BRAIN
SCIENTIFIC COMMENTARY

Seronegative autoimmune encephalitis: exploring the unknown

This scientific commentary refers to 'Seronegative autoimmune encephalitis: clinical characteristics and factors associated with outcomes' by Lee *et al*. (https://doi.org/10.1093/brain/awac166).

Autoimmune encephalitis (AE) is a severe inflammatory brain disease strongly associated with pathogenic neuronal autoantibodies targeting extracellular antigens.[1] Detection of neuronal autoantibodies in serum and CSF plays a major role in the diagnosis of AE. In addition, the efficacy of immunotherapy regimens, tumour association and prognosis are largely dependent on autoantibody subtypes.[2] However, in a substantial proportion of patients with suspected AE no autoantibody can be found, despite strong evidence of an immune-mediated disorder (e.g. compatible brain MRI, inflammatory CSF profile).[2] In 2016, Graus *et al*.[2] addressed this problem by proposing criteria for seronegative AE. This was an important development, as the 2016 criteria allow a diagnosis of AE in the absence of autoantibodies. However, descriptions of the clinical features and underlying pathogenic mechanisms of seronegative AE are limited. In this issue of *Brain*, Lee and colleagues[3] provide the first extensive description in a large cohort of the clinical features, treatment response and prognosis of seronegative AE.

The 2016 criteria distinguish between two subtypes of seronegative AE: definite autoimmune limbic encephalitis (LE) and autoantibody-negative but probable AE (ANPRA).[2] The criteria for definite autoimmune LE focus on disorders located in the limbic system and require the presence of bilateral T_2-weighted fluid attenuated inversion recovery (FLAIR) hyperintensities restricted to the mesiotemporal lobes on brain MRI. Importantly, positive antibody status is not mandatory for definite autoimmune LE, as bilateral mesiotemporal hyperintensities are considered highly specific for an immune-mediated disorder and have been described in only a limited number of alternative diagnoses.[2]

Criteria for ANPRA also allow radiological features outside the limbic system, including in other cortical and subcortical regions. Notably, neuropathological findings indicating an inflammatory cause can be used in diagnosing ANPRA. This is different from seropositive AE, in which brain biopsy is generally unnecessary. Although precise epidemiological data are not available, previous research indicates that seronegative AE accounts for a significant proportion of AE cases, emphasizing the importance of research into this subtype.[4]

In their new study, Lee *et al*.[5] describe 147 adult patients with seronegative AE identified at a specialized referral centre and with 2 years of follow-up. By applying the strict 2016 criteria for seronegative AE, the authors were able to study a well defined cohort. The proportion of AE cases found to be seronegative (60% of all AE patients) was markedly higher than in previous research. As stated by the authors, this might be due to selection of atypical or severely affected patients, since the cohort was established in a national referral centre. Disease severity was determined using the Clinical Assessment Scale in AE (CASE) and modified Ranking Scale (mRS). Diagnostic categories included definite autoimmune LE ($n = 23$) and ANPRA ($n = 117$). In addition, acute disseminated encephalomyelitis (ADEM; $n = 7$) was also considered as a subcategory in this study. This is peculiar, as ADEM is generally considered a distinct demyelinating disorder. The variety of clinical and radiological features in seronegative AE was high, especially in the ANPRA subtype, emphasizing that seronegative AE constitutes a highly heterogeneous group of disorders.

The authors show that 57% of seronegative AE patients had a good 2-year outcome (mRS <3), compared to 51% of patients with the ANPRA subtype. Outcomes for seronegative AE were worse than those for anti-NMDAR encephalitis, which apparently cannot be explained by differences in immunotherapy strategies or treatment delay.[6] The different outcomes instead suggest that other unexplored pathophysiological mechanisms may be involved in seronegative AE, for example unidentified pathogenic autoantibodies. An alternative potential explanation is the contribution of T cell-mediated neuronal cytotoxicity, as seen in syndromes with classical onconeuronal autoantibodies, which are strongly associated with cancer. Intriguingly, an underlying malignancy was identified in only three of 147 patients in the study by Lee *et al*.,[3] but T cell-mediated cytotoxicity is still likely in a subset of patients with ANPRA.

Twenty-three patients with seronegative definite autoimmune LE were described by Lee *et al*.,[3] and 2-year outcomes were better in this subgroup (78%) than among patients with ANPRA. In an earlier study of 12 patients with seronegative definite autoimmune LE, treatment outcomes were worse, with only half of patients showing improvement after immunotherapy.[7] This difference might partially be explained by the higher age at onset in the latter study.[7]

Lee *et al*.[3] present an easily assessable 2-year outcome scoring system (RAPID score), including five factors: refractory status epilepticus (RSE), age of onset ≥60, ANPRA subtype, infratentorial involvement and delay of immunotherapy ≥1 month. The RAPID score correlated particularly well with 2-year outcome in the ANPRA and LE subtypes. With a cut-off of 2, the RAPID score

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demonstrated a sensitivity of 81.3% and specificity of 66.3% in predicting a poor 2-year outcome (mRS <3). Another prediction model in AE—the anti-NMDAR encephalitis 1-year functional status (NEOS) score[8]—also includes immunotherapy delay ≥1 month, emphasizing the importance of rapid immunotherapy in the disorder. A point of discussion is the inclusion of infratentorial involvement in the RAPID score. This might lead to a self-fulfilling prophecy, since the mRS is biased towards infratentorial and pyramidal symptoms (i.e. gait disturbance). In clinical practice, the RAPID score may aid in the identification of patients with a severe disease course in seronegative AE requiring more aggressive immunotherapy strategies. However, external validation is necessary prior to clinical implementation.

The authors used a linear mixed model (LMM) to analyse immunotherapy regimens and concluded that more aggressive treatment is associated with better 2-year outcomes. Importantly, there were few serious adverse events. The beneficial effect of second-line immunotherapy has been demonstrated previously in anti-NMDAR encephalitis, with second-line treatment found to be effective if first-line immunotherapy had failed.[6] However, it remains questionable whether similar conclusions about second-line immunotherapy in seronegative AE can be drawn based on the LMM, as Lee and colleagues[3] also acknowledge in their discussion. The same patients were categorized in the same order in different treatment groups (first-line and second-line immunotherapy) and were compared with each other in the model, while time is also an important factor for follow-up prediction. The scores at individual time points are therefore not at all independent, and so strictly speaking the LMM is not allowed: it will underestimate the effect of first-line immunotherapy and overestimate the effect of second-line immunotherapy, in particular tocilizumab. For that reason, the additional analysis using repeated measures analysis of covariance (RM-ANCOVA) is more robust (Supplementary Table 8 in Lee *et al.*[3]). This demonstrated that both rituximab and tocilizumab were associated with a reduction of CASE scores 4 weeks and 8 weeks after the initiation of treatment: this provides more convincing evidence than the LMM, although the level of evidence is still low.

Lee *et al.*[3] also show that in cases of persistent disease (mRS ≥3) at 6 months, although not at 12 months, prolonged immunotherapy was associated with a favourable outcome. However, the groups were small, and in principle, selection bias might have influenced decisions to prolong treatments, although no obvious bias was identified. Overall, the authors provide some evidence for the beneficial effect of second-line and prolonged immunotherapy in selected patients.

Finally, the authors demonstrate that cerebellar atrophy on brain MRI at 6, 12 and 24 months was associated with poor 2-year outcomes. An association between cerebellar atrophy and long-term outcomes has also been shown in anti-NMDAR encephalitis.[9] However, the precise mechanism underlying cerebellar atrophy remains unknown, further illustrating the complexity of the pathomechanisms that give rise to AE. In clinical practice, these features may help in determining long-term prognosis.

By describing clinical features and treatment outcomes in a relatively large cohort of patients, the study by Lee *et al.*[3] represents a valuable step in exploring the new disease entity of seronegative AE. Replication and external validation of published results will be essential. Future research should focus on the identification of specific biomarkers that clarify relevant pathophysiological mechanisms in order to further subcategorize patients within the seronegative AE spectrum and develop targeted treatment regimens.

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Competing interests

R.W.v.S. reports no competing interests. M.J.T. has filed a patent, on behalf of the Erasmus MC, for methods for typing neurological disorders and cancer, and devices for use therein, and has received research funds for serving on a scientific advisory board of Horizon Therapeutics, for consultation at Guidepoint Global LLC, for consultation at UCB, and for teaching colleagues by Novartis. M.J.T. has received an unrestricted research grant from Euroimmun AG, and from CSL Behring.

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