The field of neuroimmunology is developing rapidly, with an ever-increasing number of new antibodies and associated phenotypes. Given the treatability of autoimmune movement disorders, there is naturally a high index of suspicion and a low threshold for antibody testing. Yet our awareness of problems with interpretation of test results, be they positive or negative ones, is lagging greatly behind — a clinical problem that is not exclusive to the field of movement disorders or antibodies. We introduce this viewpoint with an exemplary case, the sort often faced in clinical practise:

A 71-year-old woman developed “dizzy spells” at age 66 years and is now suffering from a syndrome characterized by cerebellar signs and dysautonomia (orthostatic hypotension, urinary frequency). She has a history of REM sleep behavior disorder (RBD). A brain MRI had shown a hot cross bun sign and T2 hyperintensities in the middle cerebellar peduncles. A dopamine transporter (DAT) scan has been normal. At age 70, she was noted to have very brief episodes of decreased responsiveness. LGI1 antibodies tested negative, but Caspr2 antibodies tested positive in her serum (titer on a research base 1:400). Repeat antibody testing confirmed serum positivity for Caspr2 antibodies, but cerebrospinal fluid (CSF) result was negative, and there were also no CSF-restricted oligoclonal bands (OCBs). A screening for malignancy including whole-body CT-PET was negative. A trial of intravenous methylprednisolone did not lead to any noticeable improvement. She carried a diagnosis of multisystem atrophy of the cerebellar type, and a normal DAT scan, albeit unusual, would not exclude this diagnosis.

However, could this be a case of anti-Caspr2 encephalitis? Caspr2 antibodies can cause cerebellar ataxia, and RBD has been described with antibodies targeting the voltage-gated potassium channel complex. On the other hand, the MRI, the absence of Caspr2-antibodies in the CSF, and, arguably, the absence of a response to immunotherapy would caution against this notion. This patient was one of a few cases we have seen over the last few years posing the question about the significance of an antibody test.

Neighboring disciplines like neurogenetics offer instructive parallels. In the past years, we have seen a wave of rapid developments in neurogenetics, in which technical advances and wider availability of genetic testing have fueled the identification of new genes and expanded existing genotype–phenotype correlations. To account for the increasing complexity, practically, this has led to the development of gene panels to cover various genetic causes of specific syndromes (eg, dystonia gene panel) on the one hand, and on screening approaches like whole-exome sequencing, on the other. To use either in clinical practice, knowledge about the methodological limitations (eg, incomplete gene panels; triplet repeat disorders or deletions not being captured by whole exome sequencing) is key. Apart from these more technical considerations, as a corollary of increased genetic testing, we have to face a new set of problems — the interpretation of equivocal test results...
such as variants of unknown significance (VUS). Clinicians are cautioned to base clinical decision-making on VUS or generally let a genetic test result override clinical acumen. Here we point out how the same applies in the current situation with movement disorders and antibodies, for which “antibody of unknown significance” represents a new challenge in clinical practice. We highlight the pitfalls of testing and how to best approach this issue.

Antibody of Unknown Significance

Surely antibodies are important for the diagnosis in autoimmune movement disorders, but the latter should not rely on antibody tests alone, but on the conjuncture of clinical and paraclinical findings. An expert recommendation on the diagnosis of autoimmune encephalitis highlighted the importance of the clinical assessment and other investigations. Of course, if antibody positivity, phenotype, and other investigations (eg, MRI) are compatible and alternative causes are reasonably excluded, the diagnosis of an autoimmune syndrome is fairly straightforward.

However, if the above criteria are not met, the significance of the positive test result requires critical review. On the one hand, there is an expanding clinical spectrum associated with antibodies (see Supplementary Table S2). On the other hand, there is literature suggesting that auto-antibodies may occur as part of the natural immune repertoire and be detected in healthy individuals. Neuronal autoantibodies have also been found when the final neurological diagnosis was not autoimmune. For example, GlyR antibodies were detected in Creutzfeldt-Jakob disease or genetic dystonia; NMDAR antibodies in patients with Creutzfeldt-Jakob disease or Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS); and GABAAR antibodies in genetically proven Huntington’s disease. The above examples are mostly because of methodological shortcomings (eg, type of tests used, lack of CSF testing, etc.; see below),

![FIG. 1. The groups of neuronal antibodies and their pathogenic roles, examples, treatment responses, and tumor associations (adapted from reference 5).](color figure can be viewed at wileyonlinelibrary.com)
but there is also the occasional persistence of antibodies after recovery, illustrating that, just as the proverbial swallow does not make a summer, a positive autoantibody test alone does not define disease.\textsuperscript{17,18}

In genetics, standard terminology by which laboratories classify genetic variants based on a certain set of criteria (eg, evidence from population data, computational data, functional data, segregation data) includes the term “variant of unknown significance” (VUS).\textsuperscript{19} Similarly, we could use the term “antibody of unknown significance” (AUS) in scenarios like the above. Different from VUS, criteria for AUS still have to be defined, and the onus of classifying AUS is mainly on the clinician’s side. Besides, another difference between genetics and neuroimmunology is that a “relevant variant” is pathogenic, whereas a “relevant antibody” might be pathogenic itself or indicate a primarily T-cell-mediated autoimmune (often paraneoplastic) autoimmunity (see Fig. 1).

Here we discuss some potential handles for AUS and their limitations:

**Phenotypic Compatibility and Biological Plausibility**

The first step when receiving an unexpected antibody result would be to check if such a phenotype has ever been described. A reference for the movement disorder spectrum associated with the various neuronal antibodies as well as a suggestion for comprehensive “antibody panels” can be found here.\textsuperscript{1} A second step could be checking for biological plausibility: is the antigen expressed in the brain regions presumably affected, based on the patient’s signs and symptoms?

**Appropriate Test, Antibody Titers, Repeat Testing, Confirmatory Alternative Tests**

There are various methods of antibody detection, for example, tissue-based immunohistochemistry (tIHC), cell-based assay (CBA), radio immunoprecipitation assay, Western blot, and enzyme-linked immunosorbent assay (ELISA).\textsuperscript{20} Neuronal cell-surface antibodies, for example, are often screened for with CBA, with the antigen expressed, in a conformation akin to that in vivo, on the cell surface. Such CBAs are designed to detect antibodies that are pathogenic and are validated in the original publications, but also have some limitations (both live and fixed CBA). However, some laboratories offer yet other test methods that are not designed to detect such pathogenic antibodies against conformational epitopes. For example, antibodies against the dopamine 2 receptor were originally described and detected by tIHC and CBA,\textsuperscript{21} but ambiguous (and sensu strictu, false-positive) findings resulted from testing by ELISA, a test detecting antibodies to peptides or linear epitores.\textsuperscript{22,23} Similarly, laboratories may offer tests for dopamine 2 receptor–like antibodies or “basal ganglia antibodies” that are not useful in the diagnosis of autoimmune movement disorders.\textsuperscript{24,25} Laboratories may also offer testing for IgM or IgA antibodies,\textsuperscript{10} but existing evidence would caution against ascribing them a diagnostic or pathogenic relevance.\textsuperscript{26,27}

There is good evidence for an overall correlation between neuronal surface antibody titers and clinical symptoms and course, although titers between patients may differ greatly, which makes it difficult to define a cutoff value.\textsuperscript{28} Although future studies will hopefully allow generating generally accepted values to define a “clinically relevant range” for the various antibodies in specific tests, low serum titers are known to sometimes be unspecific for some antibodies (eg, Caspr2, GABA\textsubscript{A}R, and GlyR antibodies).\textsuperscript{29} On the other hand, of course, titers might be low early in the disease, and repeated testing is needed.

Overall, replication of test results with a different methodology (eg, combining cell-based assays with brain immunohistochemistry) or referral to a reference laboratory are recommended, particularly if results are ambiguous.\textsuperscript{9,28,30,31} For example, a recent study showed that the predictive value of onconeural antibodies positive in line blot assays only is low and that positive results must be confirmed by immunohistochemistry.\textsuperscript{32} The same also applies to neuronal surface antibodies. As an interim conclusion, it is of the utmost importance to ensure the appropriate test has been selected in the first place and that results can be replicated in a second, confirmatory test.

**CSF Testing and Antibody Index**

CSF in autoimmune disease may be normal, show unspecific changes, or be a clearly inflammatory profile. The pattern differs from antibody to antibody.\textsuperscript{33} Antibodies against NMDAR, GABA\textsubscript{A}R, and DPPX associate with inflammatory CSF changes, but the basic CSF parameters with Caspr2, LGI1, GABA\textsubscript{A}R, or glycine receptor antibodies are mostly normal. Anti-IgLON5 disease more often features elevated protein, whereas anti-GAD syndromes often show CSF-restricted OCBs as the only abnormality.\textsuperscript{33} Kelch-like protein 11 antibodies often seem to have a signature of multiple (>8) CSF-restricted OCBs, whereas for some of the newer antibodies, for example, PDE10A or Septin-5 antibodies, more data are needed to allow conclusions on their CSF profile.\textsuperscript{34-36}

Overall, the basic CSF parameters alone may be of help, but are often not game-changing.

When testing for antibodies, in practice, we tend to send serum samples, and often enough, the positive or negative test results reflect the true diagnosis. However, the gold standard is testing paired serum-CSF samples to achieve the highest sensitivity and specificity and to avoid false-positive and false-negative test results.\textsuperscript{9,28,37,38}
The presence of autoantibodies not only in serum, but also in CSF would increase the specificity and suggest central nervous system (CNS) autoimmunity, and, mostly, CSF yields positive results in such cases.\(^4\) There are, however, rare cases with a classic encephalitic syndrome (eg, Morvan syndrome with thymoma and Caspr2 antibodies) with very low titers of CSF antibodies that may not be detected with one technique (eg, iIFC) but with another (eg, CBA).\(^40\) The presence of serum but not CSF antibodies in an autoimmune CNS disease seems mechanistically not intuitive and may relate to methodological issues of antibody testing, a primarily peripherally driven immunopathophysiology, in which breakdown of the CSF–blood barrier is enough, or “brain as sink” hypothesis, in which antibodies bound to brain antigens are not detectable anymore. In cases of peripheral syndromes (eg, peripheral nerve hyperexcitability with Caspr2 antibodies), CSF antibodies seem not to be a prerequisite.\(^41\)

Here we highlighted the problem and proposed some handles for practice. Of particular importance is the use of the appropriate specimen (serum and CSF), the appropriate tests, and the use of a second, confirmatory method.

Wider access to gold standard antibody testing, and the expertise of reference laboratories would probably reduce the numbers of AUS. However, there are differences regarding the sensitivity and specificity of the different assays, even in the hands of laboratories with expertise,\(^46\) and there are some inherent limitations of different techniques per se and of the commercial kits used in various laboratories. Currently, there is a lack of agreed standards regarding methodology and reporting, which hopefully will improve in the future with collaborative efforts and quality control.\(^46\) In the meantime, it remains our responsibility to critically evaluate AUS and ensure diagnoses are not based on false-positive antibody test results.

Similarly, as clinicians and researchers, we should strive for certain standards in data collection and communication (see Supplementary Table S1), which may include data on the above-mentioned points. This would facilitate, for example, meta-analysis of data of these rare diseases to allow conclusions about the diagnostic value of certain antibodies, assays, titers, and specimens.

It may be that, despite these efforts, a gray area of ambiguous test results remains. If so, future research could lead to a better understanding of immunopathophysiology and the roles of antibodies per se and which further factors define their pathogenicity, and might facilitate the development of new, widely available (eg, functional) test systems, eventually allowing further criteria akin to those for genetic variants.

## Ancillary Testing

Further investigations can help to substantiate paraclinical features of the disease. For example, brain MRI may show characteristic abnormalities (eg, basal ganglia hyperintensities in anti-LGI1 encephalitis) or electrophysiology detect subclinical disease-specific features (eg, neuromyotonia or myokymia in Caspr2 antibody–related disease).

## Trial of Immunotherapy

In the individual clinical scenario when an autoimmune etiology is plausible, a trial of immunotherapy is a valid and frequently practiced consideration. However, response to immunotherapy as a criterion also has some shortcomings because patients with autoimmune disease might not show any noticeable response,\(^29,40,42,44\) and, conversely, patients with non-autoimmune disease may experience a placebo effect.

## Conclusion and Future Directions

AUS poses a significant problem in clinical practice. Misdiagnosis as an autoimmune movement disorder may be harmful by delaying the correct diagnosis and treatment, and exposing patients to potentially harmful immunosuppression.

### References


**Supporting Data**

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.
Author Roles


B.B.: 3A
K.B.: 3B
J.D.: 3B

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