Determinants of cognition in autoimmune limbic encephalitis—A retrospective cohort study

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Abstract
Autoimmune limbic encephalitis (ALE) is the most common type of autoimmune encephalitis (AIE). Subacute memory disturbance, temporal lobe seizures, and psychiatric symptoms are clinical hallmarks of the disease. However, little is known on the factors contributing to cognitive functioning in ALE. Hence, we here investigate major determinants of cognitive functioning in ALE. In a retrospective analysis of 102 patients with ALE, we first compared verbal learning capacity, nonverbal learning capacity, and attentional and executive functioning by absence or presence of different types of neural autoantibodies (AABs). Subsequently we established three linear regression models including 63, 38, and 61 patients, respectively to investigate how cognitive functioning in these domains may depend on common markers of ALE such as intrathecal inflammation, blood-cerebrospinal fluid (CSF)-barrier function, mesiotemporal epileptiform discharges and slowing, determined by electroencephalography (EEG) and structural mesiotemporal changes, measured with magnetic resonance imaging (MRI). We also accounted for possible effects of cancer- and immunotherapy and other centrally effective medication. There was no effect of AAB status on cognitive functioning. Although the regression models could not predict verbal and nonverbal learning capacity, structural mesiotemporal neural network alterations on T2-/fluid attenuated inversion recovery (FLAIR)-signal-weighted MRI and mesiotemporal epileptiform discharges or slowing on EEG exerted a significant impact on memory functions. In contrast, the regression model significantly predicted attentional and executive functioning with CSF white blood cell count and centrally effective medication being significant determinants. In this cohort, cognitive functioning in ALE does not depend on the AAB status. Common markers of ALE significantly predict attentional and executive functioning that is significantly related to centrally effective medication and CSF.
white blood cell count, which may point toward inflammation affecting brain regions beyond the limbic system.

**KEYWORDS**
autoantibody, autoimmune limbic encephalitis, cognition, determinant, inflammation, memory, neuropsychology

1 | INTRODUCTION

Autoimmune limbic encephalitis (ALE) is the most common type of autoimmune encephalitis (AIE). Subacute memory disturbance, temporal lobe seizures, altered mental status, and psychiatric symptoms are clinical hallmarks of the disease (Brierley et al., 1960; Graus et al., 2016). Interictal surface electroencephalography (EEG) typically shows uni- or bilateral mesiotemporal epileptiform discharges and slowing with seizures without side predilection (Kaplan & Sutter, 2013). Serial magnetic resonance imaging (MRI) initially exhibits, often very subtle, uni- or bilateral volume- and T2-/fluid attenuated inversion recovery (FLAIR)-signal increases of the amygdala and anterior hippocampus (Bauer et al., 2020; Wagner et al., 2013; Wagner et al., 2015), which later on may persist or result in hippocampal sclerosis (HS) with chronic pharmacoresistant mesial temporal lobe epilepsy (MTLE; [Bien & Elger, 2007; Bien et al., 2007; Bien et al., 2000]). Cerebrospinal fluid (CSF) might exhibit rather mild inflammatory changes, including lymphocytic pleocytosis, impairment of the blood-CSF-barrier as well as intrathecal immunoglobulin (Ig) synthesis or oligoclonal bands (OCBs; Blinder & Lewerenz, 2019; Hébert et al., 2020). Serum and CSF may contain specific neural autoantibodies (AABs) that bind to either intracellular neural antigens (i.e., Hu, Ri, Ma/Ta, CV2/CRMP5, amphiphysin, and glutamic acid decarboxylase [GAD65]), or plasma membrane neural antigens (i.e., α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor [AMPA], metabotropic glutamate receptor [mGluR5], γ-aminobutyric acid B receptors [GABABR1], glycine receptors [G], dipeptidyl-peptidase-like protein 6 [DPPX], leucine-rich, glioma inactivated 1 [LG11], contactin-associated protein-like 2 [CASPR2], and neurexin-3 [NRX-3]) in seropositive ALE (Crisp et al., 2016; Lancaster & Dalmay, 2012; Melzer et al., 2012). Specific anti-neural AABs are not detected in all patients that fulfill the clinical and paraclinical criteria of ALE (Graus, 2016), which is seronegative ALE (Bien et al., 2000; Bien & Elger, 2007; Graus et al., 2018; Malter et al., 2016)). Nevertheless, in both seropositive and seronegative ALE, brain tissue specimens exhibit unexpectedly dense lymphocyte infiltrates (Bien et al., 2012; Graus et al., 2018) suggestive of encephalitis of autoimmune origin.

ALE has been recognized since 1960 with cognitive impairment, especially memory impairment, being a major clinical symptom (Brierley et al., 1960). Nevertheless, little is known about the factors contributing to cognitive functioning in ALE.

Cognitive functioning in ALE as in AIE in general is likely to be related to a complex interplay between inflammation and blood–brain–barrier (BBB) disruption as well as functional and structural neural network alteration in the mesial temporal lobe and possibly other areas of the brain (Holtmann et al., 2018). To obtain a cohort with a rather homogenous pattern of neuropsychological deficits, in which determinants of cognitive functioning can be studied, we decided to collect a sample of patients with ALE instead of AIE in general.

We first explored whether different subgroups of seropositive and seronegative ALE differ in the extent of cognitive impairment. Afterward we aimed at elucidating to which extent cognitive functioning in ALE is related to intrathecal inflammation and blood-CSF-barrier function, mesiotemporal epileptiform discharges, and slowing on EEG and structural mesiotemporal changes on MRI as well as centrally effective medication and causal treatment.

2 | METHODS

2.1 | Ethics approval

This study was approved by the ethics committee of the Westfälische Wilhelms-University of Münster, Germany (AZ 2013-350-f-S).

2.2 | Sample

We retrospectively collected clinical data of patients from the Department of Neurology of the Westfälische Wilhelms-University of Münster, Germany. To this end, we searched clinical and neuropsychological records by the term “encephalitis” and the registry of the autoantibody laboratory for AIE cases being investigated at the institution for the period spanning January 2000 to September 2019.

Cases diagnosed with preexisting neurological disorders other than AIE such as viral encephalitis, neurodegenerative disorders, glioma, or stroke and preexisting psychiatric disorders such as depression, mania, schizophrenia, and addiction were excluded from the sample to avoid confounding of cognitive functioning through non-AIE pathology.

To obtain a pure sample of patients with ALE out of the larger sample of patients with AIE, we modified the diagnostic criteria proposed by Graus et al. (2016) and applied them to our preselected AIE patient cohort. Graus et al. (2016) provide clinical diagnostic panels for “possible autoimmune encephalitis” (panel 1), “definite autoimmune
limbic encephalitis” (panel 2), and “autoantibody-negative but probable autoimmune encephalitis” (panel 7).

For a clear focus on limbic pathology we specified the criteria for these panels. In panels 1 and 7, we considered hyperintense signals on T2-/FLAIR-weighted MRI in one or both medial temporal lobes, instead of “MRI features suggestive for encephalitis,” as suggestive of ALE (Graus et al., 2016). For panel 2 we considered the criterion of “EEG with epileptic or slow-wave activity involving the temporal lobes” (Graus et al., 2016) to be met, only if both medial temporal lobes showed abnormalities independent from each other. Restricting these criteria to a clear limbic pathology, we refer to ALE as “possible autoimmune limbic encephalitis,” “definite autoimmune limbic encephalitis,” and “autoantibody-negative but probable autoimmune limbic encephalitis” in the following.

The different sub-criteria of these diagnostic panels proposed by Graus et al. were evaluated by the authors independently from each other (clinical presentation [N.M., Ch.M.]; MRI [N.M., A.D.]; seizures and EEG [L.M.L.]; CSF- and laboratory findings [J.M.R., Ch.M., S.J.R.]). Fulfillment of these adapted panel criteria was assessed afterward by Ch.M. Since these panels neither depend on each other, nor necessarily exclude each other, patients fulfilling the criteria of more than one panel were assigned to the panel encoding the highest probability of ALE. For classification according to the neural AAB type, patients with more than one AAB type were assigned to the one with the highest titer.

In cases in which patients had been admitted multiple times, only the record concerning the first admission under suspicion of ALE was considered. The corresponding neuropsychological data were analyzed. Further diagnostic data (i.e., MRI, EEG, CSF-analysis incl. Screening for well-characterized neural AABs in serum and CSF) obtained within 6 weeks before or after neuropsychological assessment were related to the results of the neuropsychological assessment.

2.3 | Neuropsychological assessment

Neuropsychological assessments were performed after full recovery from seizures as according to the self-assessment by the patients and were evaluated independently from the findings in EEG, MRI, CSF-, and laboratory studies. To assess memory we used a visual memory test and a verbal memory test, associated with the right and the left hemisphere (see Helmstaedter et al., 2001; Weidlich et al., 2011). For a comprehensive understanding of determinants of cognition in ALE we also included a screening test for attention and executive functioning.

Visual memory was assessed by the Diagnosticum für Cerebralschädigung II [diagnostic instrument for cerebral damage] (DCS II; Weidlich et al., 2011) frequently used in ALE (see e.g., Bauer et al., 2020; Helmstaedter et al., 2020; Wagner et al., 2015). In this test, pictures of nine abstract figures, each consisting of five straight lines are consecutively presented to the patient one at a time. Then the patient is asked to recall and re-construct the nine figures from memory using five wooden sticks. This procedure is repeated until a patient reproduces all nine figures correctly or for six learning trials at maximum with consecutive recall from memory. Since the DCS does not provide data for a long-term memory recall, we used the sum of correct reproductions as parameter for nonverbal memory reflecting the learning capacity of visual information (DCS Rcum score).

Verbal memory was assessed using the Verbal Lern- und Merkfähigkeitstest (VLMT, Helmstaedter et al., 2001; verbal learning test), which is a word list learning paradigm frequently used in ALE (see e.g., Frisch et al., 2013; Malter et al., 2014; Wagner et al., 2015). Patients are presented with a spoken list of 15 German nouns in 5 consecutive learning trials. After each trial, patients are asked to recall as many words from the list as possible. The total number of correctly recalled items across the five learning trials is regarded as learning capacity for verbal material. We used the total number of nonverbal items recalled across trials for nonverbal learning capacity, respectively.

Attention and executive functions were assessed using the screening test EpiTrack (Helmstaedter & Lutz, 2005) frequently used in ALE (see e.g., Frisch et al., 2013; Hansen et al., 2016; Helmstaedter et al., 2019). The EpiTrack is comprised of six well-established subtests covering attention and executive functions. The subtests include an adaptation of the Trail Making Test for motor speed and cognitive flexibility (Helmstaedter & Lutz, 2005), a response inhibition task, a digit span backward test to measure verbal working memory, a word fluency test and a maze test. These subtests yield an unweighted sum score as a proxy for attentional and executive functioning.

According to common use in clinical (Lezak et al., 2012) and scientific neuropsychological practice (e.g., Grote et al., 2016; Helmstaedter & Witt, 2012) a normative z-value of –1 was regarded as cut-off score for a cognitive deficit. To this end, we transformed the raw scores of the memory tests into standardized z-scores. An EpiTrack age-corrected score lower than 29 is rated as a deficit (Helmstaedter & Lutz, 2005).

2.4 | MRI

MRI was performed on 1.5- or 3.0-tesla scanners. Diffusion-weighted imaging with calculation of apparent diffusion coefficient maps, high-resolution sagittal, axial and coronal T1 spin-echo before and after application of gadolinium, sagittal, axial and coronal FLAIR, as well as axial and coronal T2-weighted fast-field echo, and T2-weighted turbo spin-echo sequences were performed and independently evaluated by two certified epileptologists (N.M. and A.D.) blinded for the findings in neuropsychological, electroencephalographic, CSF- and laboratory studies. Axial and coronal FLAIR sequences were rated regarding the presence or absence of unilateral (right/left) or bilateral (right > left, left > right, left = right) T2-/FLAIR-signal and volume increases of the amygdala and anterior hippocampus consistent with mesiotemporal inflammation. Cases with more diffuse cortico-subcortical FLAIR
lesions as well as gadolinium-enhancing lesions were excluded from the analysis.

2.5 | Short-term and long-term (video) EEG

We used the standard 10–20 system of surface electrode placement. During short-term (video-)EEG recordings (56.3% cases) we used additional anterior temporal (T1 and T2) electrodes to measure mesiotemporal epilepticiform discharges and slowing. For long-term (video-)EEG recordings (43.7% of cases) basal temporal electrodes FT9/FT10 and TP9/TP10 were used. The duration of the recordings ranged between 20 min and 9 days depending on the frequency and severity of clinical seizures. For reviewing the EEG records, standard longitudinal bipolar and common average montages were used. The stored interictal and ictal data were reviewed by another certified epileptologist (L.L.) blinded for the findings in neuropsychological, MRI, CSF-, and laboratory studies. The EEG records were rated for interictal epileptiform discharges and slowing as well as ictal events according to Graus et al., detected by anterior temporal electrodes (F7, F8, T1, and T2) in a unilateral (right/left) or an independent bilateral (right > left, left > right, left = right) fashion following standard guidelines (Binnie & Ebersole, 2004). Interictal epileptiform discharges were classified as bilateral, if at least one sharp-slow-wave or spike-wave complex was confined independently to the other hemisphere. Cases with only extratemporal epileptiform discharges or slowing (n = 14) were excluded from the analysis.

2.6 | Serum–CSF studies

Peripheral blood and CSF were obtained from all patients and analyzed for 30 min after retrieval. Serum and CSF supernatant were analyzed for the presence of IgG AABs against intracellular neural antigens (ANNA1 [Hu], ANNA2 [Ri], ANNA3, PCA1 [Yo], PCA2, Tr/DNER, Ma/Ta, CV2/CRMP5, amphiphysin, SOX1, ZIC4, and GAD65), as well as against surface membrane neural antigens (NMDAR, AMPAR, GABAAR, GABAbR, GR, LG1, CASPR2, DPPX, and NRX-3). Note that detection of AABs against ANNA1 [Hu], ANNA2 [Ri], PCA1 [Yo], Ma/Ta, CV2/CRMP5, and amphiphysin define a paraneoplastic form of ALE, whereas the association to malignancies is less tight in ALE with the remaining AABs (Dalmau & Rosenfeld, 2008; Graus & Dalmau, 2019).

For detection of AABs against intracellular neural antigens a combination of immunoblot and tissue-based assays was applied according to manufacturer's instructions (EUROIMMUN, Lübeck, Germany). To detect AABs against surface membrane neural antigens, we used a combination of cell-based and tissue-based assays according to manufacturer's instructions (EUROIMMUN, Lübeck, Germany).

As a measure of intrathecal inflammation, the CSF white blood cell count per mm³ was used. To quantify blood-CSF-barrier function, the serum–CSF albumin ratio (1 × 10⁻³) was used. It was corrected for the age-dependent upper normal limit according to Reiber and Peter (2001) by expressing the ratio as percentage reached of the upper normal limit for each patient.

CSF samples were negative for viral, fungal and bacterial pathogens, and an extensive serum-panel for rheumatological-vasculitic disorders (ANA, ENA, ANCA, RF, ds-DNA-abs, ACE, and phospholipid-AABs) was negative.

2.7 | Statistical analyses

The statistical analyses were performed using SPSS (version 26; IBM, USA) software. To compare cognitive functioning between groups of different neural AABs and without AABs, three one-way ANOVAs were computed with “different neural AABs and absence of AABs” as group factor and z-scores of the verbal learning capacity in VLMT, the nonverbal learning capacity in DCS II and the age corrected EpiTrack score as dependent variables. Neural AAB types with a prevalence of two or less cases in our sample were thereby subsumed into a single group, entitled “others.” In addition, we compared cognitive functioning between patients with AABs binding to intracellular neural antigens and surface membrane neural antigens by ANOVAs.

To predict cognitive functioning, we established three linear regression models for these dependent variables (z-scores of the verbal learning capacity in VLMT, the nonverbal learning capacity in DCS II and the age corrected EpiTrack score) already accounting for age as a major confounder of cognitive functioning. As independent variables we used 1. CSF white blood cell count per mm³ (intrathecal inflammation), 2. Serum–CSF albumin ratio as percentage reached of the age corrected upper normal limit (blood-CSF-barrier function), 3. Mesiotemporal EEG-alterations (only interictal or ictal epileptiform discharges (8.7% of cases) or slowing (17.5% of cases) or both (44.7% of cases); functional network alterations), 4. Mesiotemporal T2-/FLAIR-signal and volume increase on MRI (structural sign of inflammation), 5. Information on whether a preceding immunotherapy and/or cancer treatment had been finished, since causal ALE treatment could impact cognitive functioning. Moreover, we included in the regression models, 6. whether or not patients received symptomatic medication effecting central nervous system function (i.e., anticonvulsive, antidepressant, antipsychotic, or other drugs) at the time of neuropsychological assessment. Determinants of nominal format (EEG alterations, MRI alterations, cancer-, and immunotherapy and centrally effective medication) were dummy-coded to establish regression models.

Regression models were computed only for cases in which all data of the selected predictors and dependent variables (cognitive parameters) were available, to avoid imputation of data. As we wanted to analyze the impact of AAB on cognitive functioning in the largest possible sample size, we decided to do so separately from the regression models, which would have excluded cases because of missing data.
3 | RESULTS

3.1 | Sample of ALE patients

Of a total of 282 patients, that underwent neuropsychological assessment under suspicion of ALE, 36 patients fulfilled criteria of possible ALE, 5 fulfilled criteria of AAB-negative probable ALE, and 61 fulfilled criteria of definite ALE. This yielded a total of 102 patients with ALE functionally and structurally confined to the limbic system. The groups of possible ALE, probable ALE and definite ALE did not differ regarding verbal learning capacity ($F_{(2,87)} = 4.17, p = .06$), nonverbal learning capacity ($F_{(2,53)} = 2.16, p = .125$), and attention and executive functioning ($F_{(2,81)} = 1.40, p = .25$). In 52.9% of the cases, no neural AABs were detected, but the patients nevertheless met the criteria of the described panels. For the portions of distinct AAB-associated ALE subtypes please refer to Figure 1. Mean age of the sample was 55.8 years (range from 16.6 to 80.3 years), 45.1% were female and the mean education was 13.4 years (SD = 3.2) including school years and further education pursuant to the schema of the Memory Clinic, Basel (Thomann et al., 2018). The mean duration from clinical symptom onset to neuropsychological assessment was 81.4 weeks (SD = 142.8). In our sample, 35.3% of patients had finished or were still under causal treatment (e.g., steroids, apheresis, immunosuppressants, or a combination thereof) at the time of neuropsychological assessment. Causal treatment was initiated on average 81.39 weeks (SD = 141.77 weeks) after symptom onset and on average 21.44 weeks (SD = 104.84 weeks) before neuropsychological assessment. A total of 84.3% received centrally effective medication. On average, the sample of patients showed normal cognitive functioning with large interindividual variabilities. The mean score of verbal learning capacity was $z = -0.36$ (SD = 1.26, n = 90), the mean score of nonverbal learning capacity was $z = -0.78$ (SD 1.10, n = 56), and the mean EpiTrack score was 31.35 (SD = 6.89, n = 84). The quota of patients with deficits in the VLMT and DCS II with a cut-off of $z = -1$ and with an EpiTrack score corrected for age of below 29 are shown in Table 1. For percentages of affection of the mesial temporal lobes according to MRI and EEG see online Supporting Information.

3.2 | Effect of autoantibody status on cognition in ALE

The One-Way ANOVAs with different neural AABs and absence of AABs as group factor showed no main effect of these serotypes on verbal learning capacity ($n = 90, F_{(9,80)} = 1.63, p = .12$), nonverbal learning capacity ($n = 55, F_{(7,48)} = .99, p = .45$), and EpiTrack score ($n = 83, F_{(7,76)} = .89, p = .52$). Also One-Way-ANOVAs comparing AABs against intracellular to AABs against surface membrane neural antigens showed no main effect on verbal learning capacity ($n = 39, F_{(1,37)} = 1.06, p = .31$), nonverbal learning capacity ($n = 17, F_{(1,16)} = .01, p = .91$), and EpiTrack score ($n = 34, F_{(1,32)} = .02; p = .89$). Therefore, this sample can be considered as homogenous with respect to cognitive functioning.

3.3 | Impact of common marker of ALE on cognitive functioning

The regression models could not entirely predict learning capacity (see Table 2). The linear regression model with 63 patients to predict verbal learning capacity just explained $R^2 = .34$ of variance, which was not significant ($F_{(14,48)} = 1.75; p = .08$). The linear regression model with 38 patients to predict nonverbal learning capacity explained $R^2 = .51$ of variance, which was also not significant ($F_{(14,23)} = 1.71; p = .12$). In contrast, the established model with 61 patients to predict attentional and executive functioning reached significance ($F_{(14,44)} = 2.05; p = .03$), explaining $R^2 = .38$ of variance.

In detail, the following predictors reached significance:

3.3.1 | Predictors of verbal learning capacity

Verbal learning capacity was significantly influenced by EEG alterations (ictal or interictal epileptiform discharges or slowing) in the right mesiotemporal lobe (unstandardized b-weight = +1.24; $p = .02$). That is, patients with EEG alterations restricted to the right mesiotemporal lobe showed a z-score of verbal learning capacity 1.24 points higher than patients without EEG alterations in the right mesiotemporal lobe. Interestingly, neither parameters of intrathecal inflammation and blood-CSF-barrier function nor of structural neural network alteration in the mesial temporal had significant impact on verbal memory function. Moreover, neither immunotherapy nor other centrally effective treatment showed an effect.

3.3.2 | Predictors of nonverbal learning capacity

Nonverbal learning capacity was significantly related to bilateral structural alterations of the medial temporal lobes (unstandardized

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**FIGURE 1** Autoantibodies within the autoimmune limbic encephalitis sample: GAD65, glutamic acid decarboxylase 65; LGI1, leucine-rich glioma inactivated-1; CASPR2, contactin-associated protein 2; GABAr, gamma aminobutyric acid receptor b; DPPX, dipeptidylpeptidase-like protein 6
b-weight = −1.10, p = .04), showing that patients with bilateral structural alterations of the medial temporal lobes scored 1.10 points lower on the z-score of nonverbal learning capacity than patients without structural medial temporal alterations. Parameters of intrathecal inflammation and blood-CSF-barrier function as well as functional mesiotemporal network alterations on EEG had no significant impact on nonverbal memory function. Immunotherapy and other centrally effective medication showed no effect on nonverbal learning capacity either.

### 3.3.3 Predictors of Attentional and executive functioning

Attentional and executive functioning was significantly predicted by white blood cell count and centrally effective medication (unstandardized b-weight = −0.70, p = .01 for white blood cell count and unstandardized beta = −7.61, p = .05 for centrally effective medication). So, one additional white blood cell per μl in CSF statistically predicts a slight reduction of EpiTrack score of 0.67 points, and patients taking more than one centrally effective medication score 7.61 points lower on EpiTrack than patients without any centrally effective medication. For further results of the linear regression models, see Table 2.

### 4 DISCUSSION

The aim of this study was to investigate major determinants of cognitive functioning in patients with ALE. We assumed that cognitive function in ALE is related to a complex interplay between parenchymal inflammation and local BBB disruption as well as functional and structural neural network alteration predominantly within the mesial temporal lobe but possibly also beyond (Holtmann et al., 2018), by also taking cancer- and immunotherapy and centrally effective medication into account.

Given the rarity of the disease, we collected a rather large sample size. We selected patients very precisely referring to the criteria
proposed by Graus et al. by making even more stringent claims for a clear limbic pathology.

### 4.1 | Autoantibody status is not related to learning and a screening score for attention and executive functioning in ALE

We found no impact of the absence or presence of distinct AABs on verbal learning capacity, nonverbal learning capacity, and attentional and executive functioning. Although ALE with different AABs types have recently been reported to differ in white matter integrity (Wagner et al., 2016), especially regarding fiber tracts (Bauer et al., 2020), these differences were not significantly related to memory performance in ALE with GAD65-, LGI1-, and CASPR2 AABs. Moreover, Bauer et al. (2020) found a significant relation between the left-right ratio of fiber cross section of the superior longitudinal fascicle and verbal memory performance, but memory performance was also not significantly different between patients with ALE with GAD65-, LGI1-, or CASPR2 AABs.

In contrast, in earlier studies ALE with VGKC-complex AABs was found to be more impaired in memory, but exhibited a significantly better response to immunotherapy than ALE with GAD65 AABs (Frisch et al., 2013; Wagner et al., 2015). In this context, it needs to be considered that a substantial portion of ALE cases with VGKC-complex AABs was shown to be negative for AABs against LGI1- and CASPR2 as parts of the VGKC-complex (Yeo et al., 2018) and exhibit no evidence of autoimmune inflammation (van Sonderen et al., 2016). Thus, the aforementioned data remain hard to interpret retrospectively.

Klein et al. (2013) present a significantly higher frequency of cognitive impairments in ALE with LGI1 AABs than in ALE patients with CASPR2 AABs, and Vanil-Yavuz et al. (2016) state that ALE with CASPR2 AABs can be distinguished from AAB-negative ALE by cognitive dysfunction. Unfortunately, neither research groups give a definition of cognitive dysfunction or impairment nor do they report how cognition was assessed.

Despite a significantly better response to immunotherapy in ALE with paraneoplastic onco-neural AABs compared to GAD65 AABs, Hansen et al. (2016) found no significant difference in overall cognitive performance between both groups at baseline. Moreover, Helmstaedter et al. (2019) found a significantly pronounced accelerated long-term forgetting in AAB-negative compared to AAB-positive ALE patients. In addition, there were no significant differences in cognition and mood between AAB-negative ALE and ALE with AABs against intracellular (onco-neural antigens and GAD65) antigens (Helmstaedter et al., 2020). The proportion of AAB-negative and AAB-positive ALE in our cohort is in line with a sample of 80 ALE patients collected by Lee et al. (2016), from an Autoimmune Synaptic and Paraneoplastic Encephalitis Registry in Korea, in which 43.8% were seronegative. Bataller et al. (2007) for example, reported only 18% of seronegative ALE in their sample of 39 patients collected in the hospital of the University of Pennsylvania. In addition to variable numbers of AABs included in screening panels using cell-based assays and immunoblots at different centers (without or without additional screening using tissue-based assays) and variable sensitivities of the tests employed, we assume that this large variation may be due to differences in the approach to diagnose AIE in general. This may be more related to autoantibody testing at departments of general neurology and more related to typical features on (high-resolution) MRI, EEG, and neuropsychology at specialized departments of epileptology.

In summary, there is some evidence that AAB status is related to structural differences and different responses to immunotherapy in ALE. However, evidence for different cognitive profiles and different degrees of cognitive impairment between different AAB subgroups is much less clear.

In our large and carefully selected sample, there was no significant impact of AAB status on cognitive functioning as measured by VLMT, DCS II, and EpiTrack.

### 4.2 | The established multiple linear regression models could not entirely predict verbal and nonverbal learning capacity, but attentional and executive functioning in ALE

In our ALE cohort, serum–CSF albumin ratio, CSF white blood cell count, mesiotemporal epileptiform discharges and slowing on EEG, structural mesiotemporal changes on MRI, preceding cancer- and immunotherapy and centrally effective medication were not sufficient to entirely predict verbal and nonverbal learning capacity. Though, we could detect significant relations between functional and structural alterations of the mesial temporal lobes and memory functioning and could predict attentional and executive functioning with significant predictors CSF white blood cell count and centrally effective medication.

#### 4.2.1 | Memory performance is related to structural and functional alterations of the mesial temporal lobes

We used mesiotemporal T2-/FLAIR volume and signal increase as a measure of parenchymal edema and inflammation. We observed that nonverbal learning capacity is worse in ALE patients with bilateral mesiotemporal MRI alterations compared to those without any structural changes or with just right mesiotemporal alteration. This observation is in line with the common finding that nonverbal memory performance is associated with both temporal lobes (see e.g., (Hermann et al., 1992; Kelley et al., 1998)).

Consistently, Sola-Valls et al. (2020) found bilateral hippocampal T2-/FLAIR-hyperintensity on MRI to be predictive even for general cognitive long-term outcome in ALE with LGI1-AABs. In contrast, other studies found no relation between structural hippocampal alterations on MRI and figural or verbal memory performance in ALE (Bauer et al., 2020; Frisch et al., 2013; von Rhein et al., 2017). In another study, about 20–60% of patients with ALE showed no structural MRI abnormalities despite cognitive symptoms being present (Heine et al., 2018).
To resolve the obvious discrepancy between these findings, high-resolution MRI with hippocampal subfield analysis appears to be a promising tool since a relation to memory performance could be detected (Finke et al., 2017; Miller et al., 2017). In addition, a wider view may clarify structural correlates of memory impairment other than the hippocampus, as AIE was found to affect broader functional networks beyond the limbic system including the motor cortex (Navarro et al., 2016), sensorimotor system and basal ganglia (Dodich et al., 2016), the ventral and dorsal default mode network (Heine et al., 2018), or the superior longitudinal fascicle (Bauer et al., 2020). Such an approach will likely yield a more accurate prediction of memory performance in ALE from structural MRI changes.

Moreover, more direct measures of mesiotemporal parenchymal inflammation such as positron emission tomography (PET) of the translocator protein (TSPO) may exhibit a closer correlation with cognition. TSPO is overexpressed on activated microglia and reactive astrocytes (Gershen et al., 2015) and its PET-signal is markedly upregulated in neuroinflammatory diseases such as multiple sclerosis (Nutma et al., 2019) and was found to be increased ipsilateral to seizure foci in temporal lobe epilepsy (Gershen et al., 2015).

We used anterior temporal surface EEG recordings as a measure of mesiotemporal neural network function. Our finding that verbal memory performance was better in ALE patients with right mesiotemporal EEG alterations (epileptiform discharges or slowing) compared to those without any EEG alterations does not appear intuitive at first glance. One would expect worse nonverbal memory performance under this circumstance. In our cohort 8.7% exhibited mesiotemporal epileptiform discharges only, 17.5% exhibited mesiotemporal slowing only, and 44.7% displayed both. Possibly the slowing detected by surface EEG electrodes indicate epileptic activity in the depth of the mesiotemporal lobe (Lieb et al., 1976). However, it has also been proposed that slow wave activity during sleep mediates memory consolidation and other forms of cognition such as executive functions (Wilkens et al., 2018) and artificial induction of slow wave activity during sleep and wakefulness supposedly enhances cognitive functioning (Wilkens et al., 2018). Thus, we cannot exclude that mesiotemporal slow wave activity in ALE may represent a correlate of functional network adaptation during ongoing inflammation.

In contrast, interictal epileptiform discharges and clinical and subclinical seizures are reported to disrupt cognitive functioning in epilepsy in general (Drane et al., 2016). Such an impact of epileptiform discharges on cognitive functioning was also reported in ALE with a high frequency of subclinical seizures (Kanazawa et al., 2014). These effects are transient, but can have a relevant impact on cognitive functioning, if they occur in temporal association to cognitive testing (Drane et al., 2016). In our sample, EEG recordings (and other assessments) were conducted within a period of 6 weeks before or after neuropsychological assessment. Therefore, we cannot actually be sure about the EEG pattern during neuropsychological testing and may thus be unable to detect a significant relation between EEG activity and nonverbal memory and attentional and executive functioning. Furthermore, the majority of EEG data were derived from short-term recordings (56.3%) in our cohort likely leading to an overrepresentation of cases with mesiotemporal slowing and an underestimation of cases with mesiotemporal epileptiform discharges. Regarding potentially converse effects of slow wave and epileptiform discharges on cognition, this may further have distorted the findings in our cohort.

In addition, surface EEG signals in ALE (and other mesiotemporal pathologies) are significantly attenuated and distorted by the inhomo-geneous conductivity of the tissue between the mesiotemporal potential generators and recording electrodes. Such effects are largely absent in mesiotemporal magnetoencephalography (MEG) signals and it has therefore been proposed to combine surface EEG and MEG to identify sub-compartments of the temporal lobe to differentiate between subtypes of mesial temporal lobe epilepsy (Pataria et al., 2005). In summary, we demonstrate that memory function in ALE depends on structural as well as functional alterations of mesiotemporal neural networks.

### 4.2.2 CSF white blood cell count is a significant predictor for attentional and executive functioning

We used the CSF white blood cell count as a measure of intrathecal inflammation and the serum–CSF albumin ratio as a measure of focal BBB function in ALE. We found the CSF white blood cell count and centrally effective medication are significant predictors of attentional and executive functioning, whereas the serum–CSF albumin ratio did not significantly predict any of the cognitive parameters employed here. We suggest that impairment of attentional and executive functioning is due to a direct effect of inflammation beyond the mesial temporal lobe in ALE rather than an indirect effect of mesiotemporal inflammation as we could not find a significant relation to memory functioning. This could be due to a modulating effect of cytokines. Cytokines are released in various autoimmune CNS disorders and associated with cognitive functioning (McAfoose & Baune, 2009). In chronic viral CNS diseases higher levels of cytokines were associated with worse attention and executive functioning (Cohen et al., 2011). Possible mechanism of action of cytokines could be neurotransmitter dysregulation, demyelination, or glial and neuronal apoptosis (Wilson et al., 2002). Nevertheless, the association appears to be cytokine- and task specific (Cohen et al., 2011) and cytokines may also exert protective effects (McAfoose & Baune, 2009). In multiple sclerosis, a higher percentage of T cells releasing IFN-γ was found to be associated with better performance in processing speed (Heesen et al., 2010).

The networks of diverse attentional and executive functions assessed by the EpiTrack in a sum score are more widespread compared to the fronto-temporal networks involved in memory functions assessed by DCS and VLMT likely rendering more susceptibility to modulation by intrathecal inflammation. Multi-dimensional flow cytometry (Golombeck et al., 2016; Hansen et al., 2020) and integrated single cell analysis of CSF (and potentially also of peripheral blood; see e.g., Schafflick et al., 2020) together with appropriate controls may provide a more detailed measure of intrathecal immune responses in ALE and its relation to cognitive functioning, especially to memory functions. Moreover, PET-imaging of focal leukocyte permeation of the
BBB may provide a more accurate measure of the impact of local BBB disruption on cognition in ALE (Gerwien et al., 2016).

4.2.3 | Causal treatment has no effect on cognitive functioning

We found no effect of causal treatment (immunotherapy and cancer therapy) on verbal learning capacity, nonverbal learning capacity, or attentional and executive functioning in the regression analyses.

Disease duration at time of neuropsychological assessment in this sample is broad. Though its impact on cognitive functioning is not clear. Frisch et al. (2013) found no correlation between disease duration and severity of cognitive impairment in patients with GAD65 AABs in patients with VGKC AAB in the initial assessment. While Hébert et al. (2018) found a delay of more than 60 days between symptom onset to be predictive for a worse therapy outcome on cognition after more than 1 year in AIE, Szots et al. (2014) report an improvement of cognition in ALE with LGI1 AABs over a natural course of disease over 3–5 years.

Though the time interval between treatment initiation and neuropsychological assessment was sufficient for the treatment to exert an effect (see e.g., Frisch et al., 2013; Wagner et al., 2015). The outcome of immunotherapy in ALE reported in the literature seems to depend on the AAB subtype: For patients suffering from ALE with GAD65 AABs, there are consistent reports that memory performance does not improve by immunotherapy (Frisch et al., 2013; Hansen et al., 2016; Onugoren et al., 2016; Wagner et al., 2015). In paraneoplastic ALE with onco-neural AABs, improvement of attentional and executive but not memory functioning after immunotherapy has been described (Hansen et al., 2016). For ALE with VGKC-complex AABs cognitive function has been reported to improve following immunotherapy (Frisch et al., 2013; Malter et al., 2014; Wagner et al., 2015). For ALE with definitive LGI1 and CASPR2 AABs the results are mainly favorable but not that consistent (see Gadot et al., 2017; Li et al., 2016; Malter et al., 2014; Onugoren et al., 2016). The few studies with AAB-negative ALE patients have relevant limitations concerning sample selection and neuropsychological assessment (see von Rhein et al., 2017). Grau et al. (2018). It must be noted that these studies report treatment effects for selected subgroups of ALE. However, most patients suffer from cognitive impairments despite some effects by immunotherapy (see Arino et al., 2016; Frisch et al., 2013; Hansen et al., 2016; Malter et al., 2014; Onugoren et al., 2016; Wagner et al., 2015).

Taken together, there are chances for a cognitive improvement through immunotherapy in ALE, but treatment effects appear to be AAB-specific and small overall. Thus, these effects disappear when considering the whole group of ALE.

4.3 | Limitations

The following limitations of our study should be mentioned: (i) first and foremost, we mainly used retrospective data with the disadvantage of missing data. This is problematic especially when establishing a multiple linear regression model to predict nonverbal learning capacity with 6 predictors and complete data sets of just 38 patients, resulting in an overestimation of the explained variance. As another disadvantage we were limited in the choice and temporal relation of dependent variables and predictors; (ii) since different kinds of cognitive impairments have been reported to occur in ALE (Hansen, 2019), it might be possible that learning ability is not the most appropriate parameter of hippocampal impairment in ALE (see e.g., Helmstaedter et al., 2019; Lad et al., 2019). Moreover, cognitive domains other than declarative memory, attentional, and executive functioning may be even more affected in ALE, for instance affective responses to emotional stimulation (see e.g., Holtmann et al., 2018). Further investigation is needed to determine the relevant prevailing impaired cognitive functions in ALE; (iii) the parameters used as potential predictors of cognitive functioning in ALE were indirect or at least distorted measures of the pathological processes deemed relevant for cognition in ALE. This contributes to the fact that just 34–51% of the observed variance of cognitive parameters could be predicted. In future studies more direct measurements of these pathological processes should be employed as discussed above; (iv) other possible associates of cognition in ALE could be disease duration (Sunwoo et al., 2016), premorbid cognitive reserve (Sola-Valls et al., 2020), sex (Husari & Dubey, 2019), or other indirect estimators such as serum level of total or neural antigen-specific IgG (Butler et al., 2014; Yamagata & Fukai, 2020).

4.4 | Conclusion

In ALE, cognitive functioning is not influenced by AAB status. Memory functioning only partially depends on structural and functional alterations of the mesiotemporal neural networks. Surprisingly, intrathecal inflammation and function of the blood-CSF-barrier did not have impact memory functioning. In contrast, CSF white blood cell count showed a significant relation to attentional and executive functioning, which may point toward inflammation (e.g., cytokines) affecting brain regions beyond the limbic system.

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AUTHOR CONTRIBUTIONS

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