELISA, immunoblotting, and immunohistochemistry. By contrast, antibodies against neuronal cell-surface proteins have different properties that should be considered for a better understanding of the most appropriate test to use and interpretation of the results.
- Conformational antigens. **Most antibodies against neuronal cell-surface proteins** recognize target epitopes only if they are **expressed in their native conformation**. Techniques that meet this requirement are **cell-based assays** (most clinical laboratories), **immunohistochemistry of brain sections** adapted to membrane proteins (commercially available; sometimes used as a confirmatory test) and **immunohistochemistry of cultures of dissociated rodent live hippocampal neurons** (only used in research laboratories).
- Molecular precision. The NMDA receptor is a heterotetramer comprised of two GluN1 subunits and two GluN2/3 subunits. **Detection of IgG antibodies against the GluN1 subunit is a signature of anti-NMDAr encephalitis**. By contrast, antibodies against linear epitopes of GluN2 have been reported in different disorders, providing an uncertain clinical significance (e.g. lupus). Antibodies considered against **VGKC** (voltage-gated potassium channels) do not target these channels; they are directed against **LGI1 or CASPR2** proteins. These antibodies have **well defined syndrome associations**. By contrast, **antibodies named "VGKC complex antibodies"** that do not target LGI1 or CASPR2 are **not syndrome specific** and should not be used as demonstration of an immune-mediated pathogenesis.
- Immunoglobulin class. The antibodies associated with autoimmune encephalitis are IgG antibodies. IgA or IgM antibodies against any of the indicated antigens have unclear significance.

## **CSF Studies**

The investigation of CSF antibodies is important:

1.- Most patients with AE have CSF antibodies, and relevant antibodies might be found only in CSF. In anti-NMDAr, **up to 14% of patients have antibodies in the CSF, but not in the serum.**

2.- The repertoire of antibodies in the CSF and serum can be different in the same patient (eg, NMDAr in serum and CSF and GABARb only in serum), and in this setting, the antibody found in **CSF usually determines the clinical picture.**

3.- For some disorders, like anti-NMDAR encephalitis, the titer of **CSF antibodies correlates better with the clinical course** than the titer in serum.

4.- Neuronal antibody testing using serum and cell-based assays could lead to false positive or false-negative results; this problem rarely occurs with CSF analysis. While awaiting larger studies with autoantibodies, **our recommendation is to include both CSF and serum for neuronal antibody testing in patients with suspected AE.**

- **The approach of first testing serum and subsequently testing CSF (if the serum is found negative), should be avoided because it can delay the diagnosis**

- **If serum is positive and CSF negative** or if the **clinical picture does not fit** with the antibody identified, the possibilities of a **result unrelated** to the syndrome or a **false-positive** should be considered. The **laboratories should be contacted in order to reassess the samples** or use confirmatory tests like brain immunohistochemistry or cultured neurons.

- **Treatment decisions** during the course of the disease should **rely more on clinical assessment than on antibody titers.** Although the titers might correlate with the clinical course, this correlation is imperfect, and antibodies often remain detectable after clinical recovery

## **Seronegative AE**

After excluding all well characterized syndromes of AE (with or without autoantibodies) and other syndromes accompanied by well-defined autoantibodies, there is still a subgroup of possible AE that remains without a final diagnosis (panel 1). Patients in this group can be regarded as having probable AE if they fulfill criteria of Hashimoto's encephalopathy (panel 6) or the criteria shown in panel 7.

Patients with **Hashimoto's encephalopathy** are classically considered to show a **good response to steroids**. The disorder is considered immune mediated despite the unclear physiopathology and the absence of response to prednisone in the patient of the original report. Most patients are **women with a wide age range**. About **60-70% of the patients have thyroid dysfunction**. Patients usually develop seizures, myoclonus, hallucinations, and stroke-like episodes. MRI and CSF studies are normal or with non-specific findings. Because most patients respond to corticosteroids, the disorder has been recently re-named as *steroid-responsive encephalopathy with autoimmune thyroiditis* (SREAT). **Thyroid antibodies are not specific for Hashimoto's encephalopathy because they are present in up to 15% of healthy individuals** (25% in women older than 60 years).

Other poorly defined syndromes with no antibodies can be regarded as probable AE if they fulfill the criteria shown in panel 7.

- The **absence of CSF pleocytosis does not rule out AE** (60% of LGI1 encephalitis do not have pleocytosis). Normal routine CSF studies do not imply that there is no intrathecal IgG synthesis or an absence of CSF antibodies.
- AE can occur with **normal or atypical MRI findings**
  - In children, several genetic disorders, mitochondrial diseases or leukodystrophies can develop with CSF and MRI abnormalities similar to those found in AE and might also respond to steroids.

Patients who meet criteria of probable AE but do not have well-characterized autoantibodies (panel 7) should be investigated for new antibodies in serum and CSF. **The importance of these studies surpasses the clinical significance of inflammatory infiltrates in a brain biopsy**, which may suggest an inflammatory process but cannot establish the autoimmune etiology.

There are several autoimmune CNS disorders that can be considered in the differential diagnosis of AE. These are summarized in the appendix, including Rasmussen's encephalitis, primary CNS angiitis, Morvan's syndrome and other disorders of unclear cause like FIRES (febrile infection-related epilepsy syndrome).

### **Implications and directions for future research**

This position manuscript demonstrates that it is possible to proceed through a **logical differential diagnosis of AE** using criteria based on conventional clinical neurological assessment and standard diagnostic tests (MRI, EEG and CSF studies). **Levels of evidence can be achieved early and therapies implemented quickly**, with the possibility of fine-tuning the diagnosis and treatment when antibody results become available.

The **stepwise escalation of immunotherapy**, which includes first-line therapy (steroids, IVIg, plasma exchange, or both) followed, if there is no clinical response, by second-line therapy (rituximab, cyclophosphamide, or other), is often used in the treatment of anti-NMDAR encephalitis and other types of AE, **but rituximab is increasingly being considered as a first-line therapy**.

Not all AE need a similar approach. **Patients with LE and LGI1 antibodies appear to respond faster and better to steroids than patients with anti-NMDAR encephalitis**, yet the long term outcome is better in the last group.