Autoimmune encephalitis and epilepsy

Marienke A.A.M. de Bruijn

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Chapter 1

General introduction

Adapted from: Anti-NMDAR encephalitis and other glutamate and GABA receptor antibody encephalopathies

M.A.A.M. de Bruijn and M.J. Titulaer

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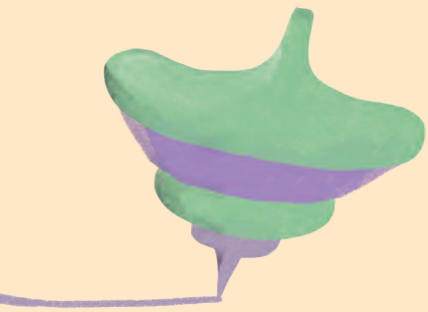

Neuronal antibodies

In the 1960s, the first reports outlining the association between malignancy and antibodies causing encephalopathy were published.[1,2] Over the next decades further cases of neurologic syndromes associated with tumors, and the first evidence for specific paraneoplastic antibodies were described. The targets of these antibodies were intracellular proteins named according to the first patient identified (for instance, Hu, Yo, Ri). These 'onconeural' antibodies are now thought to be a marker for the presence of paraneoplastic syndromes (PNS) and not to be pathogenic,[3] as the neurologic features appear to be caused by cytotoxic T cells. Patients with these antibodies can have variable neurological syndromes, including cerebellar ataxia, polyneuropathy, and limbic encephalitis. PNS are generally progressive and rarely respond to immunotherapy.[4]

The first evidence for antibody-mediated autoimmunity for central nervous system (CNS) diseases was the discovery of metabotropic glutamate receptor antibodies (mGluR1) in cerebellar ataxia.[5] However, the major turning point in this field has been the description of a new clinical entity,[6] subsequently identified as anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis.[7] Patients harbor antibodies aimed at the extracellular part of the NMDAR, and, compared to onconeural antibodies, these antibodies directly affect signal transduction in neurons. The discovery of anti-NMDAR encephalitis has led to descriptions of other antibodies targeting extracellular neuronal epitopes, including antibodies aimed at the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR),[8] the gamma-aminobutyric acid type$_B$ receptor (GABA$_B$R),[9] dipeptidyl-peptidase-like protein-6 (DPP6 or DPPX),[10] leucine-rich glioma inactivated 1 (LGI1),[11] contactin associated protein-2 (Caspr2),[11, 12] the glycine receptor (GlyR),[13] and the gamma-aminobutyric acid type$_A$ receptor (GABA$_A$R).[14] All these antibodies can cause variants of autoimmune encephalitis (AIE), and compared to the classic PNS, syndromes are less frequently associated with malignancy, occur in younger patients, even in children, and often respond to immunotherapy.[15]

A separate group are neuronal antibodies targeting intracellular synaptic proteins. The associated antigens are amphiphysin[16] and glutamic-acid-decarboxylase 65 (GAD65).[17] The pathogenic mechanism of these antibodies is unclear; some hypothesize that inflammation is caused by T cells, while others think that the antigen sometimes becomes extracellular available, and that occasional binding of antibodies leads to neurological symptoms. In anti-GAD65 associated AIE, responses to immunotherapy are described as being only moderate.[18]

This thesis gives an overview of the clinical characteristics and diagnostic possibilities in patients with autoimmune encephalitis, with special emphasis on seizure recognition and treatment. At the end of this thesis autoimmune encephalitis in children is discussed.

Autoimmune encephalitis and epilepsy

AIE is characterized by the subacute onset of memory deficits or altered mental status, often accompanied by other neurological and psychiatric symptoms, and with signs of CNS inflammation.[19] AIE is a heterogenic disease entity comprising three clinical phenotypes: limbic encephalitis, panencephaltis, and less frequently occurring brainstem encephalitis.

Limbic encephalitis is caused by inflammation of the hippocampus, mesial temporal lobe and amygdala. Hallmarks of limbic encephalitis are severe memory problems, psychiatric symptoms, decreased consciousness, and seizures. An exemplary syndrome is anti-$GABA_BR$ encephalitis (**Chapter 2**), in which almost all patients have limbic encephalitis.[9] More than half of the patients have small cell lung cancer (SCLC), and refractory seizures occur in almost all patients. Despite the underlying SCLC, approximately 75% of patients show partial to complete response to immunotherapy and (if applicable) tumor therapy, but, as in all conditions, those without an aggressive tumor have a better chance of survival.[20]

Patients with panencephalitis have a more widespread syndrome with additional involvement of the cerebellum, basal ganglia, brainstem and autonomic system. Panencephalitis is typical for anti-NMDAR encephalitis, which is the most common AIE in adults and in children,[21] with more than 1000 cases described, although the exact incidence is unknown. In more than half of patients the clinical syndrome starts with a prodromal phase, including headache, gastrointestinal symptoms, upper respiratory tract symptoms, or fever, suggesting a nonspecific viral infection. After one to two weeks this phase is followed by a second phase consisting of neuropsychiatric symptoms, autonomic dysfunction, and coma. About 75% of patients are admitted to intensive care units (ICU), and many patients are severely disabled and in need for continuous care in the acute disease phase. About 35–40% of patients have an underlying ovarian teratoma, especially found in young fertile women (55–60%). Responses to immunotherapy are good, and the majority of patients have a favorable outcome, but this could take over two years to achieve.[22]

Seizures are a common feature in AIE, and occur in 50-95% of all patients.[23] Seizures occur most frequently in patients with anti-$GABA_BR$, anti-NMDAR, anti-LGI1, and anti-$GABA_AR$ encephalitis. These provoked seizures are an early symptom of the disease. They can be the presenting symptom or can occur after the onset of behavioral or cognitive problems.

Especially anti-$GABA_BR$ encephalitis, anti-NMDAR encephalitis and the rarely occurring anti-$GABA_AR$ encephalitis are associated with severe refractory seizures, often leading to super-refractory status epilepticus, and prolonged ICU admission.[14, 20, 22] Although, many of these patients present with refractory status epilepticus, the exact incidence of neuronal antibodies in patients with new-onset status epilepticus is unknown. In this thesis we describe a prospective multicenter cohort study, including 50 patients with new-onset status epilepticus and show that a considerable part of patients have an autoimmune etiology (**Chapter 3**).

Next to these typical, severe syndromes, it has become clear that some patients only have subtle AIE signs. Examples are patients with anti-LGI1, anti-Caspr2, and anti-GAD65

encephalitis. Patients with anti-LGI1 encephalitis often have limbic encephalitis, but can also have discrete focal seizures and less noticeable cognitive or behavioral symptoms.[24] Anti-Caspr2 encephalitis involves a broad spectrum of neurological symptoms, and next to the mostly subtle, encephalitis signs patients can have other symptoms like gait instability, muscle cramps, and weight loss.[25] Patients with anti-GAD65 related AIE (**Chapter 4**) often have a more prolonged disease course with signs of cognitive or behavioral changes, refractory focal seizures, stiff person syndrome, cerebellar ataxia or overlapping phenotypes.[26, 27]

Concerning seizures in these less overt syndromes, patients with anti-LGI1 encephalitis often have subtle focal seizures and faciobrachial dystonic seizures (FBDS).[28] FBDS are exclusively seen in anti-LGI1 encephalitis, and are defined as a tonic deviation of the arm often accompanied by an ipsilateral facial contraction. The description of FBDS in anti-LGI1 encephalitis has led to earlier recognition of the syndrome. However, not all patients have FBDS, and about 20% of patients only have stereotypical temporal focal seizures, mostly with impaired awareness and cognitive (absence) or autonomic (goosebumps, gastric rising) onset.[24]

In the last years neuronal antibodies have been described in patients with epilepsy, including LGI1 antibodies, and studies report a prevalence of 8-14% in selected patient cohorts.[29-32] This observation made us hypothesis that neuronal antibodies might also occur in patients with focal epilepsy of unknown origin. To clarify this issue, we describe the results of the prospective multicenter antibodies contributing to focal epilepsy signs and symptoms (ACES) study, in which we aimed to determine the frequency of neuronal antibodies in patients with focal epilepsy of unknown origin. In addition, we have created the ACES score, which can be used to guide screening (**Chapter 5)**.

Ancillary testing

In the standard work up of patients with suspected autoimmune encephalitis, MRI, CSF analysis and EEG should be performed. MRI can show mild and nonspecific changes, best visible on fluid-attenuated inversion recovery (FLAIR) and T2 sequences. These abnormalities usually involve cortical and subcortical regions of the brain, mainly hippocampus, sometimes affecting the basal ganglia. However, in some patients MRI remains normal during the disease course. For example, in anti-NMDAR encephalitis the MRI is normal in two third of patients at disease onset,[22] while considering the total disease course, MRIs show abnormalities in half of the patients.[33] CSF analysis can show moderate lymphocytic pleocytosis, increased protein concentration, elevated IgG index, and CSF-specific oligoclonal bands.[4]

Electrographic abnormalities are often seen in patients with AIE, and only in a minority of patients EEG is normal at presentation. In anti-NMDAR encephalitis, EEGs usually show diffuse background slowing, and a quarter of patients have electrographic seizures. In severely affected patients, admitted to ICU of tertiary medical centers, about 30% of EEGs show an extreme delta brush pattern, not described in any other disorder.[34, 35] This pattern

combines an almost continuous combination of delta activity with fast activity in the beta range; it is symmetric and predominant in the frontal regions.

Antibody testing, interpretation and optimization

Results from ancillary testing can support the diagnosis of AIE, but antibody testing is required to confirm diagnosis. NMDAR antibodies were discovered in 2007 using three laboratory methods.[7] The first method was immunohistochemistry (IHC), whereby frozen and lightly fixed rat brain was incubated with patient's serum or CSF. The results revealed staining of neuropil from hippocampal structures. This test was combined with immunocytochemistry. Rat hippocampal neuronal cultures were exposed to patients' serum or CSF and bound antibodies were detected with fluorescence. The third test was a cell-based assay (CBA). Recombinant cells (generally human embryonic kidney 293 cells) were transfected with the suspected antigen. The transfected cells were incubated with patient's serum or CSF and subsequently labelled. This triple approach has been very successful in identifying most of the antibodies. IHC and immunocytochemistry share the advantage in detecting most extracellular antibodies and can therefore guide screening.[36] In addition, IHC can provide specific staining patterns: NMDAR, GAD and LGI1 antibodies show a specific pathognomonic staining pattern, while other antibodies (Caspr2, $GABA_AR$, $GABA_BR$, DPPX) show a general neuropil staining pattern (Figure 1).

A problem in neuronal antibodies detection is that some assays are difficult to read. The improvement of sensitivity and specificity of these suboptimal assays is of great value. We noticed there were sensitivity issues with the commercially available anti-$GABA_BR$ assay. Samples of some patients highly suspected for anti-$GABA_BR$ encephalitis, with limbic encephalitis, SCLC, and positive IHC and immunocytochemistry, repeatedly showed negative CBA results. Nevertheless, the $GABA_BR$ protein was pulled down using immunoprecipitation and mass spectrometry. In **Chapter 2** we describe the optimization of the $GABA_BR$ CBA by adding KCTD16, an auxiliary protein. We describe the usefulness of KCTD16 as a tumor marker and show that anti-$GABA_BR$ encephalitis patients have different clinical phenotypes, including a new phenotype; rapidly progressive dementia, without seizures.

With the increase in tests requested, the risk of false-positive results and clinically irrelevant results is rising. Concerning incorrect interpretation of results, one of the most discussed antibodies are those targeting the VGKC complex. In about half of the positive anti-VGKC samples LGI1 or Caspr2 antibodies are detected, while in the other half, with absence of LGI1 and Caspr2, an increased anti-VGKC titer is nowadays considered to be irrelevant.[37, 38] Another frequently described, probably irrelevant result, are low-titer GAD65 antibodies, whereas those also occur in patients with diabetes mellitus type 1, and even in healthy individuals. By analyzing serum and CSF of patients with anti-GAD65 we have determined cut-off values for clinical relevance. We show that only patients with neurological symptoms and anti-GAD65 titers above these determined cut-off values have well-defined clinical phenotypes.

In addition, treatment response and serological responses of these high-titer patients were evaluated (**Chapter 4**).

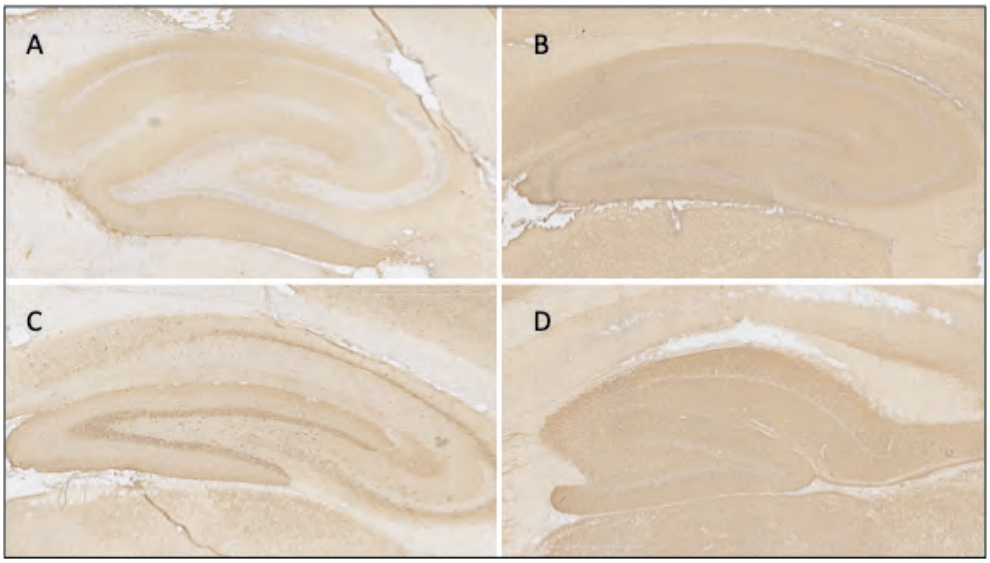

Figure 1. Immunohistochemistry showing different staining patterns. Specific staining patterns are seen in: anti-NMDAR (A), anti-LGI1 (B), and anti-GAD65 (C) encephalitis, while presence of anti-$GABA_BR$ causes a diffuse staining pattern, which can also be seen in anti-Caspr2, anti-AMPAR, and anti-DPPX encephalitis.

Differential diagnosis in adults

Diagnosing AIE is challenging and can be difficult. Although antibody testing is the gold standard, signs and symptoms are not always clear, and antibody testing is not available in the acute setting. Besides, AIE has a broad differential diagnosis. When patients present in the acute phase with (sub)acute confusion, behavioral changes, and memory deficits, it is important to exclude a structural lesion, like cerebral ischemia, cerebral hemorrhage, or cancer (cerebral lymphoma, primary brain tumor, brain metastasis, or carcinomatous meningitis). CSF analysis should rule out an infectious or viral cause of encephalitis.[39, 40] HSVE can present with comparable clinical symptoms, CSF findings, and MRI abnormalities. Other infectious types of encephalitis present with similar clinic and MRI findings, including post-transplant acute human herpes virus-6, neuroborreliosis, and neurosyphilis. Other autoimmune disorders to consider are cerebral vasculitis, caused systemic lupus erythematosus,[41] and acute demyelinating encephalomyelitis. Hashimoto encephalitis should be a diagnosis of exclusion, because antibodies against thyroperoxidase and thyroglobulin are nonspecific, and co-occurrence of thyroid peroxidase antibodies and cell surface antibodies is described in anti-NMDAR encephalitis and anti-$GABA_AR$ encephalitis.[14, 42] Patients with AIE can present with a rapidly progressive dementia. Neurodegenerative diseases, especially Creutzfeldt–Jakob disease (CJD), can present similarly. Metabolic disorders can cause a

general encephalopathy, mimicking AIE, like thiamine deficiency, and Whipple disease.[19] Anti-NMDAR and anti-AMPAR encephalitis patients can present solely with psychiatric symptoms. These types of encephalitis can be mistaken for primary psychiatric disorders, including schizophrenia or a depressive disorder. However, solely psychiatric symptoms without neurologic involvement beyond four weeks of disease are rare.[43]

Advances in AIE research have led to more insight into the etiology of AIE and better recognition of syndromes. Examples are the discovery of post-herpes simplex virus encephalitis anti-NMDAR encephalitis,[44] and the detection of specific HLA types in idiopathic anti-LGI1 encephalitis.[45] Another significant improvement in AIE research was the establishment of the clinical guideline to diagnose AIE by Graus et al. in 2016.[19] This guideline can be used in patients with possible autoimmune encephalitis (Table 1), and allows physicians to start immunotherapy before definite diagnosis in patients with definite limbic encephalitis or probable anti-NMDAR encephalitis (Table 2 and 3).

Table 1. Diagnostic criteria for possible autoimmune encephalitis

Diagnosis can be made when all three of the following criteria have been met:	
1.	Subacute onset (rapid progression of less than 3 months) of working memory deficits (short-term memory loss), altered mental status*, or psychiatric symptoms
2.	At least one of the following: a. New focal CNS findings b. Seizures not explained by a previously known seizure disorder c. CSF pleocytosis (white blood cell count of more than five cells per mm^3) d. MRI features suggestive of encephalitis†
3.	Reasonable exclusion of alternative causes#

* Altered mental status is defined as decreased or altered level of consciousness, lethargy, or personality change.
† Brain MRI hyperintens signal on T2-weighted fluid-attenuated inversion recovery sequences highly restricted to one or both medial temporal lobes (limbic encephalitis), or in multifocal areas involving grey matter, white matter, or both compatible with demyelination or inflammation.
# CNS infections, septic encephalopathy, metabolic encephalopathy, drug toxicity, cerebrovascular diseases, neoplastic disorders, Creutzfeldt-Jakob disease, epileptic disorders, rheumatological disorders, mitochondrial diseases. Additionally in children: Kleine-Levin, Reye syndrome, inborn errors of metabolism.

Table 2. 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: a. CSF pleocytosis (white blood cell count of more than five cells per mm^3) b. 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 (18F-FDG) PET can be used to fulfil this criterion. Results from prior studies suggest that 18F-FDG-PET imaging might be more sensitive than MRI to show an increase in FDG uptake in normal-appearing medial temporal lobes.
# Herpes simplex virus encephalitis, HHV-6 encephalitis, glioma, status epilepticus, neurosyphilis, Whipple, HIV.

Table 3. Diagnostic criteria for probable anti-NMDA receptor encephalitis

Diagnosis can be made when all three of the following criteria have been met:
1. Rapid onset (less than 3 months) of at least four of the six following major groups of symptoms: a. Abnormal (psychiatric) behavior or cognitive dysfunction b. Speech dysfunction (pressured speech, verbal reduction, mutism) c. Seizures d. Movement disorder, dyskinesias, or rigidity/abnormal postures e. Decreased level of consciousness f. Autonomic dysfunction or central hypoventilation
2. At least one of the following laboratory study results: a. Abnormal EEG (focal or diffuse slow or disorganized activity, epileptic activity, or extreme delta brush pattern) b. 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 mentioned symptoms accompanied by a systemic teratoma

* See table 1 and 2.

Treatment responses and long-term outcome

Treatment of AIE is aimed at both the removal of antibodies and direct targeting of clinical symptoms. Removal of antibodies can be achieved by halting the trigger of the immune response, the tumor,[46] if present, and by attacking the overactive immune system. Early detection and treatment of the underlying tumor are of the highest importance, both increasing the chance of curing the cancer, and offering the patient a better chance to respond to immunotherapy. The circulating autoantibodies are pathogenic, therefore immunotherapy is targeted at antibody removal and to diminish and deactivate B cells that are the precursors of plasma cells that excrete the antibodies. There is no doubt about the effect of immunotherapy,[22] but the types and regimens are all expert opinion only. Generally, we can discriminate between first-line immunotherapy, second-line immunotherapy, and chronic immunotherapy. First-line immunotherapy consists of high-dose corticosteroids (intravenous or oral), intravenous immunoglobulins, or intravenous plasmapheresis. Second-line therapy consists of rituximab, cyclophosphamide, or both combined. Azathioprine, or mycophenolate mofetil are most frequently used as chronic immunotherapy, but due to their slow effect they are less useful in acute phases.

Symptomatic treatment includes antiepileptic drugs (AEDs) in refractory seizures,[47] psychiatric medication in patients with extreme agitation or psychosis, and critical care management. Focusing on seizure management, seizures only rarely respond to AED treatment. However AEDs are sometimes needed to achieve seizure freedom, but due to the lack of randomized controlled trials, there are no good guidelines describing treatment recommendations and duration of treatment. In addition, it is unclear if recovered patients require maintenance AEDs. To clarify these issues, we have evaluated treatment responses and the chance to develop chronic epilepsy after resolved encephalitis in a cohort of 110 patients with seizures provoked by LGI1, NMDAR or $GABA_BR$ antibodies (**Chapter 6**). Although patients can remain comatose for months, functional outcome is often good.[22] Therefore physicians should have patience, but at the same time treatment should be aggressive.

Autoimmune encephalitis in children

About 35% of patients with anti-NMDAR encephalitis are children,[21, 48] while other antibodies have been described only rarely in children. Children with anti-NMDAR encephalitis present more often with seizures and movement disorders,[22] and the differential diagnosis is slightly different compared to adults. Usually viral encephalitis is also the most probable diagnosis in a child presenting with acute neurologic symptoms and hyperthermia, but psychosis and dyskinesias occur very rarely in children with viral encephalitis.[49] Children also present with psychiatric symptoms, therefore a primary psychiatric disorder can be considered.[33] In older children drug abuse can be suspected because of changes in personality and behavior and movement disorders. Specific disorders that should be distinguished from anti-NMDAR encephalitis in children are pediatric autoimmune neuropsychiatric disorders associated with (group A beta-hemolytic) streptococcal infection (PANDAS) and Sydenham chorea. These children often have movement disorders, for example, chorea, dystonia, and tics, as well as psychiatric symptoms, including obsessive-compulsive disorders and Tourette syndrome.[50] However, there are patients with a positive group A beta-hemolytic streptococcal titer and chorea who have positive NMDAR antibodies. Finally, Kleine–Levin syndrome should also be considered. This is characterized by hypersomnia, compulsive hyperphagia, hypersexuality, apathy, and childlike behavior. Generally, anti-NMDAR encephalitis patients present with insomnia and dysphagia, while hypersomnia and hyperphagia are seen in the recovering phase. As children present more often with refractory seizures, it is hard to distinguish from other epilepsy syndromes, like fever-induced refractory epileptic encephalopathy.[51]

The guideline to diagnose AIE[19] should be used with caution in children, because of less noticeable syndrome presentation and broader differential diagnosis. To assess the usefulness of the diagnostic Graus criteria in children and to describe the differential diagnosis in a prospective cohort of children with variable syndromes with suspicion of an autoimmune etiology, we have performed the prospective Children's Autoimmunity related to Neuropsychiatric disorders, Chorea and Epilepsy (CHANCE) study (**Chapter 7**).

The acute treatment of AIE in children is quite comparable to the treatment of adults with AIE. In addition, there are signals that children can suffer from residual deficits in multiple cognitive domains after the acute disease phase.[24, 52-55] This may lead to school drop-out, participation problems and social isolation. To draw attention to these extensive neuropsychological problems that may arise after the acute disease phase, we have described the disease course, and long-term follow-up of 28 pediatric anti-NMDAR encephalitis patients in detail, including neuropsychological examination results (**Chapter 8**).

Hypotheses

- Anti-$GABA_BR$ encephalitis is often associated with refractory seizures, but some patients have a rapidly progressive dementia without seizures. (**Chapter 2**)
- The addition of KCTD16 to the cell-based assay improves the detection of anti-$GABA_BR$. (**Chapter 2**)
- KCTD16 is a marker for the presence of a tumor in patients with anti-$GABA_BR$ encephalitis. (**Chapter 2**)
- Autoimmune encephalitis is a common cause of new-onset refractory status epilepticus. (**Chapter 3**)
- Patients with high anti-GAD65 concentrations have well-defined neurological syndromes, that partially respond to immunotherapy, supporting an autoimmune etiology. (**Chapter 4**)
- Neuronal antibodies occur in a small, but relevant proportion of patients with focal epilepsy of unknown etiology. The ACES score, based on specific clinical signs and symptoms can be used to guide screening. (**Chapter 5**)
- Immunotherapy is superior to antiepileptic drugs in the treatment of seizures in autoimmune encephalitis. (**Chapter 6**)
- The development of epilepsy after resolved encephalitis is rare. (**Chapter 6**)
- In children, autoimmune encephalitis has a broad differential diagnosis. (**Chapter 7**)
- Long-term functional outcome following pediatric anti-NMDAR encephalitis is favorable, but many children and adolescents have long-lasting neuropsychological problems. (**Chapter 8**)

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Chapter 2

The expanded clinical spectrum of anti-$GABA_BR$ encephalitis and added value of KCTD16 autoantibodies

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ABSTRACT

Objectives

In this study we report the clinical features of 32 patients with gamma aminobutyric acid B receptor ($GABA_BR$) antibodies, identify additional autoantibodies in patients with anti-$GABA_BR$ encephalitis that mark the presence of an underlying small cell lung carcinoma and optimize laboratory methods for the detection of $GABA_BR$ antibodies.

Methods

Patients (n = 3225) were tested for the presence of $GABA_BR$ antibodies using cell-based assay, immunohistochemistry and live hippocampal neurons. Clinical data were obtained retrospectively. Potassium channel tetramerization domain-containing (KCTD)16 antibodies were identified by immunoprecipitation, mass spectrometry analysis and cell-based assays.

Results

KCTD16 antibodies were identified in 23/32 patients with anti-$GABA_BR$ encephalitis, and in 1/26 patients with small cell lung carcinoma and Hu antibodies, but not in 329 healthy subjects and disease controls. Of the anti $GABA_BR$ encephalitis patients that were screened sufficiently, 18/19 (95%) patients with KCTD16 antibodies had a tumor versus 3/9 (33%) anti $GABA_BR$ encephalitis patients without KCTD16 antibodies (P = 0.001). In most cases this was a small cell lung carcinoma. Patients had cognitive or behavioural changes (97%) and prominent seizures (90%). Thirteen patients developed a refractory status epilepticus with intensive care unit admittance (42%). Strikingly, 4/32 patients had a rapidly progressive dementia. The addition of KCTD16 to the $GABA_BR$ cell-based assay improved sensitivity of the in-house fixed cell-based assay, without loss of specificity. Twenty-two of 26 patients improved (partially) to immunotherapy or chemotherapy.

Conclusions

Anti $GABA_BR$ encephalitis is a limbic encephalitis with prominent, severe seizures, but patients can also present with rapidly progressive dementia. The co-occurrence of KCTD16 antibodies points towards a paraneoplastic origin. The addition of KCTD16 improves the sensitivity of the cell-based assay.

INTRODUCTION

Autoimmune encephalitis (AIE) is a group of severe neurological disorders of which some are associated with pathogenic autoantibodies directed at neuronal membrane proteins,[1] including the metabotropic GABA$_B$ receptor (GABA$_B$R).[2] The majority of patients with anti-GABA$_B$R encephalitis present with limbic encephalitis with prominent seizures. Around 50% of the patients have an underlying small cell lung carcinoma (SCLC). Nearly all patients respond, completely or partially, to immunotherapy or a combination of immunotherapy and tumor treatment,[2-6] stressing the importance of early diagnosis and treatment. Diagnostic laboratories currently test the presence of GABA$_B$R antibodies in serum or CSF with a (commercial or in house) fixed cell based assay (CBA) in which both GABA$_{B1}$ and GABA$_{B2}$ subunits are expressed. Sensitivity of an in house developed fixed CBA was reported to be ~100% for CSF and ~67-80% for serum.[4] Alternatively, a live CBA, in which the surface of living transfected cells is stained with patient antibodies can be used (own observation). However live CBAs cannot be stored for later use and can therefore not be commercialized for widespread use. In some studies additional immunohistochemistry of rat brain or immunocytochemistry of live hippocampal neurons in culture is used for confirmation of fixed CBA.[7]

In this study we: 1) provide a detailed description of the clinical and laboratory findings of 32 patients with anti-GABA$_B$R encephalitis and show that besides limbic encephalitis anti GABA$_B$R encephalitis can also present with a rapidly progressive dementia (RPD); 2) present a novel autoantibody directed at the intracellular GABA$_B$R accessory protein potassium channel tetramerization domain containing 16 (KCTD16), which points towards a paraneoplastic origin; 3) evaluate the different laboratory methods that are available for the detection of GABA$_B$R antibodies and show that the in-house fixed GABA$_B$R-CBA can be improved by the addition of KCTD16.

MATERIALS AND METHODS

Patient inclusion

3225 samples of patients clinically suspected to have immune-mediated encephalitis were tested prospectively (May 2011 to Aug 2018) by routine diagnostic testing with immunohistochemistry and commercial CBA. Two hundred and eighty-two samples, collected for diagnostic testing of onconeuronal antibodies prior to the identification of GABA$_B$R as an autoantigen (2000-2010), were tested retrospectively with immunohistochemistry and in-house fixed CBA. Lastly, in a cohort of 384 patients with clinical suspicion of Creutzfeldt-Jakob disease, 22 patients were retrospectively diagnosed with autoimmune encephalitis by a neuropathologist.[8] These 22 CSF samples were subsequently tested with immunohistochemistry and in-house fixed CBA. All diagnostic tests were performed by the

Erasmus MC University Medical Center (Erasmus MC, Rotterdam, the Netherlands), the Dutch national referral center for paraneoplastic neurological syndromes and autoimmune encephalitis. This study was approved by the institutional review board and informed consent was obtained from patients or their relatives.

The control individuals (n = 329) included plasma or serum from 46 anonymous healthy bloodbank donors, 13 rheumatoid factor positive patients, 50 patients with SCLC without neurological symptoms (13 with limited disease, 31 with extensive disease, six with unknown disease grading),[9] 26 patients with Hu syndrome and SCLC, 21 patients with Lambert-Eaton myasthenic syndrome (LEMS), VGCC antibodies and SCLC, 50 patients with amyotrophic lateral sclerosis[10] and 123 patients clinically suspected of autoimmune encephalitis.

Clinical description

Clinical information was obtained retrospectively from medical records and telephone interviews with patients, relatives or treating physicians. Reduced consciousness was included as a symptom if not caused by a status epilepticus or induced by medication. We included the results of the first MRI, EEG and CSF examination carried out after disease onset. The number of antiepileptic drugs includes all medication known to control seizures that were administered (according to clinical letters), including intravenous drugs during intensive care unit (ICU) admittance. Seizure types were classified according to the International League Against Epilepsy Seizure Classification.[11] Severity of clinical symptoms were scored according to the modified Ranking Scale (mRS).[12] Treatment response was defined as a decrease of at least one point in mRS before and after immunotherapy. Patients were classified as having limbic encephalitis when the criteria were met as described in Graus et al.[1] Rapidly progressive dementia was scored using the NINCDS-ADRDA classification[13]. Dementia criteria needed to be met within six months after the appearance of the first cognitive symptom or the patient had died within two years after the appearance of the first cognitive symptom.

Statistical analysis

Incidence rate was calculated with 95% confidence intervals (CI), using the number of patients identified prospectively in 2015-2017, assuming a Poisson distribution, using available Dutch population data (statline.cbs.nl/statweb/). Statistical analysis was performed using IBM SPSS Statistics 21 and GraphPad Prism 6.0. The following statistical tests were used when appropriate: Fisher's Exact Test, Fisher-Freeman-Halton test, Mann-Whitney U-test, and Wilcoxon signed rank test. Because of the exploratory nature of the study, p-values between 0.01 and 0.05 should be considered with caution.

Laboratory procedures for diagnostic tests

Immunohistochemistry was performed as previously described.[14] Briefly, rat brains were fixed with paraformaldehyde, cryoprotected, snap frozen and cut into sagittal sections. Sections were incubated with patients' serum (1:200) or CSF (1:2). The staining was visualized with diaminobenzidine and slides were counterstained with hematoxylin. Antigen retrieval using sodium citrate (pH 6) of paraffin embedded SCLC tissue samples was performed prior to staining with rabbit anti-KCTD16 (1:200) (*Sigma Aldrich*).

Neuronal cultures and staining were performed essentially as described previously.[15, 16] In short, living hippocampal neurons of at least 14 days in vitro were incubated with patient serum (1:50) or CSF (1:2) and were subsequently fixed and stained with a fluorescently labelled secondary antibody.

Commercial CBA (*Euroimmun*) was used according to the manufacturer's recommendations. In short, human embryonic kidney (HEK) cells are co-transfected with unlabeled GABA$_{B1}$ and GABA$_{B2}$ and stained with patient serum (1:10) or CSF (undiluted). For in house CBAs HEK cells were transfected with GFP-GABA$_{B1}$ (*kind gift dr. Lily Jan, UCSF, San Francisco*) and GABA$_{B2}$ (*RN214644, Origene*) with or without co-transfection of FLAG-KCTD16, KCTD12 or KCTD8 (*kind gift dr. Martin Gassmann, University of Basel, Basel*) and were stained with patient serum (1:40) or CSF (1:2). The presence of KCTD antibodies was determined by fixed CBA with HEK cells transfected with the individual KCTD subunits. Titrations were performed using serial dilutions of fixed CBA. CBAs of serum and CSF, with and without co-transfection of KCTD16, were stained and scored in the same batch. For live CBA, incubation with the patient sample (serum 1:40, CSF 1:2) was performed in culturing medium prior to fixation. In addition, the samples were tested by the diagnostic immunology laboratory at the Erasmus MC University Medical Center for the presence of a panel of classic paraneoplastic antibodies (anti-Hu, Yo, Ri, Ma1, Ma2, Tr, amphiphysin, VGCC and CV2) and anti-neuronal surface antibodies (anti-NMDAR, AMPAR, GABA$_B$R, LGI1 and Caspr2).

Mass spectrometry

Immunoprecipitation and mass spectrometry analysis was performed as described previously.[17, 18] In short, protein was extracted from adult rat brains and incubated overnight with 10 µl of serum. After 16h, protA/G sepharose beads (*GE Healthcare Life Sciences*) were added. The beads were washed, boiled and supernatant was loaded on a 4-12% Bis-Tris gel (*Invitrogen*) and send for mass spectrometry analysis.

Microscopy

Immunohistochemistries were scored visually on an Olympus BX50F. CBAs and live hippocampal neurons were scored visually by two independent observers using a Nikon eclipse 80i upright microscope. Confocal images were acquired with a Zeiss LSM 700 using the 40x and 63x (oil) objectives. Images were processed using ImageJ.

RESULTS

KCTD16 antibodies are associated with an underlying SCLC

Thirty-two patients with anti-$GABA_BR$ encephalitis were identified, of which 18 were diagnosed prospectively and nine retrospectively. In the five remaining patients immunohistochemistry showed neuropil staining and live neurons showed surface labelling, but in house $GABA_BR$-CBA was initially scored negative (before optimization of the assays). In those patients antibodies to the $GABA_BR$ were detected using immunoprecipitation and mass spectrometry analysis. Next to the $GABA_BR$ subunits $GABA_{B1}$ and $GABA_{B2}$, which confirmed the presence of $GABA_BR$ antibodies in these samples, in four of five patients the intracellular $GABA_BR$-accessory proteins KCTD8, KCTD12 or KCTD16 were pulled down (Supplementary Table 1). The presence of KCTD8, KCTD12 or KCTD16 was confirmed with KCTD-only fixed CBAs (Supplementary Table 2 and Supplementary Figure 1). A subgroup of 23/32 (72%) anti-$GABA_BR$ encephalitis patients had KCTD16 antibodies. These antibodies were found in 1 out of 26 (4%) patients with SCLC and anti-Hu syndrome, whereas 329 healthy and other disease control samples (including 50 patients with SCLC without PNS, and 21 patients with SCLC, LEMS and VGCC antibodies) tested negative. Anti-$GABA_BR$ encephalitis patients with KCTD16 antibodies had an underlying tumor more frequently. Of 28 patients that underwent sufficient tumor screening (either CT thorax and abdomen or FDG-PET-CT)[19], 18 of 19 patients with KCTD16 and three of nine patients without KCTD16 antibodies had an underlying tumor ($p=0.001$) (Figure 1A). Patients with KCTD16 antibodies had significantly higher anti-$GABA_BR$ titers in CSF compared to those without KCTD16 antibodies ($p=0.01$) (Figure 1B), and tended to have a status epilepticus more frequently for which admission to the ICU was required ($p=0.045$) (Figure 1C). No other factors (anti-$GABA_BR$ titers in serum, maximum mRS during disease and response to immuno- and/or chemotherapy) differed significantly between patients with or without KCTD16 antibodies (Figure 1D,E). SCLC biopsy tissue from a patient with KCTD16 antibodies expressed KCTD16, whereas normal lung tissue (Figure 1F) and SCLC tissue from a patient without KCTD16 antibodies did not (data not shown).

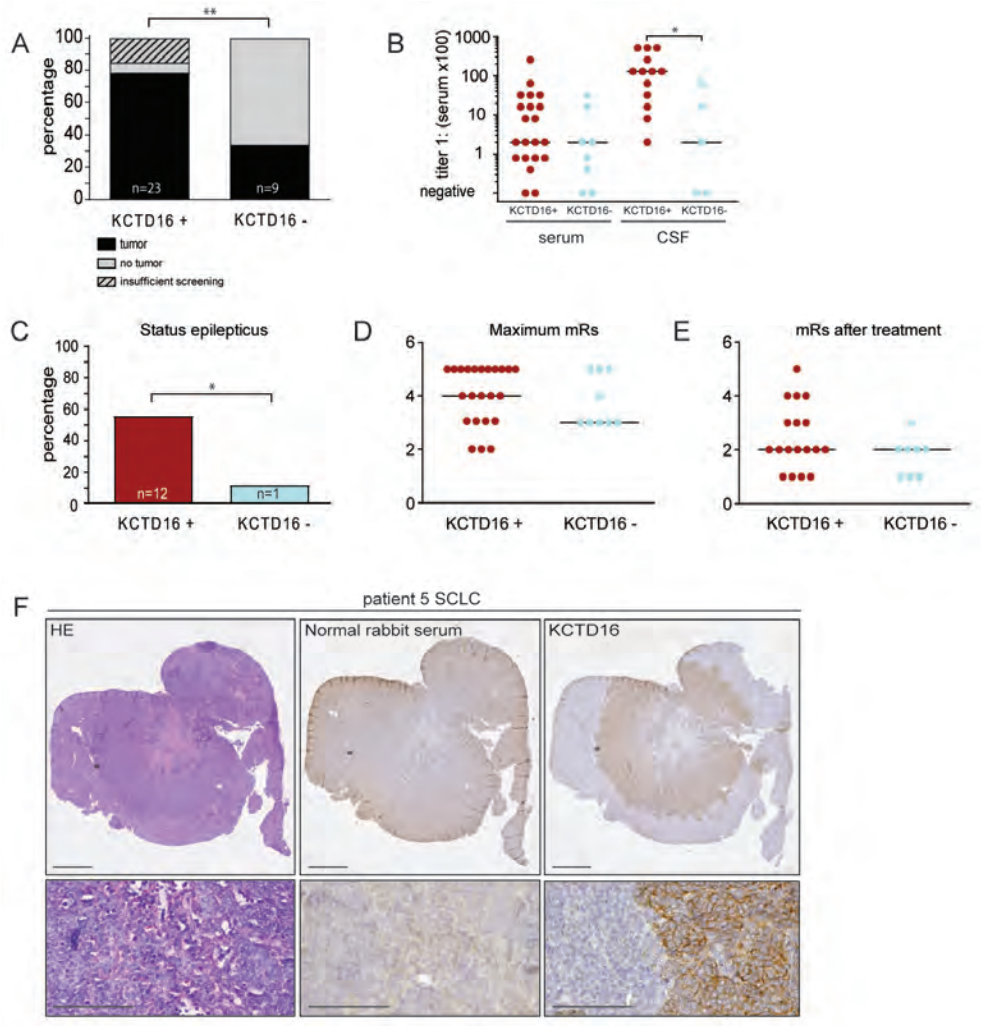

Figure 1. KCTD16 antibodies are associated with an underlying tumor. (A) Bar diagram depicting percentages of patients with or without an underlying tumor. Patients with KCTD16 antibodies more frequently have an underlying tumor. Fisher exact test, p = 0.001. (B) Scatterplot depicting serum and CSF anti-GABA$_B$R titres of patients with or without KCTD16 antibodies; lines indicate median values. GABA$_B$R antibody titres in serum do not differ between patients with or without KCTD16 antibodies, whereas antibody titres in CSF are significantly higher in patients with KCTD16 antibodies. Mann-Whitney test, p = 0.24 (serum), p = 0.01 (CSF). (C) Bar diagram depicting percentages of patients with a status epilepticus. Status epilepticus tended to occur more frequently in patients with KCTD16 antibodies when compared to patients without KCTD16 antibodies. Fisher exact test, p = 0.045 (P-values between 0.01 and 0.05 should be considered with caution). (D) Scatterplot depicting mRS at disease maximum, lines indicate median values. Maximum disease severity does not differ between patients with or without KCTD16 antibodies. Mann-Whitney test, p = 0.59. (E) Scatterplot depicting minimal mRS after treatment, lines indicate median values. Response to treatment does not differ between patients with or without KCTD16 antibodies. Mann-Whitney test, p = 0.20. (F) Immunohistochemistry of SCLC tissue from Patient 5, stained with haematoxylin and eosin (HE), normal rabbit serum and KCTD16 antibody. The image shows specific KCTD16 expression in tumor cells, which is absent in healthy lung tissue. Staining was performed on sequential slides and images were taken in the same area of the sample. Scale bars=25 mm.

Patients and clinical phenotype

The characteristics of all patients are summarized in Table 1. The detailed clinical information for individual patients can be found in Supplementary Table 3 and 4. Sixteen patients were male (50%). The median age at disease onset was 66 years. Incidence of anti-$GABA_BR$ encephalitis (calculated from January 2015-December 2017) was 0.26/1000000 inhabitants/year (95%-CI 0.14-0.44). Limbic encephalitis was the main clinical syndrome in most patients (27/32; 84%). Of the five remaining patients one only had seizures and four (13%) had a rapidly progressive dementia. All four rapidly progressive dementia patients presented with a subacute cognitive decline and hallucinations/psychosis (Table 2). Two patients had myoclonia and/or cerebellar/pyramidal disturbance of movement. Creutzfeldt-Jakob disease was seriously considered in all four patients, and one patient fulfilled criteria for probable Creutzfeldt-Jakob disease, according to the CDC Diagnostic Criteria for Creutzfeldt-Jakob Disease, 2010 (https://www.cdc.gov/prions/cjd/index.html). Overall, most patients initially presented with seizures (53%), while the others had subacute cognitive decline or behavioural changes. None of these 15 patients were initially considered to have a primary psychiatric disorder. In all fifteen patients there was a subacute onset of severe cognitive symptoms or behavioural disorders. Thirteen were directly referred to a neurologist (after visiting the emergency room or outpatient clinic), while two patients were first admitted to the department of internal medicine (one with hypertension and a cognitive disorder, and one with pneumonia and signs of delirium). During the disease course 31 of 32 patients (97%) developed cognitive or behavioural problems. Nearly all patients (90%) experienced seizures. In all cases seizures were generalized, in 15% these were clear focal to bilateral tonic clonic seizures. In addition, eight patients experienced focal seizures, five of which with impaired awareness. In five cases the type of seizures was not described. Often the seizures were refractory to antiepileptic drugs. Thirteen patients (42%) developed a refractory status epilepticus for which admittance to the ICU was required. Additional symptoms that occurred frequently were psychosis/hallucinations (32%), language/speech problems (26%), reduced consciousness (23%) and headache/vomiting (19%). The median mRS was 4 (interquartile range (IQR): 3-5; range: 2-5) at maximum disease severity. There were no differences between non-tumor (n=7) and tumor patients (n=21) and specific clinical features (seizures presenting symptom, status epilepticus, maximum mRS [pre-treatment], best mRS [post-treatment]), although the power was limited due to the sample size (Table 3).

Table 1. Patient characteristics (n=32)

Male: Female	16: 16	
Median age of onset (IQR, range)	66	(57-75, 44-85)
Presenting symptom		
Seizures	17	(53%)
Cognitive(/behavioral)[&]	6	(19%)
Behavioral/(cognitive)	9	(28%)
Clinical syndrome		
Limbic encephalitis	27	(84%)
Rapidly progressive dementia	4	(13%)
Epilepsy	1	(3%)
Symptoms (during disease course)		
Cognitive and/or behavioral	31/32	(97%)
Seizures	29/32	(90%)
▪ *Generalized*	26/26	(100%)
▪ *Focal to bilateral tonic clonic*	4/26	(15%)
▪ *Focal with impaired awareness*	5/26	(19%)
▪ *Focal*	3/26	(12%)
Hallucinations	10/31	(32%)
Language/ speech	8/31	(26%)
Reduced level of consciousness	7/31	(23%)
Headache and/ or vomiting	6/31	(19%)
Autonomic dysregulation[@]	4/31	(13%)
Focal neurological symptoms[#]	4/31	(13%)
Sleep disturbance	3/31	(10%)
Movement disorder[^]	2/31	(6%)
Cerebellar symptoms	1/31	(3%)
Super refractory status epilepticus	13/31	(42%)
Median number of AEDs (IQR, range)	2	(1-4, 0-6)
CSF (first performed, n=30)		
Pleocytosis (median, range cells/mm^3)	23/30	(17, 7-195) (76%)
Elevated protein	9/25	(36%)
Normal	1/30	(3%)
MRI (first performed, n=29)		
Mesiotemporal T2/FLAIR hyperintensities	11/29	(38%)
▪ *Bilateral*	7/11	(64%)
▪ *Unilateral*	4/11	(36%)
Mesiotemporal atrophy	1/29	(3%)
Mesiotemporal hypointensities	1/29	(3%)
Normal[$]	16/29	(55%)
EEG (first performed, n=25)		
Epileptic and encephalopathic	11/25	(44%)
Encephalopathic	8/25	(32%)
Epileptic	2/25	(8%)
Normal	4/25	(16%)
Tumor		
SCLC[±]	16/32	(50%)
Small cell bladder tumor	1/32	(3%)
Tumor, unknown type	4/32	(13%)
No tumor, sufficient screening	7/32	(22%)
No tumor, insufficient screening	4/32	(13%)
Other onconeuronal antibodies*	8/32	(25%)
Treatment		
Immunotherapy	14/31	(45%)
Tumor therapy	2/31	(6%)
Immunotherapy + tumor therapy	11/31	(35%)
No treatment	4/31	(13%)
Response to treatment	22/26	(85%)

ICU = Intensive Care Unit. AED = Antiepileptic drugs. Pleocytosis = >5 cells/ mm^3. Elevated protein = >0.58 gr/L. SCLC = Small Cell Lung Carcinoma. [&]Symptoms were scored as cognitive(/behavioral), if patients mainly had cognitive symptoms, or as behavioral/(cognitive), if patients mainly had behavioral problems. [#]Paresis arm, facial paresis, apraxia, gait instability. [^]Myoclonus (2x). [@]Bradypnea, tachypnea, asystolia, bradycardia. [$]Two of eight patients with initial normal MRI developed mesiotemporal T2/FLAIR hyperintensities later in the disease course, and one patient developed mesiotemporal atrophy later in the disease course (5 remained normal). [±]Fourteen of fifteen tumor patients (93%) smoked or had a history of smoking (in 6 patients data were not available). *Hu (2x), VGCC (2x), GABA$_A$R, GAD, SOX1, Ri, AMPAR (2x).

Table 2. Patient characteristics of the four patients with rapidly progressive dementia

	24. ▌	26. ◆ ‡	28. ⬣	31. □
Sex	M	M	F	M
Age at onset	56	77	85	72
Tumor	No	No	No	No screening
Presenting symptom	behavioral (/cognitive)	behavioral (/cognitive)	behavioral (/cognitive)	cognitive (/behavioral)
Symptoms during disease course	Subacute cognitive decline, complete loss of memory and recognition, apraxia and hallucinations, sleep disturbance	Hypertension, psychotic behavior, cognitive decline, in days followed by cerebellar ataxia and aphasia	Pneumonia, two weeks later confusion, visual hallucinations, psychotic behavior, memory deficit	Acute psychosis, within days followed by cognitive decline, only later on in disease course a few seizures and myoclonus
CSF	105 WBC, elevated protein, elevated IgG index, OCB; 14-3-3 positive; tau 12880, phospho-tau 95	18 WBC, elevated IgG index, OCB; 14-3-3 negative, tau and phospho-tau normal	-	15 WBC; 14-3-3 positive, tau 2450, phospho-tau normal
MRI	Hyperintensity mesiotemporal (bilateral)	Atrophy mesiotemporal, vascular white matter lesions	Normal	Hyperintensity mesiotemporal (unilateral)
EEG	Encephalopathic	Triphasic periodic complexes and encephalopathic	-	Normal
Autopsy brain	-	Perivascular inflammatory infiltrates consisting of B and T-cells. Local infiltration in the hippocampus and basal ganglia. No evidence for CJD	-	Perivascular lymphocyte infiltration and gliosis left hippocampus. No evidence for CJD
Max mRS	4	5	4	5
Immunotherapy	MP + IVIg + rituximab	-	MP + IVIg + rituximab + cyclophosphamide	MP
Best mRS after treatment	2	5	3	5
Treatment response	Responded to therapy	Not treated	Some response to immunotherapy	Not treated
FU (months)	7	1 †	3	4 †

SCLC = Small Cell Lung Carcinoma. WBC = White blood cells. OCB = Oligoclonal bands. CJD = Creutzfeldt-Jacob Disease, FU=follow-up. ‡ Fulfilled criteria for "probable CJD", but pathology refuted this diagnosis. † = deceased. MP = Methylprednisolone. IVIg = Intravenous Immunoglobulin

Ancillary testing

The detailed results for ancillary testing from individual patients can be found in Supplementary Table 3. CSF analysis was carried out in 30 patients, and was abnormal in 29 (97%). The findings mainly included mild pleiocytosis (76%) and increased protein level (36%). Increased IgG index and/or oligoclonal bands were reported in 9/11 patients, but were in most patients. Initial MRIs were obtained in 29 patients and were abnormal in 45% of the cases, most frequently T_2/FLAIR-hyperintensities of the mesiotemporal lobe (11/29; four unilateral and seven bilateral), one patient had atrophy of the mesiotemporal lobe and one patient had mesiotemporal hypointensity. Initial EEG results showed focal slowing (76%) often in combination with epileptic discharges (44%).

CSF analysis was available in three of four rapidly progressive dementia cases and showed a mild pleocytosis in all cases, often accompanied by oligoclonal bands or an elevated IgG index. In two of three patients 14-3-3 was present in CSF and tau was very high (with relatively normal phospho-tau, ratio > 40). In the patient that lacked 14-3-3 protein in CSF, the EEG showed triphasic complexes typical of Creutzfeldt-Jakob disease. In the other cases the EEG was either normal or showed an aspecific encephalopathy. Together with the clinical findings one patient met the criteria for "probable Creutzfeldt-Jakob disease". In two of four patients with rapidly progressive dementia diagnosis of anti-GABA$_B$R encephalitis was made post-mortem. In the remaining two, MRI and CSF abnormalities initiated the search for anti-neuronal autoantibodies and a possible underlying tumor, leading to the diagnosis autoimmune encephalitis.

Table 3. Clinical features, comparing between tumor and non-tumor patients

		Tumor (n=21)	No-tumor (n=7)	All (n=28)	
Seizures presenting symptom		13 (62%)	3 (43%)	16 (57%)	p=0.42
SE		10 (50%)*	1 (14%)	11 (41%)*	p=0.18
Max mRS (pre-treatment)	2 3 4 5	3 7 3 8	0 2 2 3	3 9 5 11	p=0.86
Best mRS (post-treatment)	1 2 3 4	5 9 3 2	2 2 1 1	7 11 4 3	p=1.00
Deceased at last FU		14 (67%)	2 (29%)	16 (57%)	
Survival in months, median (95% CI)		15 (11.04-18.96)	n.a. (< 50% deceased)	17 (7.80-26.20)	p=0.36

SE= status epilepticus, mRS= modified Rankin Scale, FU= follow-up.

* for one patient, status epilepticus was unknown.

Tumor association and response to treatment

Tumor screening with (FDG-PET) CT of thorax and abdomen was carried out in 28 patients, of whom 16 (57%) had an underlying SCLC, one patient had a small cell carcinoma of the bladder, and four (14%) had disseminated disease of a primary tumor of unknown type (in those patients no material for pathological examination could be obtained). Besides GABA$_B$R antibodies, two patients with an unknown tumor type had other SCLC-associated antibodies (anti-AMPAR and anti-VGCC). Median time to tumor diagnosis after first contact with a physician was six weeks (IQR: 2-9; range 0-62). One patient was screened by a pulmonologists for suspected lung cancer prior to the onset of the neurological symptoms, the other patients were diagnosed after the onset of neurological symptoms (96%).

Treatment data were available in 31 patients. Patients were treated with a combination of immuno- and tumor therapy 11/31 (35%), immunotherapy alone 14/31 (45%), tumor therapy alone 2/31 (6%) or remained untreated 4/31 (13%). The majority of the patients 22/26 (85%) responded to treatment, with a median best mRS of 2 (IQR: 1-3; range: 1-5) after treatment.

In 19/21 patients with seizures and treatment response, seizure freedom was reached with a median time to seizure freedom after immunotherapy of 6 days (IQR 0-22, range 0-239). In 17/21 patients with cognitive symptoms and treatment response, cognitive symptoms improved after immunotherapy, with a median of 35 days (IQR 10-104, range 10-265). Seizure freedom was achieved faster than cognitive improvement (n=20; p=0.012). In 20 patients both cognitive improvement and seizure freedom were assessed after immunotherapy. In 13 patients (65%) seizure freedom was reached earlier than cognitive improvement, while 5 patients (25%) first showed cognitive improvement. In 2 patient seizure freedom and cognitive improvement were reached simultaneously (10%). In addition, seizure freedom was achieved faster than cognitive improvement (n=20; p=0.012).

Two patients that did not respond had a poor overall physical condition and died shortly after immunotherapy before effects were assessable. Four patients did not receive treatment because of poor overall condition or because their disease presented prior to discovery of AIE, one of those showed some improvement spontaneously. Two patients relapsed after four and six months respectively. No tumor was found at relapse either.

At last follow-up, 13/32 patients were still alive (median follow up 16 months; IQR 8-27; range 2-109). Median mRS at last follow up was 2 (IQR 2-3; range 0-4). Median survival was 17 months (95%-CI 7.80-26.20), not different between patients with tumors (15 months, 95%-CI 11.04-18.96), or without (no median number as >50% survived, p=0.36; Figure 2). The two deceased patients without a tumor (out of seven) had their anti-$GABA_BR$ encephalitis diagnosis made post-mortem. One patient (#26) had a rapid progressive dementia, presumed Creutzfeldt-Jakob disease, and was not treated. He died due to neurologic deterioration. The other patient (#27) was treated with methylprednisolone and intravenous immunoglobulins for a possible autoimmune encephalitis, but had a severe syndrome with status epilepticus, autonomic dysfunction and respiratory failure caused by pneumonia. intensive care unit treatment was discontinued after one month, due to inconclusive diagnosis, older age, and a history of cognitive impairment. None of the other patients without tumor died after this acute phase. Eight of the fourteen deceased patients with tumor died of tumor progression. In the other six patients with a tumor, the cause of death was not reported Three of four patient with insufficient tumor screening had died, two due to infection, while in one patient the cause of death was not reported. In six patients no initial improvement occurred prior to death of which four were not treated with immunotherapy. Until deterioration leading to death, the patients responded (partially), resulting in a median mRS of 2 (IQR 2-4; range 1-5).

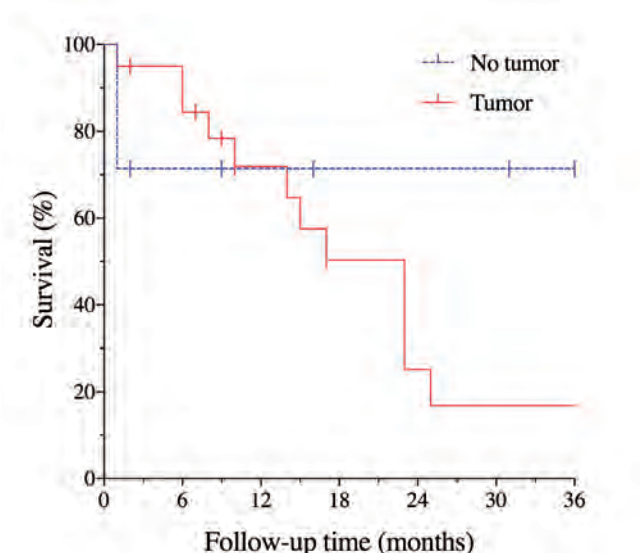

Figure 2. Kaplan Meier curve of survival comparing tumor and non-tumor patients. Showing the data of patients with (n = 21) and without tumors (n = 7). Median survival was 17 months (95% CI 7.80–26.20), not different between patients with tumors (15 months, 95% CI 11.04–18.96), or without (no median number as >50% survived, p = 0.36).

Addition of KCTD16 to GABA$_B$R-CBA improves detection

The patients' sera (n=30) and CSF (n=21) samples were tested for the presence of GABA$_B$R antibodies using a set of different laboratory techniques (Figure 3A-C) and Supplementary Table 5. All sera and CSF samples showed neuropil staining on immunohistochemistry (Figure 3A,D). All CSF samples and all but one sera tested, labelled the surface of live hippocampal neurons (Figure 3B,D). 28/30 sera and 20/20 CSF samples were anti-GABA$_B$R positive using live CBA (Figure 3C,D). With commercial CBA GABA$_B$R antibodies were detected in all but one sera (97%), however the sensitivity of the CSF samples was 84% (16/19) (Figure 3D).

Three out of 1125 serum samples tested in routine diagnostics were positive by commercial CBA without confirmation in CSF or by other laboratory tests and considered clinically irrelevant, resulting in a specificity of 99.7%. In house fixed CBA detected GABA$_B$R antibodies in 26/30 (87%) of the sera and 18/20 (90%) of the CSF samples. When GABA$_{B1}$ and GABA$_{B2}$ subunits were co-transfected with the GABA$_B$R-accessory subunit KCTD16, the sensitivity for serum improved to 29/30 (97%) and in CSF to 20/20 (100%) (Fig. 3D). The addition of KCTD8 or KCTD12 to the CBA was inferior to KCTD16 (data not shown). To validate the improvement of the fixed CBA by the addition of KCTD16, we performed serial dilution of all sera and 17 CSF samples on fixed CBA with and without co-expression of KCTD16. We observed a significant increase in titers detected with the CBA with KCTD16 co-expression (Figure 4A-C). This effect was seen in both serum and CSF (serum 23/29, 79%, p = 0.00008; CSF 14/17, 82%, p = 0.001). Titers had a median 8-fold increase (IQR

2-52, range 0-800, p < 0.0001) in serum and a median 4-fold increase in CSF (IQR 2-7, range 0-256, p = 0.001). Fold changes in serum and CSF titers did not differ significantly between patients with and without KCTD16 antibodies. Also, the addition of KCTD16 to the fixed CBA did not result in a reduction of specificity as 193 healthy and diseased control samples tested negative. No differences were found between titers of patients with and without underlying tumors (Figure 4D).

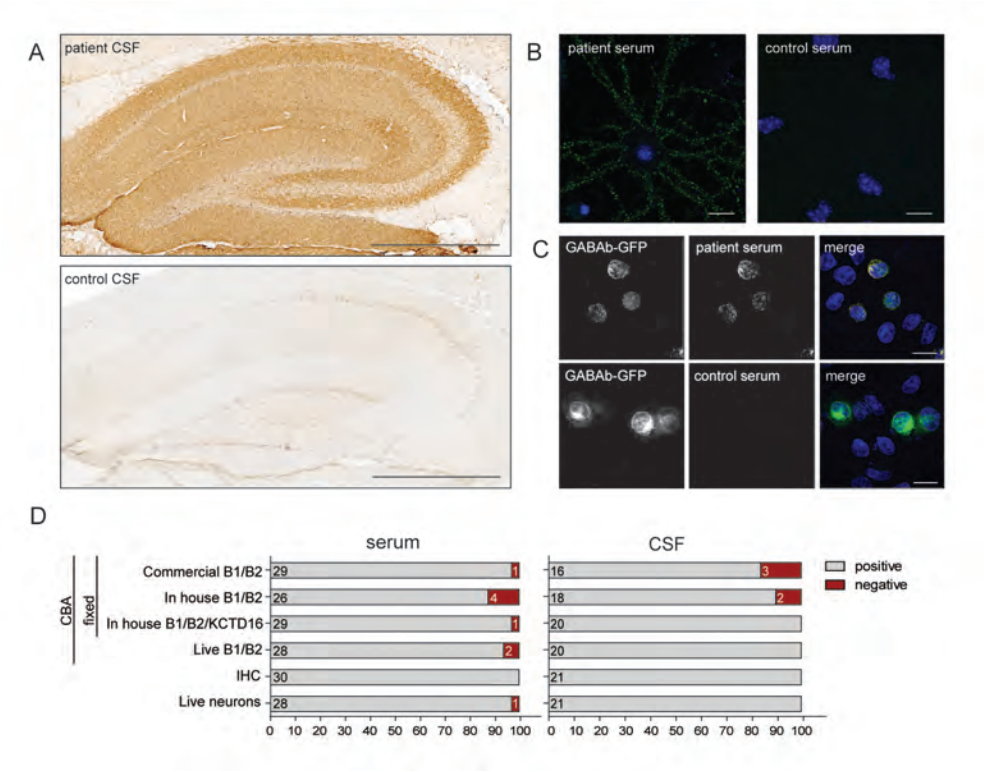

Figure 3. Diagnostic tests for $GABA_BR$ receptor antibodies. (A) Immunohistochemistry of adult rat brain stained with patient CSF or control CSF. The patient CSF shows brain-wide neuropil staining, here exemplified by an image of the hippocampus. Scale bars = 500 mm. (B) Immunocytochemistry of living rat hippocampal neurons. Labelling with the patient serum (green) results in a dot-like pattern along the neurites. Scale bars = 10 mm. (C) Live in-house CBA of HEK cells transfected with $GABA_{B1}$-GFP and $GABA_{B2}$ (green) and stained with patient serum or control serum (red). The patient serum labels the surface of cells transfected with $GABA_BR$. Scale bars = 20 mm. (D) Bar diagram representing the percentages of positive and negative tests for the different laboratory techniques that are used for the detection of $GABA_BR$ antibodies. For one patient, CSF was not available to perform CBA, but this sample did test positive for $GABA_BR$ in live CBA (Dalmau lab, Barcelona).

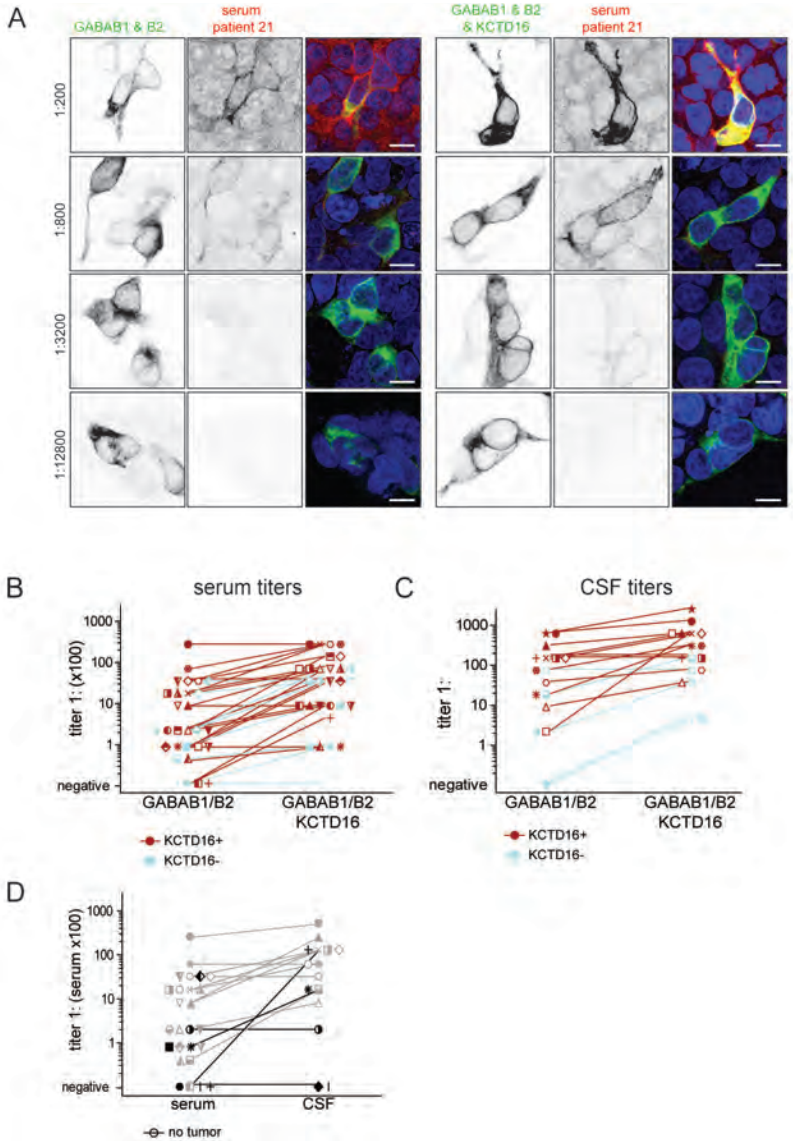

Figure 4. Endpoint titrations with fixed cell-based assay. (A) Titration of serum of an anti-KCTD16-negative patient (red) using a fixed CBA of HEK cells transfected with GABA$_{B1}$-GFP and GABA$_{B2}$ (green) with or without co-transfection of KCTD16. Staining of cells co-transfected with KCTD16 can be detected up to a dilution of 1:3200, as opposed to without KCTD16 co-transfection, up to a dilution of 1:800. (B) Serum titres detected with a fixed CBA with or without co-transfection of KCTD16. Higher serum titres are detected with the addition of KCTD16 to the CBA. Median serum titre detected with the GABA$_{B1/2}$ assay was 200 (IQR 60–1600, range 0–25 600), and with addition of KCTD16 3200 (IQR 3200–9600, range 0–64 000; p<50.0001). (C) CSF titres detected with or without co-transfection of KCTD16. Higher CSF titres are detected with the addition of KCTD16 to the CBA. Median CSF titre detected with the GABA$_{B1/2}$ assay was 64 (IQR 7–160, range 0–512), and with addition of KCTD16 128 (IQR 48–512, range 4–2048; p = 0.001). (D) Serum and CSF titres detected with a fixed CBA without KCTD16 co-transfection. Patients with an underlying tumor do not have higher titres in serum and CSF than patients without an underlying tumor. Mann-Whitney U test, serum p = 0.23, CSF p = 0.41. Symbols in B–D refer to individual patients, which are explained in greater detail in Supplementary Tables 1 and 3–5.

DISCUSSION

This study; 1) identifies $GABA_BR$ antibodies in patients with rapidly progressive dementia in the absence of seizures, in addition to the majority of patients exhibiting limbic encephalitis with prominent and severe seizures, 2) describes a novel autoantibody directed against the $GABA_BR$ accessory protein KCTD16, which is strongly associated with a SCLC, and 3) shows that the addition of KCTD16 to the fixed $GABA_BR$ CBA results in improved detection of $GABA_BR$ antibodies.

Drug-resistant generalized seizures are the most prominent clinical feature of anti-$GABA_BR$ encephalitis. About half of the patients required intensive care unit admittance to control seizures, which is more frequent than previously reported.[2, 4] Moreover, this number probably underestimates the occurrence of drug-resistant epilepsy, as only cases that required intensive care unit admittance were taken into account. In contrast with anti-$GABA_BR$ limbic encephalitis with early and severe seizures, 4 of 32 patients presented with RPD, of which only one developed seizures late in the disease course. As literature on pure cognitive decline in patients with anti-neuronal autoantibodies is sparse [20, 21], many patients with RPD are not investigated for antibodies. CSF abnormalities like 14-3-3 protein, high phospho-tau/tau ratios did not discriminate between neurodegenerative disease and anti-$GABA_BR$ encephalitis. Clues for autoimmune encephalitis, like (mild) pleocytosis, oligoclonal bands or typical MRI abnormalities might hint towards an autoimmune etiology, and were helpful in three of our cases. However, as ancillary testing might be normal and antibodies are not often thought of, patients with this treatable cause of dementia might not be diagnosed in general practice and denied treatment.

The presence of KCTD16 antibodies increases the probability of a paraneoplastic origin, and the necessity to screen more rapidly and more frequently. An underlying tumor occurred in almost all patients with KCTD16 antibodies, as opposed to 3/9 patients without KCTD16 antibodies. Two paraneoplastic anti-$GABA_BR$ encephalitis patients lacked KCTD16 antibodies but had other SCLC associated antibodies, anti-Hu and anti-VGCC respectively. However these were absent in the majority of the KCTD16 positive SCLC patients. Therefore, anti-KCTD16 seems to have additional value to other SCLC-associated autoantibodies, such as anti-SOX1 [4, 22], anti-Hu [23] and anti-VGCC [24], and can help in predicting a paraneoplastic origin of AIE. The presence of KCTD16 antibodies in one patient with anti-Hu syndrome and SCLC shows the association of anti-KCTD16 with SCLC, also outside the context of anti-$GABA_BR$ encephalitis. However, we could not detect staining against KCTD16 proteins in the SCLC of one patient without PNS, as has been published for Hu [23] and SOX1.[22] It would be of additional value to study the expression of $GABA_BR$ and KCTD16 in more SCLC of patients without PNS.

The presence of anti-KCTD16 is associated with higher $GABA_BR$ antibody titers in CSF and a more frequent severe status epilepticus. Probably, this is largely explained by the presence of an underlying tumor, which has also been associated with more severe disease

in anti-NMDAR encephalitis[7] and Lambert Eaton myasthenic syndrome.[25] Nor KCTD16, nor presence of a tumor were predictive for survival. Although shorter survival time in SCLC-GABA$_B$R patients is expected, this was not found. Likely explanations are the small number of idiopathic anti-GABA$_B$R encephalitis patients, and the lack of a diagnosis before dying, leading to no or insufficient immunotherapy. The two deceased patients without tumor died within 2 months from symptom onset, had their diagnosis made post-mortem and were offered no treatment or insufficient treatment. The other idiopathic anti-GABA$_B$R encephalitis patients survived after the acute disease phase.

The fact that anti-KCTD16 co-occurs with SCLC suggests that their formation is a result of the aberrant expression of KCTDs, by SCLC tissue. Although most patients had both anti-KCTD and anti- GABA$_B$R antibodies, suggesting aberrant expression of the GABA$_B$R -KCTD complex, KCTD antibodies were not limited to anti-GABA$_B$R encephalitis as one patient with anti-Hu syndrome and SCLC also had these. Unlike the GABA$_B$R antibodies, the KCTD16 antibodies are directed at an intracellular antigen and most likely do not have pathogenic properties, although this was not tested within the scope of this study.

We show that co-expression of the intracellular auxiliary subunit KCTD16 with GABA$_{B1}$ and GABA$_{B2}$ in a fixed CBA improves the detection of GABA$_B$R antibodies. Native GABA$_B$Rs consist of two different core receptor units GABA$_{B1a/b}$ and GABA$_{B2}$. These core receptor subunits control receptor surface expression, axonal and dendritic distribution, ligand binding and G-protein coupling.[26] In addition, the GABA$_{B2}$ subunit binds homo- or heterotetrameres of cytosolic auxiliary proteins belonging to the KCTD family (KCTD8, -12, -12b and -16). The different KCTD-family members show distinct expression profiles in the brain and bind to the intracellular part of GABA$_{B2}$ as a stable and obligatory part of the receptor at the cell surface. The KCTDs induce desensitization of K^+ currents in response to GABA$_B$R activation in a subtype specific manner.[27-30] Given the properties of KCTD proteins, there are several possible explanations for the improvement of the fixed CBA by the addition of KCTD16 (Figure 5); GABA$_B$R antibodies are directed at a conformational epitope [4] and their binding might be suboptimal when the receptor is lacking an integral component, such as a KCTD protein (Figure 5A,B).[27] Alternatively, the co-expression of KCTD16 could lead to improved clustering of GABA$_B$R on the cell surface, via binding of KCTD16 to a (currently unidentified) scaffold protein (Figure 5C). A previous study shows that the detection of low-affinity antibodies to the acetylcholine receptor in myasthenia gravis can be improved by clustering the acetylcholine receptor in CBAs.[31] Lastly, the additional KCTD16 antibodies could be a partial explanation for the improved detection, as with the addition of KCTD16 the fixed CBA now also detects anti-KCTD16 titers. However, also patients lacking anti-KCTD16 showed increased titers with KCTD16 co-expression.

Importantly, the addition of KCTD16 to the fixed CBA increases sensitivity of the assay, without loss of specificity. Despite different optimization steps, some serum samples remained difficult to score by the in house assay due to high noise levels. As the addition of KCTD16 increases the dilution the test can still be scored positive, the noise level becomes

smaller. The net result is an improvement of the signal-to-noise-ratio. With the addition of KCTD16 the fixed CBA performs as well as the live CBA with the advantage that it could be stored and is therefore suitable for use in many clinical diagnostic laboratories. For CSF samples the $GABA_BR$-KCTD16 CBA performs better than the current commercial CBA. This diminishes the chance of a missed diagnosis if only CSF is sent for testing. The main limitations of our study are due to its retrospective design and the low incidence of anti-$GABA_BR$ encephalitis when compared to anti-NMDAR encephalitis[32] or anti-LGI1 encephalitis.[33] This leads to the limited availability of clinical data and the lack of a standardized treatment regimen that could be evaluated. In addition, the retrospective identification of patients with anti-$GABA_BR$ encephalitis amongst samples collected for testing for onconeural antibodies could have led to biased results, as could be the case for the higher tumor frequency in our study when compared to previous studies.[2, 4]

Overall, our findings have three major practical implications: 1) Anti-$GABA_BR$ encephalitis should also be considered in patients with RPD. 2) KCTD16 antibodies can be used in clinical practice to determine the likelihood of an underlying SCLC in patients with PNS and can lead to early tumor diagnosis and treatment. 3) The addition of KCTD16 to the fixed $GABA_BR$ CBA increases sensitivity without loss of specificity. Early diagnosis of anti-$GABA_BR$ encephalitis is of great importance, given the fact that most patients respond to treatment.

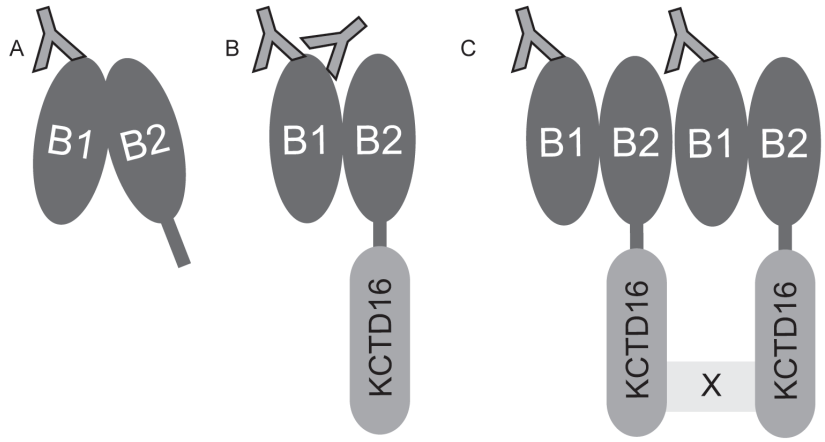

Figure 5. GABAB receptor with and without KCTD16 co-expression.
Schematic representation of the possible effect of the addition of KCTD16 to the cell based assay.
(A) $GABA_{B1}$ and $GABA_{B2}$ subunits expressed without co-expression of KCTD16. (B) $GABA_{B1}$ and $GABA_{B2}$ subunits expressed with co-expression of KCTD16 resulting in a conformational change in the $GABA_B$ receptor that allows for more efficient antibody binding. (C) $GABA_{B1}$ and $GABA_{B2}$ subunits expressed with co-expression of KCTD16 resulting in clustering of $GABA_B$ receptors via the unknown scaffold protein X resulting in more dense antibody labelling.

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SUPPLEMENTARY DATA

Table 1. Results of immunoprecipitation

	17. ◒	*18.* ◧	*19.* ⬓	*26.* ◆	*31.* ☐
Serum (duplo)	GABA$_{B1}$, GABA$_{B2}$, KCTD12	GABA$_{B1}$, GABA$_{B2}$, KCTD8, KCTD12, KCTD16, GluR1, GluR2, (GluR3, GluR4)	GABA$_{B1}$, GABA$_{B2}$, KCTD12	-	GABA$_{B1}$, GABA$_{B2}$, KCTD12
CSF	-	-	-	GABA$_{B3}$, GABA$_{A1}$, GABA$_{A2}$, (GABA$_{B1}$, GABA$_{B2}$)	GABA$_{B1}$, GABA$_{B2}$, KCTD8, KCTD12, KCTD16

Proteins pulled down in the different immunoprecipitation (IP) experiments. Proteins between brackets were pulled down in levels just above the threshold. In 12/12 other samples tested with IP, nor KCTD, nor GABA$_B$R was pulled down. No other proteins were identified consistently.

Table 2. Overview of KCTD CBA results in GABA$_B$R positive patients

	Fixed CBA positive
KCTD 8	2 / 17
KCTD 12	0 / 17
KCTD 16	23 / 32

Table 3. **Demographic features, presenting symptoms, disease course, and ancillary testing**

Patient	*Sex, age (years)*	*Tumor by imaging or pathology*	*Presenting symptom&*	*Clinical syndrome*	*Symptoms during disease course*	*KCTD16 antibodies*	*CSF (abnormal values)*	*MRI (presentation)*	*EEG*	*Autopsy brain*
1. ▼	M, 53	SCLC	Seizures	LE	Presented with seizures (focal with impaired awareness), followed by cognitive decline directly after seizures, behavioral changes and status epilepticus	Yes	195 WBC, elevated IgG index, OCB	Normal	Epileptic and encephalopathic	-
2. ◇	F, 54	SCLC	Seizures	LE	Presented with tonic-clonic seizures, within days, followed by confusion and cognitive decline	Yes	11 WBC, elevated IgG index, OCB	Normal	Encephalopathic	-
3. △	F, 56	SCLC	Seizures	LE	Presentation with tonic-clonic seizures, a week later followed by confusion and cognitive decline	Yes	7 WBC	Normal	Epileptic and encephalopathic	-
4. ⬒	M, 56	SCLC	Cognitive/ (behavioral)	LE	Subacute onset with confusion and cognitive decline followed by seizures. Nine months before LE he developed proximal weakness and autonomic symptoms, consistent with LEMS	Yes	Elevated protein	Normal	-	-
5. ▲	F, 57	SCLC	Cognitive/ (behavioral)	LE	Subacute onset with confusion and cognitive decline, within days followed by tonic-clonic seizures and status epilepticus	Yes	unknown	White matter abnormalities	Epileptic and encephalopathic	-
6. ◭	M, 57	SCLC	Seizures	LE	Initially presentation with seizures (tonic-clonic), directly followed by confusion and cognitive decline	Yes	35 WBC, elevated protein	Normal	Encephalopathic	-
7. ○	M, 58	SCLC	Seizures	LE	Initially presented with seizures (tonic-clonic), followed by cognitive decline	No	3 WBC, elevated protein	-	-	-

Patient	*Sex, age (years)*	*Tumor by imaging or pathology*	*Presenting symptom*[&]	*Clinical syndrome*	*Symptoms during disease course*	*KCTD16 antibodies*	*CSF (abnormal values)*	*MRI (presentation)*	*EEG*	*Autopsy brain*
8. ⛒	M, 58	SCLC	Seizures	LE	Initially presented with tonic-clonic seizures, within weeks followed by cognitive decline and behavioral change	Yes	2 WBC, elevated protein	Normal	-	-
9. ▼	M, 64	SCLC	Seizures	LE	Subacute onset with confusion and cognitive decline, directly followed by seizures (toni- clonic) and status epilepticus	Yes	41 WBC	Hyperintensity mesiotemporal (bilateral)	Encephalopathic	-
10. ▽	F, 64	SCLC	Cognitive/ (behavioral)	LE	Subacute onset with confusion and cognitive decline, 3 months later followed by tonic-clonic seizures	Yes	Elevated protein	Hyperintensity mesiotemporal (bilateral)	-	-
11. ●	F, 65	SCLC	Seizures	LE	Presented with tonic- clonic seizures, directly followed by cognitive decline, status epilepticus and autonomic dysfunction	Yes	13 WBC	Hyperintensity mesiotemporal (bilateral)	Encephalopathic	-
12. ▲	M, 66	SCLC	Seizures	LE	Presented with tonic-clonic seizures, two weeks later followed by confusion, cognitive decline and status epilepticus	Yes	28 WBC	Hyperintensity mesiotemporal (unilateral)	Epileptic and encephalopathic	-
13. ⬡	F, 70	SCLC	Seizures	LE	Presented with tonic-clonic seizures, directly followed by cognitive decline, hallucinations. and sleep disturbances	Yes	Normal	Hyperintensity mesiotemporal (unilateral)	Epileptic	-
14. ✕	F, 74	SCLC	Behavioral/ (cognitive)	LE	Subacute onset with confusion and cognitive decline within weeks followed by tonic-clonic seizures	Yes	11 WBC	Normal	Encephalopathic	-

Patient	*Sex, age (years)*	*Tumor by imaging or pathology*	*Presenting symptom&*	*Clinical syndrome*	*Symptoms during disease course*	*KCTD16 antibodies*	*CSF (abnormal values)*	*MRI (presentation)*	*EEG*	*Autopsy brain*
15. ▼	M, 76	SCLC	Cognitive/ (behavioral)	LE	Acute cognitive decline after 2 months followed by focal seizures with impaired awareness, and tonic-clonic seizure	Yes	5 WBC, elevated protein	Normal	Encephalopathic	-
16. ◨	F, 78	SCLC	Seizures	LE	Presented with tonic-clonic seizures, 2 days later followed by confusion, cognitive decline and status epilepticus	Yes	15 WBC, elevated IgG index, OCB	-	Epileptic and encephalopathic	-
17. ◒	M, 75	Small cell bladder carcinoma	Behavioral/ (cognitive)	LE	Subacute onset with psychosis and cognitive decline followed by seizures and autonomic dysregulation	No	13 WBC, elevated protein, OCB	Hyperintensity mesiotemporal (bilateral)	Epileptic and encephalopathic	-
18. ◧	F, 55	Yes (tumor type unknown)	Cognitive/ (behavioral)	LE	Acute cognitive decline after 1 month followed by seizures (tonic-clonic, focal with impaired awareness), psychosis and status epilepticus	Yes	14 WBC	Hyperintensity mesiotemporal (bilateral)	Normal	-
19. ⬓	F, 67	Yes (tumor type unknown)	Behavioral/ (cognitive)	LE	Subacute onset with confusion, cognitive decline and hallucinations, a week later followed by tonic-clonic seizures	No	10 WBC	Hyperintensity mesiotemporal (bilateral)	Epileptic and encephalopathic	-
20. ✱	M, 72	Yes (tumor type unknown)	Seizures	LE	Initially presented with tonic-clonic seizure, a month later followed by cognitive decline and behavioral changes	Yes	17 WBC, elevated protein	Hyperintensity mesiotemporal (bilateral)	Epileptic and encephalopathic	-
21. ◆	F, 76	Yes (tumor type unknown)	Seizures	Seizures	Tonic-clonic seizures	Yes	5 WBC, elevated IgG index, OCB	Vascular white matter lesions	Normal	-
22. ◆	M , 44	No	Seizures	LE	Initially presented with seizures, 3 days later followed by confusion, cognitive decline, aphasia and psychosis	No	22 WBC	Normal	Encephalopathic	-

Patient	*Sex, age (years)*	*Tumor by imaging or pathology*	*Presenting symptom*[&]	*Clinical syndrome*	*Symptoms during disease course*	*KCTD16 antibodies*	*CSF (abnormal values)*	*MRI (presentation)*	*EEG*	*Autopsy brain*
23. ■	F, 47	No	Seizures	LE	Presented with seizures (focal to bilateral tonic-clonic), a week later cognitive decline, behavioral changes and aphasia	No	39 WBC	Normal	Epileptic and encephalopathic	-
24. ▌	M, 56	No	Behavioral/ (cognitive)	RPD	Subacute cognitive decline, complete loss of memory and recognition, apraxia and hallucinations, sleep disturbance	No	105 WBC, elevated protein, elevated IgG index, OCB	Hyperintensity mesiotemporal (bilateral)	Encephalopathic	-
25. ◑	F, 63	No	Seizures	LE	Presentation with seizures (tonic-clonic), directly followed by cognitive decline	No	75 WBC	Hyperintensity mesiotemporal (unilateral)	-	-
26. ◆	M, 77	No	Behavioral/ (cognitive)	RPD	Hypertension, psychotic behavior, cognitive decline, in days followed by cerebellar ataxia and aphasia	No	18 WBC, elevated IgG index, OCB	Atrophy mesiotemporal, vascular white matter lesions	Triphasic periodic complexes and encephalopathic	Perivascular inflammatory infiltrates consisting of B and T-cells. Local infiltration in the hippocampus and basal ganglia. No evidence for CJD
27. +	F, 79	No	Behavioral/ (cognitive)	LE	Subacute onset with confusion and cognitive decline, within days followed by tonic-clonic seizures, status epilepticus, aphasia, autonomic dysregulation	Yes	174 WBC	Normal	Epileptic	-
28. ⬣	F, 85	No	Behavioral/ (cognitive)	RPD	Pneumonia, two weeks later confusion, visual hallucinations, psychotic behavior, memory deficit	No	-	Normal	-	-

Patient	*Sex, age (years)*	*Tumor by imaging or pathology*	*Presenting symptom*[&]	*Clinical syndrome*	*Symptoms during disease course*	*KCTD16 antibodies*	*CSF (abnormal values)*	*MRI (presentation)*	*EEG*	*Autopsy brain*
29. ◐	M, 66	No (screening not performed)	Seizures	LE	Presented with tonic-clonic seizures, 2 days later followed by focal seizures with impaired awareness, cognitive decline and behavioral changes	Yes	17 WBC	Vascular white matter lesions	Epileptic and encephalopathic	-
30. ★	F, 70	No (X-thorax only)	Behavioral/ (cognitive)	LE	Subacute onset with confusion and cognitive decline, directly followed by a seizure, episodes of reduced consciousness, and autonomic dysregulation	Yes	14 WBC, elevated IgG index	Normal	Normal	Oedema hippocampus. Perivascular lymphocyte infiltration. No evidence for CJD
31. ❑	M, 72	No (screening not perfomed)	Cognitive/ (behavioral)	RPD	Acute psychosis, within days followed by cognitive decline, only later on in disease course a few seizures and myoclonus	No	15 WBC	Hyperintensity mesiotemporal (unilateral)	Normal	Perivascular lymphocyte infiltration and gliosis left hippocampus. No evidence for CJD
32. ⬒	M, 75	No (X-thorax only)	Behavioral/ (cognitive)	LE	Subacute onset with confusion and cognitive decline followed by seizures and status epilepticus 2 weeks after symptom onset	Yes	32 WBC elevated IgG index	-	Encephalopathic	-

SCLC = Small Cell Lung Carcinoma. LE = Limbic Encephalitis. RPD= rapidly progressive dementia, WBC = White Blood Cells. OCB = Oligoclonal bands. CJD = Creutzfeldt-Jacob Disease, LEMS=Lambert-Eaton myasthenic syndrome. [‡] Fulfilled criteria for "probable CJD", but pathology refuted this diagnosis. [&] symptoms were scored as cognitive(/behavioral), if patients mainly had cognitive symptoms, or as behavioral/(cognitive), if patients mainly had behavioral problems.

Table 4. Treatment and outcome

Patient	*Status Epilepticus*	*AEDs, number*	*Max mRS*	*Immunotherapy and/or tumortherapy*	*Best mRS after treatment*	*Treatment response*	*Follow up, months*	*Cause of death*	*Other findings*
1. ▼	Yes	4	5	MP+ IVIg + chemotherapy + radiotherapy	3	Responded to therapy	103	-	-
2. ◇	No	1	4	MP	2	Responded to therapy	13	-	-
3. △	No	3	3	MP + IVIg + chemotherapy	1	Responded to therapy	10†	Tumor progression	SIADH
4. ⬒	No	1	4	Chemotherapy + radiotherapy + steroids	Unknown	Some treatment response, but extent unknown	51†	Tumor progression	Anti-Hu, Anti-VGCC
5. ▲	Yes	5	5	unknown	Unknown	Unknown	8†	Unknown	-
6. ◭	Yes	3	3	MP+ IVIg + chemotherapy + radiotherapy	1	Responded to therapy. Relapse of seizures at cancer progression	15†	Unknown	-
7. ○	No	2	3	Rituximab + chemotherapy	1	Responded to therapy	26†	Unknown	Anti-Hu
8. ✇	Yes	6	5	MP	2	Responded to therapy	2	-	-
9. ▼	Yes	3	5	PLEX + IVIg	2	Responded to therapy	8†	Tumor progression	-
10. ▽	No	1	3	Chemotherapy + radiotherapy	2	Responded to therapy	14†	Unknown	Anti-AMPAR SIADH
11. ●	Yes	3	5	MP+ IVIg + chemotherapy	2	Responded to therapy	21	-	-
12. ▲	Yes	3	5	MP+ IVIg + chemotherapy	3	Responded to therapy	9†	Tumor progression	-
13. ⬭	No	1	4	MP + IVIg	4	No response to treatment	2†	Tumor progression	Anti-Ri SIADH
14. ✕	No	2	2	MP	1	Responded to therapy	7	-	-
15. ▼	No	1	2	Chemotherapy + radiotherapy	2	Responded to therapy	24†	Tumor progression	-
16. ◨	Yes	4	5	MP + chemotherapy	4	Responded to therapy	8†	Unknown	SIADH
17. ◒	Yes	4	5	MP	2	Responded to therapy	17†	Tumor progression	-
18. ◧	Yes	3	5	MP	3	Responded to therapy	8†	Tumor progression	Anti-AMPAR
19 ⬓	No	2	3	MP + IVIg	2	Responded to therapy. Relapse after 6 months, no response to therapy	17†	Unknown	Anti-VGCC SIADH
20. ✱	No	1	3	MP + IVIg	2	Responded to therapy	7	-	-
21. ◆	No	1	2	MP	1	Responded to therapy	10	-	-
22. ◆	No	4	4	MP	2	Responded to therapy	10 (lost to follow up)	-	-

Patient	*Status Epilepticus*	*AEDs, number*	*Max mRS*	*Immunotherapy and/ or tumortherapy*	*Best mRS after treatment*	*Treatment response*	*Follow up, months*	*Cause of death*	*Other findings*
23. ■	No	2	3	MP + IVIg	1	Responded to therapy	16	-	-
24. ▌	No	-	4	MP + IVIg + rituximab	2	Responded to therapy	7	-	Anti-GAD (high) 14-3-3 positive
25. ◑	No	1	3	MP + IVIg	1	Responded to therapy. Relapse after 4 months, no response to second line immunotherapy. Complicated by HSV2 vasculitis	26	-	-
26. ◆	No	3	5	-	5	Not treated	1†	Neurologic deterioration	Anti-$GABA_A$
27. ┼	Yes	1	5	MP + IVIg	4	Responded to therapy	2†	Infection	SIADH
28. ⬣	No	-	4	MP + IVIg + rituximab + cyclophosphamide	4	Some response to therapy	3	-	SIADH
29. ◐	Yes	4	4	-	3	Not treated	12 (lost to follow up)	-	-
30. ★	No	1	5	-	4	Not treated	2†	Unknown	-
31. □	No	-	5	-	5	Not treated	4†	Infection	14-3-3 positive
32. ⬒	Yes	4	5	MP	5	No response to therapy	1†	Infection	-

MP = Methylprednisolone. IVIg = Intravenous Immunoglobulins. PLEX = Plasma exchange. ER = Emergency room. SIADH = Syndrome of inappropriate antidiuretic hormone secretion. AMPAR = **α**-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor. VGCC = Voltage Gated Calcium Channels. GAD = Glutamic Acid Decarboxylase. † = deceased.

Table 5. Laboratory tests

Patient	*Sample type*	*IHC*	*Live neurons*	*Live CBA GABA$_B$*	*Commercial CBA GABA$_B$*	*Fixed CBA KCTD16*	*Fixed CBA GABA$_B$*	*Fixed CBA GABA$_B$ + KCTD16*	*Titer fixed CBA GABA$_B$*	*Titer fixed CBA GABA$_B$ + KCTD16*	*IP-MS*
1.	serum	pos	pos	pos	strong pos	pos	pos	pos	1:80	1:800	
2.	serum	strong pos	pos	pos	pos	pos	pos	pos	1:3200	1:128000	
	CSF	pos	pos	pos	pos	pos	pos	pos	1:128	1:512	
3.	serum	pos	pos	pos	pos	pos	pos	pos	1:200	1:6400	
	CSF	pos	pos	pos	neg	pos	pos	pos	1:8	1:32	
4.	serum	pos	-	pos	pos	pos	pos	pos	-	-	
5.	serum	pos	pos	pos	pos	pos	pos	pos	1:1600	1:6400	
	CSF	strong pos	pos	pos	pos	pos	pos	pos	1:512	nd	
6.	serum	pos	pos	pos	pos	pos	pos	pos	1:80	1:80	
	CSF	strong pos	pos	nd	pos	pos	pos	pos	nd	nd	
7.	serum	strong pos	pos	pos	pos	neg	pos	pos	1:1600	1:3200	
	CSF	strong pos	pos	pos	pos	neg	pos	pos	1:64	1:64	
8.	serum	strong pos	pos	pos	strong pos	pos	pos	pos	1:6400	1:256000	
	CSF	strong pos	pos	pos	strong pos	pos	pos	pos	1:64	1:256	
9.	serum	pos	pos	pos	pos	pos	pos	pos	1:3200	1:3200	
10.	serum	strong pos	pos	pos	pos	pos	pos	pos	1:800	1:6400	
11.	serum	pos	pos	pos	pos	pos	pos	pos	1:256000	1:256000	
	CSF	strong pos	pos	pos	pos	pos	pos	pos	1:512	1:1024	
12.	serum	pos	pos	pos	pos	pos	pos	pos	1:800	1:800	
	CSF	strong pos	pos	pos	pos	pos	pos	pos	1:256	1:512	
13.	serum	pos	pos	pos	pos	pos	pos	pos	1:3200	1:25600	
	CSF	strong pos	pos	pos	nd	pos	pos	pos	1:32	1:64	
14.	serum	pos	pos	pos	strong pos	pos	pos	pos	1:1600	1:256000	
	CSF	pos	pos	pos	strong pos	pos	pos	pos	1:128	1:512	
15.	serum	pos	pos	pos	pos	pos	pos	pos	1:200	1:800	
16.	serum	pos	pos	pos	pos	pos	pos	pos	1:1600	1:6400	
	CSF	pos	pos	pos	pos	pos	pos	pos	1:128	1:128	
17.	serum	pos	pos	pos	pos	neg	pos	pos	1:200	1:1600	yes
	CSF	pos	pos	pos	nd	nd	nd	nd	Nf=d	nd	
18.	serum	pos	pos	pos	pos	pos	neg	pos	0	1:800	yes
19.	serum	pos	pos	pos	pos	neg	pos	pos	1:40	1:80	yes
	CSF	pos	pos	pos	pos	neg	pos	pos	1:16	1:128	
20.	serum	pos	pos	pos	strong pos	pos	pos	pos	1:80	1:80	
	CSF	strong pos	pos	pos	strong pos	pos	pos	pos	1:16	1:256	
21.	serum	pos	pos	pos	pos	pos	pos	pos	1:80	1:3200	
22.	serum	pos	pos	pos	pos	neg	pos	pos	1:3200	1:3200	

Patient	*Sample type*	*IHC*	*Live neurons*	*Live CBA $GABA_B$*	*Commercial CBA $GABA_B$*	*Fixed CBA KCTD16*	*Fixed CBA $GABA_B$*	*Fixed CBA $GABA_B$ + KCTD16*	*Titer fixed CBA $GABA_B$*	*Titer fixed CBA $GABA_B$ + KCTD16*	*IP-MS*
23. ■	serum	pos	pos	pos	pos	neg	pos	pos	1:80	1:800	
	CSF	pos	pos	pos	pos	neg	pos	pos	nd	nd	
24. ▌	serum	pos	pos	neg	neg	neg	neg	neg	0	0	
	CSF	pos	pos	pos	neg	neg	neg	pos	0	1:4	
25. ◐	serum	pos	pos	pos	pos	neg	pos	pos	1:200	1:6400	
	CSF	pos	pos	pos	strong pos	neg	pos	pos	1:2	1:32	
26. ◆	CSF	pos	pos	pos	neg	neg	neg	pos	0	1:4	yes
27. ┼	serum	pos	pos	pos	pos	pos	neg	pos	0	1:400	
	CSF	pos	pos	pos	pos	pos	pos	pos	1:128	1:128	
28. ⬣	serum	pos	neg	neg	pos	neg	neg	pos	0	1:80	
29. ◐	serum	pos	pos	pos	pos	pos	pos	pos	1:200	1:800	
30. ★	CSF	strong pos	pos	pos	pos	pos	pos	pos	1:512	1:2048	
31. □	serum	pos	pos	pos	pos	pos	pos	pos	1:80	1:6400	yes
	CSF	strong pos	pos	pos	pos	pos	pos	pos	1:2	1:512	
32. ⬒	serum	pos	pos	pos	pos	pos	pos	pos	1:200	1:12800	

IHC = immunohistochemistry. CBA = Cell Based Assay. IP-MS = immunoprecipitation-mass spectrometry, nd= not determined

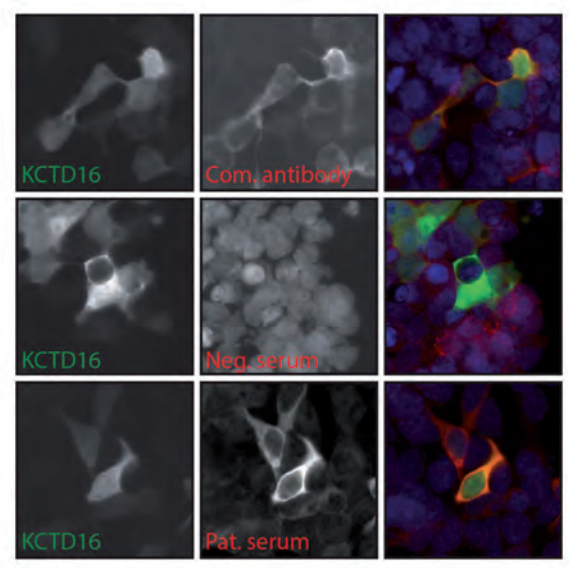

Figure 1. Reactivity of patient's serum and CSF with HEK cells expressing KCTD16**.** KCTD16 cell based assay: HEK-cells transfected with KCTD16-HA (green) and stained with commercial anti-KCTD16 antibody (top row, red), healthy control serum (middle row, red) and serum from a patient with anti-KCTD16 (bottom row, red). Both the commercial antibody and the patient serum stain KCTD16 transfected cells whereas the negative control serum does not.

Chapter 3

New-onset status epilepticus caused by autoimmune encephalitis

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In preparation

Chapter 4

Neurological syndromes associated with anti-GAD65 clinical and serological response to treatment

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ABSTRACT

Objectives

Antibodies against Glutamic Acid Decarboxylase 65 (GAD65) are associated with a number of neurologic syndromes. However, their pathogenic role is controversial. Our objective was to describe clinical and paraclinical characteristics of GAD-antibody patients and analyze their response to immunotherapy.

Methods

Retrospectively, we studied patients (n=56) with GAD65 antibodies and any neurological symptom. We tested serum and CSF with ELISA, immunohistochemistry (IHC) and cell-based assay (CBA). Accordingly, we set a cut-off value of 10,000 IU/mL in serum by ELISA to group patients into high-concentration (n=36) and low-concentration (n=20) groups. We compared clinical and immunological features and analyzed response to immunotherapy.

Results

Classical anti-GAD65-associated syndromes were seen in 34/36 patients with high-concentration (94%): stiff-person syndrome (7), cerebellar ataxia (3), chronic epilepsy (9), limbic encephalitis (9) or an overlap of two or more of the former (6). Patients with low-concentrations had a broad, heterogeneous symptom spectrum. Immunotherapy was effective in 19/27 treated patients (70%), though none of them completely recovered. Antibody concentration reduction occurred in 15/17 patients with available pre- and post-treatment samples (median reduction 69%; range 27-99%), of which 14 improved clinically. The two patients with unchanged concentrations showed no clinical improvement. No differences in treatment responses were observed between specific syndromes.

Conclusions

Most patients with high concentration anti-GAD65 (>10.000 IU/mL) showed some improvement after immunotherapy, unfortunately without complete clinical recovery. Serum antibody concentrations' course might be useful to monitor response. In patients with low anti-GAD65 concentrations, especially in those with atypical clinical phenotypes, diagnostic alternatives are more likely.

INTRODUCTION

Autoantibodies against Glutamic Acid Decarboxylase 65 (GAD65) have been linked to different types of syndromes. These antibodies are widely used as biomarkers for diabetes mellitus type 1 (DM1), as they are present in 80% of patients at diagnosis.[1,2] However, it is well known that anti-GAD65 can also be associated with specific neurologic disorders, including stiff-person syndrome (SPS), cerebellar ataxia (CA), epilepsy (Ep), and limbic encephalitis (LE).[3–6]

The pathophysiologic role of anti-GAD65 in neuroinflammation is still unclear. It is hard to understand whether there is a direct antibody-associated pathogenic effect because the target antigen is located intracellularly. Moreover, responses to immunotherapy seem to be poorer than in patients with neurologic disorders caused by most other antineuronal antibodies.[7,8]

In studies evaluating treatment effects in anti–GAD65-positive patients, methods used are variable, and patient cohorts are often restricted to one of the specific clinical phenotypes.[9–11]

In addition, some studies describing patients with neurologic symptoms and anti-GAD65 also include patients with low antibody concentrations. In these patients, clinical relevance of anti-GAD65 is questionable, because low antibody concentrations are regularly found among patients with DM1 (without neurologic symptoms) and rarely in healthy individuals.[1,2,12]

The aim of this cohort study is to evaluate the clinical relevance of low and high anti-GAD65 concentrations in patients with neurologic symptoms, to establish clinically relevant cut-off values (in serum and CSF), and to evaluate clinical and serologic treatment responses.

METHODS

Patients

We retrospectively included patients with neurological symptoms and an increased anti-GAD65 concentration detected in serum and/or CSF, from January 2015 until June 2018. Anti-GAD65 was routinely detected at the department of Immunology (Laboratory Medical Immunology) of the Erasmus University Medical Center by using Enzyme-Linked Immunosorbent Assay (ELISA) and reported as negative or positive. Clinical information was obtained from medical files. Thirty of 56 patients (54%) were seen by one of the authors.

Laboratory tests

Anti-GAD65 was determined in serum and CSF when available, using three assays. Paired serum and CSF samples were used if possible. Otherwise, serum samples drawn closest to the CSF tap were used, provided they were preimmunotherapy samples. First, automated quantitative ELISA was performed according to the manufacturer's instructions (Medizym® anti-GAD, Medipan, Berlin, Germany). Calibration curves based on 5 calibrators (5, 18, 35, 120, and 250 IU/mL) were used to infer antibody concentrations. Samples were considered positive with anti-GAD65 concentrations above 5 IU/mL. When concentrations were over 250 IU/mL, we tested serial dilutions (1:10; 1:100; 1:1,000; 1: 10,000) and chose the most reliable result (i.e., optical density value in the linear part of the calibration curve) to determine the IU/mL end concentration. Secondly, immunohistochemistry (IHC) was used as a screening method, to determine immunoreactivity of patients' serum (diluted 1:200) or CSF (diluted 1:2) against rat hippocampal brain tissue. A detailed description can be found elsewhere.[13] GAD65 antibody binding causes a characteristic staining pattern.[13] Finally, cell-based assay (CBA) (Euroimmun, Lübeck, Germany; REF: FA 1022-1005-50) was performed, according to manufacturer's instructions, using human embryonal kidney cells (HEK293) expressing recombinant GAD65. Serum was diluted 1:10 and CSF was used undiluted.

ELISA provided quantitative results. IHC and CBA were used as confirmatory qualitative techniques to determine clinically relevant cut-off values for serum and CSF. Samples positive by ELISA and confirmed with positive IHC and CBA were considered high-concentration samples.[14] Samples showing a positive staining pattern on IHC, but no typical GAD pattern, were tested more extensively with commercial and in-house CBAs using fixed or live cells (Euroimmun kits and in-house CBAs).

Defining clinical phenotypes and clinical relevance of anti-GAD65

Patients were allocated into six groups based on the clinical phenotypes described in literature: 1) stiff person syndrome (SPS), 2) cerebellar ataxia (CA), 3) epilepsy (Ep), 4) limbic encephalitis (LE), 5) overlap and 6) other. The overlap category consisted of patients who had developed more than one anti-GAD65 associated neurological syndrome over the disease course. Patients with LE, presenting with seizures or developing seizures following LE were not considered as overlap syndromes, and were all classified as LE. After determining cut-off values, patients were classified into a high-concentration or low-concentration group. Patients in the high-concentration group were studied more thoroughly to assess clinical and serological response to immunotherapy. Immunotherapy responses were evaluated by one of the authors during follow-up, or were retrospectively assessed from medical files. A seizure frequency reduction of at least 50% was considered as improvement in patients with epilepsy. For the other clinical phenotypes, modified Rankin Score (mRS), and the Scale for the Assessment and Rating of Ataxia (SARA score) were used,[15,16] when available. One point improvement in mRS or 3 points at the SARA score was considered clinically relevant. In

the absence of absolute scores, patients' and physicians' evaluations, as measured by the clinical global impression—improvement scale,[17] were taken into account.
Serological response to treatment was measured comparing pre- and post-immunotherapy ELISA-antibody concentrations. A reduction of at least 25% following immunotherapy was considered a relevant concentration reduction.

Patients with isolated DM1

In order to explore the spectrum of concentrations of anti-GAD65, we also assessed patients with DM1 without neurological symptoms. At the department of Immunology of the Erasmus University Medical Center, sera from 669 patients were tested for anti-GAD65 between January 2018 and December 2018. As the reason for testing was often unknown, filtering based on additional antibody testing for Islet Cell Cytoplasmic Autoantibodies (ICA) or tyrosine phosphatase antibodies (anti-IA2) was performed. This way, we selected samples from 198 patients that were sent specifically for a suspicion of insulin-dependent diabetes mellitus because neurologists would not request additional islet antigen antibodies. Seventy-three samples (37%) tested positive for anti-GAD65. Of these, 37 were analyzed more extensively with quantitative ELISA, IHC and CBA. Patients with high concentrations and positive IHC and CBA (n=3), were approached to identify associated neurological disorders, of whom two could be traced.

Statistics

The Fisher exact test was used to compare categorical variables between the high-concentration and low-concentration groups. Age, CSF cell count and protein count were compared with the Mann Whitney U test. To compare antibody tests, the McNemar test was used. P values less than 0.05 were considered statistically significant. We used SPSS 24.0 and Graph Path 7.0 for analysis and data visualization.

RESULTS

Laboratory tests

We identified 71 patients with neurological symptoms and at least one positive serum or CSF anti-GAD65 test result, of whom 56 were included in our study. Eleven patients could not be reached to collect informed consent, and four patients refused to participate.
The median serum concentration, measured with ELISA was 74,700 IU/mL (n=54; range 6-2,130,000) and the median CSF concentration was 2,430 IU/mL (n=43; range 0-83,800). Serum and CSF samples were drawn a median of 25 months (IQR 6-69) and 19 months (IQR 1-69) after symptom onset, respectively.
IHC and CBA showed a high concordance (Figure 1). A cut-off of 100 IU/mL showed a 100% concordance among tests in CSF. For serum, with a cut-off value of 10,000 IU/mL 100% of

low-concentration samples had a negative IHC and 97% of high-concentration samples had a positive IHC. Similarly, 100% of high-concentration samples were positive with CBA and 96% of low-concentration samples were negative. All CBAs and IHCs corresponded, except three diabetes samples with positive CBA and negative IHC. In those three samples, ELISA concentrations were between 6,220 and 15,400 IU/mL. Samples with an unspecific neuropil staining on IHC were excluded for calculations. Other antibodies were found in 1 patient in the high anti-GAD65 concentrationgroup (anti- gamma-aminobutyric acid B receptor [$GABA_BR$]) and 2 patients in the low anti-GAD65 concentrationgroup (1 anti-$GABA_BR$ and 1 anti-GlyR).

In the DM1 group without overt neurological symptoms, the median serum concentration of 68 DM1 patients (35% of 192 samples) was 86 IU/mL (IQR 51-3,670; range 15-145,400). Three samples (1.6%) showed an anti-GAD65 concentration >10,000 IUm/L and also tested positive by IHC and CBA. The two patients traceable had DM1. One also had multiple other autoimmunities (vitiligo, ITP and thyroiditis), but in both patients no neurological or psychiatric symptoms were present.

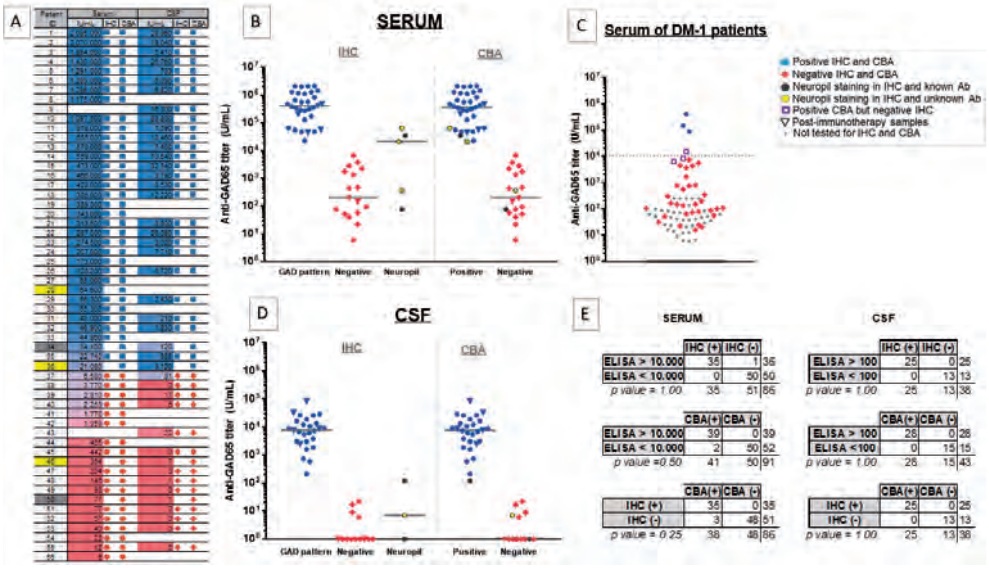

Figure 1. Comparison of laboratory techniques. ELISA concentrations of serum (A and B) and CSF (A and C) of patients with neurological disorders in comparison to IHC and CBA. Patients identified with dark gray and yellow squares in A showed a neuropil staining on IHC, instead of the typical GAD pattern. Samples with a neuropil staining and high ELISA concentration had a positive GAD65-CBA result, whereas CBA was negative for low-concentration samples. In B and D, dark gray and yellow dots are used in both IHC and CBA columns to identify these samples. In the dark gray dotted patients, a different antibody was found. Serum or CSF of the yellow dotted patients showed a neuropil staining, but no known antibody was found. Serum results from patients with antibody testing for diabetes (DM-1) are shown (C). Logarithmic transformation was used for charts. Concordance rates for ELISA, IHC, and CBA are provided for serum and CSF (E). CBA = cell-based assay; GAD = glutamic acid decarboxylase; IHC = immunohistochemistry.

Patients

Thirty-six patients were allocated into the high-concentration group (serum concentration >10,000IU/mL or CSF concentration > 100 IU/mL) and 20 patients into the low-concentration group. Clinical characteristics are shown in Table 1.

In the high-concentration group, 34/36 (94%) patients had a typical anti-GAD65 associated neurological syndrome; SPS (n=7), CA (n=3), Ep (n=9), LE (n=9), or an overlap syndrome (n=6). In the overlap group, four patients initially had drug-resistant focal Ep and developed CA, SPS or a combination of both, between two and seven years after seizure onset (Figure 2). One patient initially had CA and developed SPS five years later, and one SPS patient developed prominent cerebellar symptoms seven years after onset. One patient with LE, no seizures and associated extralimbic involvement on MRI, had concomitant $GABA_BR$ antibodies, without a tumor. Of the remaining two patients, both with DM type 2, one had a pseudo-orthostatic tremor and the other one had optic neuropathy.

Table 1. Clinical and paraclinical characteristics of high-concentration and low-concentration groups

	High-concentration (n=36)		Low-concentration (n=20)		p value
Age at onset, median (IQR; range)	29	(33; 11-80)	52	(22; 5-72)	0,23
Women, n (%)	29	(81%)	9	(45%)	0,02*
Autoimmune disorders, n (%)	18/32	(56%)	14/18	(77%)	0,21
DM1, n (%)	14/35	(40%)	8/18	(44%)	0,78
Other autoimmune disorders, n (%)	11/32	(34%)	12/18	(67%)	0,04*
Tumors#, n (%)	4/20	(20%)	2/10	(20%)	1,00
Clinical syndrome:					0,0008**‡
Typical, n (%)	34	(94%)	11	(55%)	
SPS, n (%)	7	(19%)	2	(10%)	
CA, n (%)	3	(8%)	2	(10%)	
Epilepsy, n (%)	9	(25%)	3	(15%)	
LE, n (%)	9	(25%)	4	(20%)	
Overlap, n (%)	6	(17%)	0	(0%)	
Other, n (%)	2	(6%)	9	(45%)	
CSF					
Pleocytosis, n (%)	6/27	(22%)	7/14	(50%)	0,09
Cell-count, median (IQR; range)	31	(128, 6-310)	18	(102; 11-366)	0,66
Proteins increased, n (%)	8/24	(33%)	7/12	(58%)	0,175
Protein-count, median (IQR; range)	0,38	(0,20; 0,20-1,13)	0,55	(0,54; 0,31-1,24)	0,01*
Oligoclonal bands	12/18	(67%)	3/5	(60%)	0,38
MRI (brain) abnormalities, n (%)	15/32	(46%)	5/16	(31%)	0,38
Other antibodies, n (%)†	2/35	(3%)	2/17	(6%)	1,00
Immunotherapy use, n (%)	27/35	(77%)	8/19	(42%)	0,02*

* $p < 0.05$; ** $p < 0.005$

#Tumors in the high-concentration group: intestinal lymphoma (1), breast cancer (1), prostate adenocarcinoma (1) and testicular tumor (pathology unknown) (1). Tumors in the low-concentration group: glioblastoma (1), pancreatic carcinoma (1).

‡Comparing the frequency of 'typical' versus 'other' syndromes between patients with high-concentration or low-concentration samples.

† Two patients in the high-concentration group had anti-$GABA_BR$ antibodies. In low-concentration group, one patient had anti-Glycine antibodies and one patient had anti-$GABA_BR$ antibodies.

Abbreviations: CA = cerebellar ataxia; DM1 = diabetes mellitus type 1; Ep = epilepsy; IQR = interquartal range; LE = limbic encephalitis; SPS = stiff-person syndrome.

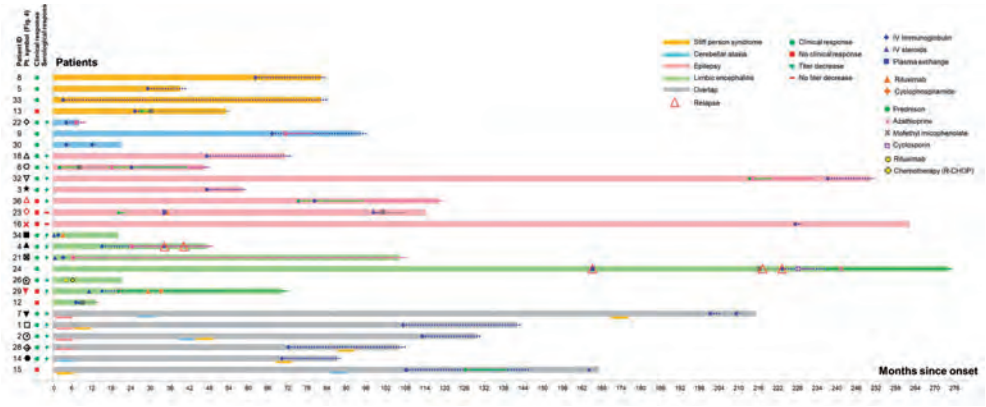

Figure 2. Patients treated with immunotherapy. Disease courses and treatment regimens of the 27 patients that were treated with immunotherapy. In patients with overlapping syndromes, the specific syndromes are indicated below the grey bar with the corresponding color at the relative time when they were diagnosed. Symbols inside the color bars show specific treatments or the moment they were initiated. A line with the same color is used for chronic (continuous line) and periodic (discontinuous line) treatments to show their duration. Symbols on the left side correspond to those in Figure 5.

In the low-concentration group 12/20 patients had an alternative diagnosis, including 1 LE with anti-$GABA_BR$ and a pancreatic adenocarcinoma, and 1 progressive encephalomyelitis with rigidity and myoclonus (PERM) with anti-GlyR without an associated tumor, different variants of subacute or chronic polyradiculoneuropathies, and other non–immune-mediated diseases (glioblastoma, hemispastic syndrome after brain surgery, multiple system atrophy, or functional disorder). The remaining eight patients had chronic Ep, otherwise seronegative LE or nonspecific ataxia and gait disorder (Supplementary Table 1). Figure 3 shows patients distributed according to their clinical phenotype and antibody concentration in serum and CSF. The median concentrations of the different clinical phenotypes (in the high-concentration group) were comparable in serum ($p = 0.210$) and CSF ($p = 0.067$). No association was found between concentration levels and clinical severity between patients (data not shown). Within the high-concentration group, the median serum/CSF anti-GAD65 ratio was 53 (IQR 171; range 7-1761, n=28). After excluding overlaps, there were no significant ratio differences between the clinical syndromes (p=0.29), although the median ratio of SPS was more than two-fold that of the other phenotypes (Figure 3C).

Table 2. **Effects of immunotherapy in patients with high anti-GAD65 concentrations**

ID	Condition	Improved	Before treatment	Best after treatment	Improved mRs/SARA	Subjective improvement	CGI-S	CGI-I
8	SPS	Yes	Walked with a walker, limited by painful spasms that worsened with movement. mRs 3	Cycles and walks independent or sometimes with walker. mRs 2	Yes, 3 to 2 / NA	Yes, much better	4 moderate	2 much improved
5	SPS	Yes	Stiff leg syndrome. Could walk without assistance. mR 2	Much better with IVIg, but still increased tonus. mR 1.	Yes, 2 to 1 / NA	Yes, much better	4 moderate	2 much improved
33	SPS	Yes	Stiff leg syndrome. Unassisted in short distances, wheelchair and walker for longer walks outside home. mRs 3	Mild stiffness. Very satisfied. 'Sometimes forgets that she is ill' mRs 1.	Yes, 3 to 1 / NA	Yes, much better	4 moderate	2 much improved
13	SPS	No	Walked unassisted, independent for daily activities of life, but limited by leg stiffness, painful cramps and lower back pain. mRs 2.	Progression halted, but no improvement. mRs 2.	No / NA	No	3 mild	4 not improved
22	CA	Yes	Walked without assistance, but tandem impossible. Diplopia and dysmetria. mRS 2	Diplopia and hand dysmetria solved, subjectively ataxia 50% improved (still needs assistance). mR 2	No / Yes, 11 to 8	Yes, improved	4 moderate	2 much improved
9	CA	Yes	Moderate ataxia, dysarthria and dysmetria. Only short distances with a walker. mR 4.	Ataxia improved, able to walk 30 m with a stick. mR 3.	Yes, 4 to 3 / Unknown	Yes	4 moderate	3 mildly improved
30	CA	Yes	Ataxia, dysarthria, nystagmus and dysmetria; needs a walker. mRS 3.	Objective improvement of static and dynamic stability measured by physical therapist. mR 2.	Yes, 3 to 2 / Unknown	Yes	4 moderate	2 much improved
18	Ep	Yes	7 seizures/day FOA -déjà-vu-, FOIA)	Clusters of days with 1-3 seizures/day, days without seizures	NA / NA	Yes, mildly	4 moderate	3 mildly improved
6	Ep	Yes	40 seizures/month (FOIA, FOA, bilateral tonic-clonic)	2-4 seizures/month (less intense)	NA / NA	Yes, much better	4 moderate	2 much improved
32	Ep	Yes	Clusters of 1-3 seizures/day every 3 weeks (FOIA)	1-2 seizures/month (FOIA)	NA / NA	Yes, much better	3 mild	2 much improved
3	Ep	Yes	5-10 seizures/month (FOIA, FOA)	2 seizures/month (FOA)	NA / NA	Yes, improved	3 mild	2 much improved
36	Ep	No	Daily seizures (FOIA daily, bilateral tonic-clonic often)	No improvement	NA / NA	No	4 moderate	4 not improved
23	Ep	No	Daily FOA (déjà-vu), 9 tonic-clonic seizures/month	Improved (déjà-vu 2-5/week, 5 tonic-clonic seizures/month), but less than 50%	NA / NA	Yes, mildly (insufficient)	4 moderate	3 mildly improved
16	Ep	No	50-60 seizures/month (FOIA)	No improvement	NA / NA	No	4 moderate	4 not improved
34	LE	Yes	Severe cognitive decline, memory severely impaired, unable to recognize family members	Initial improvement, recognized family members. Able to live at home with serious cognitive impairment. Relapse 9 months later, without improvement.	Yes, 5 to 4 / NA	Yes	6 very severe	3 mildly improved
4	LE	Yes	Debut: seizures and cognition ((FOA -déjà-vu-, FOIA, bilateral tonic-clonic, status epilepticus). Relapses: seizure frequency increase, status epilepticus, cognition.	Clear improvement: seizure-frequency reduced (almost all FOA), cognition improved. Worsens with immunotherapy reduction.	Yes, 5 to 2 / NA	Yes, improved	4 moderate	2 much improved

ID	Condition	Improved	Before treatment	Best after treatment	Improved mRs/SARA	Subjective improvement	CGI-S	CGI-I
21	LE	Yes	Debut: memory impairment, panic attacks, seizures (FOA, bilateral tonic-clonic) and status epilepticus. Then chronic epilepsy. Relapses: seizure frequency to 1-2 daily	Seizures and memory improved during first admission. Seizures almost disappeared with chronic immunotherapy (occasional FOIA).	Yes, 5 to 1 / NA	Yes, much better	4 moderate	2 much improved
24	LE	Yes	Chronic epilepsy with relapses. Relapses: seizure frequency increase (almost daily), behavioral symptoms; twice status epilepticus.	1 seizure/week, lately stable for 15 months	Yes, 5 to 2 / NA	Yes, much better	4 moderate	2 much improved
26	LE	Yes	Memory impairment and seizures; clinical deterioration, unable to walk or feed herself	No seizures, walks assisted, able to eat (immunotherapy, surgery, chemotherapy)	Yes, 5 to 3 / NA	Yes, improved	5 severe	3 mildly improved
29	LE	No	Seizures (FOIA) and behavioral symptoms. Admitted with status epilepticus.	Chronic treatment-refractory epilepsy, No improvement with immunotherapy.	No / NA	No	4 moderate	4 not improved
12	LE	No	Memory impairment and seizures (FOA -déjà-vu- 2/day, bilateral tonic-clonic twice)	2 seizures/week (FOA), memory normalized	Yes, 3 to 1 / NA	Yes, much better	4 moderate	2 much improved
7	Ep+SPS	Yes	Seizures (FOA -musicogenic-, bilateral tonic-clonic) had variable frequency. Stiffness in legs, walker. Serious progression after immunotherapy withdrawal (falls, hip fracture, continued spasm), became bedbound. mRs5	Initially: became seizure free, not much improvement in stiffness. Treatment stopped. Restarted after progression, with serious improvement. Needs walker. mR 3	Yes, 5 to 3 / NA	Yes, much better	5 severe	2 much improved
1	Ep+SPS	Yes	Seizures 15/week (FOIA). Wheelchair due to stiffness and pain. mR 4.	Seizures 1-4/week (FOIA). Able to cycle 5 Km and recovered some independence. mR 2.	Yes,4 to 2 / NA	Yes, improved	4 moderate	2 much improved
2	Ep+SPS+CA	Yes	4 seizures/month. Due to ataxia and stiffness, unable to walk at all. Wheelchair, SARA 22, mRs 4	No seizures. SARA 17. Can walk 12 m with two people. mR 4. But subjectively somewhat better than before treatment.	No / Yes, 22 to 17	Yes, improved	5 severe	3 mildly improved
28	Ep+SPS	Yes	Seizures 1/month (FOIA). Mild stiffness in right leg and arm, walked with assistance. mR 3.	Seizures unchanged (1/month). Clear but short-lasting improvement in stiffness and spasms after IVIG infusions. Able to walk without assistance. mR 2.	Yes, 3 to 2 / NA	Yes, improved	4 moderate	2 much improved
14	CA+SPS	Yes	Ataxia and dysmetria and dizziness (SARA 11,5) and stiffness. Needed a stick at home and a walker for long distances. mR 4	Dizziness disappeared. Speech. Ataxia clearly improved, stiffness mildly improved. SARA 7, stick to go out, unassisted at home. mR 2.	Yes, 4 to 2 / NA	Yes, much better	4 moderate	3 mildly improved
15	SPS+CA	No	Severe cerebellar syndrome, bedbound most of the time. mRS 5	Never improved	No / Unknown	No	6 very severe	4 not improved

Abbreviations: CA = cerebellar ataxia; CGI-I = Clinical Global Impression–Improvement scale: improvement of disease after immunotherapy rated by a clinician (1—very much improved to 7—very much worse); CGI-S = Clinical Global Impression—Severity scale: severity of the patient's illness before immunotherapy rated by a clinician (1—normal to 7—most extremely ill); Ep = epilepsy; FOA = focal onset preserved awareness seizures; FOIA = focal onset impaired awareness seizures; IVIg = IV immunoglobulin; LE = limbic encephalitis; mRS = modified Rankin Scale; NA = not applicable; SARA = Scale for the Assessment and Rating of Ataxia; SPS = stiff-person syndrome. SARA scores are only provided in patients with ataxia, whereas mRS scores are omitted for patients having Ep only.

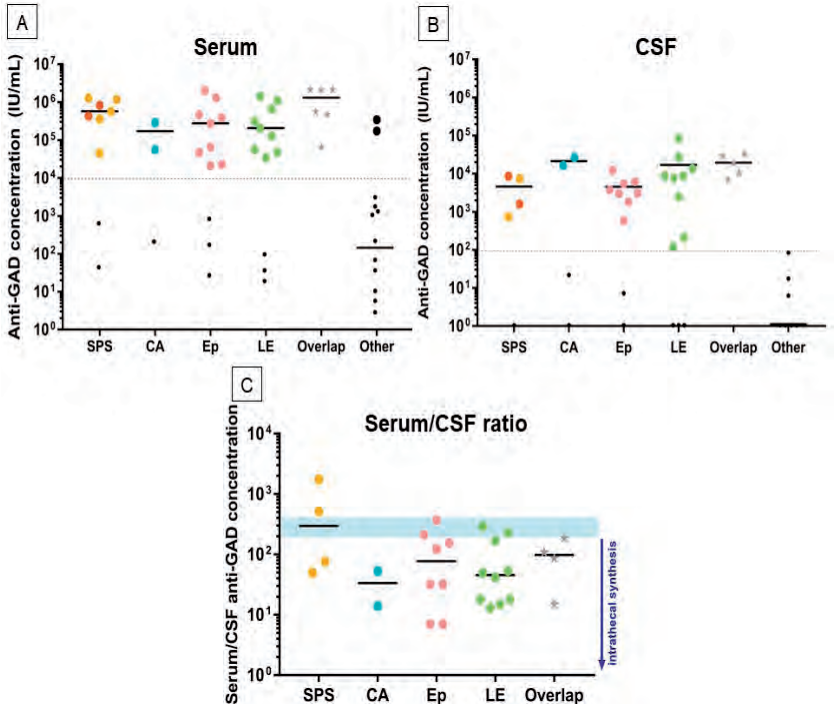

Figure 3. Antibody concentrations and clinical syndromes. Patients were grouped according to their clinical phenotype into one of the four classical anti-GAD65 associated syndrome categories, in the overlap or other category. Serum (A) and CSF (B) ELISA concentrations are shown in a logarithmic scale. Small black dots represent patients with low antibody concentrations. The symptoms of these patients are listed in the supplementary data. Orange dots in SPS column were patients with progressive encephalomyelitis with rigidity and myoclonus (PERM), which is considered an extended form of SPS. (C) Serum/CSF ratio of anti-GAD65 ELISA values within the different syndromes in the high-concentration group. The blue ribbon represents the ratio-frame where intrathecal antibody synthesis would start. CA = cerebellar ataxia; Ep = epilepsy; GAD = glutamic acid decarboxylase; LE = limbic encephalitis; SPS = stiff-person syndrome.

Twenty-seven patients from the high-concentration group were treated with immunotherapy, of them 26 (96%) received intravenous immunoglobulins (IVIg), in different treatment regimes. Most used regime was 0,4g/kg for 5 days, repeated monthly for at least two more times, to assess response. Eight patients (30%) were treated with intravenous methylprednisolone, and three patients received plasma exchange. Four patients were additionally treated with second line immunotherapy, including Rituximab (n=3), and cyclophosphamide (n=1). Chronic immunosuppression was given in 11 patients, consisting of combinations of azathioprine (n=9), oral steroids (n=8), mycophenolate mofetil (n=5) and cyclosporine (n=1). Figure 2 shows individual timelines of the high-concentration group patients treated with immunotherapy. Nineteen patients (70%) improved. Eight of 10 patients with SPS (including patients with overlap syndromes) improved according to themselves and their physicians, accompanied by the mRS score decrease in 7 of them (Table 2). All patients with CA (including patients with overlap syndromes) except one felt better and obtained a reduction in the mRS score. SARA scores improved ≥3 points in the only 2 patients in whom scores were available. In patients with chronic Ep, >50% seizure frequency reduction was

obtained in 4 of 7 patients. Immunotherapy was efficacious in 5 of 7 patients with LE, and 2 of them had relapses despite chronic treatment. Those 2 responded to more intensive therapies. Four typical patient examples are provided in Figure 4.

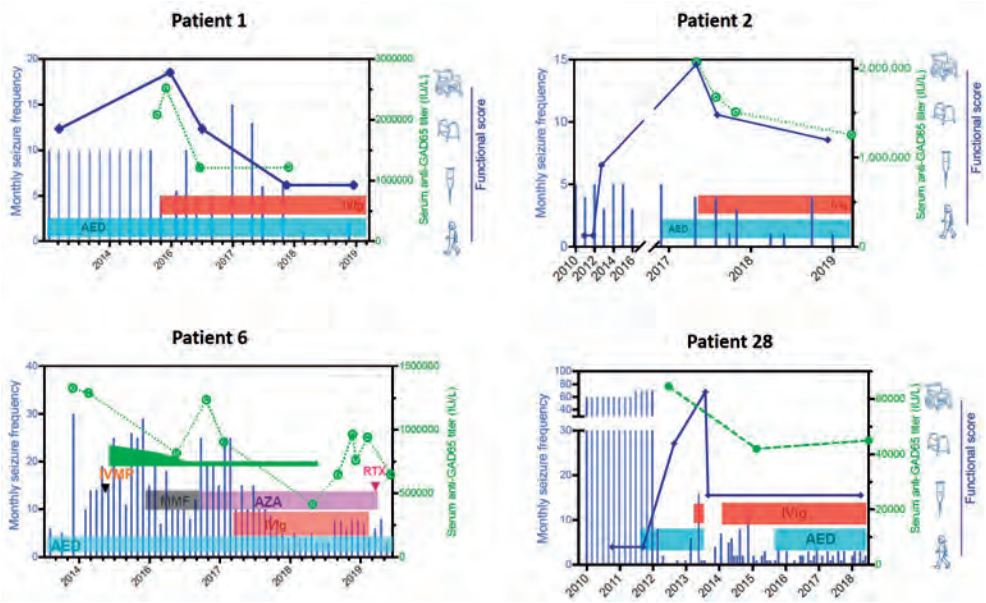

Figure 4. Disease course and treatment response of four patients in the high-concentration group. Horizontal bars containing treatment abbreviations represent the time periods of the corresponding treatments. Blue vertical lines represent seizure frequency over time. Green discontinuous line connected by green circles shows evolution of serum anti-GAD65 concentration. Thick dark-blue line connected by rhomboids represents functional status over time, according to the visual score at the right most part of the charts.
AED antiepileptic drugs; IVIg intravenous immunoglobulins; IVMP intravenous methylprednisolone; PDN prednisone, AZA azathioprine; MMF mofetil micophenolate; RTX rituximab.

In 17/36 high-concentration patients pre- and post-treatment samples were available for comparison. Antibody concentrations showed a median concentration reduction of 69% (range 27-99%) and decrease was over 25% in 15 of the 17 serum samples after immunotherapy (Figure 5). A clear clinical response was observed in 14 of these 15 treated patients. The two patients without consistent decrease in concentration showed no clinical improvement. Both had focal epilepsy for years.

In none of the patients, complete recovery was reached. Similarly, in none of the patients, antibodies disappeared in serum. In 6/17 patients with pre- and post-treatment samples serum concentrations became lower than 10.000 IU/mL Despite periodic or chronic immunotherapy, a stagnation, both clinically and serologically, was observed after initial improvement. However, after withdrawal or tapering of immunotherapy symptoms deteriorated, syndromes relapsed, or concentrations increased in six patients (Figure 2 and Figure 4). Four patients also had relapses or clinical worsening despite stable chronic or periodic immunotherapy.

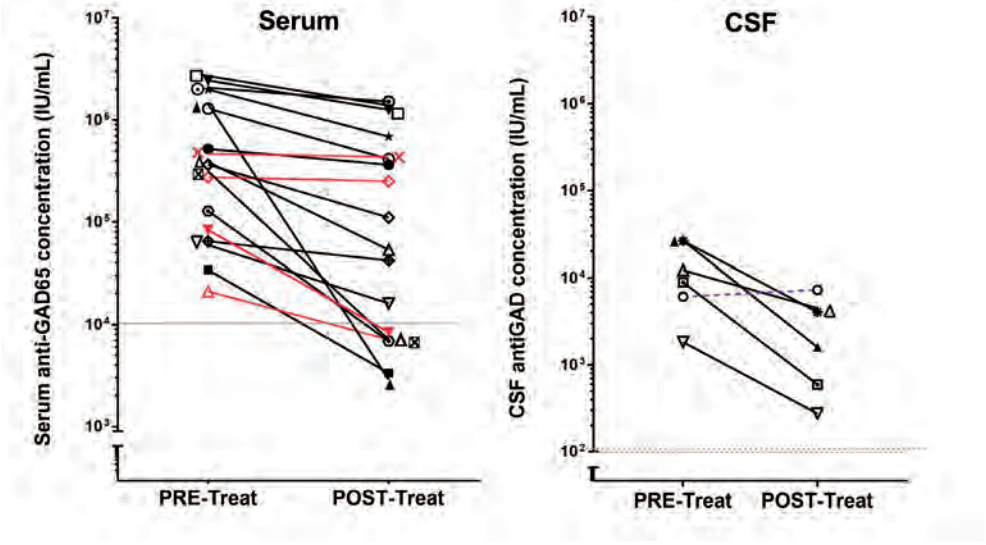

Figure 5. Antibody-concentration response to immunotherapy. Concentration responses to treatment of the 17 patients of whom pre-treatment and post-treatment samples were available. Red lines represent the patients lacking clinical improvement. The blue dashed line in the right graph represent CSF samples obtained pre-treatment and at clinical relapse. A reduction of at least 25% following immunotherapy was considered a relevant concentration reduction.

DISCUSSION

This study aimed to determine the clinical relevance of GAD65 antibodies in patients with neurological syndromes and to evaluate their responses to immunotherapy. We showed that patients with high anti-GAD65 concentrations presented with a limited number of specific neurological syndromes, whereas the neurological symptoms observed in patient with the low-concentrations were less specific, necessitating additional investigations. The laboratory tests used showed a high concordance and a clear cut-off value for the high and low antibody concentrations. Around two thirds of the patients responded to immunotherapy, although all incompletely. The clinical improvement was reflected in the reduction of anti-GAD65 concentrations in both serum and CSF.

Patients with high antibody concentrations had well-defined clinical phenotypes that have been classically associated to GAD65 antibodies, such as stiff-person syndrome, cerebellar ataxia, epilepsy or limbic encephalitis. Additionally, one out of five patients had overlap syndromes. Remarkably, in these patients the relapse tended to present with different phenotypes. Another interesting feature was a different, not previously described phenotype, including pseudo-orthostatic tremor and optic neuropathy in two patients in the high-concentration group. Unfortunately, these two patients were not seen by one of the authors, so diagnosis and absence of more typical symptoms should be taken with caution.

From a different perspective, patients with low antibody concentrations presented with a diversity of neurological syndromes. Some of them had a phenotype that is similar to the classically anti-GAD65-associated syndromes, which was probably the reason why anti-GAD65 was requested in these patients. However, frequently other diagnoses were identified in the workup or during the follow-up, like neurodegenerative diseases or tumors. In these cases, positive ELISA was not confirmed by IHC or CBA, and concentrations were low and comparable to those seen in samples tested for type 1 diabetes mellitus. In these patients, extensive work-up to identify other diseases is warranted, including requesting other antibodies. Anti-$GABA_BR$ antibodies were identified in a few patients with high and low-concentrations of GAD65 antibodies, in line with previous studies. [18] Around 40% of the patients in both high and low-concentration groups had been diagnosed with it DM before the onset of neurological symptoms, which is also in line with previous studies [19–22]

To define which antibody concentration should be considered high and relevant, we compared three different laboratory techniques and found highly concordant results. ELISA concentrations above 10.000 IU/mL for serum and 100 IU/mL for CSF can be detected by IHC and CBA, with comparable results. CBA, as expected, is more specific, especially if a different antibody is concomitantly present in the tested sample (leading to an unspecific IHC staining pattern). Another cut-off value (of 2,000) has been suggested before, [13,23] but these studies used a radioimmunoassay (RIA) with different test calibration, currently not widely used anymore.

The role of GAD65 antibodies in neuroinflammation is debatable and still unknown. An obvious theory would be that antibodies block GAD65, interfering with GABA synthesis and impairing the inhibitory GABAergic circuits. This would result in a hyperexcitability state of the central nervous system. There are studies supporting this theory, demonstrating a reduced GABA concentration in the CSF and in the cerebral cortex of patients with SPS.[24,25]. However, GAD65 is located intracellularly and as such not accessible for extracellular antibodies. As an explanation, it has been hypothesized that GAD65 might transiently appear on the cell surface, in the synaptic cleft during the process of neurotransmission and exocytosis.[26] In rats, in vivo injection of GAD65 antibodies from SPS patients induced electrophysiological changes in myelinated neurons, whereas GAD65 antibodies from DM1 patients did not.[27] On the other hand, injection of GAD65-specific T cells in mice caused death even in mice without B-cells.[28] This suggests that the mechanism might be comparable to paraneoplastic syndromes, in which T-cell mediated responses primarily lead to symptoms.[29]Similarly, despite several efforts, there are no convincing successful animal models to support the pathogenicity of GAD65 antibodies.

Moreover, studies report poor responses to immunotherapy targeting different GAD65-syndromes,[6,11,30] as compared to other neurological disorders caused by antibodies targeted to extracellular neuronal structures, again questioning the direct pathogenic role of anti-GAD65. Although the mechanisms remain questionable, our results revealed that many patients show improvement after immunotherapy (mostly IVIg), coupled to a simultaneous

concentration decrease. Many patients show improvement after immunotherapy (mostly IVIg), coupled to a simultaneous concentration decrease. Many patients improve not immediately after initiating treatments, but might need some months of treatment before improvement. This might explain some lack of treatment-response from the literature. Repeated doses or combinations of different treatments might be considered before classifying a patient as a non-responder. Nevertheless, some of the patients do not respond at all to immunotherapy. In addition, it is humbling that even in responders, improvement is generally incomplete and often patients suffer from stagnation in both the clinical, as well as the serological response after a few months with chronic immunotherapy. Unfortunately, no patient completely recovered. A possible explanation might be a combination of functional and structural neuronal damage, as different research groups have demonstrated in CA [9,30,31] Immunotherapy helps to restore cell-function, but cell death is non reversible.

Despite clinical stabilization with immunotherapy, treatment-intensity reductions or withdrawals frequently result in clinical progression (in CA) or relapses (in SPS, LE and epilepsy). A few patients have relapses despite stable chronic immunotherapy. Fortunately, these patients usually respond to more intense treatments.

The limitations of this study are mainly linked to its retrospective design. In addition, not all patients were seen by one of the authors. Accordingly, it was sometimes difficult to assess outcome after treatment. Objective parameters like mRS, SARA score and seizure frequency were used when applicable. As treatments were open label, a placebo effect or regression to the mean could explain part of the results. However, most patients had stable (or slowly progressive) disease for a longer period, and showed a serious deviation from the time course before. Similarly, the association with concentrations also suggests some real effects. For future studies, it would be of additional value to study treatment outcome prospectively by using standardized questionnaires, examinations, serological follow-up and neuropsychological assessments.

To summarize, in patients with classical syndromes (stiff-person, CA, and encephalitis with seizures), detection of high concentrations of anti-GAD65 is practically diagnostic of an anti–GAD65-related syndrome. However, depending on the syndrome (e.g., LE), physicians should consider the possibility of concurrent antibodies, such as $GABA_BR$. These classical syndromes should be treated with prolonged immunotherapy (as the current study suggests). On the other hand, the presence of atypical syndromes (or nonspecific encephalitic syndromes with seizures) or a typical syndrome but with low-concentration anti-GAD65 should raise concern for another underlying disease (anti-GAD65 as bystanders). In these patients, more extensive investigations for alternative diagnoses should be considered.

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Table 1. Clinical and paraclinical characteristics of patiens in the low-concentration group

No	Gender	Age at onset	DM	Autoimmune disorders	Clinical description	CSF	MRI	Serum concentration (U/mL)	CSF concentration (U/mL)
37	M	72	No	No	Behavioral changes for six months. Admitted for 2 seizures, with normal LP and MRI. Eight months later gait disturbance, paraparesis and bladder dysfunction (polyradiculitis).	360 cells/ uL; proteins 1244mg/L	Brain and spine normal	6,580	81
38	F	44	Yes	Thyroid disease	Six months after a properly treated Lyme disease (bite, skin lesion and general malaise), she developed a gait disorder (fluctuating and changing) and burning pain in legs. Examination and ancillary tests were normal. Considered to be functional.	0 cells/uL	Normal	3,770	1
39	F	55	Yes (LADA)	Thyroid disease	ICU admission in coma. Normal brain MRI, mild CSF pleocytosis, slow activity in EEG, everything else normal. Suspected to be epileptic. Empirically treated with antibiotics, aciclovir and ivMP. Recovery in a few days.	Pleocytosis	Normal	2,810	17
40	M	61	Yes	Thyroid disease	Patient with left hemipyramidalism and a long-back musculature dystonia as sequelae of meningioma surgery. Transient worsening of spasms and stiffness in left limbs.	Proteins 66 mg/dL	Surgery sequelae	2,259	6
41	M	0	Yes	Ant -TPO	Unclassified inborn syndrome with cerebral hemangiomas, epilepsy and autism.	-	Hemangiomas and cerebellar atrophy	1,770	-
42	F	23	Yes	No	Cramps and stiffness in lower-back and legs after exercising or standing for long. SPS-like phenotype.	-	Spine MRI normal	1,359	-
43	M	-	Yes	No	Progressive cerebellar syndrome. No more details were provided.	-	-	-	22
44	F	40	Yes	Arthropaty	Muscular fatigue and stiffness after exertion. EMG shows a combination of axonal polyneuropathy and mild myopathy	-	Spine MRI normal	465	-
45	M	69	No	No	Idiopathic Late Onset Cerebellar Ataxia and autonomic dysfunction; possibly MSA-C.	Normal	Normal	442	1
46	F	6	Yes	Antitpo	Epilepsy, focal-onset non-motor (normal EEG).	Normal	Normal	364	7
47	F	47	No	No	Limbic encephalitis with beharvioral and psychotic symptoms and seizures. Classified as possible Hashimotto's encephalopathy (Anti-TPO 3,100, anti-Tg 229) after thorough investigtions.	20 cells/uL, proteins 100 mg/dL	Normal	204	1

No	Gender	Age at onset	DM	Autoimmune disorders	Clinical description	CSF	MRI	Serum concentration (U/mL)	CSF concentration (U/mL)
48	M	46	Unknown	Unknown	CIDP (chronic inflammatory demyelinating polyneuropathy)	11 cells/uL, proteins 92 mg/dL		146	1
49	M	39	No	No	PERM with positive anti-glycine receptor antibodies.	15 cells/ uL, proteins normal.	Brainstem encephalitis	93	1
50	F	63	No	No	Limbic encephalitis and treatment resistant status epilepticus. Probably paraneoplastic, associated to a pancreatic carcinoma. Neuropil staining and anti-$GABA_BR$ antibodies.	5cells/uL, mild protein increase	Normal	77	1
51	F	34	No	Alopecia	Focal onset epileptic seizures that start at age 34. Confusion lasts a few days and resolves spontaneously. Never treated with immunotherapy.	30 cells/uL, OCB positive.	Normal	77	1
52	F	68	No	Unknown	Episodes of focal onset non-motor seizures (temporal bilateral). Chronic epilepsy of unknown source.	Normal	Normal	57	1
53	M	46	Yes	No	Seronegative limbic encephalopathy with low-concentration anti-GAD65 antibodies and no other findings.	11 cells/ Ul, proteins normal	Bilateral hyperintense signal in limbic regions.	40	1
54	M	71	No	Unknown	Guillain-Barré syndrome.	2 cells/uL, proteins 139 mg/dL.	Spine MRI normal	22	-
55	M	56	No	No	Symptomatic epilepsy due to right parietal glioblastoma	Normal	Glioblastoma	12	1
56	M	52	No	Pernicious anemia	Suspected Miller-Fisher syndrome with dysarthria, severe ataxia and areflexia. Non-conclusive EMG and no LP performed. Spontaneous recovery within weeks.	-	Normal	6	-

Chapter 5

The Antibodies Contributing to focal Epilepsy Signs and symptoms (ACES) score

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ABSTRACT

Objectives

Diagnosing autoimmune encephalitis (AIE) can be difficult in patients with less fulminant diseases, like epilepsy. However, recognition of these less severe syndromes is important, as patients require immunotherapy. This study aims to identify antibodies in patients with focal epilepsy of unknown etiology, and to create a score to preselect patient requiring testing.

Methods

In this prospective, multicenter, cohort study, adults with focal epilepsy of unknown etiology, without recognized AIE, were included, between December 2014-2017, and followed for one year. Serum, and if available CSF, were analyzed using different laboratory techniques. The ACES score was created using factors favoring an autoimmune etiology of seizures (AES), as determined by multivariable logistic regression. The model was externally validated and evaluated using Concordance (C) statistic.

Results

We included 582 patients, with median epilepsy duration of 8 years (IQR 2-18). Twenty patients (3.4%) had AES, of whom 3 anti-Leucine-rich-Glioma-Inactivated 1, 3 anti-Contactin-Associated Protein-like2, 1 anti-N-methyl-D-Aspartate Receptor, and 13 anti-Glutamic Acid Decarboxylase-65 (ELISA-concentrations>10,000IU/ml). Risk factors for AES were: temporal MRI-hyperintensities (OR 255.3, 95%-CI 19.6-3332.2; p<0.0001), autoimmune diseases (OR 13.31, 95%-CI 3.1-56.6; p=0.0005), behavioral changes (OR 12.3, 95%-CI 3.2-49.9; p=0.0003), autonomic symptoms (OR 13.3,95%-CI 3.1-56.6;p=0.0005), cognitive symptoms (OR 30.6, 95%-CI 2.4-382.7; p=0.009), and speech problems (OR 9.6, 95%-CI 2.0-46.7; p=0.005). The internally validated C statistic was 0.95, and 0.92 in the validation cohort (n=128). Assigning each factor one point, an ACES score ≥2 had a sensitivity of 100% to detect AES, and a specificity of 84.9%.

Conclusions

Specific signs point towards AES in focal epilepsy of unknown etiology. The ACES score (with a cut-off value ≥2) is useful to select patients requiring antibody testing.

INTRODUCTION

Autoimmune encephalitis (AIE) associated with neuronal antibodies is a severe but treatable neurological disease. Seizures occur frequently in patients with AIE (50-95%), often in combination with other symptoms, such as cognitive symptoms, behavioral changes and autonomic dysfunction.[1-4] Seizures are often resistant to antiepileptic drugs (AEDs), while the response to immunotherapy is good.[5, 6] Most patients have fulminant encephalitis with prominent seizures.

Neuronal antibodies have also been reported in patients with epilepsy (14-31%).[7-9] Results from these studies have ensured that patients, with less rapidly progressive encephalitis, are being recognized as well. Nevertheless, in most of these studies, patients had short epilepsy duration, and most of them had signs and symptoms of encephalitis. Interestingly, some of the mentioned studies report patients with epilepsy without fulminant encephalitis or even any sign of encephalitis. To complicate interpretation, part of these studies describe a variety of antibodies, some pathogenic, but others with questionable clinical relevance.[10, 11]

An important category are neuronal antibody positive epilepsy patients without other encephalitis signs, because underdiagnosis is likely. It is essential to recognize these patients early and to perform antibody testing in preselected patients. At the same time, testing needs to be rigorous, confirming results using different tests, to avoid false positives or clinically irrelevant results. Similarly, it is important to limit the amount of patients that require testing, for the sake of specificity and cost-effectiveness.

The aim of our prospective, multicenter study was to identify neuronal antibodies in a comprehensive cohort of patients with focal epilepsy of unknown etiology, and without, or with unrecognized, signs of encephalitis. We have developed a clinical score, based on the prospectively collected data of patients with focal epilepsy of unknown etiology, that can be used to guide autoimmune etiology of seizures (AES) screening. This ACES score has subsequently been validated in a second, external cohort.

METHODS

Study design, participants, and definitions

In this prospective, multicenter, observational cohort study, adults with focal epilepsy of unknown etiology were included by epileptologists between December 2014 and December 2017. Patients included in this study had been referred to (tertiary) epilepsy centers by neurologists who had no particular suspicion of AIE. Patients were included in the Netherlands, from tertiary epilepsy centers and from dedicated epilepsy centers in academic hospitals, and one general hospital with an epilepsy referral center. We requested all epileptologists 1) to include patients with epilepsy with or without additional symptoms, but without suspicion of encephalitis, and 2) to exclude patients highly suspected of having

AIE. Patients with epilepsy with known infectious, genetic, or metabolic etiologies were excluded. Patients with a structural lesion on brain MRI at inclusion were excluded, while patients with mesial temporal sclerosis, or with T2/FLAIR hyperintensities mainly in the mesial temporal lobe, both unilateral and bilateral, were not, as these MRI findings might be associated with AIE.[12]

ILAE guidelines were used to define focal seizures, an unknown etiology of epilepsy and drug-resistant epilepsy.[13] The study was registered at clinicaltrials.gov (NCT02802475).

Patients were classified as 1) definite AES if a known neuronal antibody was detected in serum and/or CSF, and results were confirmed with a different laboratory test; 2) probable AES as a known neuronal antibody was detected in serum and/or CSF, but we were unable to confirm the finding, or when the diagnostic criteria for seronegative AIE were met;[14] 3) possible AES, if serum or CSF showed similar staining patterns on IHC, but without known antibody; 4) non-AES.

Procedures

The including epileptologist or the coordinating investigator (MB) completed a case record form at enrollment, containing information about: patient characteristics, epilepsy characteristics, presence of autoimmune associated clinical signs or symptoms (disorders of memory, sleep, behavior, speech, movement, or of the autonomic system), previous and current medication (including AEDs), current level of functioning (measured with modified Rankin Scale [mRS[15]]), and results from prior ancillary testing (including MRI, EEG, and if available CSF analysis). Blood was drawn, and if CSF analysis was performed this was collected as well.

Patients were prospectively followed for one year (last follow-up in December 2018). At one, four, eight, and twelve months after inclusion the treating physician or coordinating investigator collected data about seizure type, monthly seizure frequency, types of AEDs used, and mRS. Final diagnosis was obtained at last follow-up.

Laboratory methods

All samples were screened for immunoreactivity with immunohistochemistry (IHC), as previously described.[16] Subsequently, all samples with a positive or questionable IHC result were tested more extensively. A combination of laboratory techniques was used, depending on the staining pattern and the clinical phenotype. Commercial cell-based assay (CBA; *Euroimmun, Lübeck, Germany*) was used to detect anti-N-methyl-D-aspartate receptor (anti-NMDAR), anti-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (anti-AMPAR), anti- γ-aminobutyric acid B receptor (anti-$GABA_BR$), anti-leucine-rich glioma-inactivated 1 (anti-LGI1), anti-contactin-associated protein-like 2 (anti-Caspr2), and anti-glutamic acid decarboxylase 65 (anti-GAD65). Anti-GAD65 was tested with Enzyme-linked immunosorbent assay (ELISA; *Medizym anti-GAD 96, Medipan GMBH, Berlin, Germany)* as well, to quantify antibody concentration. An in-house CBA with live cells was used to

detect anti-γ-aminobutyric acid A receptor (anti-$GABA_AR$), anti-$GABA_BR$, anti-AMPAR, and anti-dipeptidyl-peptidase like protein-6 (anti-DPPX). Radio-immuno-assay (RIA; *VGKC Antibody Assay RIA, DLD Diagnostika, Hamburg, Germany*) was used to detect voltage gated potassium channel complex antibodies (anti-VGKC), immunoblotting was used to detect onconeural antibodies (anti-Hu/Yo/Ri/Tr/amphyphysin/CV2/Ma1/Ma2; Euroimmun AG, Lübeck, Germany). We considered serum anti-GAD65 relevant if the ELISA concentration was above 10,000 IU/ml. Only samples tested positive by ELISA and a compatible positive staining on IHC were considered positive.[17] In addition, all serum samples were screened for the presence of anti-Glycine receptor (anti-GlyR) with an in house CBA with live cells, as these antibodies cannot be visualized with IHC.[18,19] All positive results were confirmed in separate experiments.

Samples scored questionable or positive on IHC, but without a known antibody, were tested more extensively using immunocytochemistry with live hippocampal cell cultures.[16]

A subgroup of patients considered for epilepsy surgery, was side by side tested by CBA, ELISA and RIA for antibodies, as a part of a standardized protocol. In addition, patients with an ACES score of 2 or more were tested by commercial CBA and ELISA post-hoc.

Statistical analysis

Dutch cohort

Patients were divided into three groups, based on antibody test results: 1) antibodies targeting extracellular neuronal antigens, 2) high-concentration anti-GAD65, and 3) no autoimmune etiology of seizures (AES). Comparisons between the three groups (extracellular-AES, GAD-AES and non-AES) were performed with the Fisher-Freeman-Halton test, the Pearson Chi-Square test or the Kruskal Wallis test, when appropriate. To correct for multiple testing, we only considered p-values below 0.002 to be relevant (Bonferroni). Exploratory, post-hoc in between analysis using the Fisher-Exact and Mann-Whitney U test was used to visualize group differences.

The patients with probable or possible AES were not included in the analysis, and are only described exploratory, as their etiology could not be confirmed with certainty. We repeated analyses assigning these patients to the non-AES group to assess the effects of exclusion.

A multivariable logistic regression model was used to identify independent risk factors pointing towards AES (comparing both extracellular-AES and GAD-AES to non-AES). Antibody status was used as dependent variable. Missing values (0.8% of the total number of values) were imputed. Multiple imputation was used to create five sample sets, with antibody status included as covariate. We excluded the factor "family history of autoimmune diseases" as we identified overt recall bias (lack of reliable information in many patients without AES). The univariable regression and multivariable regression models were constructed using the imputed data. Variables with a p-value below 0.007 in univariable analysis (corrected for multiple testing) were included in the multivariable logistic regression model. To derive the ACES score, variables were eliminated using a backward LR method with cut-off p-value

of 0.05. The selected risk factors were used on the original and imputed data to check the accuracy. Each risk factor was assigned one point to create the ACES score (range: 0 to 6 points). Sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) were calculated using the non-imputed dataset. Percentages were visualized with 95%-confidence interval (CI) assuming a Poisson distribution.
The systematic difference in direction between the APE/APE2 and ACES scores were compared using McNemar's paired test. Internal validation with bootstrapping was used to estimate the degree of optimism in the ACES score. In addition, Firth logistic regression was used. We performed a sensitivity analysis to assess the validity of our model allocating the excluded patients with probable or possible AES to the non-AES group (to be conservative).

Czech cohort
External validation was performed in a Czech cohort consisting of 128 temporal lobe epilepsy patients.[20] In the validation cohort, samples were tested by commercial CBAs for anti-NMDAR, anti-AMPAR, anti- $GABA_BR$, anti-Caspr2, and anti-LGI1 (Euroimmun, Lübeck, Germany) and immunoblot (Ravo, PNS 11 Line Assay), confirmed by RIA for anti-GAD65 (CentAKanti-GAD65M, Medipa). All positive and questionable samples had been tested by IHC (FL). For uniformity, all questionable and positive samples were additionally tested by CBA, ELISA and IHC in Rotterdam (MT). All six risk factors used in the ACES score had been collected prospectively in the Czech cohort, and were retrieved from the database to calculate the ACES score.
The model performance in this validation cohort was described by discriminatory value, using the Concordance (C) statistic (area under the curve of the receiver operating curve) on the ACES model. Statistical analysis was performed using IBM SPSS Statistics 21, R statistical software version 3.6.2 (*rms* library), and Prism 8.0.1 (GraphPad) for Windows.

Ethics committee approval
The Institutional Review Board of the Erasmus MC University Medical Center approved the study protocol (MEC-2014-463). Written informed consent was obtained from all patients.

RESULTS

Patients – Dutch cohort
Informed consent was obtained in 618 patients, of whom 36 (6%) were excluded (Figure 1). Of 582 included patients with focal epilepsy of unknown etiology, 48% were male. The median age at inclusion was 44 years (IQR 29-58; range 18-89). Ten percent of all included patients had a history of autoimmune diseases. Median epilepsy duration was 8 years (IQR 2-18; range 0.1-75), while 66 patients (11%) had an epilepsy duration of less

than one year. Patients were treated with a median of one AEDs at inclusion (IQR 1-2; range 0-5).

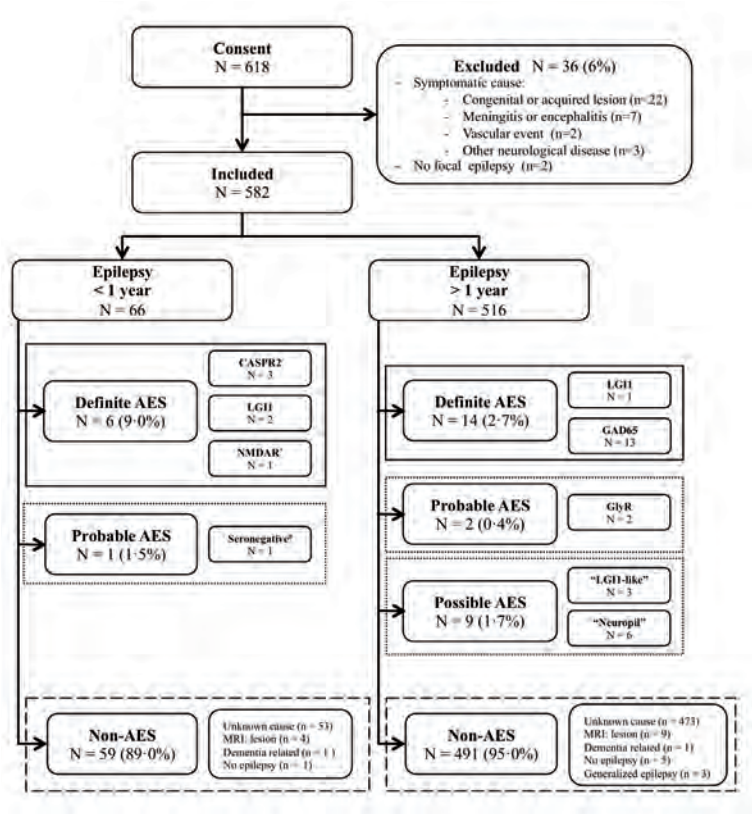


Figure 1. Flowchart with follow-up diagnosis of all included patients.
*This patient had focal onset epilepsy with sporadically occurring focal to bilateral tonic-clonic seizures, and had a recently discovered esophagus carcinoma. Eleven months after inclusion his seizure frequency increased and he developed psychotic symptoms. CSF was positive for anti-NMDAR, confirmed with live hippocampal neurons.
#According to the criteria.[20]
Abbreviations: LGI1= leucine-rich glioma-inactivated 1, CASPR2= Contactin-associated protein-like 2, NMDAR= N-methyl-D-aspartate receptor, GAD65= Glutamic Acid Decarboxylase 65, GlyR= Glycine receptor, MRI= magnetic resonance imaging.

The MRI was normal in 532 patients (91%), while T2/FLAIR hyperintensities of the mesial temporal lobe were observed in 14 patients (2%), and 36 patients (6%) had mesial temporal sclerosis. The EEG showed epileptic discharges in 389 patients (67%). In 362 of these 389 patients EEG reports were available. In 23% (n=83) seizures had a multifocal onset, while in 77% (n=279) there was a focal seizure onset, including temporal (39%; n=108), fronto-temporal (39%; n=110) or extra-temporal (20%; n=57) localization.

Results of laboratory studies

Serum was collected from all patients, and from 46 patients (8%) CSF was collected. In 20 of all 582 patients (3.4%) neuronal antibodies related to AES were detected (definite AES), including anti-LGI1 (n=3), anti-Caspr2 (n=3), anti-NMDAR (n=1), and high-concentration anti-GAD65 (n=13). Of the 66 patients with epilepsy less than one year at inclusion, six had antibodies (9%; Figure 1).

Three patients of all 582 patients were scored as probable autoimmune. One patient, with a neuropil staining pattern on IHC, fulfilled the criteria of seronegative AIE,[14] while in serum of the other two patients anti-GlyR were detected using CBA. Nine patients had a positive IHC, but no known antibody was identified, nor was immunocytochemistry positive in any of these samples; these patients were considered possible autoimmune. Serum and CSF of three of these nine patients showed a similar "LGI1-like" staining pattern on IHC. In the other six patients, IHC showed a diffuse neuropil staining pattern. No additional antibodies were detected in the patients with questionable IHC staining patterns and negative live neurons (n=36). In addition, 186 samples were tested by commercial antibody testing, partly in parallel, and often as part of screening for epilepsy surgery (n=104). The other samples were tested because of high ACES scores (ACES ≥ 2, n=82; post hoc testing). No antibodies were found in these samples, in line with negative screening by IHC.

Comparison between AES and non-AES

Comparison of patients with antibodies against extracellular antigens (LGI1, CASPR2, NMDAR; n=7), patients with anti-GAD65 (n=13), and non-AES patients (n=550; Supplementary Table 1), showed that patients with AES (both antibodies against extracellular antigens and GAD65) more frequently had drug-resistant epilepsy (p=0.002), uni- or bilateral T2/FLAIR hyperintensities of the mesial temporal lobe (p<0.0001), higher mRS at inclusion (p<0.0001), and more often cognitive symptoms (p<0.0001) and behavioral changes (p<0.0001; Figure 2A). Post hoc in between analysis (Figure 2) showed that patients with antibodies against extracellular antigens, were older at disease onset, more frequently male, had shorter epilepsy durations, and more frequently had autonomic symptoms, than patients with anti-GAD65 or non-AES patients (Figure 2A and 2B). Patients with high-concentration anti-GAD65 were female, more often had other autoimmune diseases, speech problems (word finding difficulties, non-fluent speech), and muscle stiffness, than patients with antibodies against extracellular antigens or non-AES patients (Figure 2A and 2C).

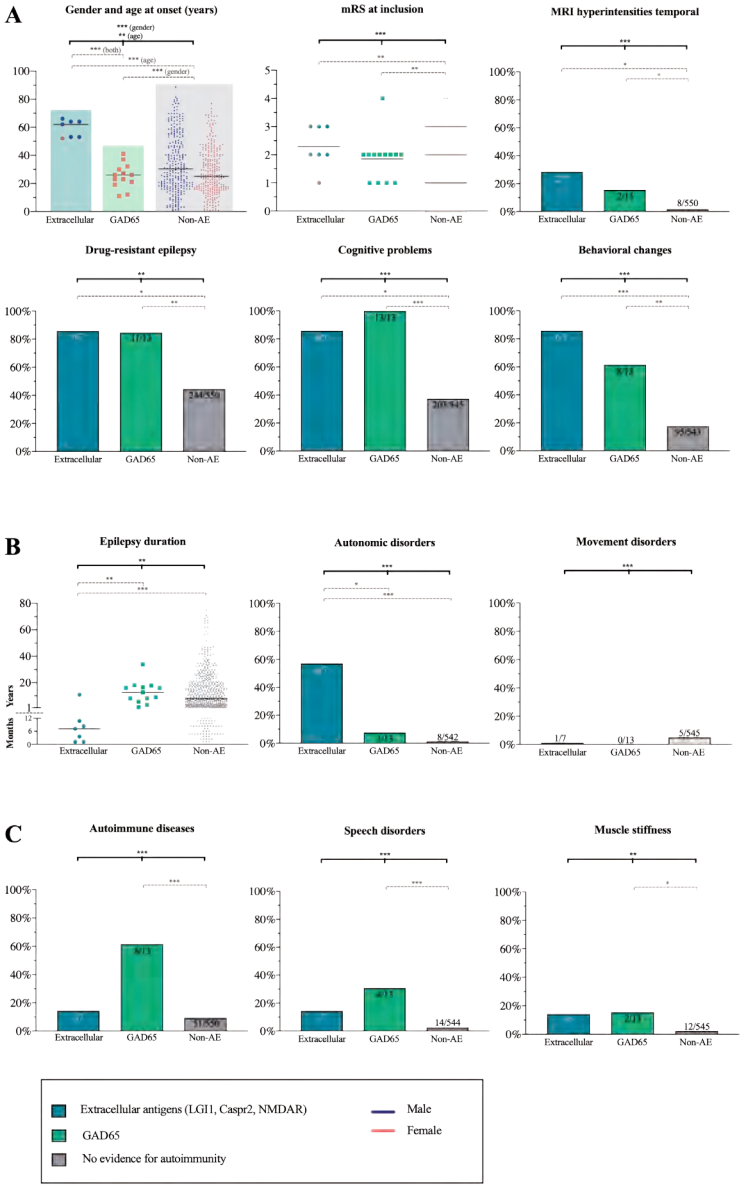


Figure 2. Overview of the characteristics, signs and symptoms that occurred more often in patients with neuronal antibodies.
*p-value between 0.05 and 0.005, **p-value between 0.005 and 0.0001, *** p-value below 0.0001.
The top p-values correspond to the values visualized in Table 1. The lighter-colored lines visualize significance of the post-hoc in between analysis (raw data are visualized in the supplemental data).
Figure A shows the factors that differed significantly between autoimmune etiology of seizures (AES) and non-AES. Figure B shows the values that differed between the extracellular antigen group and the non-AES group, and Figure C shows the values that differed between the GAD65 and non-AES group.
In order of appearance, the y-axis shows: 1) years (age at onset), 2) cumulative percentage (all bar diagrams), 3) months and years (0-12, 1-80 respectively; epilepsy duration). In the bar diagrams the x-axis shows the raw values.
Abbreviations: AES = autoimmune etiology of seizures; GAD65= Glutamic Acid Decarboxylase 65, mRS= modified Rankin scale, MRI= magnetic resonance imaging.

The ACES score

Eight variables showed a statistically significant association with antibody status in univariable analysis: drug-resistant epilepsy, autoimmune diseases, behavioral changes, cognitive symptoms, muscle stiffness, speech problems, autonomic symptoms, and MRI hyperintensities of the mesial temporal lobe (Table 1 and Fig 2). The multivariable model included the following factors: autoimmune diseases, behavioral changes, cognitive symptoms, speech problems, autonomic symptoms, and MRI hyperintensities of the mesial temporal lobe (Table 1). The internally validated C statistic was 0.95. To correct for optimism, penalized modeling was performed showing similar odds ratios for all factors, all remaining significant and independent risk factors (data not shown).

The external validation cohort consisted of 128 temporal lobe epilepsy patients of whom 7 had neuronal antibodies (5.5%; compared to 3.4% in the Dutch cohort; p=0.30). Data of the Dutch and Czech cohort are compared in Supplementary Table 2 and results of the Czech antibody positive patients are shown in Supplementary Table 3. The C statistic of our model for the external validation cohort was 0.92, showing that the (overall) discriminative performance is similar in the validation cohort.

The six independent risk factors were used to create the ACES score, each factor assigning one point. Evaluating different cut-off values for the ACES score (Figure 3) in the original cohort, 19.4% (95%-CI 11.4-29.6) of the patients with a score ≥ 2 had antibodies (PPV), while none of the patients with less than two risk factors had antibodies, resulting in a NPV of 100% (95%-CI 81.4-100). Sensitivity of an ACES score of ≥ 2 was 100% (95%-CI 81.4-100) and specificity 84.9% (95%-CI 67.9-100; Table 2). No patient with an ACES score ≥ 2, but without AES (n=83) was identified by commercial tests, outside the planned screening algorithm (Fig 3B). In addition, we reviewed data of 55 Dutch anti-NMDAR, 43 anti-LGI1, 19 anti-GAD65 and 14 anti-Caspr2 patients with seizures. All of them, except one patient with anti-LGI1 (ACES score 1), had an ACES score of 2 or more. Using the same cut-off of the ACES score, 37 patients in the Czech validation cohort had an ACES score ≥ 2 (29%). All patients with AES were identified (Figure 3C). [20]

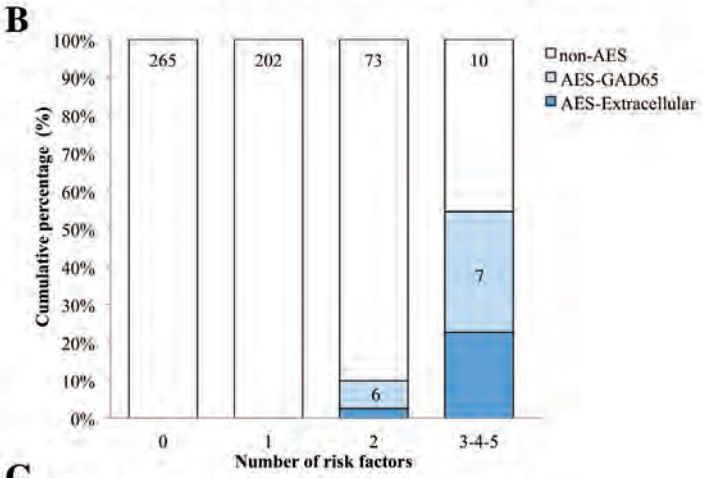
Results from the sensitivity analysis (allocating the 12 patients with probable or possible AES [3 and 9 patients, respectively] to the non-AES group) showed comparable odds ratio's (a sensitivity of 100% and specificity of 84.7%)).

Comparing the ACES score (of both cohorts) to the prior published APE/APE2 score,[8, 21] the APE/APE2 score detected 8/10 patients with antibodies against extracellular antibodies, and 7/17 patients with anti-GAD65. All scores performed well in patients with a short history of epilepsy, but the ACES score scored better in patients with chronic epilepsy (p=0.0015, Figure 4).

A

ACES SCORE

	Point
Cognitive symptoms	1
Behavioral changes	1
Autonomic symptoms	1
Speech problems	1
Autoimmune diseases	1
Temporal MRI hyperintensities	1



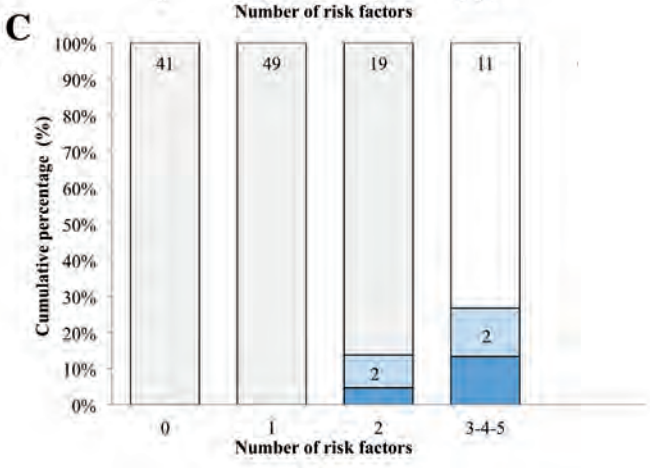


Figure 3. AES patients as distributed by the ACES score.
The number of AES patients by ACES score, provided for both the original, Dutch cohort (A) and the Czech validation cohort (B).
Only the data of the patients with antibodies targeting extracellular antigens, anti-GAD65, and no evidence for autoimmunity are shown. The numbers in the bar diagrams correspond with the numbers of patients of each specific group. All patients with an ACES score of ≥2 without AES were tested by commercial CBA and ELISA post-hoc, and were all negative.
Abbreviations: AES= autoimmune etiology of seizures, GAD65= Glutamatic Acid Decarboxylase 65.

Table 1. Results from univariable and multivariable analysis

	Univariable analysis			Multivariable analysis		
	Odds ratio (95%-CI)[a]		p-value[a]	Adjusted odds ratio (95%-CI)[a]		p-value[a]
Drug-resistant epilepsy	7·1	(2·1-24·5)	0·002	-		-
Autoimmune diseases	8·2	(3·2-20·7)	<0·0001	13·3	(3.1-56·6)	0·0005
Behavioral changes	11·0	(4·1-29·3)	<0·0001	12·6	(3·2-49·9)	0·0003
Cognitive symptoms	31·8	(4·4-229·9)	0·001	30·6	(2·4-382·7)	0·009
Muscle stiffness	7·3	(1·9-28·7)	0·004	-		-
Speech problems	12·8	(4·1-40·0)	<0·0001	9·6	(2·0-46·7)	0·005
Autonomic symptoms	22·1	(6·4-75·5)	<0·0001	23·3	(3·8-143·3)	0·001
MRI hyperintensities temporal	16·9	(4·6-62·1)	<0·0001	255·3	(19·6-3332·2)	<0·0001

[a] Numbers shown are from the cohort data after imputation.
The table only shows the data of patients with AES (n=20; antibodies against extracellular antigens (LGI1, CASPR2, and NMDAR, and antibodies against GAD65) and with no evidence for autoimmunity (n=550).
Abbreviations: mRS= Modified Rankin Scale, MRI= Magnetic Resonance Imaging.

Table 2. Testing properties of the ACES score using different cut-off values.

Cut-off[a]	N (=570)	PPV (95%-CI)	NPV (95%-CI)	Sensitivity (95%-CI)	Specificity (95%-CI)
≥ 1 point	305 (53%)	6·5 (2·8-13·1)	100 (81·4-100)	100 (81·4-100)	48·2 (36·5-63·6)
≥ 2 point	103 (18%)	19·4 (12·2-29·7)	100 (81·4-100)	100 (81·4-100)	84·9 (67·9-100)
≥ 3 point	22 (4%)	54·5 (41·4-70·5)	98·5 (80·5-100)	60 (45·8-77·2)	98·2 (80·5-100)
≥ 4 point	1 (0·2%)	100 (81·4-100)	96·7 (78·7-100)	5 (1·6-11·7)	100 (81·4-100)

[a] The six factors include: autoimmune diseases, behavioral changes, cognitive symptoms, speech problems, autonomic symptoms, MRI hyperintensities temporal.
Abbreviations: PPV= positive predictive value, NPV= negative predictive value, CI= confidence interval.

Clinical characteristics of the Dutch cohort

Looking very carefully, additional subtle signs or symptoms of encephalitis/encephalopathy were present at inclusion in 19/20 patients with AES. All patients with anti-LGI1 had subtle characteristic signs, including cognitive symptoms, insomnia, hyponatremia and behavioral changes, and two of three patients had faciobrachial dystonic seizures (FBDS).[22] Two of three patients with anti-Caspr2 had other core signs or symptoms,[23] albeit mild, including cognitive symptoms, peripheral nerve hyperexcitability, insomnia, autonomic symptoms, and cerebellar symptoms, and one patient developed neuropathic pain after inclusion. In both the anti-LGI1 and anti-Caspr2 patients some signs were identified in retrospect (insomnia, FBDS, peripheral nerve hyperexcitability, cerebellar symptoms), while others were observed, but remained unrecognized as AES-related (because of their subtleness, and concomitant start of AEDs). During follow-up, one patient with focal epilepsy with sporadically occurring tonic-clonic seizures and recently diagnosed esophagus carcinoma developed hallucinations, refractory epilepsy and psychosis. Anti-NMDAR was detected in his CSF 11 months after inclusion, while his serum was negative at baseline, but also at 11 months after inclusion.

All 13 women with anti-GAD65 had a serum concentration above 10.000 IU/ml (median serum concentration 466,000 IU/ml, median CSF concentration 4,600 IU/ml). Five anti-GAD65 patients had diabetes mellitus type 1 (38%). All patients had focal onset seizures,

most frequently with sensory onset (déjà vu episodes; 9/13). Interestingly, in four patients temporal seizures were clearly provoked by music. Oligoclonal bands unique to the CSF were tested in eight patients, of whom five tested positive.

In the "probable autoimmune" group, one patient developed frequent seizures. Shortly after, he suffered from abdominal symptoms and weight loss, which turned out to be Crohn's disease. He then continued to deteriorate with progressive cognitive symptoms. He was treated with steroids and adalimumab, leading to (slow) cognitive improvement and cessation of seizures. He met the "seronegative AIE" criteria.[14] The two patients with anti-GlyR were a middle-aged male with drug-resistant epilepsy, and a young woman with sporadic seizures without other signs.

In the "possible autoimmune" group, all three women with a similar "LGI1-like" pattern on IHC had daily drug-resistant focal onset seizures. In two of these three women AEDs were withdrawn because of ineffectiveness. The patients with neuropil staining on IHC had variable clinical phenotypes. All clinical characteristics of the patients with definite, probable and possible AES are shown in the Supplementary Table 4 and 5.

In 550 patients there was no evidence for autoimmunity at last follow-up. Of these 550 patients, 96% were diagnosed as focal epilepsy with unknown etiology, while in the other patients ancillary testing led to another diagnosis, including a symptomatic lesion on revised or new MRI (n=13), generalized epilepsy (n=3), dementia and seizures (n=2), or no epilepsy (n=2).

Immunotherapy responses

All six patients with anti-LGI1 and anti-Caspr2 encephalitis were treated with immunotherapy (Figure 5A-B), and became seizure free (one anti-LGI1 encephalitis patient became seizure free at 13 months, just outside the study period), as well as the anti-NMDAR encephalitis patient. Ten of the 13 anti-GAD65 patients were treated with immunotherapy; five of them had a remarkable decrease of their seizure frequency (Figure 5C, 50-95 %), deviating from their seizure frequency the year before, while in the other five patients no seizure frequency reduction was observed (Figure 5D). Three patients refused immunotherapy (reasons: less severe disease [n=2] or fear of side-effects [n=1]).

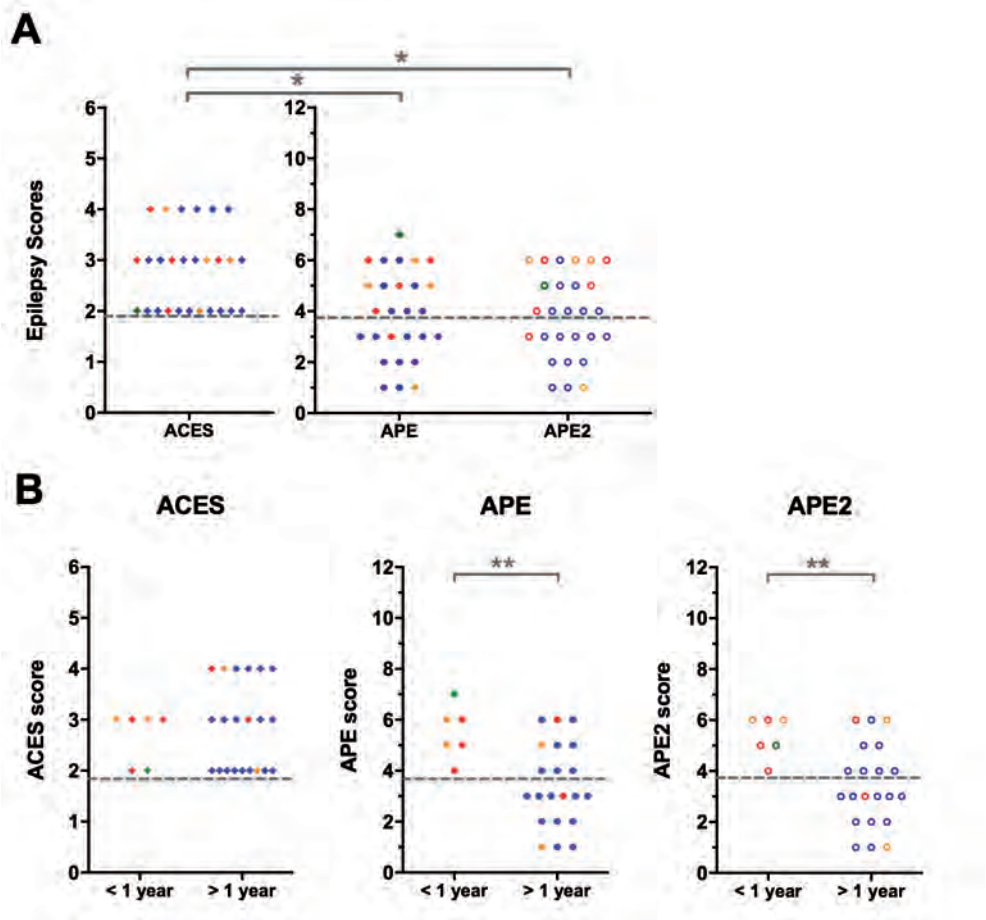


Figure 4. Comparison of the ACES score with the APE and APE2 score.

Visualization of the epilepsy scores per patient, combining the Dutch and Czech cohorts, show that all patients are identified by the ACES score, while a considerable part is not identified by the APE or APE2 score (A). Dissection in duration of epilepsy shows that the difference in performance in caused by the patients with epilepsy for over a year (B). * $p < 0.05$; $p < 0.01$. The colors refer to the antibodies identified: red CASPR2; orange LGI1; green NMDAR; and blue GAD65.

Abbreviations: ACES= antibodies contributing to epilepsy signs and symptoms score; APE= Antibody Prevalence in Epilepsy and Encephalopathy score; CASPR2= contactin-associated protein 2; LGI1= leucin-rich glioma-inactivated protein 1; NMDAR= N-methyl-D-aspartate receptor; GAD65= glutamic acid decarboxylase 65.

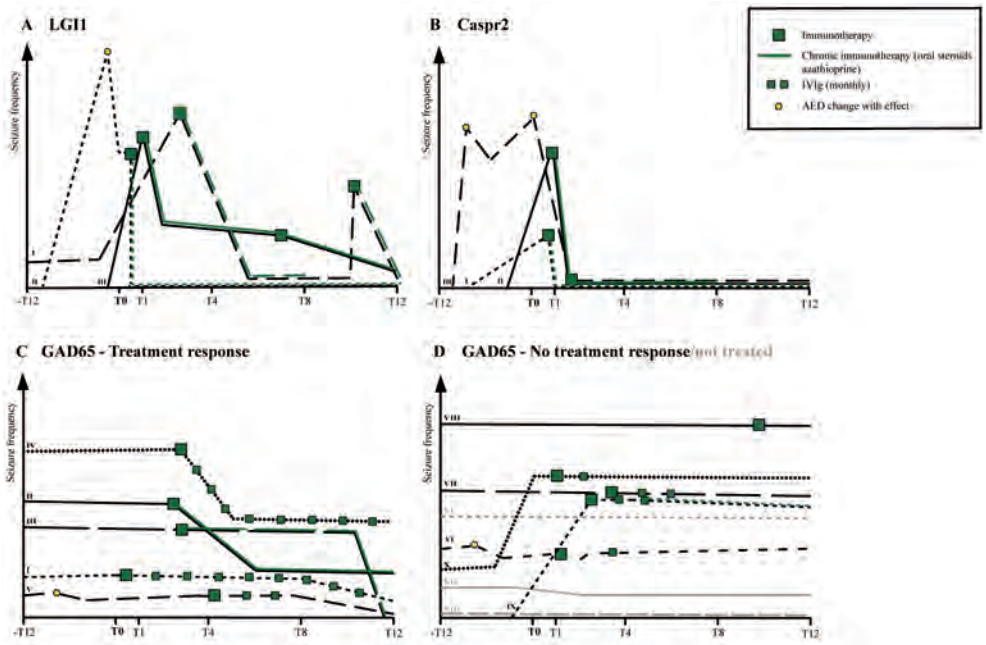


Figure 5. Individual treatment responses of patients with anti-LGI1, anti-Caspr2 and anti-GAD65.
The figures visualize the seizure frequency over time per antibody: (A) LGI1, (B) CASPR2, and GAD65 (C) with treatment response, and (D) without treatment response (both black), or untreated (grey). The Roman numerals correspond to the numbers shown in the Supplementary Table 4. All patients were treated with AEDs. The figures only show AED changes that led to a decrease in seizure frequency.
Abbreviations: AED = anti-epileptic drugs; -T12: twelve months before inclusion, T0= inclusion date, T1= 1 month after inclusion, T4= 4 months after inclusion, T8= 8 months after inclusion, T12= 12 months after inclusion.

DISCUSSION

In this prospective, multicenter cohort study we show that a small, but relevant, proportion of patients with focal epilepsy of presumed unknown etiology have neuronal antibodies. All patients with AES had unrecognized signs of encephalitis. Identifying these patients is crucial, because seizures respond better to immunotherapy than to AEDs, reflected by seizure-freedom in all patients with antibodies against extracellular proteins after immunotherapy. For recognition of AES patients, we have provided, and validated, a simple clinical score, that helps physicians in selecting patients that should be screened for neuronal antibodies. In the current study, neuronal antibodies were found in 3.4% of patients, while other studies describe higher numbers over 10%.[7, 8] The most important issue explaining this discrepancy is the difference in patient selection. The patients included in our study were referred to epilepsy centers by neurologists who had no suspicion of AIE. In retrospect, most of the patients with AES had subtle signs or symptoms of AIE, but these signs were unrecognized as being related to AIE. For example, insomnia, muscle stiffness, or mild cognitive problems were often considered as side-effects of AEDs. Besides, patients were only included in the AES group when substantial evidence for autoimmunity for the detected antibodies was available. In a smaller prospective cohort of patients with temporal lobe epilepsy, the same antibodies were identified as in the ACES study,[20] while others found a greater variability of antibodies.[7, 8] Some of the described antibodies are pathogenic, like anti-NMDAR, and anti-$GABA_BR$, but occurred in highly selected patients with clear encephalitis. However, experiments to confirm antibody results were lacking in some studies, while it is an essential necessity when screening large cohorts, to avoid false positive results. Prior studies also describe antibodies with a debatable role in neuro-inflammation, including low-concentration anti-GAD65, and double negative anti-VGKC, nowadays considered clinically irrelevant by most.[10, 11]

The Czech study[20] was used to externally validate the results. This study was chosen as it was of a considerable size, investigated chronic epilepsy as well (median duration even longer) and did not include patients referred for overt encephalitis. Different to our study, all patients had temporal epilepsy and had MTS more often. Aside, the epidemiological and clinical characteristics were largely similar. Reassuringly, the antibody frequency and type of antibodies (anti-LGI1, anti-Caspr2, anti-GAD65) were within the same range.

We found anti-LGI1, anti-Caspr2, and anti-GAD65. This is a comprehensible observation, because the clinical phenotypes of patients with these syndromes can be more prolonged, less aggressive, and diagnosis is more likely to be delayed or missed than in patients with clear encephalitis[1, 12, 22] Although clinical phenotypes related to these antibodies can be less serious, immunotherapy is superior to AEDs,[5, 17] which makes diagnosis in an early phase important. Thirteen women had high anti-GAD65 concentrations. Earlier performed laboratory studies were unable to reveal the pathogenicity of anti-GAD65.[23] However, patients with high-concentrations have overlapping and well-defined neurological syndromes, and

comparable seizure characteristics, not observed in low-concentration patients.[17] Almost 75% of the high-concentration anti-GAD65 women had déjà vu episodes. In addition, a quarter had musicogenic epilepsy, meaning temporal seizures clearly triggered by specific music or songs, while the prevalence of musicogenic epilepsy in other non-AES patients is very low. Musicogenic epilepsy has been described before in anti-GAD65 encephalopathy.[24] Concerning immunotherapy responses, in contrast to the patients with anti-LGI1 or anti-Caspr2, no patient became seizure free. However, in half of the treated patients a long-term epilepsy frequency reduction, up to 95% was observed, while these patients had been refractory to AEDs for a long time. This makes regression to the mean or natural history as explanation for the epilepsy frequency reduction highly unlikely. In support, seizure response was accompanied by a serological response.

The finding that only anti-LGI1, anti-Caspr2 and anti-GAD65 occurred suggests that screening for these antibodies only is generally sufficient in patients with focal epilepsy of unknown etiology without clear encephalitis. If anti-LGI1 is detected in serum and the phenotype fits, diagnosis can be made and treatment can be started.[12] If serum is positive for anti-Caspr2, CSF should be tested as well.[22] If the anti-GAD65 ELISA concentration is above 10.000 IU/mL, this can be considered clinically relevant.[17] This is roughly comparable to a RIA titer of 20 nmol/L[8] or 2000 U/mL.[24] In case of deterioration of symptoms or suggestive signs or symptoms antibody testing should be expanded, taking into account the clinical phenotype.

We identified six independent risk factors, pointing towards AES. The assessed risk factors were pre-defined before the study started. The ACES score was created assigning all factor one point instead of creating a value-weighted score. The easier the score, the more likely it is used in clinical settings. In addition, as our score is meant to identify those that need antibody testing, our primary aim was a sensitive score. Although a weighted score would provide better model performance, it would not increase the sensitivity (already optimal if at least two factors of the ACES score were present). Lastly, some of the odds ratios showed large confidence intervals due to the low frequency in the control group of the original cohort. By not assigning a weighted value we lower the risk for overfitting. Using a cut-off value of two for the ACES score, all antibody positive patients were identified, but antibody testing was unrevealing in 14%. It should therefore not be used to diagnose AES, but to guide selection of patients for antibody screening.

The factors included in the ACES score partially overlap with those used in the APE/APE2 score.[8,21] Nevertheless, there are important differences, which makes sense as, judging by the published papers of Dubey et al., the scores serve different populations. Our study included patients with focal epilepsy, all without overt encephalitis. In addition, our ACES score was validated in another chronic epilepsy cohort, and showed very good discrimination. In this study we confirm that the APE/APE2 score is useful to detect patients in a (sub) acute setting with antibodies against extracellular neuronal proteins. However, almost 70%

of chronic patients would have been missed using this score, underlining the importance of a score which is applicable to patients with variable disease courses.

Some patients were classified as "probable or possible autoimmune". Data of these patients were not used in the main analysis, but inclusion would not have changed the results. Two patients categorized as "probable autoimmune" had anti-GlyR. Clinical characteristics of these two patients showed no notable similarities. Testing serum of these patients consistently showed a positive result on live CBA, but we were unable to analyze CSF or to confirm our findings with additional techniques. Except for these two epilepsy samples, we have not identified any positive sample in 1206 patients outside published clinical phenotypes.[19, 25] Although anti-GlyR can be directly pathogenic,[18] we prefer to be careful in describing unconfirmed findings. The "possible autoimmune" category contained three patients with similar clinical characteristics and drug-resistant epilepsy. Both serum and CSF showed an unknown pattern on IHC. We are currently analyzing these samples using immunoprecipitation and mass spectrometry. In six other patients, IHC showed a diffuse neuropil staining However, live hippocampal neuron staining was negative, CSF was not available for confirmation, and patients had different clinical phenotypes, all questioning the clinical relevance.

Our study has limitations. Due to the small number of antibody-positive patients, there is risk of overfitting. Therefore, we also assessed a penalized model. In addition, external validation in a second, independent, foreign cohort showed comparable test characteristics. Concerning data extraction, this was performed thoroughly; but it is possible that recall bias occurred, especially in the variables that are not discussed in the standard intake. An example is "family history of autoimmune diseases". We therefore decided to exclude this variable from the analysis. Another limitation was the use of only serum in most patients. Although highly sensitive to test for anti-LGI1,[12] anti-Caspr2,[22] anti-GAD65, and anti-$GABA_BR$,[26] it is somewhat less sensitive to screen for anti-NMDAR[27] or anti-AMPAR.[28] Testing only serum poses the risk of missing a small proportion of patients. However, both anti-NMDAR and anti-AMPAR encephalitis tend to present more fulminantly,[2] and the syndromes would probably have revealed themselves during follow-up, as exemplified by the only patient, who was antibody negative (in serum), at inclusion, but who developed a panencephalitis eleven months after inclusion with anti-NMDAR in CSF. In retrospect, it is unclear, because of the lack of CSF at inclusion, if this patient had anti-NMDAR causing his seizures before deterioration, or if he coincidentally developed anti-NMDAR encephalitis later on. The presence of esophageal carcinoma in this case underlines the importance of screening for tumors in these patients.[29] Samples were screened with immunohistochemistry, and questionable and positive samples were tested more thoroughly (CBAs, ELISA, live hippocampal neurons). This approach was chosen because of the high sensitivity of IHC.[12, 17, 22, 26-28, 30, 31] Although we cannot guarantee that we have identified all patients, previous publications support our claim, that this approach seems most reliable and sensitive to test

large cohorts of patients with low prior chances, as also enforced by testing 186 samples negative by IHC with additional CBA and ELISA without revealing additional antibodies.
To conclude, patients with AES present differently and have specific, though not always discriminative characteristics. Recognition of these patients can be difficult, but is important for treatment decisions. An ACES score ≥ 2 can be used to identify patients with higher risk of having AES, and to select those that require antibody testing.

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SUPPLEMENTARY DATA

Table 1. Comparisons between patients with autoimmune etiology of seizures and with no evidence for autoimmunity

	AES (n=20)				Non-AES (n=550)		p-value[a]
	Extracellular antigens[b] (n=7)		GAD65 (n=13)				
Sex (male)	6	(86%)	0		270	(49%)	0.0003***
Ethnicity							0.97
• Caucasian	7	(100%)	12	(92%)	513	(93%)	
• Arabic	0		0		17	(3%)	
• African	0		1	(8%)	10	(2%)	
• Asian	0		0		6	(1%)	
• Hindustan	0		0		2	(0.5%)	
• South American	0		0		2	(0.5%)	
Age at onset, median in years (IQR; range)	62	(53-64; 38-66)	26	(20-31; 11-41)	27	(16-44; 0-88)	0.002**
Epilepsy duration, median in years (IQR; range)	0.6	(0.1-0.9; 0.1-10)	12	(6-17; 1.3-33)	8	(2-19; 0.1-75)	0.001**
≤ 1 year	6	(86%)	0		60	(11%)	
>1 year	1	(14%)	13	(100%)	490	(89%)	
Seizure frequency last month, median (IQR; range)	60	(0-1000; 0-2400)	5	(0-70; 0-400)	1	(0-4; 0-600)	0.015*
mRS at inclusion, median (IQR; range)	2	(2-3; 1-3)	2	(1-2; 1-4)	1	(0-2; 0-4)	<0.0001***
0	0		0		179/547	(33%)	
1	1	(14%)	4	(31%)	180/547	(33%)	
2	3	(43%)	8	(62%)	151/547	(28%)	
3	3	(43%)	0		35/547	(6%)	
4	0		1	(8%)	2/547	(0.4%)	
Focal onset seizures	7	(100%)	13	(100%)	449	(82%)	0.11
With normal awareness	2	(29%)	5	(38%)	131/436	(30%)	0.81
With impaired awareness	6	(86%)	11	(85%)	349/436	(80%)	0.86
With motor onset	2	(29%)	2	(15%)	98/437	(22%)	0.77
With non-motor onset	6	(86%)	13	(100%)	368/436	(84%)	0.30
Autonomic	3	(43%)	0		63/436	(14%)	0.034*
Behavior arrest	1	(14%)	2	(15%)	98/436	(22%)	0.73
Cognitive	3	(43%)	3	(23%)	96/436	(22%)	0.42
Emotional	0		0		10/436	(2%)	0.78
Sensory	1	(14%)	9	(69%)	135/436	(31%)	0.009*
Focal onset to bilateral TCS	3	(43%)	9	(69%)	416	(76%)	0.12
Drug-resistant epilepsy	6	(86%)	11	(85%)	244	(44%)	0.002**
Autoimmune diseases	1	(14%)	8	(62%)	50/549	(9%)	<0.0001***
Thyroid disease	0		2	(25%)	21	(41%)	
Rheumatoid arthritis	0		0		9	(18%)	
Diabetes mellitus type 1	0		5	(63%)	4	(8%)	
Psoriasis	1	(100%)	0		4	(8%)	
Crohn/Colitis	0		0		3	(6%)	
Other	0		1	(13%)	9	(18%)	
Family history of epilepsy	2	(29%)	2	(15%)	115/481	(24%)	0.74
Family history of AID	1	(40%)	7	(54%)	20/481	(4%)	<0.0001***
Co-morbidities							
Psychiatric disorders	0		3	(23%)	103	(19%)	0.41
Malignancy	1	(14%)	0		18	(3%)	0.22

	AES (n=20)				Non-AES (n=550)		p-value[a]
	Extracellular antigens[b] (n=7)		GAD65 (n=13)				
Other complaints							
Behavioral changes	6	(86%)	8	(62%)	95/543	(17%)	<0.0001***
Cognitive symptoms	6	(86%)	13	(100%)	203/545	(37%)	<0.0001***
Movement disorders	1	(14%)	0		5/545	(1%)	0.003**
Muscle stiffness	1	(14%)	2	(15%)	12/545	(2%)	0.002**
Sleep disorders	3	(43%)	2	(15%)	51/541	(9%)	0.011*
Speech problems	1	(14%)	4	(31%)	14/544	(3%)	<0.0001***
Autonomic symptoms	4	(57%)	1	(8%)	8/542	(2%)	<0.0001***
Ancillary testing							
MRI hyperintensities temporal	2	(29%)	2	(15%)	8	(2%)	<0.0001***
Mesial temporal sclerosis	0		0		35	(6%)	0.51
EEG temporal epilepsy	2/2	(100%)	5/12	(42%)	101/337	(30%)	0.072
EEG frontotemporal epilepsy	0/2		2/12	(17%)	103/337	(31%)	0.38
EEG extra-temporal epilepsy	0/2		0/12		57/337	(17%)	0.24
EEG multifocal epilepsy	0/2		5/12	(42%)	75/340	(22%)	0.21

*p-value < 0.05, ** p-value <0 .005, ***p-value < 0.0005.

[a] Only p-values below 0.002 were considered relevant.

[b] LGI1, CASPR2, and NMDAR.

The table only shows the data of patients with AES (antibodies against extracellular antigens (LGI1, CASPR2, and NMDAR, and antibodies against GAD65) and with no evidence for autoimmunity. Data of patients with probable or possible AES are shown in the supplemental data.
Abbreviations: AES= autoimmune etiology of seizures, TCS= tonic clonic seizures, IQR= interquartile range, mRS= modified Rankin Scale, AID= autoimmune disease, MRI= magnetic resonance imaging, EEG= electroencephalography.

Table 2. Characteristics of the original cohort versus the Czech cohort (validation cohort)

	Dutch AES (n=20)	Dutch Non-AES (n=550)	Czech AES (n=7)	Czech Non-AES (n=121)
Gender (M)	6 (30%)	270 (49%)	3 (43%)	55 (45%)
Age at onset, m in years (IQR; range)	31 (23-53; 11-66)	27 (16-44; 0-88)	44 (26-64; 19-68)	25 (12-37; 0-80)
Epilepsy duration, m in years (IQR; range)	8 (0-16; 0-33)	8 (2-19; 0.1-75)	4 (2-24; 1-39)	14 (6-22; 0-65)
Seizure frequency last month, m (IQR; range)#	13 (0-88; 0-2400)	5 (0-70; 0-400)	1 (1-5;0-10)	2 (0-5; 0-30)
Focal onset seizures	20 (100%)	449 (82%)	6 (86%)	115 (95%)
Focal onset to bilateral tonic-clonic seizures	12 (60%)	416 (76%)	6 (86%)	90 (74%)
Drug-resistant epilepsy	17 (85%)	244 (44%)	5 (71%)	71/120 (59%)
Autoimmune diseases	9 (45%)	50/549 (9%)	3 (43%)	11 (9%)
Co-morbidities				
Psychiatric disorders	3 (15%)	103 (19%)	2 (29%)	35 (29%)
Malignancy	1 (5%)	18 (3%)	1 (14%)	9 (7%)
Other complaints				
Behavioral changes	14 (70%)	95/543 (17%)	3 (43%)	41/120 (34%)
Cognitive symptoms	19 (95%)	203/545 (37%)	7 (100%)	35/120 (29%)
Movement disorders	1 (5%)	5/545 (1%)	0	9/120 (8%)
Muscle stiffness	3 (15%)	12/545 (2%)	1 (14%)	0
Sleep disorders	5 (25%)	51/541 (9%)	0	10/120 (8%)
Speech problems	5 (25%)	14/544 (3%)	1 (14%)	1/120 (1%)
Autonomic symptoms	5 (25%)	8/542 (2%)	1 (14%)	6/120 (5%)
Ancillary testing				
MRI hyperintensity temporal	4 (20%)	8 (2%)	5 (71%)	30 (25%)
Mesial temporal sclerosis				
EEG temporal ep	0	35 (6%)	3 (43%)	44 (36%)
EEG frontotemporal ep				
EEG extra-temporal ep	7/14 (50%)	101/337 (30%)	4 (57%)	53/120 (44%)
EEG multifocal ep	2/14 (14%)	103/337 (31%)	1 (14%)	40/120 (33%)
	0	57/337 (17%)	0	1/120 (1%)
	5/14 (36%)	75/340 (22%)	2 (29%)	23/120 (19%)

Abbreviations: IQR= interquartile range, m=median, mRS= modified Rankin Scale, AID= autoimmune diseases, MRI= magnetic resonance imaging, EEG= electroencephalography, AES= autoimmune etiology of seizures, ep=epilepsy.

the seizure frequency was assessed retrospectively in the Czech cohort.

Table 3. **Characteristics of patients with an autoimmune etiology of seizures (validation cohort, n=7)**

Antigen	Sex, age inclusion	Duration epilepsy (y)	Description epilepsy	MRI	EEG	CSF	Treatments and responses	Antibody screening	Antibody testing	Other symptoms/ characteristics
LGI1 -1	F, 66	2	Sporadic FIAS, FBTCS, seizure free after immunotherapy without AED	Normal	EEG - interictal, slow activity FT bilat	Normal	LTG: effective, seizure free, but immediately followed by MP 3g + oral steroids: seizure free IVIg - substitution for Ig deficiency	IHC: LGI1 pattern	LGI1 +, CASPR2-	Mild cognitive impairment (normalized after immunotherapy), orthostatic hypotension IgA, IgG deficiency
CASPR2-1	M, 68	4	Sporadic FIAS, FBTCS, seizure free after immunotherapy, ongoing AED	Hyperintense lateral temporal bilat	EEG - interictal FT bilat	Normal	VPA:effective, not seizure free LEV: effective, seizure free MP + oral steroids: seizure free	IHC: neuropil	CASPR2 +, LGI1 -	Cognitive disorder, behavioral changes
CASPR2 -2	M, 58	39	Pharmacoresistant epilepsy even after surgery	HS bilateral + hyperintense lateral T bilat	EEG - ictal, T left	Normal	CBZ, LTG, PHE, TPM, GBP, VPA - pharmacoresistant, N/A for adverse events Stereotactic electrocoagulation amygdalohippocampal left - partial effect MP + oral steroids: not effective	IHC: neuropil	CASPR2 +, LGI1 -	Severe psychiatric comorbidity, dysphasia - acute onset 20y ago - possible LE history
GAD65-1	F, 29	1	Pharmacoresistant epilepsy, FIAS, FBTCS	hyperintense medial T left	EEG - ictal, T left	Normal	LEV: effective, not seizure free, LTG: blurry vision, CBZ, CLB, PGB:: effective, not seizure free IVIg - no response	IHC: GAD pattern	GAD65 + Titer: 701,000	Cognitive disorder, autoimmune thyroiditis
GAD65-2	F, 73	5	Pharmacoresistant epilepsy, FIAS (acute onset in 65y)	Hyperintense mesial + lateral temporal left	EEG - ictal, T left	OCB+, otherwise normal	LEV: effective, not seizure free PGB:effective, not seizure free MP + oral steroids: no response	IHC: GAD pattern	GAD65 + Titer: 1,160,000	Late onset diabetes mellitus after corticosteroids, AI thyroiditis, cognitive disorder, behavioral disorder, speech problems, cerebellar symptoms

Antigen	Sex, age inclusion	Duration epilepsy (y)	Description epilepsy	MRI	EEG	CSF	Treatments and responses	Antibody screening	Antibody testing	Other symptoms/ characteristics
GAD65-3	F, 28	2	Pharmacoresistant epilepsy, FIAS (acute onset 26)	Normal	EEG - interictal slow activity FT left	OCB+, otherwise normal	LTG: allergic reaction LEV: effective, not seizure free MP: responder	IHC: GAD pattern	GAD65 + Titer: 618,000	AI thyroiditis, DM1, cognitive disorder
GAD65-4	M, 44	24	Pharmacoresistant epilepsy, FIAS, FBTCS	Hippocampal sclerosis bilat	EEG - interictal slow FT left to bilat	N/A	CBZ, TGB, LEV: effective, not seizure free	IHC:GAD pattern	GAD65 + Titer: 130,000	Cognitive disorder

Abbreviations: Np= not performed, WBC= white blood cells, OCB= oligoclonal bands, VPA= valproic acid, LEV= levetiracetam, CBZ= carbamazepine, CLB= clobazam, LCM= lacosamide, LTG= lamotrigine, PHT= phenytoin, TPM= topiramate, ESM=ethosuximide, ZNS= zonisamide, IVIg= intravenous immunoglobulins, MP= methylprednisolone, AZA= azathioprine, PLEX= plasmapheresis, IHC= immunohistochemistry, CBA= cell-based assay.

Table 4. **Characteristics of patients with an autoimmune etiology of seizures (original cohort, n=20)**

Antigen	Sex, age inclusion	Duration epilepsy	Description epilepsy	MRI	EEG	CSF	Treatments and responses	Antibody screening	Antibody testing	Other symptoms/ characteristics
LGI1 I	F, 63	10 years	Only sporadically tonic-clonic seizures, 1 month before inclusion increasing frequency with focal seizures with impaired awareness and autonomic onset (2/day) and frequent faciobrachial dystonic seizures	Normal	Normal	Np	VPA: liver dysfunction LEV: behavioral disorder LCM: psychotic behavior CBZ: nightmares CLB, LTG: effective, not seizure-free MP + oral steroids: seizure-free	IHC: LGI1 pattern (serum) GlyR –	VGKC 431 Caspr2 -, LGI1 + (serum)	Cognitive disorder, obsessive behavior, insomnia, hyperhidrosis At end of follow-up relapse while tapering oral steroids
LGI1 II	M, 54	10 months	Focal seizures with impaired awareness and motor onset (80/day)	Normal	Normal	WBC 1, Protein 0.43, Glucose 3.2	LEV: ineffective CBZ: effective, not seizure-free Prednisone + AZA: seizure-free	IHC: LGI1 pattern (serum and CSF) GlyR –	VGKC 1035 Caspr2-, LGI1 + in serum, in CSF negative	Cognitive disorder, behavioral disorder, erectile dysfunction, during follow-up hyponatremia.
LGI1 III	M, 64	1 month	Focal seizures with impaired awareness and autonomic onset (4/day), and faciobrachial dystonic seizures.	Hyperintense mesiotemporal, bilateral	Epileptic temporal, unilateral (left)	WBC 0, Protein 0.5, Glucose 4.0	LEV: ineffective PLEX + prednisone: seizure-free at end of follow-up	IHC: LGI1 pattern (serum) GlyR –	VGKC 460 Caspr2 -, LGI1 + (serum)	Cognitive disorder, behavioral disorder
Caspr2 I	M, 54	8 months	tonic-clonic seizures, and focal seizures with normal awareness and cognitive onset (1/day)	Normal	Focal epileptic temporal, bilateral	WBC 6 Protein 0.41, Glucose 3.5, IgG index 0.6, OCB -	VPA: ineffective IVIg + MP + oral steroids: seizure-free	IHC: neuropil (serum and CSF) GlyR –	VKGC 417 LGI1-, Caspr2 + (serum and CSF)	Subtle Morvan syndrome (cognitive symptoms, insomnia, neuropathic, muscle cramps, orthostatic hypotension), and cerebellar symptoms

Antigen	Sex, age inclusion	Duration epilepsy	Description epilepsy	MRI	EEG	CSF	Treatments and responses	Antibody screening	Antibody testing	Other symptoms/ characteristics
Caspr2 II	M, 65	10 months	Focal seizures with impaired awareness and autonomic onset (2/day)	Normal	Epileptic centroparietotemporal, unilateral (left)	WBC 4 Protein 0.39, Glucose 3.8, IgG index 0.51, OCB -	LEV: ineffective and behavioral disorder VPA, CLB: ineffective MP + IVIg + oral steroids: seizure-free within a day	IHC: neuropil (serum and CSF) GlyR –	VGKC 650 LGI1-, Caspr2 + (serum and CSF)	Mild memory disorder, insomnia, cerebellar symptoms, during follow-up orthostatic hypotension
Caspr2 III	M, 67	7 months	Focal seizures with impaired awareness and cognitive onset (12/day)	Hyperintense mesiotemporal, bilateral	Epileptic temporal, unilateral (left)	WBC 3, Protein 0.80, Glucose 3.7, IgG index 0.49	LEV: effective, but behavioral disorder CBZ: seizure-free MP: started after seizure-freedom	IHC: neuropil (serum and CSF) GlyR –	VGKC 601 LGI1-, Caspr2 + (serum and CSF)	Neuropathic pain during follow-up
NMDAR	M,63	1 month	Tonic-clonic seizures (2x), seizure-free in the 11th FU month tonic-clonic seizures, and focal seizures with impaired awareness and sensory onset (4/month)	Normal	Normal	WBC 6, Protein 0.53, Glucose 3.9	CBZ: effective until deterioration. VPA: effective during deterioration, seizure-free. Steroids and IvIg: started after seizure-freedom	IHC: negative (serum), NMDAR pattern (CSF). GlyR - LN +	Onconeural -, NMDAR- (serum), NMDAR+ (CSF)	2015: metastatic esophagus carcinoma At 11 months FU: hallucinations, anxiety, insomnia psychotic behavior
GAD65 I	F, 50	8 years	Focal seizures with impaired awareness and behavior arrest (3-5/ month)	Hyperintense mesiotemporal, unilateral, and cerebellar atroph.	Epileptic temporal, unilateral (left)	WBC 2, Protein 0.26, Glucose 3.7, IgG index 0.78, OCB +	CBZ, LEV: ineffective Monthly IVIg: > 50% seizure reduction, no seizure-freedom	IHC: GAD pattern (serum and CSF) GlyR–	GAD serum titer: 2,070,00 CBA+ GAD CSF titer: 19040 CBA+	Cognitive problems, word finding difficulties, cerebellar ataxia

Antigen	Sex, age inclusion	Duration epilepsy	Description epilepsy	MRI	EEG	CSF	Treatments and responses	Antibody screening	Antibody testing	Other symptoms/ characteristics
GAD65 II	F, 32	12 years	Focal seizures (60/ month) with impaired awareness and sensory (deja vue), and cognitive onset, sporadically tonic-clonic seizures	Normal	Delta activity temporal, bilateral	WBC 1, Protein 0.41, Glucose 3.0, IgG index 0.61 OCB +	LCM, LTG, PHT, ESM, VPA: ineffective CLB, ZNS: some effect MP + steroids + AZA: > 50% seizure reduction, no seizure-freedom	IHC: GAD pattern (serum and CSF) GlyR –	GAD serum titer: 1,289,000 CBA+ GAD CSF titer: 7,320 CBA+	Cognitive disorder, word finding difficulties
GAD65 III	F ,39	16 years	Focal seizures (4/month) with impaired awareness and sensory onset (deja vue)	Normal	Epileptic frontotemporal, bilateral	WBC 1, Protein 0.40, Glucose 3.2	LEV, TPM: ineffective Oral steroids, AZA: seizure free after 8 months	IHC: GAD pattern (serum and CSF) GlyR–	GAD serum titer: 59,455 CBA+ GAD CSF titer: 1,830 CBA+	Cognitive disorder, anxiety, word finding difficulties
GAD65 IV	F, 38	8 years	Focal seizures (30/ month) with impaired awareness and sensory onset (deja vue), sporadically tonic-clonic seizures	Normal	Epileptic frontotemporal bilateral	WBC 2, Protein 0.21, IgG index 0.56, OCB +	LTG, LEV: ineffective CLB: effective, not seizure-free Monthly IVIg: > 50% seizure reduction, no seizure-freedom	IHC: GAD pattern (serum and CSF) GlyR–	GAD serum titer: 2,720,000 CBA+ GAD CSF titer: 28,960 CBA+	Cognitive disorder, stiff person syndrome,
GAD65 V	F, 29	3 years	Focal seizures (5/month) with normal awareness and sensory onset (deja vue), sporadically tonic-clonic seizures	Normal	Epileptic frontotemporal, unilateral (right)	WBC 1, Protein 0.28, Glucose 3.5, IgG index 0.49, OCB -	LTG: short-term seizure reduction. Monthly IVIg: > 50% seizure reduction, no seizure-freedom	IHC: GAD pattern (serum and CSF) GlyR –	GAD serum titer: 1,900,000 CBA+ GAD CSF titer: 5,410 CBA+	Cognitive disorder, depressive mood

Antigen	Sex, age inclusion	Duration epilepsy	Description epilepsy	MRI	EEG	CSF	Treatments and responses	Antibody screening	Antibody testing	Other symptoms/ characteristics
GAD65 VI	F, 33	5 years	Frequent focal seizures with impaired awareness and sensory onset (deja vue), sporadically tonic-clonic seizures	Normal	Epileptic frontotemporal, bilateral	WBC 18, Protein 0.37, Glucose 4.0	VPA, LTG, CBZ, LEV: ineffective MP + oral steroids: ineffective IVIg: ineffective	IHC: GAD pattern (serum and CSF) GlyR – LN –	GAD serum titer: 21,080 CBA+ GAD CSF titer: 3,120 CBA+	Cognitive disorder, word finding difficulties, borderline personality disorder.
GAD65 VII	F, 36	15 years	Focal seizures (4/month) with normal awareness and sensory (deja vue), and motor onset (automatisms), tonic-clonic seizures	Normal	Epileptic frontotemporal, bilateral	WBC 1, Protein 0.29, Glucose 3.3, IgG index 0.59, OCB +	LTG, CLB: ineffective Monthly IVIg: ineffective	IHC: GAD pattern (serum and CSF) GlyR –	GAD serum titer: 1,286,000 CBA+ GAD CSF titer: 6,820 CBA+	cognitive disorder, anxiety, stiff person syndrome
GAD65 VIII	F, 33	15 months	Focal seizures (400/ month) with impaired awareness and autonomic onset, also tonic-clonic seizures	Hyperintense mesiotemporal and amygdala, unilateral (right)	Epileptic frontotemporal bilateral	WBC <5, Protein 0.28, Glucose 3.7, OCB -	LEV, CBZ, CLB, LMT: ineffective IVIg (once): no improvement	IHC: GAD pattern (serum and CSF) GlyR –	GAD serum titer: 233,000 CBA+ GAD CSF titer: 14,800 CBA+	cognitive disorder, agitation
GAD65 IX	F, 20	8 years	Focal seizures (300/ month) with impaired awareness and sensory (deja vue), and behavioral arrest onset, sporadically tonic-clonic seizures	Hyperintense mesiotemporal, bilateral	Epileptic frontotemporal, bilateral	WBC normal, Protein normal, Glucose normal, IgG index 0.68 OCB +	VPA, CBZ, LTG, CLB, Diacomit: ineffective IVIg: ineffective	IHC: GAD pattern (serum and CSF) GlyR –	GAD serum titer: 469,500 CBA+ GAD CSF titer: 3,020 CBA+	Cognitive disorder

Antigen	Sex, age inclusion	Duration epilepsy	Description epilepsy	MRI	EEG	CSF	Treatments and responses	Antibody screening	Antibody testing	Other symptoms/ characteristics
GAD65 X	F, 44	17 years	Focal seizures (80/ month) with impaired awareness and sensory (deja vue), cognitive, and motor onset (automatisms), sporadically tonic-clonic seizures	Normal	Epileptic temporal, unilateral (right)	WBC 1, Protein 0.47, Glucose 3.0, OCB -	CBZ, PHT, LTG, TPM, LEV, VPA, LCM, CLB: all ineffective IvIg (twice): ineffective	IHC: GAD pattern (serum and CSF) GlyR – LN –	GAD serum titer: 466,000 CBA+ GAD CSF titer: 3,790 CBA+	Cognitive disorder
GAD65 XI	F, 45	33 years	Focal seizures (20/ month) with impaired awareness and autonomic onset (musicogenic), sporadically tonic-clonic seizures	Normal	Epileptic temporal (right)	WBC 1, Protein 0.19, Glucose 5.6 IgG index 0.47, OCB -	CBZ, LTG, LEV, CLB: ineffective No immunotherapy	IHC: GAD pattern (serum and CSF) GlyR –	GAD serum titer: 14,540 CBA+ GAD CSF titer: 3,910 CBA+	Cognitive disorder
GAD65 XII	F, 55	18 years	Focal seizures (seizure-free at inclusion) with impaired awareness and behavior arrest (musicogenic), sporadically tonic-clonic seizures	Hyperintense mesiotemporal, unilateral (right)	Epileptic temporal bilateral	Np	VPA: effective No immunotherapy	IHC: GAD pattern (serum) GlyR –	GAD serum titer: 65,000 CBA+	Cognitive disorder, orthostatic hypotension
GAD65 XIII	F, 39	10 years	Focal seizures with impaired awareness and cognitive onset (1/ month), sporadically tonic-clonic seizures	Normal	Abnormalities frontotemporal bilateral	Np	CLB: effective VPA: effective, but cognitive problems No immunotherapy	IHC: GAD pattern (serum) GlyR –	GAD serum titer: 362,000 CBA+	Cognitive disorder, agression

Abbreviations: Np= not performed, WBC= white blood cells, OCB= oligoclonal bands, VPA= valproic acid, LEV= levetiracetam, CBZ= carbamazepine, CLB= clobazam, LCM= lacosamide, LTG= lamotrigine, PHT= phenytoin, TPM= topiramate, ESM=ethosuximide, ZNS= zonisamide, IVIg= intravenous immunoglobulins, MP= methylprednisolone, AZA= azathioprine, PLEX= plasmapheresis, IHC= immunohistochemistry, CBA= cell-based assay.

Table 5. **Characteristics of patients with probable or possible autoimmune etiology of seizures (n= 12)**

Category	Sex, age inclusion	Duration epilepsy	Description epilepsy	MRI	EEG	CSF	Treatment and responses	Antibody screening	Antibody testing	Other symptoms/ characteristics
Probable autoimmune etiology										
Seronegative	M, 61	8 months	Focal seizures (10/month) with impaired awareness and behavior arrest	Hyperintensee mesiotemporal, unilateral (right)	Abnormalities frontotemporal, bilateral	WBC normal, Protein normal, Glucose normal. OCB+	LEV, CBZ: ineffective Oral steroids + AZA: seizure-free	IHC: neuropil (serum and CSF) GlyR - LN - (serum and CSF)	Onconeural-, VGKC-, NMDAR-, Caspr2-, GABAbR-, GABAaR-, DPPX-, AMPAR-, LGI1-.	Cognitive symptoms. Onset of symptoms after cessation of Adalimumab treatment
GlycineR	M, 40	2 years	Focal seizures with normal awareness and motor onset (1/week), tonic-clonic seizures	Normal	Epileptic, focal	Np	LEV, VPA, LTG: ineffective No immunotherapy	IHC: negative (serum) GlyR + LN -	Np	Drug and alcohol abuses
GlycineR	F, 18	4 years	Sporadically tonic-clonic seizures	Normal	Epileptic, multifocal	Np	LTG: effective No immunotherapy	IHC: negative (serum) GlyR + LN -	Np	Fatigue, muscle stiffness
Possible autoimmune etiology										
LGI1-like	F, 50	14 months	Focal seizures with impaired awareness with cognitive onset (30/month), tonic-clonic seizures	Normal	Epileptic frontotemporal, bilateral epileptic	Np	LEV: not-effective, aggression CBZ: not-effective, headache LTG: not-effective PMP: not effective	IHC: LGI1 like pattern (serum) GlyR - LN - (serum)	Caspr2-, LGI1- (serum)	Cognitive disorder, behavioral changes

Category	Sex, age inclusion	Duration epilepsy	Description epilepsy	MRI	EEG	CSF	Treatment and responses	Antibody screening	Antibody testing	Other symptoms/ characteristics
LGI1-like	F,64	9 years	Focal seizures (6/ day) with impaired awareness autonomic, and motor onset (automatisms.	Hyperintense mesotemporal, unilateral (left)	Epileptic frontotemporal, bilateral	WBC 2, Protein 0.21, Gluc 3.1, IgG index 0.39, No OCB	CBZ, OXZ, LCM, LEV, VPA, ZNS, TPM: ineffective IVIg ineffective	IHC: LGI1 like pattern (serum and CSF) GlyR - LN – (serum and CSF)	VGKC-, Caspr2-, LGI1-, GAD65: 22	Depression, cognitive disorder
LGI1-like	F, 53	4 years	Focal seizures (1/ day) with impaired awareness and motor onset (automatisms).	Normal	Epileptic frontotemporal, unilateral (left)	WBC 3, Protein 0.30, Gluc 4.0, IgG index 0.28 No OCB	VPA, LEV, OXZ: all ineffective. No immunotherapy	IHC: LGI1 like pattern (serum and CSF) GlyR- LN+ (serum)	VGKC-, GAD65-, NMDAR-, GABAbR-	Cognitive disorder
Neuropil	M, 67	2 years	Focal seizures (1/3 months) with impaired awareness and autonomic (vertigo), and behavior arrest onset.	Normal	Epileptic frontotempora,l unilateral (left)	Np	LEV: ineffective LCM: effective, not seizure-free. No immunotherapy	IHC: neuropil (serum) GlyR- LN-	Caspr2-, LGI1- (serum)	Mild cognitive complaints
Neuropil	F, 49	17 months	Focal seizures (3/ day) with normal awareness and motor onset (automatisms), no tonic-clonic seizures.	Mesial temporal sclerosis right	Epileptic frontotemporal unilateral (right)	Np	CBZ: effective, not seizure-free	IHC: neuropil (serum) GlyR- LN-	Caspr2-, GABAbR-, AMPAR-, GABAaR-	

Category	Sex, age inclusion	Duration epilepsy	Description epilepsy	MRI	EEG	CSF	Treatment and responses	Antibody screening	Antibody testing	Other symptoms/ characteristics
Neuropil	M, 63	20 years	Focal seizures with normal awareness and sensory onset, since 1 year increased frequency with also tonic-clonic seizures (2x)	Normal	Normal	Np	LEV: effective No immunotherapy	IHC: neuropil (serum) GlyR- LN-	Caspr2-, GABAbR-, AMPAR-, GABAaR-	
Neuropil	F, 85	2 years	Focal seizures (3/month) with impaired awareness and sensory onset.	Hyperintense mesiotemporal, unilateral (right)	Epileptic frontotemporal, unilateral (right)	Np	VPA, LEV: side-effects. LTG: effective, seizure-free No immunotherapy	IHC: neuropil (serum) GlyR- LN-	Caspr2-, GABAbR-, AMPAR-, GABAaR-	Cognitive disorder, anxiety disorder
Neuropil	M, 61	3 years	Focal seizures (1/month) with normal awareness and motor onset (tonic), sporadic tonic-clonic seizures.	Normal	Normal	Np	CBZ, LTG: ineffective LCM: effective, not seizure-free	IHC: neuropil (serum) GlyR- LN-	VGKC 77, Caspr2-, LGI1-, GABAbR-, AMPAR-, GABAaR-	Mild cognitive complaints
Neuropil	F, 25	3 years	Focal seizures (1/month) with impaired awareness and sensory onset, sporadic tonic-clonic seizures.	Normal	Epileptic, multifocal	Np	VPA: ineffective. CBZ: effective, but not seizure-free	IHC: neuropil (serum) GlyR- LN-	Caspr2-, GABAbR-, AMPAR-, GABAaR-	Posttraumatic stress disorder

Abbreviations: WBC= white blood cells, OCB= oligoclonal bands, VPA= valproic acid, LEV= levetiracetam, CBZ= carbamazepine, OXC= oxcarbazepine, LCM= lacosamide, LTG= lamotrigine, PMP= perampanel,TPM= topiramate, ZNS= zonisamide, IVIg= intravenous immunoglobulins, AZA= azathioprine, IHC= immunohistochemistry, LN= live neurons, Np= not performed.

Chapter 6

Evaluation of seizure treatment in anti-LGI1, anti-NMDAR, and anti-$GABA_BR$ encephalitis

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ABSTRACT

Objectives

This nationwide cohort study evaluates seizure responses to immunotherapy and antiepileptic drugs (AEDs) in patients with anti-leucine-rich glioma-inactivated 1 (LGI1), anti-N-Methyl-D-Aspartate Receptor (NMDAR), and anti-Gamma-Aminobutyric-Acid-B Receptor ($GABA_BR$) encephalitis.

Methods

Anti-LGI1, anti-NMDAR, and anti-$GABA_BR$ encephalitis patients with new-onset seizures were included. Medical information about disease course, AEDs and immunotherapies used, effects, and side effects, were collected. Outcome measures were: 1) seizure freedom while using AEDs or immunotherapy, 2) days to seizure freedom from start of AEDs or immunotherapy, and 3) side effects.

Results

Of 153 autoimmune encephalitis (AIE) patients (53 LGI1; 75 NMDAR; 25 $GABA_BR$), 72% (n=110) had epileptic seizures, and 89% reached seizure freedom. At least 53% achieved seizure freedom shortly after immunotherapy, and 14% achieved seizure freedom while using only AEDs ($p<0.0001$). This effect was similar in all types ($p=0.0004$; $p=0.0005$; $p=0.013$ respectively). Median time to seizure freedom from AEDs start was 59 days (IQR 27-160), and 28 days from start of immunotherapy (IQR 9-71, $p<0.0001$). Side effects were psychotic behavior and suicidal thoughts by the use of levetiracetam, and rash by the use of carbamazepine. Carbamazepine was more effective than levetiracetam in reducing seizures in anti-LGI1 encephalitis ($p=0.031$). Only one patient, of 86 surviving patients, developed epilepsy after resolved encephalitis.

Conclusions

Epilepsy after resolved encephalitis was rare in our cohort of AIE patients treated with immunotherapy. In addition, seizure freedom is achieved faster and more frequently after immunotherapy. Therefore, AEDs should be considered as add-on treatment, and similar to treatment of other encephalitis symptoms, immunotherapy is crucial.

INTRODUCTION

The discovery of N-Methyl-D-Aspartate Receptor (NMDAR) antibodies[1] has led to the description of several other antibodies to extracellular neuronal antigens. Binding of these antibodies leads to cerebral dysfunction, which often manifests as limbic encephalitis characterized by cognitive decline, behavioral changes and seizures. Seizures occurs most frequently in autoimmune encephalitis (AIE) with: leucine-rich glioma-inactivated 1 (LGI1),[2] NMDAR antibodies,[3] and Gamma-Aminobutyric-Acid-B-Receptor ($GABA_BR$) antibodies.[4]
The description of seizures in AIE has led to a new field of interest in epileptology with challenging issues in diagnosis and treatment. Concerning diagnosis, patients can present with seizures without notable other encephalitis signs,[5-7] leading to diagnostic difficulties and treatment delay. Undesirable, as treatment delay is associated with a poorer outcome.[3] Therefore, it is essential to consider an autoimmune etiology in presence of specific clinical clues. Moreover, faciobrachial dystonic seizures (FBDS)[2] are considered pathognomonic for anti-LGI1 encephalitis. Alternatively, the subacute onset of drug-resistant seizures might be a common, but unfortunately indiscriminative feature.
Another challenging issue is to achieve seizure freedom rapidly. Seizures often seem unresponsive to antiepileptic drugs (AEDs), while responses to immunotherapy are considered good. Nevertheless, seizure freedom is not always achieved while using immunotherapy alone and AEDs are sometimes needed as well.
However, the overall efficacy of AEDs in these patients and whether any particular AEDs should be preferred is unclear. Therefore, the aim of this nationwide observational cohort study was to evaluate the responses to AEDs and immunotherapy in these syndromes, including safety, and to describe the risk for epilepsy after resolved encephalitis.

METHODS

Patients

The department of neurology of the Erasmus MC University Medical Center is the national referral site for patients with suspected AIE and the department of immunology is the national referral site for antineuronal antibody testing. We identified all Dutch adults and children with AIE with LGI1, NMDAR, or $GABA_BR$ antibodies. Patients were identified between August 1999 and May 2017, although 78% was identified after 2010. Antibodies were detected in serum, or in cerebrospinal fluid, and confirmed with both cell-based assay and immunohistochemistry.[8] Patients with new-onset seizures during their active disease course were included.

Seizures

Medical information about disease course, seizure type, status epilepticus, types of AEDs and immunotherapies used, and side effects of the different treatments were collected during a visit to our clinic (n=77), from interviews with patients and relatives by phone (n=27), and from medical files (n=49). Clinical characteristics, including all encephalitis signs, of a part of the patients have been published before.[9, 10] To provide an overview of the clinical signs, we allocated patients into two groups: 'epileptic seizures plus', and encephalitis. No patients had only epileptic seizures without any other neurological symptoms at thorough examination. 'Epileptic seizures plus' contained the patients with prominent seizures and only subtle other encephalitis signs, which were initially unrecognized or considered side effects of AEDs. Examples are mild cognitive complaints, behavioral disorders or subtle movement disorders. Limbic encephalitis was defined as an encephalitis with predominant clinical involvement of the limbic system (short-term memory loss, difficulty forming new memories, behavioral disorder) or MRI fluid-attenuated inversion recovery (FLAIR)/T2 abnormalities in the medial temporal lobes.[11]

The guidelines and new epilepsy classification of the International League Against Epilepsy (ILAE) were used to define epileptic seizures,[12] epileptic seizures with an immune etiology,[13] status epilepticus,[14] drug-resistant epileptic seizures,[15] and to classify seizures. [12,13, 16] Epileptic seizures with an immune etiology were defined as at least two seizures, not provoked by other factors, occurring more than 24 hours apart resulting directly from an immune disorder, and with evidence of autoimmune mediated central nervous system inflammation.[12, 16] Drug-resistant epileptic seizures were defined as failure to achieve seizure freedom, despite of treatment with two tolerated, adequately dosed AEDs. Seizures were classified as focal or tonic-clonic. Moreover, focal seizures were classified as seizures with or without impaired awareness. FBDS were defined as frequent attacks (>8/day) with a dystonic posture of the arm, often combined with a facial contraction, lasting less than 30 seconds.[2] Refractory status epilepticus was defined as a status epilepticus continuing even after adequate treatment. Seizure-freedom was defined as no clinical signs of seizures, meaning no seizures observed and no reporting of focal seizures (including auras) or tonic-clonic seizures by patients or physicians. At follow-up, seizures needed to be absent for at least 3 months.

Effectivity of AEDs was scored as: ineffective, some effect, seizure freedom, or unknown effect. As it was no formal prospective study some effects were difficult to assess precisely, and we could therefore not use frequently used variables like 50% seizure reduction. We only scored some effect when it was noted specifically as a considerable reduction. Level of functioning was measured with the modified Rankin Scale (mRS).[17]

Primary outcome measures were: 1) seizure freedom achieved while using AEDs and while using immunotherapy, 2) days to seizure freedom from start of AEDs and from start of immunotherapy, 3) development of epilepsy after resolved encephalitis, and 4) reported side effects.

Statistics

Comparisons between 2 groups were performed with the Mann-Whitney U test (days to seizure freedom after start of epileptic seizures). Comparisons between multiple groups were performed with the Kruskall-Wallis test (age at onset, days to seizures after disease onset), the Fisher-Freeman-Halton test (comparing effects of different AEDs), and the one-way ANOVA (sex, seizures presenting symptom, type of seizures at presentation and during disease course, and (refractory) status epilepticus).

The chances to achieve seizure freedom (during first disease episode) were compared by McNemar's test, only in patients using both AED and immunotherapy before seizure freedom to avoid confounding by indication. For each patient individually, achievement of seizure freedom after the different treatments is shown visually in the figures. McNemar's test was also used to compare AED treatment responses in patients receiving multiple AEDs. The Wilcoxon signed rank test was used to compare the days to seizure freedom from start of AEDs and from start of immunotherapy. For this test only responses of patients that were treated with both AEDs and immunotherapy before seizure freedom were evaluated.

P-values below 0.05 were considered significant. We used SPSS 21.0 (SPSS Inc) for Windows and Prism7 (GraphPad) for Windows for statistical analysis.

RESULTS

Patient and seizure characteristics

We identified 153 patients with AIE, including 53 patients with LGI1 antibodies, 75 patients with NMDAR antibodies, and 25 patients with $GABA_BR$ antibodies. Among these cases, 72% of patients (n=110) had epileptic seizures with an immune origin (87% LGI1; 57% NMDAR; 84% $GABA_BR$), while 14 additional patients (9%) had only one seizure. Table 1 shows seizure characteristics per antibody. Patients with NMDAR antibodies were younger ($p<0.0001$) and only in this group there was a female predominance ($p<0.0001$). Fourteen patients were categorized as having 'epileptic seizures plus' (of whom 10/46 [22%] with LGI1 antibodies, 4/43 [9%] with NMDAR antibodies, and 0/21 with $GABA_BR$ antibodies), the others had limbic encephalitis or panencephalitis.

Table 1. Patient and seizure characteristics

	LGI1 (n=46/53)	NMDAR (n=43/75)	$GABA_BR$ (n=21/25)	p-value
Sex, M (%)	30 (65%)	7 (16%)	10 (48%)	<0.0001
Tumor (%)*	3 (7 %)	10 (23%)	14 (67%)	<0.0001
Median age at onset [IQR, range]	65 (58-69, 9-84)	20 (16-30, 3-73)	64 (56-75, 43-78)	<0.0001
Median days to seizures after disease onset [IQR, range]	0 (0-31, 0-365)	0 (0-14, 0-151)	0 (0-3, 0-37)	0.31
Seizures presenting symptom (%)	28 (61%)	21 (48%)	16 (76%)	0.11
Type of seizures at presentation				
Focal seizures	22 (48%)	13 (33%)	5 (24%)	0.10
Faciobrachial dystonic seizures	15 (32%)	0	0	<0.0001
Tonic-clonic seizures	9 (20%)	29 (67%)	16 (76%)	<0.0001
Type of seizures during disease course				
Focal seizures	39 (83%)	32 (74%)	8 (38%)	0.0001
With impaired awareness	28 (72%)	14 (42%)	7 (88%)	0.033
Without impaired awareness	15 (38%)	18 (55%)	1 (13%)	0.033
Motor	0	17 (51%)	0	
Autonomic	10 (26%)	0	0	
Sensory	3 (8 %)	1 (3 %)	0	
Cognitive	1 (3 %)	0	1 (13%)	
Emotional	1 (3 %)	0	0	
FBDS	25 (53%)	0	0	
Tonic-clonic seizures	26 (55%)	34 (79%)	21 (100%)	0.0002
Status epilepticus	10 (22%)	15 (35%)	13 (62%)	0.006
Refractory status epilepticus	7/46 (15%)	9/43 (21%)	10/21 (48%)	0.014
Relapses	14 (30%)	6 (14%)	5 (24%)	0.18
Including seizures	10/46 (22%)	5/43 (12%)	4/21 (19%)	0.44

* Tumors: Anti-LGI1 encephalitis: one patient had a thymoma, 1 patient a mesothelioma and 1 patient had rectal carcinoma in situ (detected 2 months before onset of neurologic disease). Anti-NMDAR encephalitis: 8 patients had a ovarian teratoma, 1 patients had a Merkel cell carcinoma, and 1 patient had a renal oncocytoma. anti-$GABA_BR$ encephalitis: all 14 patients had a small cell lung carcinoma.
Abbreviations: FBDS= faciobrachial dystonic seizures.

FBDS only occurred in patients with LGI1 antibodies (53%). All patients with $GABA_BR$ antibodies had tonic-clonic seizures, compared to 55% of patients with LGI1 antibodies (p=0.0002), and 79% of patients with NMDAR antibodies. Status epilepticus occurred frequently (n=38, 34%), in particular in patients with $GABA_BR$ antibodies (62%, p=0.006), of which 26 (68%) had a refractory status epilepticus. Five patients (4%) died during status epilepticus.

Median follow-up time from onset of seizures was 27 months (IQR 15-49, range 0-149 months), twenty-four patients had died (22%). Twenty-five patients (23%) had a relapse of the encephalitis, among them 76% again had seizures (10 LGI1; 5 NMDAR; 4 $GABA_BR$). At last follow-up, 66% of patients alive had a mRS of 0-2 (LGI1 78%; NMDAR 74%; $GABA_BR$ 24%).

Seizure treatment

Of all 110 patients with new-onset epileptic seizures with an immune origin, 91% were treated with one or more AEDs (LGI1 80%, NMDAR 98%; $GABA_BR$ 100%). The median delay between seizure onset and start of AEDs was 3 days (IQR 0-31). This delay was higher in patients with anti-LGI1 encephalitis (median of 64 days, IQR 0-178, p<0.0001). During their disease course, patients were treated with a median of two AEDs (IQR 1-3, range 0-9). Moreover, 71 patients (65%) were treated with 2 or more AEDs. AEDs were continued for a median period of 8 months after diagnosis (IQR 4-18, range 0-102 months).

Most patients were treated with immunotherapy (92%), all but one with first-line immunotherapy (combination of methylprednisolone and/or intravenous immunoglobulins and/or plasmapheresis), and 17% with additional second-line immunotherapy (rituximab and/or cyclophosphamide; Table 2). The patients not treated with immunotherapy received only AEDs (n=9). Twenty-one percent of patients were treated with chronic immunotherapy, including azathioprine (n=15) or mycophenolate (n=8), of them 19 (83%) had LGI1 antibodies. Fifteen of 19 anti-LGI1 patients were treated with chronic immunotherapy after the initial episode. Two of these anti-LGI1 patients (13%) developed a relapse, necessitating adaptation of the chronic immunotherapy. Thirty-one anti-LGI1 patients did not receive chronic immunotherapy after the initial episode. Of these, 11 developed a relapse (35%). Four patients had only started chronic immunotherapy after relapse. One of these four patients developed multiple relapses that halted after administration of Rituximab.

The majority of patients with anti-NMDAR and anti-$GABA_BR$ encephalitis were treated with both AEDs and immunotherapy (NMDAR 93%; $GABA_BR$ 81%). This percentage tended to be lower in patients with anti-LGI1 encephalitis (71%, p=0.051). Among anti-LGI1 encephalitis patients, more were treated with immunotherapy (91%) than with AEDs (80%). The median treatment delay between symptom onset and start of immunotherapy was 30 days (IQR 11-93), which was highest in the anti-LGI1 group (median of 96 days, IQR 48-290, p <0.0001). Patients with anti-LGI1 encephalitis and focal seizures had a longer treatment delay (p=0.007) than patients without focal seizures, while this delay was not observed in patients with anti-LGI1 encephalitis and FBDS (p=0.20).

Table 2. Overview of all patients treated with immunotherapy

Group	Treatment*	Number of patients	%
LGI1 (n=42/46)	Oral prednisone only	7	17
	ivMP only	3	7
	ivMP + oral prednisone	11	26
	IVIg only	2	5
	ivMP + IVIg	3	7
	IVIg + oral prednisone	1	2
	ivMP + IVIg + oral prednisone	12	29
	ivMP + Plex + RTX	1	2
	ivMP + IVIg + RTX	1	2
	ivMP + IVIg + oral prednisone + RTX	1	2
Suggested treatment (since early 2015): ivMP (5d1000mg) + IVIg (5d0.4g/kg) + oral taper (start 60mg/d) + Azathioprine 2x75mg/d). If insufficient, Rituximab might be added.			*70*
NMDAR (n=41/43)	ivMP only	7 (2 OT and resection)	17
	ivMP + oral prednisone	2	5
	ivMP + IVIg	11 (3 OT and resection)	27
	ivMP + IVIg + oral prednisone	5	12
	ivMP + IVIg + Plex	3	7
	IVIg + oral steroids + Plex	1	2
	IVIg + RTX	1	2
	ivMP + IVIg + RTX	2	5
	ivMP + oral prednisone + IVIg + RTX	1	2
	ivMP + Plex + RTX	1 (1 OT and resection)	2
	ivMP + IVIg + Plex + RTX	1	2
	ivMP + IVIg + RTX + Cyclo	6 (2 OT and resection)	15
Suggested treatment (since early 2014): ivMP (5d1000mg) + IVIg (5d0.4g/kg). If effective ivMP repetition after 4 and 8 weeks (3d1000mg). If ineffective second-line immunotherapy RTX (1000 mg, 2 courses, 14 days apart + Cyclo (15mg/kg, 3 courses, 14 days apart, continued 500mg/14 days or 1000 mg/28 days). In children, RTX only is preferred.			79
$GABA_BR$ (n=18/21)	ivMP only	4 (1 SCLC, chemo)	22
	ivMP + oral prednisone	3 (1 SCLC, chemo)	17
	ivMP + IVIg	5 (4 SCLC, chemo [n=4], radiation [n=2], resection [n=1])	28
	ivMP + IVIg + oral prednisone	4 (1 SCLC, chemo)	22
	RTX	1 (1 SCLC, chemo)	6
	ivMP + IVIg + Cyclo	1	6
Suggested treatment (since early 2014): ivMP (5d1000mg) + IVIg (5d0.4g/kg). Treatment after hyperacute phase depends on improvement and tumor status. Options: ivMP repetition after 4 and 8 weeks (3d1000mg),or second-line immunotherapy can be considered (see anti-NMDAR) if improvement is mediocre.			70

* Tumor therapy in addition to immunotherapy when a tumor is found.
Abbreviations: ivMP= intravenous methylprednisolone, IVIg= intravenous immunoglobulins, Plex= plasmapheresis, RTX= rituximab, Cyclo= cyclophosphamide, OT= ovarian teratoma, SCLC= small cell lung carcinoma.

Seizure freedom and treatment effects

Figure 1, 2 and 3 visualize timelines of all patients with epileptic seizures per antibody. Seizure freedom was achieved in 89% of all 110 patients. Of these 98 patients, 14% (n=14) achieved seizure freedom while using only AEDs, while in 52 patients seizure freedom was achieved shortly after the start of immunotherapy (53%). Comparing the 68 patients receiving both AEDs and immunotherapy before seizure freedom was reached, the chance to achieve seizure freedom was higher after the use of immunotherapy than after the use of AEDs (immunotherapy, n=44, AEDs n=3, p<0.0001). This also applied for the groups separately (LGI1 p=0.0001; NMDAR p=0.0005; $GABA_BR$ p=0.013).

The median time to achieve seizure freedom after the start of AEDs was 59 days (IQR 27-160), and 28 days from start of immunotherapy (IQR 9-71, p<0.0001). This decrease in days to seizure freedom after the use of immunotherapy was observed in all three syndromes (LGI1 p<0.0001; NMDAR p<0.0001; $GABA_BR$ p=0.001).

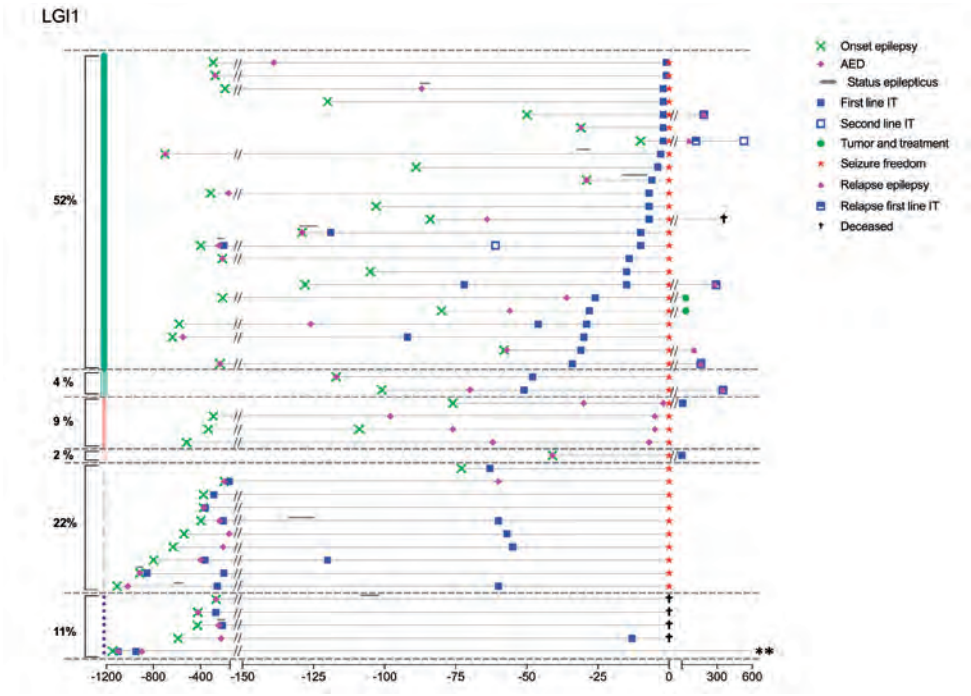

Figure 1. Timelines (in days) of anti-LGI1 encephalitis patients with epileptic seizures. The percentages shown on the left correspond to patients: 1) reaching seizure freedom after the use of immunotherapy (green), 2) reaching seizure freedom probably after the use of immunotherapy (triple green), 3) reaching seizure freedom after the use of AEDs (red), 4) reaching seizure freedom probably after the use of AEDs (double red), 5) that could not be categorized (grey stripes), and 6) that did not reach seizure freedom (black dots).

If patients were treated with another immunomodulating treatment > 1 month after the initial treatment (for example IvIg after prednisolone) this is shown as a new blue square. Treatment with an additional AED or dosage increase after > 1 month is shown as a second purple diamond. Relapses are only shown if patients had seizures.

Median time of follow-up from onset was 33 months (IQR 19-52, range 8-119). Median time of seizure freedom was 23 months (IQR 14-40, range 4-102). The median interval between start of AEDs and start of immunotherapy was 57 days (IQR 27-152).

**Timeline of the only patient that developed epilepsy after resolved encephalitis. The symbols in this timeline are not fitted to scale. The onset of seizures was in 2009, the patient was treated with prednisone (and AEDs), leading to reversibility of cognitive signs, but he still has temporal epilepsy.

Abbreviations: AED= antiepileptic drug, IT= immunotherapy.

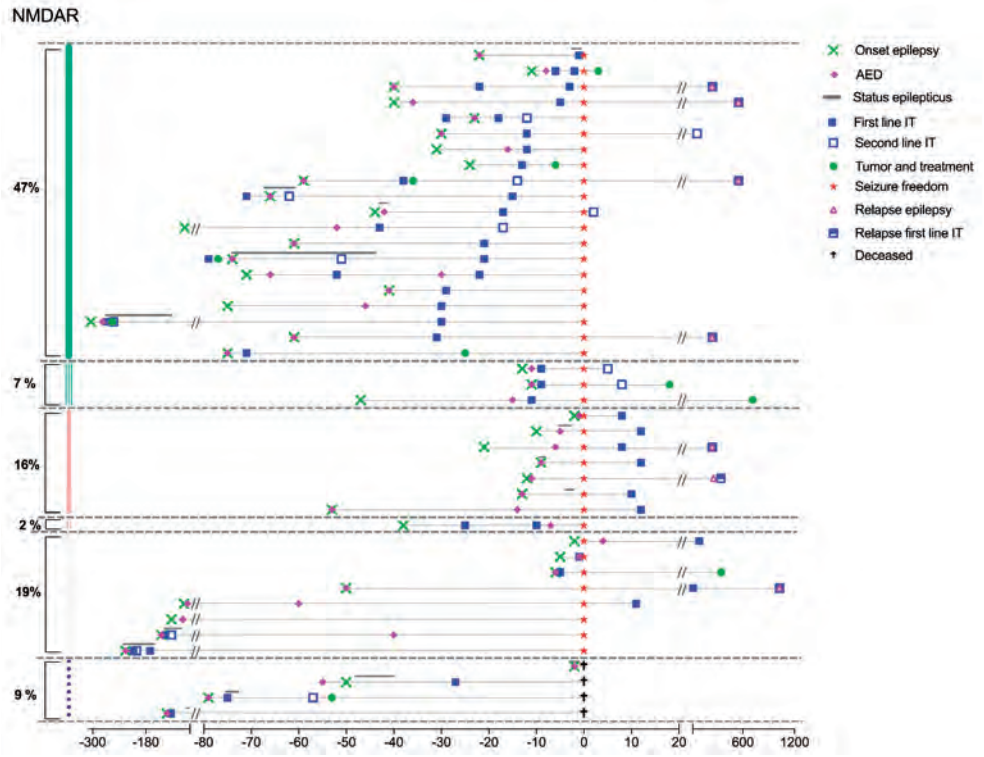

Figure 2. Timelines (in days) of anti-NMDAR encephalitis patients with epileptic seizures. See legend Figure 1. Median time of follow-up was 37 months (IQR 15-59, range 1-149). Median time of seizure freedom was 31 months (IQR 15-58, range 4-129). The median interval between start of AEDs and start of immunotherapy was 14 days (IQR 4-24).

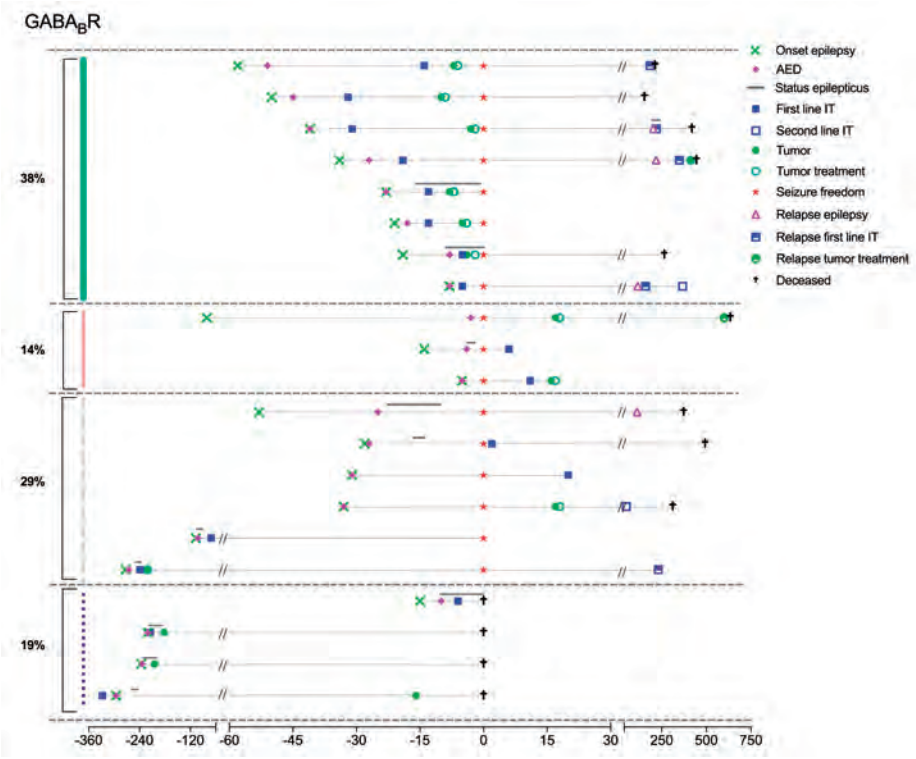

Figure 3. Timelines (in days) of anti-GABA$_B$R encephalitis patients with epileptic seizures. See legend Figure 1. Median time of follow-up was 15 months (IQR 9-21, range 0-109). Median time of seizure freedom was 15 months (IQR 9-20, range 5-100). The median interval between start of AEDs and start of immunotherapy was 10 days (IQR 7-28).

Seizure freedom was achieved faster in women than in men (p<0.0001), attributed to patients with anti-NMDAR encephalitis (p=0.038). No differences were observed in days to seizure freedom between patients with paraneoplastic[18] (n=27) or non-paraneoplastic encephalitis (n=83; p=0.085). In patients with focal seizures it took longer to achieve seizure freedom (p<0.0001), while presence of tonic-clonic seizures did not influence the interval to seizure freedom (p=0.081). In patients with LGI1 antibodies, the presence of FBDS did not shorten the interval to seizure freedom (p=0.20).

Eleven patients did not reach seizure freedom. Ten patients had deceased, due to the encephalitis, before reaching seizure freedom, while only one patient with anti-LGI1 encephalitis (3% of surviving patients with seizures and anti-LGI1 encephalitis) developed temporal epilepsy after resolved encephalitis. Median time of seizure freedom in AIE patients (after initial episode or last relapse) was 22 months (IQR 14-45, range 4-129). 14 of these patients (14%) were still using AED, while seizure free. We have evaluated the proportion of patients that continued to have seizures at 6,12 and 24 months after the initiation of immunotherapy (Figure 4). At 6 months, seizure freedom was achieved in 79% of patients, of these 73 patients, 38 (52%) still used AEDs. At 12 months, 96% of patients had reached seizure freedom, of whom 34% still used AEDs while seizure free. At 24 months, only one

patient had developed epilepsy after resolved encephalitis (2%), the other 46 patients (98%) were seizure-free, among them 4 (9%) were treated with AEDs. Fourteen patients developed a relapse with epileptic seizures within these two years (seven while using AED), and 12 became seizure free again within days or weeks after restarting immunotherapy.

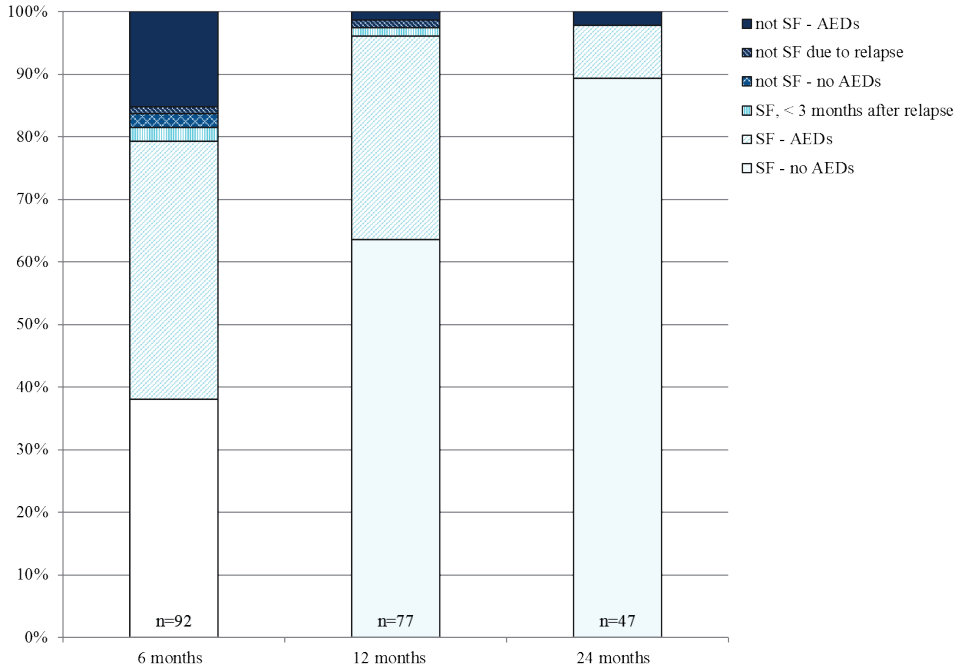

Figure 4. Evaluation of the patients that are at risk to develop epilepsy after resolved encephalitis at 6, 12 and 24 months after the initiation of immunotherapy. The figure shows the cumulative percentages of the patients that reached or did not reach seizure freedom, and the use of antiepileptic drugs. Patients with a relapse less than 3 months before the time point at 6, 12, or 24 months, or with a relapse at 6, 12 or 24 months are also shown in the figure. Fourteen patients developed a relapse with epileptic seizures within 24 months after the start of immunotherapy, in seven of them despite continuous AED treatment. At relapse the median seizure duration was 12 days (IQR 4-29, range 3-92). Eleven of these fourteen patients became seizure free within days or weeks after restarting immunotherapy, 2 patients became seizure free after three months, and 1 patients developed epilepsy after resolved encephalitis. Abbreviations: SF=seizure free, AEDs= antiepileptic drugs.

AED Effects and side effects

Prescribed AEDs were: levetiracetam (66%), valproic acid (53%), carbamazepine (32%), phenytoin (30%), clobazam (15%), lacosamide (7%), oxcarbazepine (6%), and lamotrigine (5%). Topiramate and phenobarbital were only used sporadically.

Responses to these most prescribed AEDs and side effects are visualized in Figure 5. Although some response was seen in all three groups, seizure freedom was only infrequently achieved. Carbamazepine appeared to have the best effect to reduce focal seizure frequency relatively in anti-LGI1 encephalitis (Figure 5D), while FBDS hardly responded to AEDs (Figure 5E). In those anti-LGI1 patients treated with both levetiracetam and carbamazepine (n=15), carbamazepine appeared more effective to reduce seizure frequency than levetiracetam ($p=0.031$).

Side effects were frequently reported by patients with anti-LGI1 encephalitis (37%), and less by patients with anti-NMDAR (18%) and anti-$GABA_BR$ (15%) encephalitis. Patients with LGI1 antibodies frequently had a rash by the use of carbamazepine (7/22, 32%). Most reported side effects by the use of valproic acid were memory deterioration (n=3) and tremor (n=2). Side effects of levetiracetam were rash (n=3) and serious behavioral changes (n=14; 19%), including two patients with anti-LGI1 encephalitis with severe psychotic behavior and suicidal thoughts.

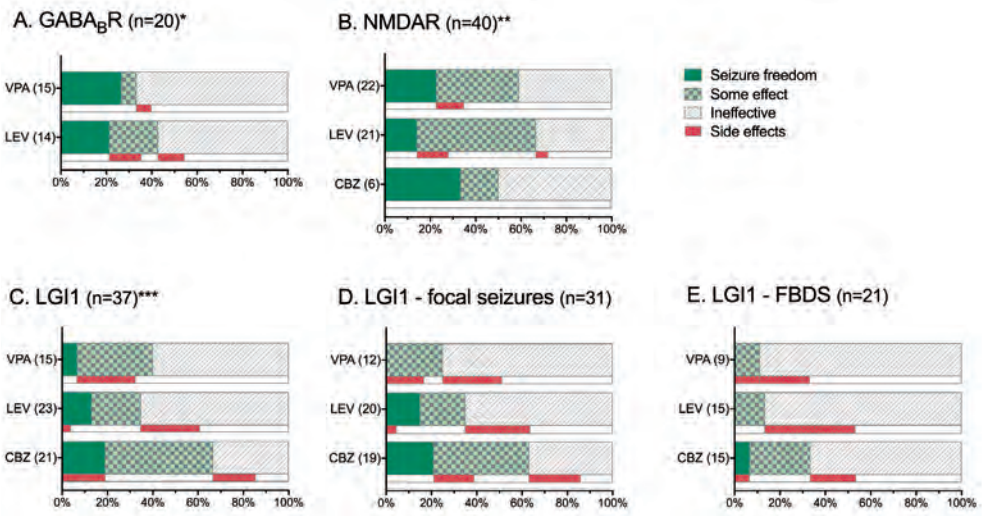

Figure 5. Response percentages of most prescribed antiepileptic drugs and side effects in patients with A) anti-$GABA_BR$, B) anti-NMDAR, or C) anti-LGI1 encephalitis, D) focal seizures in patients with anti-LGI1 encephalitis, and E) FBDS in patients with anti-LGI1 encephalitis. 'Some effect' was scored if noted specifically as a considerable reduction of seizures. In some patients, responses to specific AEDs were not assessable, due to concomitant use of immunotherapy or missing data.

*20/21 patients with anti-$GABA_BR$ encephalitis were treated with LEV (n=16) or VPA (n=15), 11 patients were treated with both LEV and VPA. Responses of 2 patients treated with LEV were not assessable.

**40/42 patients with anti-NMDAR encephalitis were treated with LEV (n=28), VPA (n=24) or CBZ (n=10), in 17 patients these AEDs were combined. Responses of seizures of 2 patients treated with VPA, 7 patients treated with LEV, and 4 patients treated with CBZ were not assessable.

***37 patients with anti-LGI1 encephalitis were treated with LEV (n=29), VPA (n=19) or CBZ (n=22), in 25 patients combinations of these AEDs were used. Responses of seizures of 4 patients treated with VPA, 6 patients treated with LEV, and 1 patient treated with CBZ were not assessable. Comparing patients treated with both LEV and CBZ (most prescribed, n=15), CBZ was more effective (p=0.031).

In the LGI1 group only four patients were treated with oxcarbazepine, 1 patient reached seizure freedom, 1 patient showed some effect and 2 had no effect.

Treatment responses of patients with anti-LGI1 encephalitis are also shown for focal seizures (D) and FBDS (E). FBDS hardly responded to VPA, LEV or CBZ, while focal seizures responded somewhat better to carbamazepine.

Abbreviations: FBDS= faciobrachial dystonic seizures, VPA = valproic acid, LEV= levetiracetam, CBZ = carbamazepine.

DISCUSSION

This nationwide observational cohort study evaluates seizure responses to immunotherapy and AEDs in patients with anti-LGI1, anti-NMDAR, and anti-$GABA_BR$ encephalitis. We show that seizure freedom is achieved faster and more frequently after the use of immunotherapy than after the use of AEDs. In some patients, AEDs might decrease seizure frequency or lead to seizure freedom, but the effect is limited and incomparable to the effect of immunotherapy. After immunotherapy, the development of epilepsy after resolved encephalitis is rare in our cohort of AIE patients treated with immunotherapy.

These results emphasize the usefulness of immunotherapy in the treatment of epileptic seizures with an immune etiology caused by extracellular neuronal antibodies. In all groups there was a clear decrease in days to seizure freedom after the use of immunotherapy. It is customary to start AEDs before immunotherapy, so only comparing intervals between start of different treatments and seizure freedom would not be entirely fair. To avoid this confounding, we additionally compared the effects of AEDs and immunotherapy in patients who used both, and in which the responses to the individual treatment could be determined. This showed a clear preference for immunotherapy, which is in line with prior research in anti-LGI1 encephalitis, showing the positive effects of early immunotherapy on epileptic seizures and cognition.[5, 19]

The effects of different treatment options were visualized (Figure 1-3), showing that seizure freedom was frequently preceded directly by the initiation of immunotherapy and that patients treated earlier on in disease course seemed to reach seizure freedom faster. This effect was most remarkable in patients with anti-LGI1 encephalitis, wherein almost half of the patients became seizure free within a week after immunotherapy, while they had been refractory to AEDs for longer periods. We did not analyze the effects of tumor treatment separately, because it was always accompanied by immunotherapy. Yet, we visualized that in patients with paraneoplastic encephalitis, both tumor treatment and immunomodulation often preceded seizure freedom. Mechanistically, both immunotherapy and tumor treatment are causal treatments, while AEDs are symptomatic treatments.

Our study shows that seizures of most patients were AED-resistant. Seizure freedom was achieved in the minority patients while using only AEDs, and adjustments in treatment regime or dosage increase of AEDs did not affect the chance to achieve seizure freedom. Additionally, these patients often had a milder disease course without status epilepticus. The AED resistant character of seizures is a confirmation of observations in other studies.[6, 19, 20] In addition, the often accompanying (subtle) cognitive symptoms also favor treatment with immunotherapy. Therefore, it seems better to use AEDs only as add-on symptomatic treatment.

After treating the acute phase of the encephalitis, the continued use of AEDs is debatable. In our study, AED therapy was successfully discontinued in most patients after resolution of encephalitis. Chronic AED use does not appear to be necessary in most AIE patients long

term. This is in line with previous studies studying separate subtypes of AIE.[5, 9, 20] Although mesiotemporal sclerosis has been described in 25-50% of follow-up MRIs in patients with anti-LGI1 encephalitis, only a few develop epilepsy after resolved encephalitis.[9, 21] For this reason, some argue against the implementation of the term 'epilepsy with immune origin'[13] (new ILAE classification) in the acute phase, reserving this for the situation after the encephalitis has been treated.[22] Additionally, side effects of AEDs, like memory disturbances, might disturb recovery after AIE, especially in combination with other drugs influencing brain functions, even more questioning the necessity for long-term AED use. Lastly, half the patients who experienced a clinical relapse with epileptic seizures developed this relapse despite using AED and almost all patients became seizure free again within days or weeks after restarting immunotherapy. However, prospective studies comparing different treatments in the chronic disease phase are lacking.

The AED-resistant character of seizures and crucial role of immunotherapy in treatment of seizures stress the importance of considering AIE as cause of epileptic seizures in patients with acquired drug-resistant seizures. Due to increased awareness, patients with a fulminant disease course with coma and status epilepticus, most frequently caused by $GABA_BR$ or NMDAR antibodies, are regularly diagnosed early on in disease course. On the other hand, almost a quarter of the patients with anti-LGI1 encephalitis did not had a full-blow encephalitis, but seizures with only subtle encephalitis signs, which were often unrecognized by referring physicians. The unrecognition leads to diagnostic and treatment delay.[9] In our study, this is reflected by 1) the longest treatment delay, 2) the longest interval between start of AEDs and immunotherapy, 3) a lower percentage of patients treated with AEDs, and 4) the observation that the presence of focal seizures extends the time to seizure freedom. As FBDS have gained much attention, better recognition and earlier treatment are to be expected. A longer delay until diagnosis and appropriate treatment in those with focal seizures shows that we should also look beyond FBDS to reduce delays and improve outcomes.

Concerning responses to most prescribed AEDs, in our cohort, physicians preferred the use of levetiracetam. However, patients often had serious behavioral changes and two patients with anti-LGI1 encephalitis developed a severe psychosis and suicidal thoughts. Additionally, levetiracetam might exaggerate symptoms of AIE, especially behavioral disorders. Focal seizures of anti-LGI1 patients responded relatively better to carbamazepine, while FBDS hardly responded to any AED. Only a few patients were treated with oxcarbazepine, a drug with a comparable mechanism of action as carbamazepine. Individual results of treatment with oxcarbazepine seem promising and comparable to the effect of carbamazepine, but need confirmation in larger patient groups. Lacosamide, a similar drug, was only used infrequently and as add-on, therefore assessment of the effects was impossible. A recent study describes that only 10% of patients with Voltage-Gated-Potassium-Channel (VGKC)-complex and Glutamic-Acid-Decaboxylase 65 (GAD65) antibodies reached seizure freedom by the use of specific AEDs.[23] Carbamazepine, lacosamide and oxcarbazepine led most

frequently to seizure-free outcome, while levetiracetam was ineffective in all patients. This is in line with our results, but difficult to compare as not all VGKC-complex antibodies are pathogenic [24] and as the pathogenicity of anti-GAD65 is still unclear,[25] and incomparable to the pathogenicity of antibodies to extracellular antigens.

Side effects were reported most frequently by patients with LGI1 antibodies, and less by the other patients, probably due to a more fulminant disease course in patients with anti-$GABA_BR$ and anti-NMDAR encephalitis. One third of patients with LGI1 antibodies treated with carbamazepine suffered from a rash. Rash is a common side effect of carbamazepine and occurs most in patients with a specific pro-immunogenic HLA types.[26] Recently, a strong correlation with specific HLA types (HLA DR7 and DRB4) was found in patients with LGI1 antibodies.[27, 28] Yet, these types do not correspond to the HLA types of patients that are prone to rash by the use of carbamazepine. An alternative explanation for the high percentage of rash within the LGI1 group might be the rapid dosage increase because of frequent, drug-resistant seizures.

Although this is the largest cohort, and a nationwide study, regarding seizure responses to different treatments in patients with AIE and epileptic seizures, there are some limitations associated with the retrospective design of this study. Concerning data collection, effects and side effects were not always accurately documented. Patients were treated with a variety of AEDs and immunotherapies, and not per protocol, so comparisons are more difficult. However, the visualization of individual data in timelines are convincing that the differences between effects of AEDs and immunotherapy are real. We were not able to compare different treatment regimens (different AEDs and immunotherapies) due to small group sizes. Especially side effects are difficult to evaluate systematically in a retrospective design. Cognitive decline and behavioral disorders are hallmark symptoms of AIE making it more difficult to categorize symptoms as disease progression or side effects of treatment. Additionally, severe disease courses with coma and long-term intensive care stay make a proper evaluation of treatment effects (and side-effects) difficult. Nevertheless, by treating these patients and by interviewing most patients, relatives and treating physicians important effects and side effects were still assessable and results from this study may help to compose treatment recommendations.

We would suggest to use AEDs with sodium channel blocking properties (like carbamazepine or potentially oxcarbazepine), as first add-on next to immunotherapy in the symptomatic treatment of patients with anti-LGI1 encephalitis and seizures as it seems to have at least some effect in reducing focal seizures. However, due to the frequent occurrence of rash, often leading to discontinuation of therapy, it is essential to be cautious with rapid dosage increase. On the other hand, levetiracetam seems not preferable in the treatment of autoimmune epileptic seizures as the effects are limited and it can induce or exaggerate serious behavioral disorders.

From this nationwide study, we can conclude that immunotherapy, is most important in the treatment of epileptic seizures in patients with anti-LGI1, anti-NMDAR and anti-$GABA_BR$

encephalitis. The overall effect of AEDs in the symptomatic treatment of epilepsy in these patients is limited and antibody-dependent. Specific AEDs should be considered to use as add-on therapy to control seizures, but not as primary and long-term treatment.

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Chapter 7

Pediatric autoimmune encephalitis recognition and diagnosis

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ABSTRACT

Objectives

The aims of this study were: 1) to describe the incidence of autoimmune encephalitis (AIE) and acute disseminated encephalomyelitis (ADEM) in children, 2) to validate the currently used clinical criteria to diagnose AIE, and 3) to describe pitfalls in the diagnosis of pediatric autoimmune and inflammatory neurological disorders.

Methods

This study cohort consists of three patient categories: 1) children with antibody-mediated AIE (n=21), 2) children with ADEM (n=32), and 3) children with suspicion of an autoimmune etiology of their neurological symptoms (n=60). Baseline and follow-up clinical data were used to validate the current guideline to diagnose AIE. In addition, patient files and final diagnoses were reviewed.

Results

One-hundred three of the 113 included patients fulfilled the criteria of possible AIE. Twenty-one children had antibody-mediated AIE, of whom 19 had anti-NMDAR, 1 had anti-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (anti-AMPAR) and 1 had anti-leucine-rich glioma inactivated-1 (anti-LGI1) encephalitis. Finally, thirty-four children had ADEM, and two children had Hashimoto's encephalopathy. Mean incidence rates were 1.54 children/million (95%-CI 0.95-2.35) for antibody-mediated AIE and 2.49 children/million (95%-CI 1.73-3.48) for ADEM. Of the other 48 children, treating physicians' diagnoses were reviewed. In 22% (n=6) of children initially diagnosed as having an autoimmune (AI)/inflammatory etiology (n=27), no support for AI/inflammation was found.

Conclusions

Besides anti-NMDAR encephalitis and ADEM, other AIEs are rare in children. The current guideline to diagnose AIE is also useful in children. However, in children with non-specific symptoms, it is important to review data critically, to perform complete work-up, and to consult specialized neuroinflammatory centers.

INTRODUCTION

Autoimmune encephalitis (AIE) has expanded the already comprehensive list of pediatric neuroinflammatory disorders of the central nervous system. Anti-N-Methyl-D-Aspartate Receptor (anti-NMDAR) encephalitis and acute disseminated encephalomyelitis (ADEM) are the most frequently described cause of autoimmune encephalitis in children,[1-4] and disease courses have been studied in detail, including treatment responses, functional recovery,[1, 4] and long-term neuropsychological outcome.[5]

Next to anti-NMDAR, other neuronal antibodies have been described only sporadically in children,[6-8] while in adults, reported incidence of these antibodies has increased dramatically.[9,10] This could indicate that, besides anti-NMDAR encephalitis, neuronal antibodies occur less frequent in children, or that these syndromes are unrecognized.

In 2016 Graus et al.[11] have described criteria to diagnose antibody-mediated AIE, ADEM and other related autoimmune encephalitides, including, Bickerstaff's brainstem encephalitis, Hashimoto's encephalopathy, and autoantibody negative (seronegative) AIE, in adults and in children. These criteria allow physicians to start first-line immunotherapy in patients with typical limbic encephalitis or probable anti-NMDAR encephalitis before definite antibody diagnosis. As already stated by the authors, the criteria should be used with caution in children, because the differential diagnosis is more widespread.

This prospective, observational, cohort study describes the incidence of pediatric antibody-mediated AIE and ADEM in the Netherlands since 2015. In addition, the diagnostic criteria of Graus et al.[11] are validated using data of prospectively collected cohorts of children with AIE, ADEM and children with neurological symptoms and suspicion of an autoimmune etiology. Finally we describe pitfalls in the diagnosis of pediatric autoimmune and inflammatory neurological disorders.

METHODS

Patients

This study cohort contains data of three patient groups, included between January 2015 and December 2018 in the Netherlands. The first group consists of all Dutch children, aged 0-18 years, diagnosed with antibody-mediated (definite) autoimmune encephalitis. Antibodies were detected in serum and CSF, using commercial cell based assays (CBAs; *Euroimmun, Lübeck, Germany*). Antibodies were confirmed with immunohistochemistry. All children were included after diagnosis and are being followed prospectively since. The second group consists of all Dutch children with ADEM diagnosed according to the International Pediatric Multiple Sclerosis Study Group (IPMSSG) criteria.[12] who were prospectively included in the nationwide, multicenter PROUDkids study.[13] The third group consists of children with a suspected autoimmune etiology of their neurological symptoms. These children were

prospectively included in the observational, multicenter, "Children's Autoimmunity Related to Neuropsychiatric symptoms, Chorea and Epilepsy" (CHANCE) study. The CHANCE study was a multicenter study, with national accrual, but no means to be complete. Inclusion criteria were: age below 18 years at symptom onset, and one of the following clinical phenotypes: 1) limbic encephalitis, 2) new-onset status epilepticus, 3) acute encephalopathy, or 4) neuropsychiatric symptoms combined with symptoms of basal ganglia dysfunction. All serum samples, and if available CSF samples, were screened for neuronal antibodies using immunohistochemistry[14] and cell based assays (CBAs; *Euroimmun, Lübeck, Germany*). Questionable or positive samples were tested with conformational laboratory techniques, including live hippocampal neurons,[15] in-house CBAs, and Enzyme-Linked Immuno Sorbent Assay (ELISA). Antithyroid autoantibodies (anti-TPO) were detected by fluorescence enzyme immunoassay (FEIA) on the Phadia 250 system using EliA according to the manufacturer's instructions (*Thermo Fishers Scientific, Freiburg, Germany*).
Data about medical history, disease course, treatment responses, and final diagnoses were collected. Data were collected from interviews with patients, from treating physicians or were retrieved from patient files.

Definitions
The criteria of Graus et al.[11] were used to define possible autoimmune encephalitis, definite autoimmune limbic encephalitis, probable anti-NMDAR encephalitis, Bickerstaff's brainstem encephalitis, Hashimoto's encephalopathy, and seronegative but probable autoimmune encephalitis. The IPMSSG criteria[12] were used to define ADEM.
Final etiology was classified as: I) Definite autoimmune encephalitis (AIE), including children with antibody-mediated AIE and ADEM without new lesions of follow-up imaging. II) Probable AIE, according to the diagnostic criteria.[11] This category consisted of children with ADEM without follow-up MRI and of children with Hashimoto's encephalopathy. III) Possible AE/inflammatory, included children not fulfilling any of the diagnostic criteria panels, but with support for autoimmunity or inflammation. This category consisted partially of children with clinically defined acquired AE/inflammatory disorders, like Rasmussen encephalitis or Sydenham's chorea, and partially of children with MRI or CSF abnormalities pointing towards an AE/inflammatory etiology (pleocytosis, elevated protein, or oligoclonal bands in CSF, MRI lesions in the temporal lobe), with exclusion of other causes, and not fulfilling the criteria of seronegative AIE.[11] IV) Unknown etiology and no support for AE/inflammatory, including children without MRI or CSF abnormalities pointing towards and AE/inflammatory etiology. V) Other diagnosis and no support for AE/inflammatory. RN, MT, and MdB reviewed follow-up etiologies. Definite diagnoses were determined by consensus.

Statistics

The annual incidence rate (from 2015-2018) was calculated with 95%-confidence intervals (CI), assuming a Poisson distribution. Available data of the Dutch pediatric population were used (StatLine; statline.cbs.nl/statweb/). Comparisons were performed using the Chi-square test or the Kruskall Wallis test.

RESULTS

Patient characteristics

We included 113 patients. Twenty-one patients had definite autoimmune encephalitis (19%), including 19 (90%) children with anti-NMDAR encephalitis. Among them, twelve had an idiopathic etiology (63%), six children recently had herpes simplex virus encephalitis (HSVE; 32%), and one girl had an ovarian teratoma (5%) detected shortly after disease onset. The other two children with neuronal antibodies had anti-leucine-rich glioma-inactivated protein 1 (anti-LGI1) encephalitis (n=1; 5%), and anti-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (anti-AMPAR) encephalitis (n=1; 5%). Thirty-two children diagnosed with ADEM (28%) were included from the PROUDkids cohort. The other sixty patients (53%) were included from the CHANCE study

Incidence

The annual incidence rates of antibody-mediated AIE and ADEM of four consecutive years (2015-2018) are shown in Table 1. Mean incidence rate were 1.54 children/million (95%-CI 0.95-2.35), and 2.49 children/million (95%-CI 1.73-3.48) for AIE and ADEM, respectively.

Table 1. Annual incidence of pediatric autoimmune encephalitis and acute disseminated encephalomyelitis

Year	AIE – incidence children/million (95%-CI)		ADEM - incidence children/million (95% CI)		Number of Dutch pediatric inhabitants
2015	1.46	(0.47-3.40)	2.62	(1.20-4.98)	3.429.193
2016	1.76	(0.64-3.82)	2.05	(0.82-4.22)	3.416.581
2017	1.76	(0.65-3.84)	3.23	(1.16-5.78)	3.404.098
2018	1.18	(0.32-3.02)	2.07	(0.83-4.26)	3.386.096
2015-2018	1.54	(0.95-2.35)	2.35	(1.61-3.31)	3.408.992

Abbreviations: AIE= autoimmune encephalitis, ADEM= acute disseminated encephalomyelitis, CI= confidence interval.

Validation of autoimmune encephalitis criteria

Of all 113 patients included, 103 (89%) fulfilled the criteria of possible autoimmune encephalitis (Figure 1). Demographical data are described in table 2. Children with AIE were more often female (p=0.023), and children with ADEM were younger (p<0.0001). Ten patients included in the CHANCE cohort did not fulfill the criteria, and were excluded, because of the absence of working memory deficits or psychiatric symptoms (n=6), or because of longer duration

of symptoms (n=4). These ten children had: epilepsy without additional symptoms (n=4), psychiatric disorders (n=3), mild encephalopathy with reversible lesion in the splenium (MERS; n=1), Niemann-Pick disease type C (n=1), or Rasmussen encephalitis without epilepsy (n=1).

Table 2. Demographical data and comparisons between groups

	AIE (n=21)	ADEM (n=32)	CHANCE (n=60)	p-value
Gender, M (%)	5 (24%)	19 (59%)	22 (37%)	0.023*
Onset age, median (IQR; range)	14 (8-16; 3-18)	4 (2-6; 1-16)	9 (5-13; 0-17)	<0.0001*
Prodromal symptoms	13 (62%)	24 (75%)	36 (60%)	0.16
Seizures	13 (62%)	2/31 (6 %)	22 (37%)	<0.0001*
Immunotherapy	21 (100%)	31 (97%)	32 (53%)	<0.0001*
CSF				
Pleocytosis	14/19 (74%)	23/27 (85%)	9/49 (18%)	<0.0001*
Protein	1/18 (6 %)	3/24 (13%)	5/46 (11%)	0.76
OCB	2/3 (66%)	10/26 (38%)	2/24 (8%)	0.014

*p-value < 0.05

Abbreviations: AIE= autoimmune encephalitis, ADEM= acute disseminated encephalomyelitis, CHANCE= Children's Autoimmunity Related to Neuropsychiatric symptoms, Chorea and Epilepsy, IQR = interquartile range, CSF= cerebrospinal fluid, OCB= oligoclonal bands.

Of the 103 children shown in the flowchart, one child had definite limbic encephalitis according to the criteria. This was a nine-year old boy who presented with tonic-clonic seizures originating in the left temporal lobe, followed by refractory status epilepticus. He was treated with valproic acid, midazolam and phenytoin. After status epilepticus he developed faciobrachial dystonic seizures (FBDS)[16] and hyperactive behavior. He was considered to have anti-LGI1 encephalitis, later confirmed in his serum and CSF. He was treated with intravenous methylprednisolone (ivMP). Because of ongoing FBDS he was treated again with ivMP and additionally with myophenolate mofetil, which led to seizure freedom and complete recovery.

The brain MRI showed demyelinating features in 34 children (33%). In all these children encephalopathy and other symptoms appeared reversible. In 22/34 children the brain MRI was repeated, and in none of them new lesions were visible. These children were diagnosed as definite ADEM (22/103; 21%). In 31/34 myelin oligodendrocyte glycoprotein (MOG) antibodies were tested. Twelve of thirty-one (39%) ADEM children were MOG positive, four children had a relapsing disease course (ADEM-optic neuritis [N=3], and multiphasic demyelinating encephalomyelitis [n=1]).

Fourteen of the 68 remaining patients fulfilled the criteria of probable anti-NMDAR encephalitis, of whom 11 had NMDAR antibodies, while the other three had no NMDAR antibodies (Table 3). Of the children with anti-NDMAR encephalitis, 8 children had an idiopathic etiology and 3 children recently had HSVE.

Nine additional patients had neuronal antibodies, without fulfilling the criteria of probable anti-NMDAR encephalitis. Eight turned out to have definite anti-NMDAR encephalitis, of which four with an idiopathic etiology (50%) but with less symptoms, three post-HSVE

(38%), and one girl had an ovarian teratoma triggering the antibody production (13%). The other patient was a 17-year old girl with anti-acetylcholine receptor (anti-AChR) positive bulbar myasthenia gravis and a thymoma, which was surgically removed. She developed severe memory problems and mood changes within days, accompanied by clinical signs of polyneuropathy. Laboratory results showed AMPAR antibodies in her serum and CSF, and an elevated anti-CV2 titer in serum (>12800). She was treated with ivMP and intravenous immunoglobulins (IVIg) resolving both the encephalitis and polyneuropathy.
No patient had Bickerstaff's brainstem encephalitis. All remaining 48 children with possible AIE were tested for TPO antibodies. Six out of 48 children had an increased anti-TPO titer, of whom two met the criteria for Hashimoto encephalopathy. One of the other four patients (Table 4) had diabetes mellitus type 1 and co-occurrence of low-titer anti-Glutamic Acid Decarboxylase 65 (anti-GAD65), considered clinically irrelevant.

Follow-up etiology of patients with possible autoimmune encephalitis

Nine of the 46 children (20%) were diagnosed by their treating physician with seronegative or probable AIE, while none of these children fulfilled the criteria of seronegative AIE. Four of these nine children had a pleocytosis in CSF, but no MRI abnormalities in the mesial temporal lobe. In the other five children brain MRI and white blood cell count in CSF were normal. However, complete CSF analysis, including IgG index and oligoclonal bands was not performed.
Concerning follow-up etiology based on treating physicians' diagnosis, these 46 children had: 1) a possible AE/inflammatory etiology (n=27), 2) no support for AE/inflammatory and another etiology (n=9), 3) no support for AIE /inflammatory and unknown etiology (n=10). After revising the data, in six children (22%) initially considered as possible AE/inflammatory, no support for an AI/inflammatory etiology was found.

Table 3. Patients without NMDAR antibodies fulfilling the criteria "probable anti-NMDAR encephalitis"

Gender, onset age	Clinical presentation and ancillary testing	Criteria	Follow-up etiology after revision
M,15	Flu, followed by confusion, hemiparesis and aphasia, somnolence, and tonic-clonic seizures. anti-TPO increased 1920 IE/ml, and subclinical hypothyreoidism. FU: improvement after ivMP. Treated with levothyroxine. The disease relapsed 2 months later (status epilepticus), again treated with ivMP.	-Abnormal behavior -Speech dysfunction -Seizures -Decreased level of consciousness -EEG: diffuse slowing	Probable AI (Hashimoto encephalopathy)
F,5	Flu, followed by confusion, aphasia, focal seizures, decreased consciousness, memory impairment, status epilepticus. FU: two months seizure-free after ivMP and IVIg, followed by drug-resistant focal seizures.	-Abnormal behavior -Speech dysfunction -Seizures -Decreased level of consciousness -EEG: diffuse slowing -MRI: hyperintensities on T2/FLAIR bilateral in insula, external and extreme capsule	Possible AI/ inflammatory
F,3	Flu, recurrent tonic posture of body and discomfort, developmental regression, dysarthria, ataxia, somnolence, chorea. FU: stabilization after ivMP (repeated twice). Improvement in months.	-Abnormal behavior -Speech dysfunction -Decreased level of consciousness -Movement disorder -EEG: diffuse slowing -CSF: pleocytosis	Possible AI/ inflammatory

Abbreviations: FU= follow-up, ivMP= intravenous methylprednisolone, IVIg= intravenous immunoglobulins, TPO= thyroid autoantibodies, EEG= electroencephalography, CSF= cerebrospinal fluid, AIE= autoimmune encephalitis.

Table 4. Patients with increased anti-TPO not fulfilling the criteria of Hashimoto encephalopathy

Gender, onset age	Clinical presentation	Laboratory results	Follow-up etiology after revision
F, 16	Malaise and fever, followed by nausea, dysarthria and ataxia.	-IHC: serum and CSF negative -anti-NMDAR negative in CSF -anti-VGKC 141, anti-LGI1 and Caspr2 negative -anti-TPO 57	Possible AI/ inflammatory
M, 9	Somnolence, apathy, fever, progressive facial weakness and respiratory problems.	-IHC: serum and CSF negative -anti-GQ1B neg anti-GlyR negative -anti-TPO 50	Possible AI/ inflammatory
F, 5	Flu, followed by confusion, aphasia, focal seizures, decreased consciousness, memory impairment, and status epilepticus.	-IHC: serum and CSF negative -anti-NMDAR negative in CSF -anti-TPO 35	Possible AI/ inflammatory
F,5	History of DM 1. Malaise, followed by recurrent attacks of delayed response, sometimes accompanied by non-rhythmic upper limb movements.	-IHC: serum and CSF negative -anti-NMDAR negative in serum and CSF -anti-GAD65 364 IU/ml -anti-TPO 454 IU/ml, but no thyroid dysfunction	No support for AI, other etiology#

#Attacks related to DM1 associated hypoglycemia.
Abbreviations: DM1= diabetes mellitus type 1, IHC= immunohistochemistry, CSF= cerebrospinal fluid, GAD65= Glutamic Acid Decarboxylase 65, TPO= thyroid autoantibodies, NMDAR= N-Methyl-D-Aspartate Receptor.

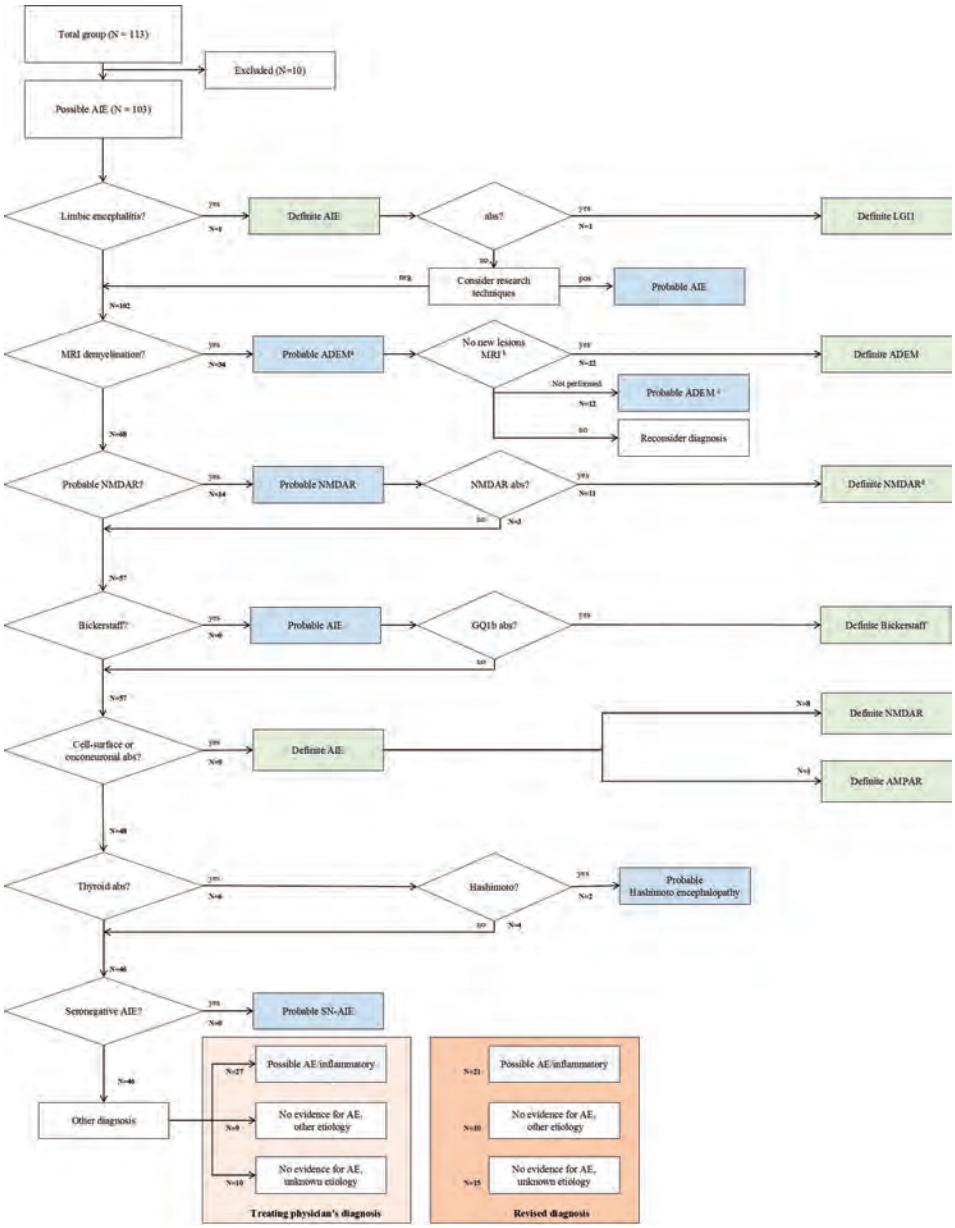

Figure 1. Flowchart showing the validation of the diagnostic criteria of autoimmune encephalitis.

[a] According to the IPMSSG criteria.

[b] In 21 of the 22 patients without new lesions on the second MRI anti-MOG was tested, in 8/21 antibodies were present (38%).

[c] In 10 of the 12 patients that had no follow-up MRI anti-MOG was tested, of them 40% (n=4) tested positive.

[d] Of whom 8 had an idiopathic etiology, and 3 recently had herpes simplex virus encephalitis.

In blue: probable diagnosis, first-line immunotherapy can be started. In green: definite diagnosis.

Abbreviations: AIE= autoimmune encephalitis, AE= autoimmune etiology, LGI1= leucine-rich glioma-inactivated protein 1, MRI= magnetic resonance imaging, ADEM= acute disseminated encephalomyelitis, NMDAR= N-Methyl-D-Aspartate Receptor, GQ1b= Ganglioside Q1b, AMPAR= α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, SN-AIE= seronegative autoimmune encephalitis.

Differential diagnosis of possible autoimmune encephalitis

In Table 5, exemplary cases of the included patients with autoimmune encephalitis and with other diagnoses are shown. These cases show overlapping features, which may suggest AIE, but also signs and symptoms pointing toward another diagnosis.

Table 5. Mimics of autoimmune encephalitis in children[27]

Sydenham's chorea	Boy (16) presented with *chorea* and *nocturnal agitation* after laryngitis weeks. Ancillary testing was normal, while anti-DNase titer was increased (298 IE/ml). Cardiac ultrasound showed mitral valve regurgitation. He was treated with ivMP and penicillin.
Neuropsychiatric systemic lupus erythematosus	Girl (16) with anti-ANA, anti-ENA, anti-RNP and anti-dsDNA positive polyarthritis and neutropenia, had *memory problems and behavioral changes*, followed by hypersomnia *and reduced awareness*. She was suspected to have bacterial meningitis. CSF showed *a mild pleocytosis*. Because of negative cultures she was treated with ivMP, oral steroids and mycophenolate mofetil. She recovered after ivMP. The disease relapsed two years later.
Rasmussen encephalitis	Boy (6) developed *daily focal seizures* with impaired awareness and behavior arrest. He had over 20 focal seizures a day, refractory to antiepileptic treatment, combined with *aggressive behavior*. His MRI showed lesions in the right hemisphere and atrophy. CSF analysis was normal. He was treated with ivMP, and responded. He had a hemispherectomy six months later because of recurrent seizures, and he is seizure-free since. Pathology examination showed infiltration of T lymphocytes and cavitation in the cortex.
PANDAS	Girl (10) developed *a complex motor tic disorder* and *childish behavior* with overnight explosion one month after laryngitis. Ancillary testing showed an increased anti-AST titer (400 IE/ml), and positive throat culture for streptococ. She was treated with IVIg, which reduced the tics only moderately. She was treated with clonidine and with amoxicilline for five years. Her tics improved, but worsen during illnesses.
Klein Levine syndrome	Girl (16) fell of a horse. Three days later she developed *disinhibited behavior* and hypersomnolence. MRI and CSF analysis were normal. She recovered within 5 weeks without treatment. Seven months later she had a comparable, episode after a cold, again symptoms were reversible.
Narcolepsia	Girl (9) developed excessive daytime sleepiness, *emotional behavior, irritability*, and collapses while being emotional, after scarlet fever. MRI and CSF analysis were normal. Hypocretin-1 was absent is CSF, and a multi-sleep latency test showed severe daytime sleepiness. She was treated with sodium oxybate and is doing well since.
Gilles de la Tourette	Boy (10) developed *motor facial tics* and vocal tics, *hyperactive behavior and irritability*. Brain MRI was normal. He was not treated, and has a stable disease, but tics worsen during illness.
Hashimoto encephalopathy	Boy (15), had *acute confusion*, aphasia, central facial palsy, and a right sided sensory disorder. He was treated with thrombolysis because of suspicion of cerebral ischemia. MRI was normal and showed no abnormalities on DWI. He developed *focal seizures and status epilepticus*. EEG showed sharp activity parieto-temporo-occipital. He was treated with ivMP because of an increased anti-TPO titer (1920 IE/ml) and subclinical hypothyroidism. He recovered completely, but disease relapsed 2 months later.

Italic: signs or symptoms that can point towards autoimmune encephalitis.
Underlined: signs and symptoms contributing to another diagnosis.
Abbreviations: MRI=magnetic resonance imaging, EEG= electroencephalography, CSF= cerebrospinal fluid, ivMP= intravenous methylprednisolone, DWI= diffusion-weighted imaging, AEDs= antiepileptic drugs, PANDAS= Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections, IVIg= intravenous immunoglobulins, TPO= thyroid autoantibodies, ADEM= acute disseminated encephalomyelitis, MOG= myelin oligodendrocyte glycoprotein, NMDAR= N-Methyl-D-Aspartate Receptor, AMPAR= α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, LGI1= leucine-rich glioma-inactivated protein 1.

DISCUSSION

This prospective observational cohort study shows that besides anti-NMDAR encephalitis and ADEM, the prevalence of other autoimmune encephalitides is very low in children. Furthermore, we describe that these autoimmune disorders show a stable incidence over the last four years. In addition, this study validates the criteria currently used to diagnose autoimmune encephalitis and shows their usefulness to detect paediatric antibody-mediated AIE, ADEM, and Hashimoto's encephalopathy in an early stage. In our cohort a substantial number of children were diagnosed and treated as having an autoimmune/inflammatory etiology of their neurological symptoms, while in more than 20% of them the support for autoimmunity or inflammation was lacking.

The vast majority of children with definite AIE described in this cohort had anti-NMDAR encephalitis, while only two children had other neuronal antibodies (anti-LGI1 and anti-AMPAR). These antibodies have been described only sporadically in paediatric cases,[17] next to other neuronal antibodies, including anti-$GABA_BR$,[7] anti-$GABA_AR$,[6] anti-GlyR,[18] and anti-GAD65.[19] The high prevalence of anti-NMDAR encephalitis in children compared to the very low prevalence of other antibodies is largely explained by epidemiological factors. In our cohort, in more than 40% of the children with anti-NMDAR encephalitis antibody production was triggered by HSVE or an ovarian teratoma, both occurring more in children and young adults.[1, 20] The other antibody-mediated AIE syndromes are not associated with these factors, and are usually idiopathic or associated with malignancy, not occurring in childhood.[11]

No neuronal antibodies were identified in our prospectively collected cohort of children with possible autoimmune encephalitis (CHANCE cohort), while others identified neuronal antibodies in 4-10% of children with selected neurological symptoms or syndromes (i.e. epilepsy,[21] demyelinating disorders[22]). However, pathogenicity of most of the detected antibodies in these studies is unproven, including double-negative voltage-gated potassium channel antibodies (anti-VGKC, without anti-LGI1 or anti-Caspr2) and low-titer anti-GAD65.[23-25]

We describe that the current guideline to diagnose autoimmune encephalitis is of additional value to correctly diagnose autoimmune related neurological conditions in children. One of the most important panels in the current guideline is 'probable anti-NMDAR encephalitis.' If children fulfil these criteria, immunotherapy can be started before definite antibody diagnosis. In our cohort, almost 70% of children with anti-NMDAR encephalitis with an idiopathic etiology could be identified by the use of these criteria, while 50% of post-HSVE anti-NMDAR encephalitis children fulfilled the criteria of 'probable anti-NMDAR encephalitis.' As the criteria were meant to identify patients for initiation of treatment before antibody results are available, the identification of 70% of idiopathic patients is relevant and important. The criteria are less important in post-HSVE anti-NMDAR encephalitis as in most children with deterioration of symptoms promptly leads to NMDAR antibodies testing

because of increased knowledge of this syndrome.[20] One third of children with idiopathic or paraneoplastic anti-NMDAR encephalitis did not fulfilled the criteria of probable anti-NMDAR encephalitis, these children had less symptoms, and most of them had milder disease courses than the ones who did fulfilled the criteria. This findings emphasis the importance of also considering this disease in children with unexplained neuropsychiatric disorders without many additional signs.

An important difficulty broached in this study was that in one fifth of the children diagnosed with an autoimmune or inflammatory etiology, no support for autoimmunity or inflammation was found. In many of these children improvement after immunotherapy was considered as a criterion favouring autoimmunity. An unjustified conclusion, because many diseases can (temporarily) respond to immunotherapy, or the observed response may even be the natural course of the disease.[26] However, there will always be a small level of uncertainty, which makes it even more important to perform complete work-up in these children; MRI and CSF analysis, including IgG index and oligoclonal bands. In the diagnosis of these syndromes, it is important to look for signs and symptoms favouring autoimmunity or inflammation, but the differential diagnosis of possible autoimmune encephalitis is broad, and other causes should also be considered, especially in children with aspecific signs.

This study was limited because of the number of patients included. However, it is the first nationwide study describing annual incidence of paediatric antibody-mediated AIE. In the CHANCE cohort coverage was well, but there was no nationwide coverage, and children may have been selected towards an autoimmune etiology, as samples of patients with a higher suspicion for AE are often referred to our center for antibody testing. Another limitation is that in most patients CSF analysis was incomplete, oligoclonal bands and IgG index were often lacking. Occasionally this resulted in difficulties to adequately revise diagnosis. In doubt, we preferred to be cautious by diagnosing children with an autoimmune of inflammatory disorder, because of the therapeutic and prognostic implications.

From this study we can conclude that autoimmune encephalitis seems to be recognized properly in children. The majority of children have anti-NMDAR encephalitis or ADEM, while other AIE syndromes occur only sporadically in children. The current guideline to diagnose AIE syndromes seems to be a useful tool to detect children with an autoimmune etiology of neurological symptoms. However, especially in children not fulfilling any of the current guideline panels, it is important to be critical before diagnosing them as having an autoimmune or inflammatory etiology of their neurological symptoms. In addition it is essential to perform complete diagnostic work-up, and to consult specialized autoimmune/inflammatory tertiary centers if in doubt.

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Chapter 8

Long-term neuropsychological outcome following pediatric anti-NMDAR encephalitis

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ABSTRACT

Objectives

To provide detailed long-term outcome data of children and adolescents following pediatric anti-NMDAR encephalitis, to identify neuropsychological impairments, and to evaluate the influence of these factors on quality of life (QoL).

Methods

All Dutch children diagnosed with anti-NMDAR encephalitis were identified. Patients currently 4 years or older were included in the follow-up study, consisting of a visit to our clinic for a detailed interview and a standardized neuropsychological assessment. The following domains were included: attention, memory, language, executive functioning, QoL and fatigue. Primary outcome measures were *z* scores on sustained attention, long-term verbal memory, QoL, fatigue, and working memory.

Results

Twenty-eight patients were included. The median Pediatric Cerebral Performance Category (PCPC) at last visit was 1 (interquartile range 1-2, range 1-4) and 64% (18/28) of patients returned consistently to their previous school level. Twenty-two patients were included in the cross-sectional part of the long-term follow-up study. Median follow-up time was 31 months (interquartile range 15-49, range 5-91). There were problems with sustained attention (z = -2.10, 95%-CI= -2.71 to -1.46, p<0.0001), and fatigue (z = -0.96, 95%-CI= -1.64 to -0.28, p=0.008). Cognitive deficits were not correlated with QoL, while fatigue was strongly correlated with QoL (r =0.82, p<0.0001).

Conclusions

Although follow-up is often reported good following pediatric anti-NMDAR encephalitis, many patients have cognitive problems and fatigue, even up until adolescence resulting in academic achievement problems and lower QoL. For physicians it is essential to be aware of these problems, to provide valuable advice to patients and caregivers in the acute and follow-up phase, and to consider early neuropsychological counseling.

INTRODUCTION

Anti-N-methyl-D-Aspartate Receptor (anti-NMDAR) encephalitis is an autoimmune disorder, initially described in 2007.[1] Increased awareness has led to more frequent diagnoses and currently more than 1000 patients have been reported, of whom 35% are children.[2] The disease course can be severe with intensive care unit (ICU) admission in 75% of children. Nevertheless, if treated with adequate immunotherapy, outcome is considered favorable in 85% of children.[2]

However, there are signals that actual recovery might be less positive than initially reported. Small studies in both adults and children describe substantial deficits in multiple cognitive domains and also behavioral problems.[3-9] Given these findings, it seems that despite apparent good outcome full neuropsychological recovery is certainly not always achieved.

Functioning can be studied from different perspectives[10] including, activities and participation. Outcome of anti-NMDAR encephalitis is currently measured in terms of activities with relatively crude measures, such as the modified Rankin Scale (mRS),[11] while participation and QoL are also of major importance especially in children and adolescents. Neuropsychological deficits can seriously affect participation and career choices as transition into adulthood might call for full cognitive abilities.

Therefore, the aim of this nationwide Dutch cohort study was to provide more insight into long-term outcome following pediatric anti-NMDAR encephalitis, with special emphasis on neuropsychological outcome, and to evaluate whether these neuropsychological factors influence QoL.

METHODS

Patients

The Departments of Neurology and Pediatric Neurology of the Erasmus MC University Medical Center - Sophia Children's Hospital, Rotterdam, the Netherlands, are national referral sites for patients with suspected autoimmune encephalitis. In addition, the Department of Immunology is the national referral site for antineuronal antibody testing of samples from patients with suspected autoimmune encephalitis. Therefore, we had the opportunity to identify all Dutch children diagnosed with anti-NMDAR encephalitis, from January 2008 until March 2017, aged 0 to 18 years at disease onset. NMDAR antibodies were confirmed in serum and/or CSF by both commercial cell-based assay and immunohistochemistry.

Clinical information

Data about disease course were obtained from medical records and from detailed interviews with patients and caregivers during a visit to our clinic. Neurological level of

function was determined using the Pediatric Cerebral Performance Category (PCPC) scale (Supplementary Table 1).[12]

Standard protocol approvals, registrations, and patient consents

The institutional review board of the Erasmus MC University Medical Center approved the study protocol. Informed consent was obtained from adult patients and for children from their parents, and if applicable, also from children aged 12 to 18 years.

Cross-sectional follow-up study

All patients currently aged 4 years or older were approached to participate in the follow-up study, as neuropsychological testing and the questionnaires required a minimal age for participation. Patients were invited for a visit to our clinic, in which current complaints and level of functioning were discussed. In addition, patients underwent a standardized neuropsychological assessment. If a visit was not possible, current problems were discussed by phone and questionnaires were sent to us by mail and checked in additional calls if necessary.

Neuropsychological assessment

The neuropsychological assessment consisted of a selection of the Cambridge Neuropsychological Test Automated Battery (CANTAB Research Suite 6.0, Cambridge Cognition Ltd., Cambridge, UK), additional neuropsychological tests and questionnaires (Supplementary Table 2). Tests and questionnaires were selected based on own experiences and on disorders found in prior studies, were administrated in their Dutch versions, and are reliable and validated in the Netherlands. The tests and questionnaires were administered to assess skills in 6 domains:

1. Attention: Reaction Time (CANTAB), Dutch Dot Cancellation Test (Bourdon-Vos).[13]
2. Memory: Paired Associated Learning (CANTAB), Rey Auditory Verbal Learning Test (RAVLT).[14]
3. Language: Boston Naming Test,[15] Token Test.[16]
4. Executive functioning: Intra-extra Dimensional Set Shift, Spatial Span, Stockings of Cambridge (all CANTAB), Word Generation (NEPSY-II [A Developmental Neuropsychological Assessment, Second Edition]),[17] Behavior Rating Inventory of Executive Function questionnaire (BRIEF-Self-report and BRIEF-Adult),[18] Strength and Difficulties Questionnaire (Self-report and parent-proxy report).[19]
5. Quality of Life: Pediatric Quality of Life Inventory 4.0 (PedsQL Self-Report and PedsQL Parent Proxy-Report).[20]
6. Fatigue: PedsQL Multidimensional Fatigue Scale questionnaire (PedsQL-MFS Self-Report and PedsQL-MFS Parent Proxy-Report).[21]

Statistical analysis

For group comparisons we used the Mann-Whitney *U* test (age), the Fisher exact (sex, immunotherapy), Fisher-Freeman-Halton extension (PCPC), and the Kruskal Wallis one-way analysis of variance (character profiles). Results of neuropsychological assessments were compared with normative data of healthy individuals, corrected for age, sex and educational level. Normative data for the CANTAB were obtained by CANTAB, Cambridge, UK. Scores were converted into standardized *z* scores for comparison. For statistics, *z* scores were set on minimum of -3 and maximum of +3 to prevent statistical differences by outliers (winsorization). In the graphs, the uncorrected *z* scores are shown, but corrected *z* scores were used for statistics. Displayed correlations were also calculated with corrected *z* scores. The *z* scores were analyzed using a one-sample *t* test (test value = 0). Primary outcome measures were sustained attention (Dutch Dot Cancellation Test-attention fluctuations), long-term verbal memory (RAVLT-delayed recall), fatigue (PedsQL-MFS Self-Report – total score), QoL (PedsQL Self-Report – total score), and working memory (BRIEF – Self-Report – working memory). Primary outcome measures were considered significant if $p < 0.017$ (Bonferroni). For the secondary outcome measures of the neuropsychological assessment p values < 0.005 were considered significant. Values between 0.005 and 0.05 should be interpreted carefully and considered exploratory. The relationship between our primary outcome measures and QoL were computed with a two-sided Pearson correlation coefficient. SPSS version 21.0 (IBM Corp., Armonk, NY) was used for statistical analyses, as well as GraphPad Prism 7 for Windows (GraphPad Software, La Jolla, CA) for Windows.

RESULTS

Clinical characteristics

Thirty children were identified, of whom 28 were included (for patient selection, see Figure 1). Twenty-one patients were female (75%), mainly in those aged 12 years or older (89%). Median age of onset was 14 years. Eighteen patients (64%) reported a prodromal phase, including headache, blurred vision or upper respiratory infection. Three children (11%) developed anti-NMDAR encephalitis 3 to 7 weeks after a herpes simplex virus (HSV) type 1 encephalitis. In addition to those three, one patient had a preexistent mild psychomotor developmental delay. The others were healthy before disease onset.

Most children presented with behavioral disorders (36%), or seizures (36%), less frequently with speech disorders and movement disorders. In 2 of 28 patients (7%), hemiparesis was the presenting symptom, only occurring in children younger than 12 years (Figure 2A). All patients presented to the initial physician with a maximum of three symptoms, while at maximum disease severity 21 patients had developed more than 4 symptoms (Figure 2, B and C). The numbers of symptoms between treatment and diagnosis were often comparable. Four patients developed one additional symptom after start of treatment; i.e. hypoventilation

(n=3), and bradycardia (n=1). One patient developed seizures after diagnosis but before treatment, with a delay between diagnosis and treatment of 2 days (patient 16). One patient developed seizures 3 days after diagnosis and 9 days after initiation of treatment (patient 9). Median time from symptom onset to maximum PCPC (maximum disease severity) was 30 days. Forty-six percent of patients (13/28) were treated in the ICU with a median stay of 13 days. Total hospital stay was more than a month in 78% of patients. All patients were treated with first-line immunotherapy. Forty-six percent of patients received either rituximab (n=12) or cyclophosphamide (n=1). In 14 of 28 patients (50%), treatment was started before diagnosis, in 6 of 28 patients (21%), treatment was initiated on the day of diagnosis and in 8 of 28 patients (29%), treatment was started after diagnosis. For all clinical characteristics see Table 1, and supplementary data.

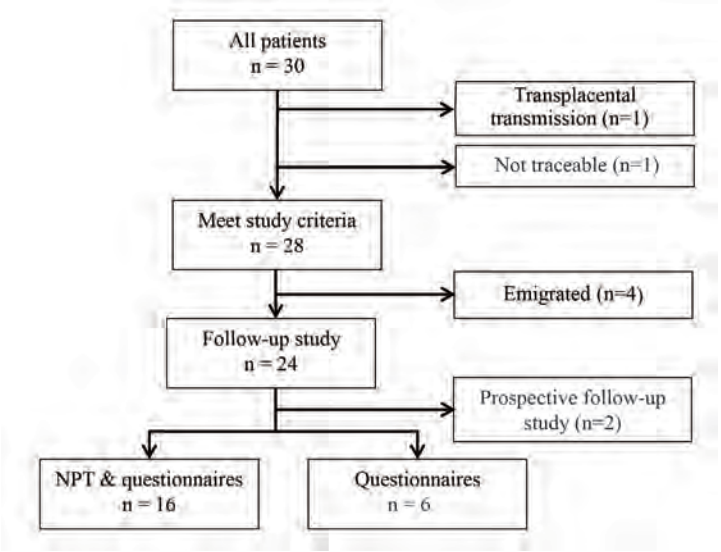

Figure 1. Flowchart of patient selection. One patient was excluded because he was younger than 4 years (transplacental transmission of anti-NMDAR),[31] and one patient was untraceable. Twenty-four patients participated in the follow-up study, of whom 2 are followed prospectively. Sixteen of the 22 participants completed the full neuropsychological assessment, 6 patients only completed the questionnaires, 3 visited our clinic and 3 were contacted by phone due to geographical distance. NPT = neuropsychological testing.

Table 1. Patient characteristics

Sex, feM	21/28	(75)
Age < 12 y	4 / 9	(44)
Age ≥ 12 y	17/19	(89)
Age at onset, y	14	(7-17; 1-17)
Prodromal phase	18/28	(64)
Days to start of treatment	21	(9-65; 3-510)
Days to antibody diagnosis	27	(13-61; 13-184)
Days to maximum disease severity	30	(15-43; 2-94)
Maximum PCPC		
3: Moderate disability	1/28	(4)
4: Severe disability	16/28	(57)
5: Coma/vegetative state	11/28	(39)
ICU stay, d	13	(4-34; 1-45)
Hospital stay, d	55	(33-67; 3-141)
MRI abnormal[a]	10/27	(37)
CSF abnormal[a]	21/27	(78)
EEG abnormal at presentation[a]	26/27	(96)
Ovarian teratoma suspected	4/21	(19)[b]
First-line IT	28/28	(100)
Methylprednisolone	27/28	(96)
Plasmapheresis	6/28	(21)
Immunoglobulins	21/28	(75)
Interval first- and second-line IT, d	18	(14-41; 6-200)
Second-line IT	13/28	(46)
Rituximab	12/28	(43)
Cyclophosphamide	1/28	(4)
Cell-based assay anti-NMDAR serum[a,c]	16/24	(67)
Cell-based assay anti-NMDAR CSF[a]	27/27	(100)

Abbreviations: anti-NMDAR = anti-N-methyl-D-aspartate receptor; ICU= intensive care unit; IT=immunotherapy; PCPC= pediatric cerebral performance category.
Data are n/n (%) or median (interquartile range; range).
[a] Additional details are shown online.
[b] For girls ≥ 12 years: 4/19 (21%). All 4 girls underwent resection; three had a teratoma, one a follicle cyst.
[c] In one patient, only serum was available; cell-based assay, immunohistochemistry and live neurons were all positive.

Outcome

Three patients had a relapse, 3, 5, and 35 months after first symptoms. One patient had a higher PCPC during the relapse than during the initial disease episode, leading to the initiation of rituximab. At hospital discharge, the median PCPC was 3 (interquartile range 2-3, range 1-4). Seventeen patients were discharged home, although 10 concurrently started with an outpatient rehabilitation program. Eleven patients (39%) were transferred directly to an inpatient rehabilitation center. Median rehabilitation time was 98 days (IQR 58-194, range 34-578). The median PCPC at last visit was 1 (IQR 1-2, range 1-4). Twenty-six patients (93%) resumed school after admission or rehabilitation. In 6 of the 26 patients who resumed school (23%) the current education level was lower, including 5 patients with special educational needs. During follow-up, 3 patients stopped school prematurely because of fatigue (n=2) or anxiety (n=1). Overall, 18 of 28 patients (64%) returned consistently to their previous school level.

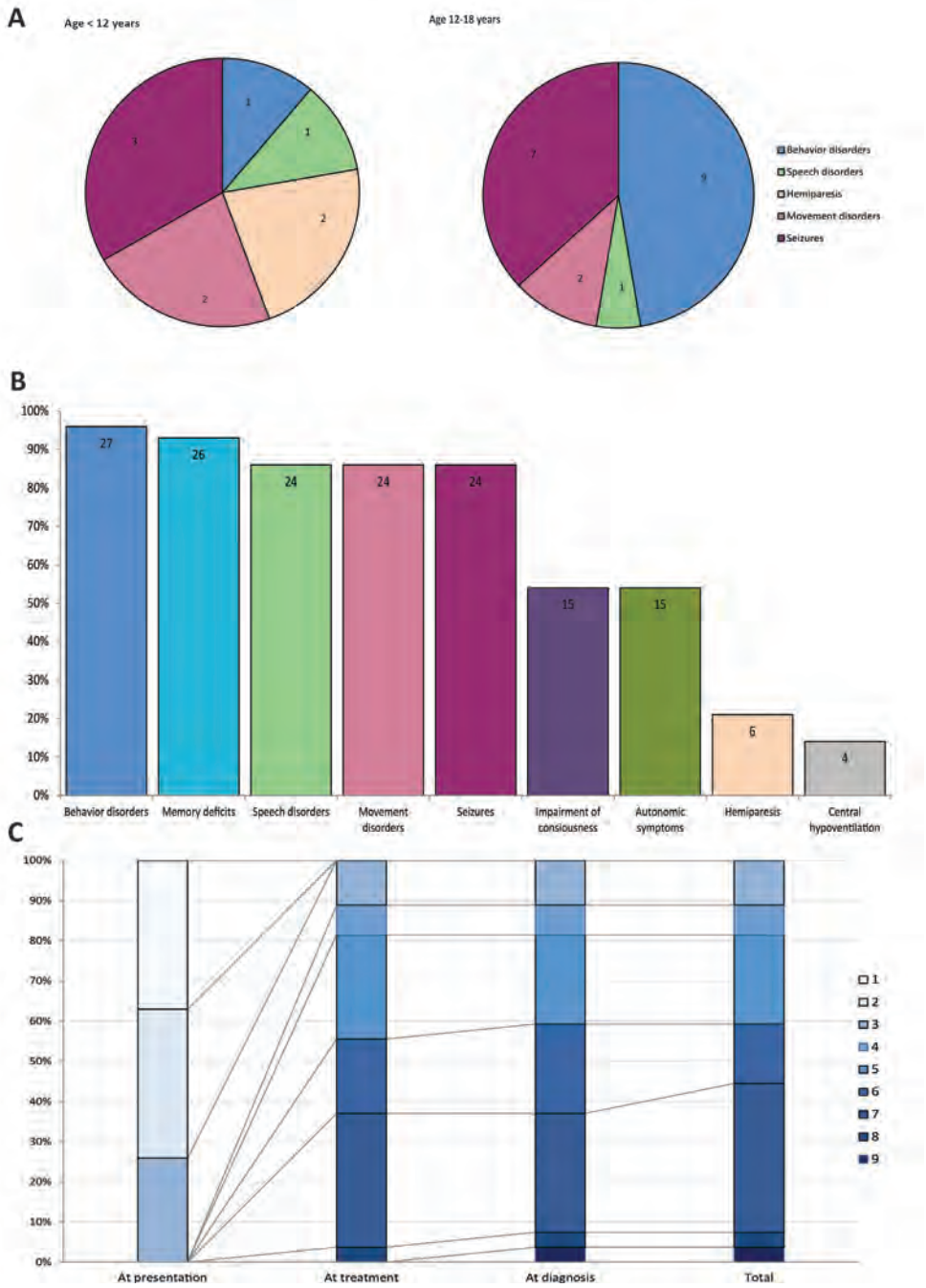

Figure 2. Patient symptoms. (A) Distribution of presenting symptoms of patients younger than 12 years and of patients aged 12-18 years. (B) Cumulative symptoms during disease course. (C) Number of core symptoms at presentation, treatment, antibody diagnosis and total number of symptoms.

Cross-sectional follow-up study

Twenty-two patients participated in the follow-up study, with a median follow-up time after symptom onset of 31 months (IQR 15-49, range 5-91). Nineteen were seen at our clinic, while three had an interview by phone. All 22 patients completed questionnaires, while 16 patients completed the full neuropsychological assessment (figure 1). Individual information is shown online in Supplementary Table 3. Median age at last visit was 17 years (IQR 12-19, range 4-25). Three patients had post-HSV encephalitis anti-NMDAR encephalitis, 2 with a follow-up PCPC of 3 (patient 14 and 18) and 1 with a PCPC of 4 (patient 19). One patient had a PCPC of 4 because of spasticity and vocal cord paralysis (patient 6).

Neuropsychological outcome

Characteristics of the 16 patients that underwent full neuropsychological assessment were similar to those of the other patients (n=13; Supplementary Table 4). Patients had lower sustained attention scores (z = -2.10, $p_{uncorrected}$<0.0001; table 2), and these were consistent among almost all patients. The mean score on long-term verbal memory tended to be lower (z = -0.68, $p_{uncorrected}$=0.031). Patients reported more fatigue (z = -0.96, $p_{uncorrected}$= 0.008) and QoL tended to be lower (z = -0.87, $p_{uncorrected}$= 0.032), while working memory was not different (z = 0.24, $p_{uncorrected}$ = 0.23). Results were similar when the 3 patients with anti-NMDAR encephalitis post-HSV encephalitis were excluded (1 full neuropsychological assessment, 2 only completed questionnaires; data not shown).

There was a strong correlation between self-reported fatigue and QoL (r= 0.82, p<0.0001; Figure 3), also as reported by parents (Parent Proxy-Report – total score; r= 0.70, p = 0.004). There were no significant correlations between QoL and fatigue and the cognitive domains sustained attention and long-term verbal memory (Figure 3). Treatment delay, follow-up time, age of onset, ICU stay, maximum PCPC, and PCPC at follow-up were not correlated with sustained attention, long-term verbal memory, or fatigue (Supplementary Figure 1). Sustained attention and long-term verbal memory were also not correlated with QoL scores as reported by parents (sustained attention: r=0.20, p=0.62; long-term verbal memory: r=0.45, p=0.27).

Among the secondary outcome measures (Supplementary Table 5 and 6), the mean z score on the domain speed was lower (Dutch Dot Cancellation Test – reaction time; z = -1,53, $p_{uncorrected}$=0.002). Scores on the domains visual memory (Paired Associated Learning – total errors; z = -0.90, $p_{uncorrected}$=0.016), short-term verbal memory (RAVLT – Trials 1-5); z = -0.76, $p_{uncorrected}$=0.023) and naming (Boston Naming Test – total score; z = -0.78, $p_{uncorrected}$=0.019) were low, but between 0.05 and 0.005. Results of the questionnaires completed by parents were comparable to those of children (Supplementary Table 7).

Patients and parents mentioned similar difficulties in the detailed interview (17/22). Regarding school or work performance the most notable problems were word finding difficulties (24%), dyslexia (12%), and attention and concentration deficits (18%). Other problems were impulsiveness (18%), anxiety (18%), and indecisiveness (12%). Concerning the disease period, 21 of 22 patients (95%) had a persistent (fragmented or complete) amnesia.

Based on our own observations during the visits to our clinics, we could differentiate three frontal lobe syndrome profiles, using the character descriptions by parents and the main complaints of the patients themselves. This way, we allocated the patients visiting our clinic, into three groups: (1) passive (apathy, n=5), (2) moderate (no signs of a frontal lobe syndrome, n=6), and (3) active (impulsive, n=7). The median scores on QoL and fatigue were compared between these groups (visualized in Supplementary Figure 2). Among the passive patients the school drop-out rate was 80% (4/5), while for the active patients, school resumption was achieved in all 7, of whom 2 did not retain previous school level.

Table 2. Results of primary outcome measures

Domain; test; measure	No.	z Score, mean	95% CI	z Score < 0, n (%)	z Score <-2, n (%)	P Value
Sustained attention; DDCT; Attention fluctuations	16	-2.10	-2.71 to -1.48	15 (94)	10 (63)	<0.0001*
Long-term verbal memory; RAVLT; Delayed recall	15[a]	-0.68	-1.29 to -0.07	12 (80)	3 (20)	0.031
Fatigue; PedsQL-MFS; Self-report; Total score	21[b]	-0.96	-1.64 to -0.28	16 (76)	5 (24)	0.008*
Quality of life; PedsQL; Self-report; Total score	21[b]	-0.86	-1.64 to -0.08	15 (71)	7 (33)	0.032
Working memory; BRIEF; Working memory	19[b]	0.24	-0.17 to 0.65	8 (42)	0	0.23

Abbreviations: BRIEF = Behavior Rating Inventory of Executive Function; CI = confidence interval; DDCT= Dutch Dot Cancellation Test; MFS= Multidimensional Fatigue Scale; PedsQL= Pediatric Quality of Life; RAVLT= Rey Auditory Verbal Learning Test.

*p < 0.017 (Bonferroni).

[a] Of one patient (no. 6) no data are shown as the test was aborted because of vocal cord paralysis.

[b] No normative data available for test results of the youngest patient(s).

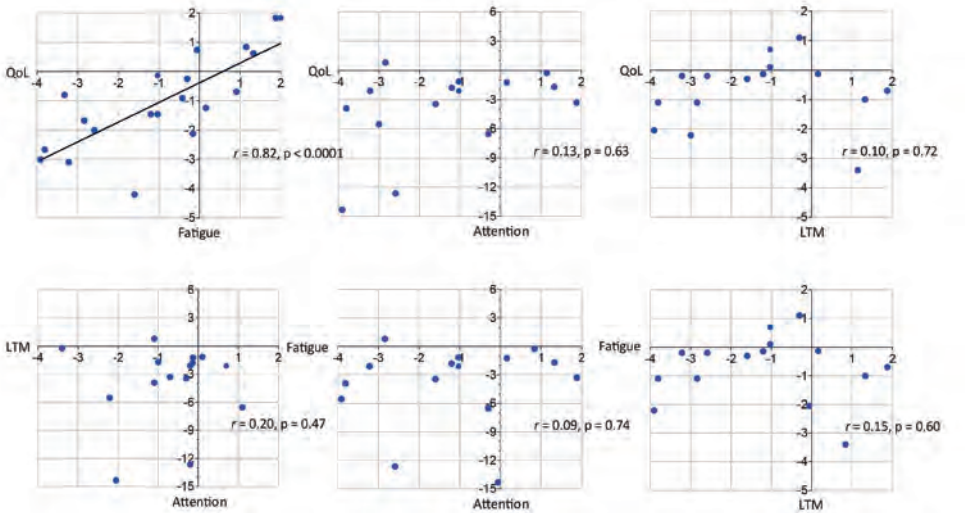

Figure 3. Overview of correlations between primary outcome measures.

Outcome measures: sustained attention (Dutch Dot Cancellation Test- Attention fluctuations), long-term verbal memory (RAVLT-Delayed recall), fatigue (PedsQL-MFS Self-report–Total score), QoL (PedsQL Self-report–Total score), working memory (BRIEF-Working memory). In all graphs, results of uncorrected *z* scores are shown, but the correlations are calculated with corrected *z* scores (maximum 3, minimum -3).

Abbreviations: LTM= long-term verbal memory. Anti-NMDAR = anti-N-methyl-D aspartate receptor; BRIEF = Behavior Rating Inventory of Executive Function; HSV = herpes simplex virus; LTM = longterm verbal memory; MFS = Multidimensional Fatigue Scale; PedsQL = Pediatric Quality of Life; QoL = quality of life; RAVLT = Rey Auditory Verbal Learning Test.

DISCUSSION

We have demonstrated that, despite good functional recovery (according to the mRS or PCPC), persistent cognitive deficits are common in young children and adolescents following pediatric anti-NMDAR encephalitis, and that important parameters for good outcome, such as treatment delay or age of onset, do not specifically affect neuropsychological outcome. Other interesting and important findings are that patients reported more fatigue, and that patients with fatigue also reported a poorer QoL, while poorer cognitive outcome did not affect QoL.

Fatigue has not been evaluated before in patients with anti-NMDAR encephalitis. However, it is known to be a common disabling symptom in pediatric aquired brain injury,[22, 23] making our results that fatigue was associated with poorer QoL plausible. This finding is supported further by the frequent reporting of fatigue by patients as the most disabling symptom often hampering normal participation.

Remarkably, poorer cognitive outcome did not influence QoL, possibly because QoL questionnaires comprise general topics, while patients often reported specific task-related problems, which might be underestimated in current questionnaires. In addition, patients becoming accustomed to a new "stable" situation and reduced awareness might be other explanations. The latter is less likely because parents' QoL scores were comparable.

Predictors of good functional outcome as treatment delay, maximum PCPC, and ICU stay were not correlated with QoL, fatigue or sustained attention. This supports our statement that "good" outcome certainly not always means "good" total recovery. NMDAR antibodies are considered to compromise signal transmission, leading to problems in multiple functional networks, corresponding to the extent of symptoms. Finke et al.[24] showed that a reduced connectivity of the anterior hippocampus and the anterior default mode network was associated with poorer memory in anti-NMDAR encephalitis. In addition, this reduced connectivity is also described in a broad spectrum of other neurological conditions.[25-28] These connections seem most vulnerable, which may explain the discrepancy between good outcome and poor memory recovery. A follow-up study testing patients by serial neuropsychological tests combined with fMRI will be essential to examine the correlation between cognitive functioning and this reduced connectivity over time, and to examine whether this process is reversible.

Most anti-NMDAR encephalitis follow-up studies concentrate on the neurobehavioral problems of disinhibition. However, frontal lobe syndromes are more widespread, and little is known about passive patients during rehabilitation and follow-up. Our data suggest that these "passive" patients might be more at risk to develop problems with normal participation because these patients showed more school drop out and reported more fatigue. This observation needs confirmation in future research, but may have important consequences for rehabilitation programs.

For cognitive outcome, we particularly observed lower scores of the domain sustained attention and speed. Possibly these cognitive deficits are most prominent and should considered during cognitive rehabilitation. However, there was no correlation between the different cognitive test results, which underlines that the occurring cognitive deficits are diverse and probably different parts of the brain are affected. Short-term verbal memory and language scores were also lower. Apparently these domains are more vulnerable to dysfunction of the NMDAR. These findings are partially in concordance with earlier findings,[3-9] although these previous published studies describe more diverse cognitive deficits, with additional deficits in executive functioning. However, these studies are difficult to interpret and to compare properly to our results, because of limited patient numbers, unstandardized methods and because some patients were assessed in the acute disease phase. By using standardized performance-based measures, such as CANTAB, we found no prominent problems in executive functions. Nevertheless, by using rating measures (questionnaires, interviews), patients reported substantial difficulties in performing activities of daily living. An explanation for this disconnection is that performance-based measures and rating measures do not assess the same aspects in cognitive and behavioral functioning. Rating measures assess whether goals in activities of daily living are reached, and have higher ecological validity.[29] Next to the BRIEF (and other rating measures we performed), the BADS-C (Behavior Assessment of the Dysexecutive Syndrome in Children) might be a useful addition.

The present study, with national coverage, detailed description of clinical data and the use of a systematic neuropsychological assessment, provides broad, valuable results, likely to be externally valid. This study exclusively pertains to pediatric anti-NMDAR encephalitis, also a valuable aspect, because in comparison to adults there are differences in disease onset, treatment decisions, and social functioning. First, children present more often with seizures or behavioral changes,[30] whereas adults mostly present with psychiatric symptoms or memory dysfunction,[2] which may lead to different intervals to diagnosis and treatment. Second, treatment decisions can be age-dependent and may affect outcome; i.e., physicians tend to be more aggressive in children, starting immunotherapy early while simultaneously being more careful with cyclophosphamide. Third, neuropsychological problems can seriously affect participation as successful transition into adulthood calls for full cognitive, emotional and behavioral abilities.

We had the unique opportunity to include all Dutch children with anti-NMDAR encephalitis. Nevertheless, despite national coverage and increasing incidence, anti-NMDAR encephalitis is a rare disease. Therefore, to include a sufficient number of patients with a reasonable follow-up time, a retrospective study design was inevitably but with the associated problems. The first issue is missing data. The amount of missing data was minimized by contacting treating physicians, parents, and patients. Regarding selection bias (between patients participating and nonparticipating in the follow-up study), we found no difference in clinical characteristics. Furthermore, clinical characteristics are in line with previous studies.[2, 30] The

results of the participants thus seem to be a good representation for the total group and results are probably generalizable.

Overall, our findings highlight the importance of awareness of persisting neuropsychological deficits and excessive fatigue following pediatric anti-NMDAR encephalitis. With a considerable median follow-up time (almost 3 years) our results clearly indicate that neuropsychological deficits can be prolonged. Currently, disease outcome is assessed with parameters measuring impairment and disabilities (mRS, PCPC), and treatment decisions are based on these parameters. Our results show that neuropsychological parameters measuring participation and QoL are also important and should be considered when assessing outcome, because these factors can substantially affect participation and well-being. Therefore, physicians should inform patients and parents correctly about the occurrence of prolonged neuropsychological problems. In addition, they should provide good accessibility to neuropsychological counseling in rehabilitation centers immediately following the acute disease course and during follow-up.

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SUPPLEMENTARY DATA

Diagnosis

Initial diagnoses were viral encephalitis, psychosis, specific epilepsy syndromes, post-infectious encephalitis, and autoimmune encephalitis. In five patients a first onset psychosis was suspected. Therefore, these five patients were initially admitted to a psychiatry department. Six patients were originally sent home after visiting the emergency department or after a short admission, all presenting with a fist generalized seizure or status epilepticus.

Ancillary testing

At disease onset, brain MRI was normal in 63% of patients. Abnormal findings were: an increased signal on T2 and/or FLAIR weighted images in mesiotemporal regions (n=4), basal ganglia (n=2), or diffuse in cortical regions (n=2), or post-HSVE abnormalities (n=3).
Except for one patient (4%) with a normal EEG at presentation, all other EEGs were abnormal. Most frequently it showed focal slowing (61%), occasionally combined with typical epileptic patterns (18%). In three patients (11%) the first EEG showed a status epilepticus. Only in patients that required ICU treatment the EEG showed an extreme delta brush pattern at presentation.
CSF analysis was done in all patients. In 61% there was mild pleocytosis. Other abnormalities were increased protein or IgG index, and the presence of oligoclonal bands (OCB).
Using commercial CBA, all available CSF samples were positive (n=27) for NMDAR IgG (NR1) antibodies. In16/24 serum samples (67%) antibodies were detected Additionally a specific hippocampal staining pattern was seen in 21/22 serum samples and 27/27 CSF samples.

Additional treatment

Sixty-two percent of patients were treated with anti-epileptic drugs (AEDs). Three of them were treated with > 2 AEDs without seizure freedom during disease course (one with post-HSVE anti-NMDAR encephalitis).

Table 1. Pediatric Cerebral Performance Category[11]

Score	Category	Description
1	Normal	Normal; at age-appropriate level; school-age child attending regular school classroom.
2	Mild disability	Conscious, alert, and able to interact at age-appropriate level; school-age child attending regular school classroom but grade perhaps not appropriate for age; possibility of mild neurological deficit.
3	Moderate disability	Conscious; sufficient cerebral function for age-appropriate independent activities of daily life; school-age child attending special education classroom and/or learning deficit present.
4	Severe disability	Conscious; dependent on others for daily support because of impaired brain function.
5	Coma or vegetative state	Any degree of coma without the presence of all brain death criteria; unaware, even if awake in appearance, without interaction with environment; cerebral unresponsiveness and no evidence of cortex function; possibility of some reflex response, spontaneous eye-opening and sleep-wake cycles.
6	Brain death	Apnea, areflexia, and/or electroencephalographic silence.

Table 2. Description of neuropsychological assessment

Test	Domain	Description
CANTAB		
Spatial span	Executive functions	White squares on the screen briefly change color in a variable sequence. Participants must remember the sequence and touch squares in the same order. The sequence length is growing throughout the test.
Stockings of Cambridge	Executive functions	Three colored balls are displayed on the screen. The participant must move the balls in the lower display to match the arrangement in the upper display.
Intra-extra dimensional set shift	Executive functions	Participants must first use feedback to learn a rule involving two dimensions. When feedback implies that the rule has changed, the participant must shift attention to the previously irrelevant dimension.
Paired associated learning	Visual memory	Boxes are displayed on the screen and automatically opened in a random order and show a number of patterns. Thereafter all patters are consecutively shown in the center of the screen. The participant must select the box where the figure was located.
Reaction time	Attention	The participant must hold down a button until a yellow spot appears on the screen and then touch the yellow spot.
Additional tests		
Dutch dot cancellation test	Speed and sustained attention	Twenty-four dotted figures on 33 lines are shown. The participant must strikethrough certain figures as rapidly as possible.
Rey Auditory Verbal Learning Test (RAVLT)	Verbal memory	Fifteen words are repeated over 5 different trials. Participants must repeat the words every trial, and recall them 30 min later.
Boston naming test	Word finding	The participant must name line drawings.
Word Generation (NEPSY II)	Executive functions	The participant must name as many animals as possible in 60 sec.
Token test	Word comprehension	There are multiple colored circles and squares shown on the table. The participant must perform certain tasks, i.e. 'place the green circle on the red square.'

Abbreviations: CANTAB = Cambridge Neuropsychological Test Automated Battery.

Table 3. **Demographics and follow-up characteristics**

No	Sex, age	Prodromal phase	Symptoms during disease course	Treatment	Max. PCPC	Hospital stay (days)	Age at FU	FU (months)	PCPC FU	Current complaints
1	F, 17	Headache	Hyperactivity, aggression, suspicious, disinhibition , memory disorder, mutism, TCS, bradycardia, hypotension	MP, IvIg Resection OT	4	27	18	15	1	No complaints
2	F, 17	Headache	TCS, focal seizures, hallucinations, cognitive decline	MP, IvIg	3	3	23	69	2	Easily irritated and angry, fatigue, difficulties concentrating
3	F, 17	Headache, nausea	Anxiety, confusion, cognitive decline, focal seizures, mutism, dystonia, indifference	MP	4	67	25	91	2	Unemployed due to fatigue, word finding difficulties, inactive
4	M, 6	Upper respiratory tract symptoms	TCS, status epilepticus, indocile, hallucinations, insomnia, dystonia, catatonia, fluctuations in saturation	MP, IvIg, Rituximab	4	64	8	27	3	Fatigue, tantrums, dyslexia, concentration problems
5	F, 3	Upper respiratory tract symptoms	Hemiparesis, hallucinations, focal seizures, insomnia, cognitive decline, dystonia, chorea, mutism	MP, IvIg, Plex, Rituximab	5	55	6	25	1	Obsessive behavior, irritable, tantrums
6	F, 14	No	Focal seizures, childish behavior, disorientation, mutism, dyskinesia, hyperhidrosis, blood pressure fluctuations, hypoventilation	MP, IvIg, Plex	5	56	16	34	4	Spasticity, vocal cord paralysis, memory deficits
7	F, 17	Blurred vision	Anxiety, decorum loss, aggressive, insomnia, mutism, TCS, catatonia, weight loss, apathy	MP, IvIg	4	103	23	77	1	Attention disorder
8	F, 17	No	Collaps after use of cannabis, mutism, childish behavior, affective disorder, apathy, fever, cognitive decline, TCS	MP, IvIg, Rituximab	4	31	18	10	1	No complaints
9	F, 13	Headache, nausea	More emotional, anxiety, childish behavior, cognitive decline, less spontaneous speech, TCS, catatonia	MP, IvIg Resection OT	4	34	17	45	1	More irritable, sometimes word finding problems, forgetful
10	F, 4	No	Focal seizure, hemiparesis, obsessive behavior, emotional fluctuations, confusion, chorea, insomnia, mutism	MP, IvIg	4	58	10	69	1	Dyslexia, word finding problems
11	F, 9	Headache	More emotional, aggressive, agitated, hallucinations, mutism, cognitive decline, insomnia, catatonia, cardiac arrhythmia, hypoventilation	MP, IvIg, Plex, Rituximab Resection OT	5	111	13	45	1	Word finding difficulties
12	F, 17	No	Anxiety, problems in organization, insomnia, dyskinesia, psychosis, loss of decorum, fever, cognitive decline, mutism, TCS, hypoventilation	MP, IvIg, Plex	5	67	20	34	2	no job because of fatigue, depression

No	Sex, age	Prodromal phase	Symptoms during disease course	Treatment	Max. PCPC	Hospital stay (days)	Age at FU	FU (months)	PCPC FU	Current complaints
13	F, 17	No	Loss of decorum, insomnia, agitation, delusions, hallucinations, focal seizures, TCS, cognitive decline, mutism, catatonia, dyskinesia	MP, Plex	5	57	21	51	1	No complaints
14	F, 16	Herpes encephalitis	Decreased consciousness, obsessive behavior, aggression, disinhibition, dyskinesia, dystonia, mutism, fever	MP, IvIg, Rituximab	5	34	18	17	3	Impulsive
15	F, 16	Headache	TCS, childish behavior, disinhibition, aggression, cognitive decline, mutism	MP, Rituximab	4	27	17	6	1	Indecisive, anxiety
16	F, 14	No	TCS, psychosis, bradyphrenia, insomnia, dystonic posture	MP	4	3	15	14	2	Impulsive, anxiety, Indecisiveness
17*	F, 15	Headache	TCS, focal seizures, hallucinations, anxiety, less spontaneous speech	MP	4	67	18	34	1	Anxiety with agoraphobia
18*	M, 7	Herpes encephalitis	Dyskinesia, cognitive decline, mutism, TCS, focal seizures, insomnia	IvIg	5	106	12	50	4	Refractory epilepsy, cognitive decline
19*	M,1	Herpes encephalitis	Status epilepticus, childish behavior, chorea, mutism, cognitive decline	MP, IvIg Plex Rituximab	5	137	4	41	3	Cognitive decline, Unable to walk, dysphasia
20*	F, 14	Headache	Status epilepticus, agressive, disinhibition	MP	5	12	15	7	1	No complaints
21*	M, 14	Headache	Hemichorea, less spontaneous speech, catatonia, behavior disorder	MP, IvIg, Rituximab	5	47	15	5	1	No complaints
22*	M,7	Headache, fever	Cognitive decline, behavior disorder, speech disorder	MP, IvIg, Rituximab	4	8	9	23	3	irritable, insecure

*Only completed the questionnaires.
Abbreviations: PCPC= Pediatric Cerebral Performance Category, FU= Follow-up, TCS= Tonic-clonic seizures, MP= Methylprednisolone, IvIg = Intravenous immunoglobulins, OT= Ovarian teratoma, Plex = Plasmapherisis

Table 4. Patients assessed by neuropsychological testing compared to the other patients

	NPT group (n = 16)	Other patients (n = 12)	p-value
Female gender			
< 12 years	3/4	1/5	0.20
≥ 12 years	12/12	5/7	0.12
Age at onset in years, median [IQR, range]	16 (10-17, 4-17)	11 (7-15, 1-16)	0.059
Maximum PCPC			0.67
3; moderate disability	1	0	
4; severe disability	9	7	
5; coma/vegetative state	6	5	
First-line immunotherapy (%)	16/16	12/12	
Second-line immunotherapy (%)	7/16	6/12	1.00

Abbreviations: NPT= neuropsychological testing, PCPC = Pediatric cerebral performance category, IQR= interquartile range.

Table 5. Results of the neuropsychological assessment

Domain - test – measure	N	*Z* score	P-value	95% CI
CANTAB				
Executive functions - spatial span - span length	16	-0.54	0.016†	-1.61 to -0.19
Executive functions - stocking of Cambridge - problems solved in minimal moves	16	-0.30	0.27	-0.86 to 0.26
Executive functions - intra-extra dimensional set shift - total errors	14[a]	-0.27	0.32	-0.84 to 0.30
Visual memory - paired associated learning - total errors	15[a]	-0.90	0.016 †	-1.61 to -0.19
Attention – reaction time - mean simple reaction time	11[b]	-0.32	0.37	-1.07 to 0.43
Additional tests				
Sustained attention - Dutch dot cancellation test - S.D. reaction time*	16	-2.10	< 0.0001	-2.71 to -1.4
Speed – Dutch dot cancellation test - reaction time	16	-1.53	0.002#	-2.40 to -0.66
Long-term verbal memory -RAVLT - total recall*	15	-0.76	0.031	-1.29 to -0.07
Short-term verbal memory -RAVLT– trial 1-5	15[a]	-0.76	0.023†	-1.40 to -0.15
Word finding - Boston naming test - total score	16	-0.78	0.019†	-1.40 to -0.15
Executive functions – Word generation- Total score	15[a]	-0.14	0.60	-0.71 to 0.42
Word comprehension -token test - total score	16	0.57	0.044†	0.17 to 1.13

*Primary outcome measures.

†Secondary outcome measures with $p_{uncorrected}$ between 0.05 and 0.005, should be considered carefully.

#Secondary outcome measure with p-value < 0.005.

[a] Results of patient of whom the test was aborted earlier are not shown.

[b] No normative data available for children < 14 years of age.

Abbreviations: S.D.= standard deviation, RAVLT= Rey Auditory Verbal Learning Test.

Table 6. Results of the questionnaires

Questionnaire - measure	N	*Z* score	P-value	95% CI
PedsQL – Self-report[a]				
Physical functioning	21	-0.58	0.11	-1.28 to 0.13
Emotional functioning	21	-0.57	0.12	-1.30 to 0.17
Social functioning	21	-083	0.013†	-1.46 to 0.20
School functioning	21	-0.76	0.056	-1.53 to 0.023
Total functioning*	21	-0.86	0.032	-1.64 to -0.08
PedsQL – MFS – Self-report[a]				
General fatigue	21	-1.03	0.003†	-1.66 to -0.41
Sleep fatigue	21	-0.54	0.069	-1.130 to 0.05
Cognitive fatigue	21	-0.80	0.019†	-1.46 to -0.14
Total fatigue*	21	-0.96	0.008†	-1.64 to -0.28
BRIEF – Self-report[ab]				
Inhibition	19	-0.26	0.27	-0.74 to 0.22
Cognitive flexibility	19	0.33	0.22	-0.22 to 0.88
Emotional Control	19	0.19	0.45	-0.34 to 0.73
Initiation	6[c]	0.083	0.89	-1.35 to 1.51
Working memory	19	0.24	0.23	-0.17 to 0.65
Planning and organization	19	0.23	0.38	-0.30 to 0.75
Monitoring	19	0.15	0.58	-0.41 to 0.70
Behavior Rating Inventory	19	-0.06	0.84	-0.52 to 0.63
Metacognition Index	19	0.24	0.35	-0.28 to 0.77
Total	19	0.18	0.45	-0.31 to 0.68
SDQ – Self-report[a,d]				
Total difficulties	10	0.002	0.94	-0.06 to 0.07
Emotional symptoms	10	0.40	0.40	-0.13 to 0.93
Behavior problems	10	-0.20	0.59	-1.01 to 0.61
Hyperactivity-inattention	10	-0.21	0.22	-0.68 to 0.25
Peer problems	10	0.06	0.06	0.73 to 0.85
Prosocial behavior	10	0.39	0.39	-0.03 to 0.95

*Primary outcome measures.

†Secondary outcome measures with $p_{uncorrected}$ between 0.05 and 0.005, and should be considered carefully.

[a] Only for patients aged > 4 years.

[b] For the results of the youngest patients no normative data were available

[c] Only for patients aged 17 years or older.

[d] Only for children aged 11 to 17 years.

Abbreviations: PedsQL = Pediatric Quality of Life, MFS= Multidimensional FatigueScale, BRIEF = Behavior Rating Inventory of Executive Function, SDQ = Strengths and Difficulties Questionnaire.

Table 7. Results of the questionnaires completed by parents

Questionnaire - measure	N	*Z* score	P-value	95% CI
PEDSQL – Parent Proxy-report[a]				
Physical functioning	15	-1.12	0.087	-2.42 to 0.18
Emotional functioning	15	-0.82	0.024[†]	-1.51 to -0.13
Social functioning	15	-1.19	0.009[†]	-2.04 to -0.34
School functioning	15	-0.87	0.047[†]	-1.73 to -0.01
Total functioning	15	-1.34	0.004[#]	-2.15 to -0.52
PEDSQL – MFS- Parent Proxy-report[a]				
General fatigue	15	-0.80	0.054	-1.61 to 0.01
Sleep fatigue	15	-0.59	0.15	-1.44 to 0.25
Cognitive fatigue	15	-0.80	0.032[†]	-1.52 to -0.08
Total fatigue	15	-0.90	0.025[†]	-1.67 to -0.13
BRIEF Parent Proxy-report[b]				
Inhibition	13	0.26	0.52	-0.61 to 1.13
Cognitive flexibility	13	0.77	0.065	-0.05 to 1.56
Emotional Control	13	0.65	0.077	-0.08 to 1.38
Working memory	13	0.62	0.064	-0.04 to 1.27
Planning and organization	13	0.25	0.30	-0.24 to 0.74
Behavior Rating Inventory	13	0.45	0.16	-0.20 to 1.11
Metacognition Index	13	0.42	0.15	-0.18 to1.02
Total	13	0.58	0.075	-0.07 to 1.24
SDQ Parent Proxy-report[c]				
Total difficulties	10	1.02	0.012[†]	0.28 to 1.77
Emotional symptoms	10	1.05	0.054	-0.02 to 2.12
Behavior problems	10	0.85	0.039[†]	0.06 to 1.24
Hyperactivity-inattention	10	0.21	0.65	-0.80 to 1.22
Peer problems	10	0.69	0.11	-0.20 to 1.59
Prosocial behavior	10	-0.54	0.32	-1.72 to 0.63

[#] Secondary outcome measures with $p_{uncorrected} < 0.005$

[†] Secondary outcome measures with $p_{uncorrected}$ between 0.05 and 0.005, and should be considered carefully.

[a] Only completed by parents of children currently 4- 17 years.

[b] Only completed by parents of children currently 6-17 years.

[c] Only completed by parents of children currently 4-16 years.

Abbreviations: PedsQL = Pediatric Quality of Life, MFS= Multidimensional FatigueScale, BRIEF = Behavior Rating Inventory of Executive Function, SDQ = Strengths and Difficulties Questionnaire.

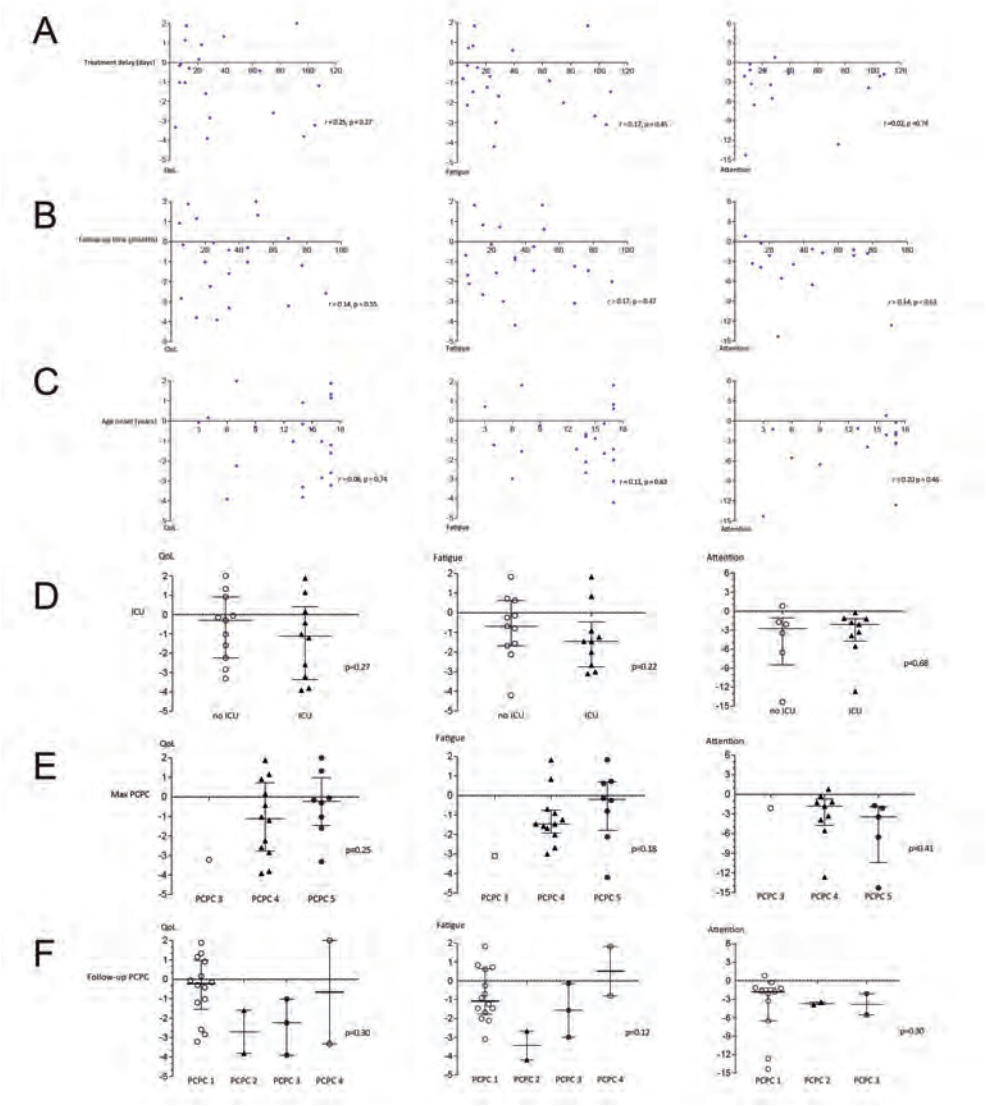

Figure 1. Correlations between functional measurements: A. treatment delay, B. follow-up time, C. age of onset, D. ICU admission, E. maximum PCPC and F. PCPC at follow-up, and the primary outcome measures QoL, fatigue and attention (Quality of life – Self-report – Total score, PedsQL-MFS- Self-report – total score), Sustained attention – Dutch dot cancellation test - S.D. reaction time, respectively). LTM= long-term verbal memory, ICU= intensive care unit, PCPC= Pediatric Cerebral Performance Category.

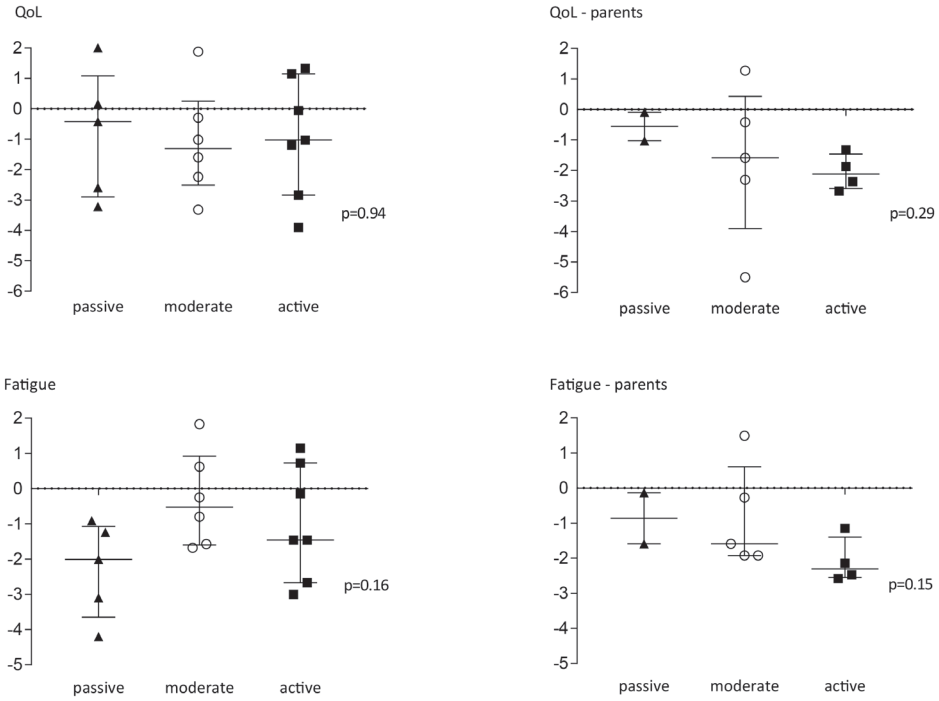

Figure 2. QoL and fatigue scores with patients allocated in character category (passive, moderate, active), based on types of frontal lobe syndromes for patients and parents.

Chapter 9

General discussion

Autoimmune encephalitis (AIE) has emerged the last decade as a severe, but treatable neurological condition. Seizures usually occur early in the disease course, making the recognition of these provoked seizures, including their relation to encephalitis, an important aim in AIE diagnosis. Mostly, these seizures are part of a multi-symptomatic aggressive disease course. However, phenotypes vary widely, and some patients can have less fulminant and protracted disease courses. This thesis focuses on the diagnosis and treatment of AIE syndromes associated with seizures. In this chapter the results from the previous chapters are interpreted, along with discussing practical clinical questions and overarching issues.

Interpretation of laboratory results

Increasing AIE incidence rates[1,2] and growing numbers of antibody requests, indicate better awareness and recognition of AIE. Nevertheless, under-recognition of specific syndromes still occurs, especially in patients with longer and less severe disease courses. Most of these milder syndromes are characterized by prominent, but due to their subtlety sometimes unremarked, seizures.[1,3,4] This makes recognition of these provoked seizures, as part of AIE, a difficult, but rewarding task, as seizures seem to respond best to immunotherapy.[1,5] In daily practice this means that some epilepsy patients probably are undertreated with AEDs only, while they would profit from immunotherapy.

The hypothesis of under-diagnosis and under-treatment was the most important reason to perform the Antibodies Contributing to focal Epilepsy Singns and Symptoms (ACES) study, in which aims were: to describe the frequency of neuronal antibodies in both patients with new-onset status epilepticus of unknown cause, and in patients with focal epilepsy of unknown etiology. We were interested in these two groups particularly because of an increase of reports of patients with predominant status epilepticus or focal epilepsy, turning out to be antibody-mediated.[6-8]

Before the inclusion period started we critically considered the methodology of the study, including the selection of laboratory tests. Judging on earlier published antibody screening studies in patients with epilepsy syndromes,[7,9-11] the mostly used technique is to perform commercial fixed cell-based assays (CBAs) to test for each antibody separately. Sometimes, but more frequently not, results are confirmed with additional laboratory techniques (a combination of immunohistochemistry and live hippocampal neurons).[12] Results of the studies not using conformational testing certainly provide an overestimation of findings, as the interpretation of CBAs can be difficult, and results can be false-positive. Scoring performance depends on the observers' experience, but also on the staining intensity, which is assay-dependent.[13] Therefore, confirmation of positivity requires a second laboratory technique to confirm true positivity, but also to reject false positive results. The use of only CBAs in screening studies, without confirmation, makes it impossible to interpret results correctly and to determine the "real" antibody-status of patients.

We have weighted the screening method of first performing multiple CBAs against screening with immunohistochemistry first, followed by confirmation with live neurons and

specific CBAs. The last method was chosen, because immunohistochemistry has the best sensitivity to identify samples containing antibodies when compared to testing antibodies using different CBAs.[14,15] Performing IHC first limits the number of tests and decreases the chance to find false positives or clinically irrelevant "positives". In addition, IHC is easier to read than most CBAs. A high sensitivity is an important requirement for a screening test, because missing "positive" patients is most undesirable. Using immunohistochemistry not all positively tested samples turn out to be truly positive, but the false positive samples are filtered out by the use of additional laboratory techniques. These additional techniques were selected based on both IHC staining patterns and patients' clinical features. An example showing the usefulness of IHC above commercial CBAs, also described in this thesis, is the detection of $GABA_BR$ antibodies (chapter 2); the fixed CBA turned out to be less sensitive than immunohistochemistry, leading to adaptations of the assay. Similar problems have been described testing serum for NMDAR antibodies by commercial CBA.[15] An important issue applying to the commercially available CBAs is that cells are strongly fixed before incubation, to guarantee the long-term use and possibility for transportation. An disadvantage of fixing cells before incubation with patients' serum or CSF, is that fixation might cause conformational changes to the antigen tested for, leading to antigen-binding difficulties, and false negative outcomes.

Another reason to perform immunohistochemistry first is that it is a useful technique to identify new antibodies. Using this approach, we have identified two patients in the status epilepticus cohort with unknown antibodies and AIE, and three patients in the focal epilepsy cohort with similar clinical characteristics and a corresponding IHC staining pattern, confirmed by testing CSF. With regard to the last three patients, they were middle-aged women with refractory focal temporal seizures. An attempt was made to identify the target antigen by using immunoprecipitation and mass spectrometry in two of them. Unfortunately, we were unable to identify the target antigen. This may imply that no analogous antibody is present, that antibody titers were insufficient to identify the protein among the noise caused by other proteins present in the precipitate, that our technique was not appropriate, or that the test was not performed correctly. Given the corresponding IHC staining pattern and comparable clinical features, it is conceivable that their seizures are related to autoimmunity, and a common antibody is a realistic possibility. Retrospectively, it is difficult to determine the exact reasons why our search was unsuccessful. Technical difficulties may be an issue, but the same technique was used before to diagnose patients with anti-DNER,[16] anti-$GABA_BR$, anti-$GABA_AR$, and anti-Contactin1.[17,18] Concerning the used technique, one issue may be that rat lysate was used for immunoprecipitation. An alternative would be to use human brain lysate, minimizing the risk to miss interesting human proteins.

Next to the use of unconfirmed laboratory results, a second issue questions the utility of results of earlier published studies reporting high prevalence of antibodies in patients with epilepsy: the description of antibodies with questionable clinical relevance. Currently we know more about the pathogenicity of neuronal antibodies, and several studies have

disputed convincingly the clinical relevance of some. Among the most discussed antibodies, are those targeting the voltage gated potassium channel complex (VGKC). These antibodies were first described in patients with acquired myotonia, Morvan's syndrome and limbic encephalitis.[19,20] Later on it became clear that clinical phenotypes of patients with increased anti-VGKC are more widespread, and that the laboratory test measuring anti-VGKC titers, a radio-immuno-assay, actually measures much more than only VGKC antibodies. In 2016, van Sonderen et al.[21] observed that only patients with additional anti-LGI1 or anti-Caspr2 had consistent phenotypes, that responded to immunotherapy. The other "double negative" patients had different clinical symptoms, most of them without evidence for autoimmunity. They concluded that an increased VGKC antibody titer in absence of anti-LGI1 or anti-Caspr2 was clinically irrelevant, which was confirmed by others.[22] These studies were an important step ahead in the field op antibody research. Not only do these studies shed light on the debatable role of anti-VGKC in neurological syndromes. These studies also ensure that we should be thoughtful and critical in estimating the value and usefulness of current literature, as many publications report anti-VGKC in patients with "presumable" autoimmune epilepsy, even in absence of (or not tested for) anti-LGI1 and anti-Caspr2. Rejecting this antibody as pathogenic decreases antibody frequencies considerably in earlier studies. The last years, the amount of issues with the VGKC test has even increased, as laboratories have diminished the threshold to consider a VGKC test positive, because anti-Caspr2 is associated with lower VGKC titers. Therefore, a lower threshold increases the sensitivity, but in the same time the likelihood to identify anti-LGI1 or anti-Caspr2 in those considered VGKC-positive has diminished.

Another antibody with questionable clinical relevance is anti-GAD65. The main problem is that the target antigen is located intrasynaptically, and that direct pathogenicity of anti-GAD65 is unproven despite different attempts.[23,24] In addition, anti-GAD65 occurs in patients with neurological disorders, but also in patients with diabetes mellitus type 1, other autoimmune diseases and even in healthy individuals. On the other hand, there are characteristics pointing towards an association between anti-GAD65 and neurological autoimmunity. First, syndromes seem to be well defined, including limbic encephalitis, stiff-person syndrome, cerebellar ataxia, and epilepsy.[4,25,26] Secondly, most studies show intrathecal synthesis in patients with anti-GAD65.[27,28] Finally, as confirmed in this thesis, more than half of the patients respond to long-term immunotherapy.[25,29] The results shown hopefully contribute to better interpretation of anti-GAD65 concentrations in patients with neurological symptoms, as we describe a cut-off value that can be used in GAD65 antibody testing to evaluate if symptoms are associated to anti-GAD65. Despite overt treatment response in patients treated with immunomodulation, no patient became seizure free, and improvement stagnated even after chronic immunotherapy. This could imply that anti-GAD65 associated neurological syndromes are partially caused by antibody effects, but also reflect irreversible processes, like T-cell activation, leading to scarring and neurodegeneration. Interestingly, in most patients, brain MRI is normal, even up until years after the disease course and also

in patients without treatment. It would be of additional value to study connectivity using functional MRI. Earlier studies evaluating cognitive outcomes in patients with anti-NMDAR and anti-LGI1 encephalitis showed a decreased connectivity in structures that are essential in plasticity,[30,31] also in patients with (relatively) normal follow-up MRIs.

Diagnosing autoimmune epilepsy

Beyond doubt neuronal antibodies should be tested in all patients with unexplained status epilepticus, as more than 30% of these patients have an autoimmune etiology.[8,32,33] However, simply testing for all neuronal antibodies is not enough to identify all patients with an autoimmune etiology of seizures. In this thesis we confirm that a large part of patients with new-onset status epilepticus had an autoimmune etiology, while only half of those had neuronal antibodies. The others fulfilled the criteria of seronegative AIE,[34] or neuronal antibody presence was determined using research techniques. One of the major difficulties in diagnosing seronegative AIE, is that clinical findings and findings from ancillary testing can seem similar at firs glance, while the underlying processes differ. For example, MRI changes caused by status epilepticus can be almost comparable to those of limbic encephalitis. The main difference is that MRI abnormalities caused by status epilepticus often involve more than only the mesial temporal lobe and usually show diffusion-weighted changes caused by cytotoxic edema.[35,36] Especially when patients with temporal MRI lesions caused by status epilepticus additionally have postictal behavioral changes, these features can be mistaken for AIE. In order to further research distinguishable and overlapping features between the two, we are devoting another study to this topic. Brain MRIs of the patients of our status epilepticus are being reviewed blindly by a neuroradiologist.

In clinical practice, there is often a trade-off between diagnosing as many AIE-related status epilepticus patients, as early as possible, while at the same time excluding other treatable causes of status epilepticus. This requires rapid neuro-imaging and CSF examination (including oligoclonal bands [OCB]), mainly to exclude structural lesions or treatable infectious causes, but also to collect more evidence for autoimmunity. Antibody testing often delays immunotherapy, but it is required for AIE definite diagnosis. This is quite undesirable, because early treatment improves outcome.[3,37] In my opinion, antibody testing should remain the golden standard in diagnosis. Not only for confirmation of etiology, but also to adapt treatment regimens based on specific syndromes, to determine the need for intensive tumor screening, and to inform patient and relatives about relapse chances and prognosis. Nevertheless, if treatable causes are excluced, and there are signs supporting autoimmunity (pleiocytosis in CSF, OCB, temporal hyperintensities on MRI), it seems justifiable to start first line immunotherapy in refractory status epilepticus patients, before definite antibody results. Besides, in the previous years it has become clear that not all AIE patients harbor known antibodies, so under specific and strictly applied circumstances, a positive antibody result is not necessary to diagnose autoimmunity (so-called seronegative AIE).

The diagnosis of seronegative AIE is increasingly made, both in children and adults, as also described in this thesis. As several mimics for AIE have been identified over the last years, with sometimes, serious consequences for the patients involved, referral to centers of expertise for neuro-immunology should be considered lightly in these specific cases.
Today, little is known about biomarkers in AIE, and it has no role in diagnosing AIE. One exception being CXCL13 in anti-NMDAR encephalitis.[38] However, this B-cell attracting cytokine is not specific to antibody-mediated autoimmunity as it is also increased in CSF of patients with neuroborreliosis[39] or multiple sclerosis.[40]
The frequency of neuronal antibodies in patients with focal epilepsy of unknown etiology was much lower than in the status epilepticus cohort (3.4%; chapter 5), as expected. Earlier studies report higher percentages (between 10 and 30%) of patients with focal epilepsy, but showed overt selection towards including patients with antibody-mediated disease causing provoked seizures.[10,11] In these studies, patients were included if samples were referred for antibody testing and patients were selected based on criteria favoring an autoimmune etiology of seizures (AES). This selection bias in combination with description of clinically irrelevant antibodies (anti-VGKC, low-concentration anti-GAD65), questions the reliability of these studies. Therefore, earlier described prevalence rates of neuronal antibodies in patients with focal epilepsy are almost certainly serious overestimations. As shown in this thesis, the prevalence of AES in patients with focal epilepsy is tenfold lower than in the status epilepticus cohort, which makes testing all patients for antibodies a time-consuming, expensive and unnecessary task. Though, not testing for antibodies at all, and missing this minority of AES patients is undesirable, as these patients should be treated differently. In almost half of patients with AES seizure freedom is expected shortly after immunotherapy, while considerable seizure reduction is achieved in additional patients. This urges the need for tools to select patients that require antibody testing.
This thesis shows that all reported patients with AES had more than only seizures, but that these symptoms were often unrecognized as being part of an underlying disease. In some patients other symptoms (like behavioral changes and cognitive problems) were considered side effects of AEDs. In others, symptoms were dismissed as functional or psychiatric (stiff-person syndrome; complex partial seizures in anti-LGI1 encephalitis or anti-GAD65 encephalopathy), and in some patients, symptoms were unrecognized at all (i.e. hyponatremia and FBDS in anti-LGI1 encephalitis; neuropathic pain in anti-Caspr2 encephalitis). To draw more attention to recognition of these additional symptoms we have described these characteristics in detail in this thesis. In addition, we have created the Antibodies Contributing to focal Epilepsy Signs and symptoms (ACES) score. This score comprises six independent risk factors, and seems useful to accurately predict an autoimmune etiology of seizures, especially when used in outpatient consultation in tertiary epilepsy centers. The score was externally validated in a foreign temporal epilepsy cohort, showing almost perfect discrimination. The ACES score is a clinical score that can be used

to guide antibody screening, and use of this score reduces the number of patients that require screening with more than 80%.

The ACES score is more useful, in outpatient consultation, than the earlier created Antibody Prevalence of Epilepsy (APE) score, also shown in this thesis.[11,41] The APE score was created using a biased patient cohort, including mainly cases with subacute disease courses, some even with clear encephalitis. The APE score consists mainly of AIE-based signs and symptoms, like subacute cognitive deterioration, psychotic behavior, pleocytosis in CSF, reflecting the selected cohort of subacute encephalitis patients. Additionally, FBDS are also included in this score, while FBDS are frequently unrecognized, as shown in our thesis. Therefore, including them in a score is unrealistic. Besides, if FBDS are present patients should be tested anyway, independent of the presence of any other sign. We have shown that the APE score was not useful in our cohort, reflecting the lack of applicability of this score to diagnose AIE syndromes with less prominent and less fulminant encephalitis signs, like anti-GAD65 encephalopathy. In addition, the creators of the original APE score claimed that using this score could be sufficient to diagnose autoimmune epilepsy, bypassing antibody testing. This is risky, because it may lead to over-diagnosis and over-treatment with chronic immunotherapy, especially when physicians with less experience in these diseases use the score. The creators of this score probably have realized this, because in the recently updated APE2 score, definite autoimmune epilepsy diagnosis can only be made based on antibody results.

To draw more attention to autoimmune encephalitis as cause of status epilepticus or focal epilepsy, some of the results of this thesis have been implemented in the immunology module of the Dutch epilepsy guideline. Currently the guideline recommends determining specific neuronal antibodies in all patients with new-onset status epilepticus, after securing vital parameters and exclusion of structural lesions and fatal infections. Concerning chronic focal epilepsy, the guideline recommends testing for neuronal antibodies in patients with faciobrachial dystonic seizures, and in patients with other signs and symptoms of encephalitis or encephalopathy, or with MRI hyperintensities in the mesial temporal lobe on T2/FLAIR weighted images. In the coming years, hopefully the ACES score can be added to the module.

Diagnosing AIE-related seizures ideally requires optimal knowledge of different AIE phenotypes, resulting in correct and specified antibody requests. This would be the ideal situation, in ways of effectiveness, time management and cost-efficiency. However, as more than ten antibodies can cause epilepsy, and there is an increase in the number of seronegative patients, it is very difficult for general neurologists to recognize all these rare syndromes separately. A frequently discussed topic is whether syndrome-specific testing with selected antibody-panels would be of additional value. Symptom-guided antibody panels might be beneficial as the use of panels can avoid testing the wrong antibodies or testing incompletely. However, there is still the risk of an atypical phenotype or a clinically irrelevant result. Therefore, consultation of expertise centers in doubt is advised, especially

because conformational testing can be necessary to confirm results or identify unknown associations.

As in epilepsy, in psychosis neuronal antibody screening and diagnosing patients with autoimmune variants is also a hot topic. The same screening studies have been performed with the same diagnostic issues, including doubtful interpretation of results, and lack of conformational testing.[42,43] Nevertheless, immunotherapy trials are performed based on these questionable findings, even with second line treatments like Rituximab.[44] Treating these patients with immunotherapy should be avoided, because side effects of immunosuppression are dangerous and unwanted. Next to the potential danger of side effects of immunosuppression, negative trials based on insufficiently strict inclusion criteria can also harm the trust in the positive effect seen in patients with true AIE. Trials should therefore be based on strict research criteria. The findings from this thesis can help to tailor immunotherapy trials in epilepsy patients. With the findings from this thesis in mind, better scientifically based immunotherapy trials can be performed in epilepsy patients.

Treatment evaluation and long-term outcome

Immunotherapy is started in patients with probable or definite autoimmune encephalitis. However, as described in this thesis, patients do not always have typical encephalitis, and in some patients symptoms can be confused with other neurological disorders, like viral encephalitis, Creutzfeldt-Jakob disease, psychosis, or new-onset epilepsy (Chapter 2, chapter 3 and chapter 7)). Therefore, it is common that other therapies, including antibiotics, anti-viral drugs, or antiepileptic drugs are started before immunotherapy. A part of these therapies are withdrawn after antibody diagnosis, but some are continued, like AEDs, while scarce evidence supports their ineffectiveness.[5,45,46] In chapter 6, we show that seizures of patients with anti-LGI1, anti-NMDAR and anti-$GABA_BR$ encephalitis are often resistant to AED treatment. Seizure freedom was achieved in 14% of patients after AEDs only, while in more than 50% seizure freedom was reached shortly after start of immunotherapy. Especially in anti-LGI1 encephalitis, focal seizures disappeared within hours to days after the start of immunotherapy, while seizures had been resistant to AEDs for months. The drug-resistant character of seizures in AIE is probably explained by ongoing synapse dysfunction and excitation of neurons caused by circulating antibodies. It is clearly not beneficial to leave the underlying process provoking the seizures untreated.[3] Antibodies (IgGs) can survive for weeks, so deletion of these antibodies does not explain the early seizure freedom sometimes even hours after immunotherapy, especially as seen in anti-LGI1 encephalitis. Potentially methylprednisolone, which is often started first, reduces immunoactive cells surrounding the inflammation directly, causing a decrease of seizure frequency.

We found that carbamazepine was the most useful AEDs as add-on to immunotherapy in anti-LGI1 encephalitis. This was confirmed by another smaller cohort study.[5] In our still limited experience, other sodium channel blockers showed similar effects as carbamazepine to treat focal seizures in anti-LGI1 encephalitis, although the numbers were too small to

draw firm conclusions. It is unclear if carbamazepine affects signal transduction in anti-LGI1 encephalitis patients. Carbamazepine binds preferentially to voltage-gated sodium channels of presynaptic neurons. It avoids the influx of sodium and prevents repetitive firing of action potentials.[47] LGI1 is part of the voltage-gated potassium channel complex, which is located in the presynaptic cleft. This complex is involved in returning cells to their depolarized state, which is regulated by the influx of potassium.[48,49] A plausible explanation for the effect of carbamazepine might be that it indirectly affects the VGKC channel.

The relative ineffectiveness of AEDs in treatment of seizures in patients with AIE questions their continued use after resolved encephalitis. In addition, the probability to develop chronic epilepsy after encephalitis is discussed broadly. Some state that many patients require long-term immunotherapy because of the development of chronic "autoimmune epilepsy", while in our cohort only one of the 86 surviving patients was not seizure-free after resolved encephalitis. There are several issues explaining this discrepancy. First, it is not always clear if the "autoimmune epilepsy" described in other studies was diagnosed based on well performed antibody testing, and some studies also describe epilepsy after viral encephalitis (with necrotizing lesions), a completely different etiology with different long-term seizure risk. Another issue is that the type of antibody present determines if treatment should be continued after the acute phase. Patients with antibodies targeting extracellulair neuronal proteins generally do not require long-term therapy to treat seizures, while patients with anti-GAD65 encephalopathy should be treated more chronically with monthly iVIG. Therefore, the use of AEDs should be evaluated critically in all patients, especially in those after resolved encephalitis. It seems unnecessary to continue AEDs for more than six months to one year after recovery, also because the use of AEDs, in patients with antibodies against extracellular antigens, causes unwanted side effects in these vulnerable patients. These side effects, including cognitive problems and behavioral disorders, can interfere with recovery. In addition, some AEDs might even exaggerate symptoms of encephalitis, like levetiracetam.

In line with the observation that the development of epilepsy after resolved encephalitis is rare, most patients show good functional recovery after AIE.[1,37] Outcome depends largely on the type of syndrome and involved antibody. Factors associated with poorer outcome, include intensive care unit admission, treatment delay, and presence of a tumor.[37] In anti-NMDAR encephalitis and anti-LGI1 encephalitis, more than 85% of patients show "good functional recovery" two years after disease onset. In our anti-$GABA_BR$ encephalitis cohort we observed that most patients responded to immunotherapy and tumor therapy, but that survival was lower because more than 50% of the patients had a small cell lung carcinoma, in line with earlier studies.[50,51] Despite good functional recovery, we describe that many patients reside with cognitive deficits, fatigue and anxiety after anti-NMDAR encephalitis. In this thesis we focus on the neuropsychological outcome following pediatric anti-NMDAR encephalitis, because the pediatric brain is vulnerable to damage and in children and adolescents it is even more important to study the effect on participation. We found that only 65% of children returned to their previous school level (chapter 8). Moreover, many children

and adolescents had participation problems because of anxiety and fatigue. Another finding was that a substantial number of children had frontal lobe syndromes after the disease, including passive/apathetic behavior and active/impulsive behavior. Unexpectedly, the children with passive/apathetic behavior had a higher school drop out rate than the active/impulsive children. The problems described are similar to those occurring in other acquired brain disorders.[52,53] The exact mechanism causing the problems is unclear, but it seems to involve disrupted processes between associated brain areas.[30,54-56] For examples, after anti-LGI1 encephalitis patients complain about problems in spatial recognition, confirmed with neuropsychological assessment.[1] This is probably due to disruption of connectivity between the parahippocampal gyrus and parietal cortex, structures that are both essential for orientation. The findings emphasize the importance of long-term follow-up of patients, and especially children, after the acute disease. Currently, the best strategy to deal with these problems is unknown. Therefore, it would be of additional value to evaluate cognitive rehabilitation programs, and to study neuropsychological outcome at different stages after the disease.

Future perspectives

Important progress has been made in the field of AIE. Translational research has led to increased recognition of syndromes, optimization of existing diagnostic assays, and identification of new antibodies. The downside of growing attention for AIE is that the number of described cases without new findings has expanded, that many studies are published describing clinically irrelevant antibodies in a variety of clinical syndromes, and that some trials are performed based on unsupported findings. To prevent these unwanted situations, researchers should join forces and collaborate internationally. A valuable reason to collaborate abroad is that despite increasing incidence, AIE is still a rare disease entity, and especially in patients with less-occurring antibodies it is of additional value to collect epidemiological and outcome data from different countries. Another important step ahead would be the formation of biobanks, containing patient information, serum, CSF, but also isolated peripheral blood mononuclear cells. These samples could be used to identify biomarkers and to study treatment responses.

The findings described in this thesis contribute to previous important work focusing on seizure characteristics in autoimmune encephalitis. Today, physicians are more aware of the different syndromes and antibody testing is embedded in regular diagnostics of patients with unexplained encephalitis, or refractory status epilepticus. Future research should move its focus towards answering clinical questions, evaluating treatment regimens and predicting seizure outcome. Because of ongoing controversy about the development of epilepsy after resolved encephalitis new research should focus on studying seizure frequency, EEGs (without the use of AEDs), antibody-titers and ratios, and MRIs, at disease onset, and at different intervals after immunotherapy. In addition, TMS-EEG, a technique that measures

cortical connectivity and excitability, seems to be a useful tool to study treatment responses, measured as an increasing excitation threshold.
After the acute disease phase, many patients and relatives report the importance of contact with other affected patients to share experiences. An important goal for the next years would be to create a Dutch platform or patient association to connect patients and to arrange informative meetings.

In conclusion, major advances have been made in AIE research the last years. Syndromes are recognized better and earlier, diagnostic methods have improved, treatment is usually started rapidly after or, in specific cases, even before definite diagnosis, and there is more attention for problems that may occur after resolved AIE. Furthermore, guidelines have been created and are used frequently to solve important patient related issues. The coming years will show us if the current diagnostic AIE criteria and screening tools (i.e. the ACES score) are sufficient to diagnose most patients, and if current treatment regimens are the best achievable. Important steps ahead would be to identify more epidemiological factors of AIE, and linking them to disease pathogenesis, to predict outcome and the necessity for more intensive treatment, but also to create evidence based therapeutic advise for both the acute disease phase and during follow-up.

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Chapter 10

Summary / Samenvatting

SUMMARY

Since the description of antibody-mediated neurological syndromes, there has been an exponential increase in the number of patients diagnosed with these disorders. Antibody-mediated disorders are diverse, ranging from polyneuropathy to cerebellar ataxia, and encephalitis. This thesis focuses on antibody-mediated, or autoimmune encephalitis (AIE). This syndrome is characterized by the subacute onset, within days to weeks, of disabling cognitive and behavioral disorders, often combined with other neurological symptoms, including seizures. These provoked seizures frequently occur early in the disease course. Generally, seizures caused by AIE are refractory to symptomatic treatment with anti-epileptic drugs (AEDs), and many patients develop status epilepticus. The most common antibody-mediated causes of status epilepticus are anti-NMDAR and anti-$GABA_BR$ encephalitis. Patients with anti-$GABA_BR$ encephalitis usually have severe encephalitis often accompanied by status epilepticus. In these patients there is a high tumor prevalence, as about 50% of the patients have small cell lung cancer (SCLC). In **chapter 2** we describe the clinical features of all Dutch anti-$GABA_BR$ encephalitis patients. We show that not all patients have seizures, and describe a novel clinical phenotype: rapid progressive dementia, without seizures or status epilepticus. To study the frequency of different antibodies in epilepsy syndromes, we prospectively collected 50 patients with status epilepticus. In all, the initial examinations did not identify a cause for the status. In **chapter 3** we describe that almost 40% of these new-onset status epilepticus patients actually have autoimmune encephalitis. This finding emphasizes the importance of antibody testing in all patients with new-onset status epilepticus, after excluding structural brain lesions, or life-threatening and treatable bacterial or viral infections. Another result form this study was that patients with status epilepticus caused by AIE were younger and more often had a super-rectory status epilepticus. In addition, in all patients results from ancillary testing (MRI, CSF) showed signs of inflammation.

The last years it has become clear that not all patients develop severe encephalitis. A part of the patients have a less aggressive, more protracted disease course. Most of these patients have frequent, though often subtle seizures, with only mild cognitive complaints or behavioral changes. Recognizing these seizures, as part of autoimmune encephalitis is important, as earlier initiation of immunotherapy leads to better outcome. Currently, patients with these milder syndromes run more risk of under-diagnosis and under-treatment, because physicians are more unfamiliar with these syndromes. One example is anti-GAD65 encephalitis. In **chapter 4** we describe 56 patients with increased anti-GAD65 concentrations and neurological syndromes. Patients with very high anti-GAD65 concentrations have well-defined neurological symptoms. In those, we observed a clinical improvement (for example: seizure frequency reduction), together with an antibody titer reduction, in a majority of the patients treated with immunomodulation.

Common other causes of these less severe syndromes are anti-LGI1, and anti-Caspr2 encephalitis. To bring these milder syndromes to the attention we have described 582 patients with focal epilepsy of unknown etiology in **chapter 5**. Using a carefully chosen screening technique to detect antibodies (immunohistochemistry) combined with confirmational techniques, we identified an autoimmune etiology of seizures (AES) in ~3% of the cohort. The detected antibodies were anti-LGI1, anti-Caspr2 and anti-GAD65. All patients with AES had specific signs and symptoms, like behavioral changes, autoimmune diseases and specific MRI lesions, occurring less in the patients without AES. Remarkably, these other signs and symptoms were often unrecognized as being related to AIE. By use of these characteristics we have created the ACES score based on six independent risk factors, pointing towards AES (Table 1). This score can be used to select patients that require antibody testing. We show that with a cut-off value of two, meaning only testing patients with ACES score of 2 or higher, all patients with antibodies are found (sensitivity 100%), while 80% of the patients screened do not have antibodies (positive predicitive value ~ 20%). We have used a Czech focal epilepsy patient cohort to show that the ACES score performs equally well in another dataset, assuring external validity.

Table 1. **ACES Score**

Risk factor	Points
Cognitive symptoms	1
Behavioral symptoms	1
Autonomic symptoms	1
Speech problems	1
Autoimmune diseases	1
Temporal MRI hyperintensities	1
Total	**6**

Anti-NMDAR encephalitis is the most described AIE in adults, but also in children. This condition is characterized by the subacute onset of cognitive problems, behavioral changes, complex movement disorders, seizures, decreased spontaneous speech, autonomic dysfunction, and central hypoventilation. Anti-NMDAR encephalitis can have a paraneoplastic etiology, and especially in young women there is a strong association with ovarian teratoma. Anti-NMDAR encephalitis has a peak-incidence in adolescence, which is remarkable, as other syndromes usually occur in older patients.

Results from ancillary testing, including MRI, EEG and CSF analysis, are important to evaluate critically if AIE is suspected, not only to support the diagnosis, but even more important to exclude other causes. The most frequently occurring abnormalities associated with AIE are: hyperintense T2/FLAIR MRI lesions in the mesial temporal lobe, pleocytosis, elevated protein or oligoclonal bands in CSF, and diffuse slowing or epileptic activity on EEG. However, ancillary testing results can be completely normal, and most findings from ancillary testing do not discriminate between etiologies. Therefore, antibody testing

is required to diagnose definite AIE. In case of positive or questionable antibody-testing results, it is important to use confirmational techniques, because of sensitivity issues of individual diagnostic methods.

Difficulties in antibody testing can relate to sensitivity. An example is the fixed (commercially available) cell-based assay (CBA) used to detect $GABA_BR$ antibodies (anti-$GABA_BR$). Especially testing CSF can lead to false-negative results. To optimize anti-$GABA_BR$ detection, we show that adding KCTD16 to the anti-$GABA_BR$ CBA improves test sensitivity (**chapter 2**). KCTD16 is an auxiliary intracellular protein, and is important for clustering of the $GABA_{B2}$ receptor. In addition, we describe the possibility of KCTD16 as tumor marker, as KCTD16 occurred significantly more frequently in patients with SCLC than in patients without a tumor. It is important to realize that not all positive antibody results are clinically relevant. Exemplary, GAD65 antibodies occur in patients with diabetes, systemic autoimmune diseases, neurological symptoms, and even in healthy individuals. In **chapter 4** we conclude that solely in patients with very high anti-GAD65 concentrations neurological symptoms are well defined and symptoms are likely to be caused by an autoimmune phenomenon.

Patients with AIE should be treated with immunotherapy as soon as possible, together with tumor treatment, if applicable. Next to treating the cause of encephalitis, symptoms should also be treated as immunotherapy alone can be insufficient. Therefore, it is common that patients are additionally treated with symptomatic medications, like anti-psychotics or AEDs. In **chapter 6** we have visualized the responses of seizures to AEDs and immunotherapy. In our cohort of 110 patients with anti-LGI1, anti-$GABA_BR$ or anti-NMDAR encephalitis we show that more than half became seizure free shortly after start of immunotherapy, while only 14% reached seizure freedom shortly after AEDs. Therefore, AEDs should be used as add-on treatment and not as monotherapy in these patients. Despite the drug-resistant character of seizures in the acute phase of the disease, only a few patients develop epilepsy after resolved encephalitis. This observation underlines the importance of critically reviewing the use of AEDs during recovery and after stabilization for each patient individually, and advocates withdrawal of AEDs after encephalitis. After the acute disease phase, more than 80% of the patients with AIE recover, with apparently a limited amount of functional deficits. Nevertheless, many patients struggle with invalidating cognitive and behavioral problems in the recovery phase.

In this thesis, AIE in children is described separately. Not only as children with AIE present in another way, but also because the differential diagnosis in children is broader. However, it is important to think of AIE in children with subacute cognitive or behavioral changes and seizures. Especially, as almost a third of the patients with anti-NMDAR encephalitis are children. In **chapter 7** we show that anti-NMDAR encephalitis is the most occurring autoimmune related neurological syndrome in children after acute disseminated encephalomyelitis (ADEM). In addition, we have validated the previously published AIE diagnostic criteria with a prospectively collected cohort of children with presumed autoimmune neurological conditions. For better recognition of syndromes, we have

described AIE mimics in children. The treatment of AIE in children is broadly similar to the treatment in adults, although no formal randomized trials have been conducted. In **chapter 8** we describe that long-term outcome is often complicated by neuropsychological sequelae in children after anti-NMDAR encephalitis. We have studied outcome of 28 children with a considerable follow-up time. Our study shows that only 65% of children resume school at their previous school level. In addition, many patients complained of fatigue. Using a standardized neuropsychological assessment, we show that most frequent cognitive problems concerned memory- and attention tasks. Therefore, monitoring these children, and also adults, after AIE is important, to diagnose neuropsychiatric problems early and to start targeted counseling and treatment if necessary.

SAMENVATTING

Wanneer het eigen afweersysteem lichaamseigen weefsel aanvalt spreken we van een autoimmuun ziekte. Dankzij de bloed-hersen-barrière, een denkbeeldige muur tussen de hersenen en de rest van het lichaam, is het moeilijker voor het afweersysteem om de hersenen 'aan te vallen'. Desondanks kunnen autoimmuun ziekten ook voorkomen in de hersenen. Dit komt omdat sommige afweercellen de bloed-hersenen-barrière kunnen passeren, waardoor er een afweerreactie tegen de hersenen ontstaat. Deze afweerreactie kan veroorzaakt worden door cellen die directe onomkeerbare schade aan de hersenen veroorzaken (T-cellen), maar vaker binden antistoffen aan hersen-specifieke eiwitten of receptoren (neuronale antistoffen) waardoor er neurologische klachten ontstaan. De oorzaak van neurologische autoimmuun ziekten is soms bekend, maar meestal onbekend. Bekende oorzaken voor de vorming van deze neuronale antistoffen zijn een tumor op afstand (paraneoplastisch) of een doorgemaakte virale hersenontsteking.
Een van de meest voorkomende klinische syndromen is een autoimmuun hersenontsteking, of autoimmuun encefalitis (AIE). Dit syndroom wordt gekenmerkt door het binnen dagen tot enkele weken ontstaan van geheugen en/of gedragsveranderingen, maar vaak ook epileptische aanvallen. Deze epileptische aanvallen ontstaan meestal vroeg in het ziekte beloop, zijn moeilijk behandelbaar, en leiden daarom regelmatig tot een langdurige epileptische aanval (status epilepticus). Een status epilepticus is een levensbedreigende aandoening waarbij patiënten vaak ondersteuning nodig hebben op de intensive care. Enkele van de meest voorkomende AIE syndromen die een ernstige status epilepticus kunnen veroorzaken zijn anti-NMDAR en anti-$GABA_BR$ encefalitis. Patiënten met anti-$GABA_BR$ encefalitis hebben vaak een ernstige AIE met status epilepticus en geheugen- en gedragsproblemen, waarbij 50% van de patiënten een kleincellige longtumor heeft. In **hoofdstuk 2** beschrijven we de klinische kenmerken van alle Nederlandse anti-$GABA_BR$ encefalitis patiënten. We beschrijven een nieuw klinisch ziektebeeld, namelijk patiënten met een snel progressieve dementie zonder epilepsie. Om te achterhalen hoe vaak antistoffen de oorzaak zijn van status epilepticus, hebben we prospectief gegevens van 50 patiënten met status epilepticus verzameld. Bij alle patiënten gaven de eerste onderzoeken (bloedonderzoek, CT-scan, eventueel MRI-scan) geen verklaring voor de status epilepticus. In **hoofdstuk 3** beschrijven we dat bij bijna 40% van deze patiënten de status epilepticus werd veroorzaakt door een autoimmuun encefalitis. Deze bevinding ondersteunt het bepalen van neuronale antistoffen bij alle patiënten met een nieuw-ontstane status epilepticus, nadat ruimte-innemende processen (zoals een hersentumor) en behandelbare bacteriële of virale oorzaken zijn uitgesloten.
Er zijn ook AIE syndromen met een milder klinisch beloop. Bij deze patiënten is het stellen van de diagnose lastig, waardoor er regelmatig vertraging is in het starten met de juiste behandeling. Deze milder verlopende ziektebeelden worden vaak gekenmerkt door zeer frequent optredende, doch onopvallende, epileptische aanvallen. Een voorbeeld is anti-

GAD65 encefalitis. In **hoofdstuk 4** beschrijven we 56 patiënten met verhoogde anti-GAD65 concentratie en neurologische klachten. We vonden dat alleen patiënten met zeer hoge anti-GAD65 concentraties specifieke neurologische klachten/ziektebeelden hadden. Ook beschrijven we dat de met immuuntherapie behandelde, hoge anti-GAD65 concentratie groep, een verbetering liet zien van neurologische klachten (bijv. epileptische aanvallen) gecombineerd met een afname van het aantal antistoffen in het bloed van meer dan de helft van de patiënten. Andere voorbeelden van deze, minder opvallende AIE syndromen zijn anti-LGI1- en anti-Caspr2 encefalitis. In **hoofdstuk 5** beschrijven we bijna 600 patiënten met focale epilepsie van onbekende oorzaak. Met antistof screening en bevestigende laboratorium technieken vonden we neuronale antistoffen in ongeveer 3% van het cohort. De patiënten met epilepsie en neuronale antistoffen hadden specifieke klinische kenmerken en symptomen, die veel minder vaak voorkwamen bij patiënten zonder antistoffen (voorbeelden: gedragsveranderingen, andere autoimmuun ziekten en specifieke MRI afwijkingen). Deze kenmerken hebben we gebruikt om de ACES score te maken (Tabel 1). Een klinische score waarmee bepaald kan worden of iemand met focale epilepsie op antistoffen getest moet worden. We hebben een studie met 128 Tsjechische focale epilepsie patiënten gebruikt om de score te valideren, hiermee laten we zien dat de ACES score ook op deze groep goed toepasbaar was.

Tabel 1. **ACES Score**

Risico factor	Punten
Geheugenstoornis	1
Gedragsverandering	1
Autonome symptomen	1
Spraak problemen	1
Autoimmuun ziekten	1
Temporale MRI hyperintensiteiten	1
Totaal	**6**

De meest beschreven, en best bekende AIE is anti-NMDAR encefalitis. Deze aandoening kenmerkt zich door een in dagen tot weken ontstaan van geheugen- en gedragsveranderingen, bewegingsstoornissen, epileptische aanvallen, verminderde spontane spraak, autonome dysfunctie en centrale hypoventilatie. De aandoening kan paraneoplastisch zijn, waarbij er vooral bij jonge vrouwen regelmatig een goedaardig eierstokgezwel (ovarium teratoom) wordt gevonden wat de ziekte veroorzaakt. Opvallend is dat de ziekte met name voorkomt bij jong-volwassenen, terwijl andere AIE syndromen vaker ontstaan rond de middelbare leeftijd.
Aanvullend onderzoek helpt bij het stellen van de diagnose AIE. De meest voorkomende afwijkingen passend bij AIE zijn MRI afwijkingen in de temporaal kwab, een verhoogd celgetal of oligoklonale banden in het hersenvocht, of een vertraagd of epileptiform EEG. Veel te vaak wordt er gezegd dat neurologische klachten niet autoimmuun zijn, omdat

het aanvullend onderzoek 'normaal' is. Dit is niet terecht, want zowel MRI als onderzoek van het hersenvocht kunnen normaal zijn bij AIE patiënten. Dit maakt het aantonen van antistoffen de gouden standaard om de diagnose AIE te stellen. Hiervoor worden verschillende laboratorium technieken gebruikt. De keuze van de techniek is afhankelijk van het type antistof. Bij een positieve of twijfelachtige uitslag is het belangrijk om bevestigende technieken te gebruiken. Het niet gebruiken van bevestigende technieken kan er namelijk toe leiden dat patiënten foutief gediagnosticeerd worden en worden behandeld (of juist niet worden behandeld) met immuuntherapie.
Antistof testen moeten goed beoordeelbaar zijn om een diagnose te kunnen stellen. De ervaring leert namelijk dat sommige testen (assays) moeilijker te beoordelen zijn dan anderen, en dat antistoffen niet altijd gevonden worden (fout-negatief, lagere test sensitiviteit). Een voorbeeld is de assay die wordt gebruikt om $GABA_BR$ antistoffen te vinden. We laten zien dat vooral hersenvocht vals-negatief kan zijn (**hoofdstuk 2**). Daarnaast laten we zien dat het toevoegen van anti-KCTD16 aan de anti-$GABA_BR$ assay zorgt voor een hogere test sensitiviteit. Aanvullend beschrijven we dat KCTD16 gebruikt zou kunnen worden als tumormarker, omdat dit antigen veel vaker voorkomt bij patiënten met dan bij patiënten zonder tumor.
Niet alle antistof uitslagen zijn klinisch relevant. Een voorbeeld is een verhoogde concentratie GAD65 antistoffen (anti-GAD65). Antistoffen tegen GAD65 kunnen voorkomen bij gezonde personen, patiënten met suikerziekte, bij patiënten met andere autoimmuunziekten, maar ook bij patiënten met neurologische klachten. In **hoofdstuk 4** stellen we vast dat alleen patiënten met zeer hoge anti-GAD65 concentraties specifieke neurologische klachten/ziektebeelden hebben.
Immuuntherapie is de belangrijkste behandeling van AIE. Het doel van de behandeling is het remmen van het eigen afweersysteem. Bij patiënten met een tumor is het behandelen van de tumor eveneens essentieel. Daarnaast is het belangrijk om klachten te behandelen, zoals psychose (anti-psychotica) of epilepsie (anti-epileptica). In **hoofdstuk 6** laten we zien dat epileptische aanvallen vaak niet goed behandeld kunnen worden met anti-epileptica en dat immuuntherapie vaker en sneller leidt tot aanvalsvrijheid. We beschrijven ook dat de kans op het ontwikkelen van epilepsie na behandelde encefalitis klein is, wat maakt dat langdurige behandeling met anti-epileptica waarschijnlijk niet nodig is. Het is daarom belangrijk om patiënten op te volgen na de ziekte en de anti-epileptica na het bereiken van aanvalsvrijheid na niet al te lange tijd te stoppen. Meer dan 80% van de patiënten met AIE herstelt tot een niveau waarbij er weinig beperkingen zijn in het lichamelijk functioneren (bijv. lage kans op rolstoelafhankelijkheid). Desondanks kampt een groot deel van de patiënten in de herstelfase met vervelende geheugenklachten en vermoeidheidsklachten die invloed hebben op het dagelijks functioneren.
In deze thesis worden kinderen met AIE apart beschreven, omdat kinderen met deze ziekten zich op een andere manier presenteren, maar ook omdat de differentiaal

diagnose (dit wil zeggen de andere aandoeningen waar je aan moet denken bij soortgelijke klachten) veel breder is. Desondanks moet er wel altijd gedacht worden aan AIE bij een kind met geheugenklachten, gedragsveranderingen of epilepsie. De meest belangrijke vorm van AIE die wordt gezien op de kinderleeftijd is anti-NMDAR encefalitis. In **hoofdstuk 7** bevestigen we dat op de kinderleeftijd anti-NMDAR encefalitis na acute disseminated encefalomyelitis (ADEM) de meest voorkomende autoimmuun aandoening van de hersenen is. Daarnaast beschrijven we ziektebeelden waarbij de klachten en bevindingen bij aanvullend onderzoek kunnen lijken op AIE. De behandeling van AIE bij kinderen komt in grote lijnen overeen met de behandeling van volwassenen, hoewel er door de zeldzaamheid nog geen onderzoeken zijn verricht waarbij patiënten blind het ene middel of het andere hebben gekregen. In **hoofdstuk 8** beschrijven we een cohort van 28 kinderen met anti-NMDAR encefalitis, gemiddeld meer dan 2 jaar na de ziekte. Ook kinderen herstellen functioneel goed, dat wil zeggen dat veel kinderen geen hulmiddel nodig hebben om te lopen. Echter blijkt uit onze studie wel dat 35% van de kinderen het opleidingsniveau van voor de ziekte niet meer haalt. Daarnaast werd er vaak vermoeidheid gemeld en bleken er bij neuropsychologisch onderzoek veelvuldig problemen te zijn met geheugen- en aandachtstaken. Dit maakt vervolgen van deze kinderen en jongvolwassenen, na AIE belangrijk, om zo neuropsychiatrische problemen in een vroeg stadium vast te stellen en gericht te behandelen.

Appendices

DANKWOORD

Bij elk ontvangen proefschrift lees ik als eerste het dankwoord. Al vaak heb ik me voorgesteld hoe leuk het zou zijn om dit zelf te mogen schrijven, en nu is het zover! In 2014 besloot ik om in het diepe te springen en 'ja' te zeggen tegen dit promotietraject, hier heb ik nooit spijt van gehad. Er zijn heel wat mensen die bij hebben gedragen aan de tot stand koming van dit proefschrift en daar ben ik hen heel veel dank voor verschuldigd. Een aantal van hen wil ik persoonlijk bedanken.

Allereerst mijn co-promotor dr. Maarten Titulaer. Beste Maarten, aan het begin van dit traject had ik geen idee waar ik in stapte. De ziekte anti-NMDAR encefalitis had ik slechts een enkele keer voorbij horen komen tijdens overdrachten en onderwijsmomenten. Desalniettemin leek de autoimmuun neurologie me waanzinnig interessant. Na een telefonische 'sollicitatie' en een verdere kennismaking op de wetenschapsdagen vertrok ik in 2014 naar Rotterdam, voor mijn promotietraject. Ik werd erg hartelijk ontvangen en werd door jou direct betrokken bij het beoordelen van antistof testen en bij klinische consultaties. Ik blijf me verbazen over je kennis; je bent werkelijk een lopende encyclopedie. Jouw kritische noot waar nodig en streven naar perfectie hebben er mede voor gezorgd dat dit proefschrift een werk is waar ik ontzettend trots op ben. Dank dat je me alle mogelijkheden hebt gegeven om mezelf te ontwikkelen de afgelopen jaren.

Mijn promotor prof. dr. Peter Sillevis Smitt wil ik graag bedanken voor het geven van de mogelijkheid om te promoveren in Rotterdam, voor het vertrouwen en voor de overleg momenten. Tijdens mijn academische stage hebben we een aantal keer samen neuro-oncologie poli gedaan, erg leerzaam!

De aanwezige leden van de promotiecommissie wil ik bedanken voor de bereidheid om tijdens mijn verdediging van gedachten te wisselen over mijn proefschrift. In het bijzonder wil ik prof. dr. B.C. Jacobs, prof. dr. M.A.J. de Koning-Tijssen en prof. dr. K.P.J. Braun bedanken voor het beoordelen van mijn proefschrift.

De kans om tijdens mijn opleiding tot neuroloog onderzoek te gaan doen heb ik te danken aan mijn voormalig opleider in Tilburg, dr. Paul de Kort. Beste Paul, ik kan me mijn voortgangsgesprek nog goed herinneren in het voorjaar van 2014. Ik was net begonnen aan het 2e jaar van mijn opleiding en dit promotietraject kwam ter sprake. Je was meteen enthousiast en zei dat ik deze kans moest grijpen. Dank voor je vertrouwen en je blijvende interesse. Ook gaat mijn dank uit naar mijn huidig opleider in Tilburg, dr. Thies van Asseldonk en plaatsvervangend opleider dr. Gerwin Roks. Beste Thies en Gerwin, dank voor jullie adviezen, vertrouwen en flexibiliteit. Thies, ook dank voor je wijze raad 'kinderen kan je beter krijgen tijdens je opleiding', dat is me goed gelukt en daar heb ik nog iedere dag plezier van.

In dit rijtje kan Nathalie Synhaeve niet ontbreken, oud collega-AIOS en inmiddels neuroloog in het ETZ. Lieve Nathalie, een mooi traject lag klaar in het young stroke onderzoek, maar ik koos voor Rotterdam. Enorm fijn dat jij het young stroke onderzoek hebt opgepakt en mij hierbij bent blijven betrekken. Ongelooflijk hoe hard jij hieraan hebt gewerkt, met als resultaat voor jou een heel vlotte promotie en onze gezamenlijke Van Buchem prijs.

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Laboratorium onderzoek was een belangrijk onderdeel van deze thesis. Ik begon met twee linker handen in het lab. Mede door de begeleiding van Esther en Mariska is het toch goed gekomen, dank jullie wel! Ook ander hulptroepen in het lab, bedankt! Melanie Ramberger met de hulp bij het $GABA_BR$ project en Esther de Graaff voor alle uitleg en hulp in Utrecht. Veel dank ook aan dr. Marco Schreurs en Sanae van de afdeling medische immunologie voor de fijne samenwerking de afgelopen jaren.

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ABOUT THE AUTHOR

Marienke de Bruijn is geboren op 7 december 1986 in Oud en Nieuw Gastel, in Nederland. In 2005 behaalde ze haar diploma (Gymnasium, Norbertus College te Roosendaal). In 2005 begon zij aan haar Geneeskunde opleiding aan de Erasmus Universiteit in Rotterdam. Ze sloot haar studie af met een oudste co-schap neurologie en een onderzoeksstage naar cognitieve uitkomsten van young stroke patiënten in het Elisabeth TweeSteden ziekenhuis, onder supervisie van dr. Paul de Kort. Na het behalen van haar artsenexamen in oktober 2011 werkte zij als ANIOS neurologie in het Amphia Ziekenhuis te Breda en in het ETZ te Tilburg. In 2013 startte zij met haar opleiding tot neuroloog in het ETZ, bij voormalige opleider dr. Paul de Kort en huidig opleider dr. Thies van Asseldonk. Vanaf juli 2014 tot mei 2019 combineerde zij haar opleiding met een promotietraject bij. Prof. dr. Peter Sillevis Smitt en dr. Maarten Titulaer in het Erasmus MC te Rotterdam. In 2020 won zij de dr. Jan Meerwaldt, runner-up prijs voor haar werk. Zij is nu bezig met de laatste jaren van haar opleiding, die zij in 2023 hoopt af te ronden. Marienke woont samen met Dirk van der Weijden in Tilburg. Zij hebben twee dochters, Saar (2015) en Fiene (2018).

LIST OF PUBLICATIONS

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de Bruijn MA, Titulaer MJ. Anti-NMDAR encephalitis and other glutamate and GABA receptor antibody encephalopathies. In: Pittock SJ, Vincent A. *Handbook of Clinical Neurology, Autoimmune Neurology. 2016, Elsevier, pp 199-217.*

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*Both authors contributed equally to this work.

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PhD PORTFOLIO

	Year	ECTS
General courses		
Research Integrity Course, Erasmus MC University Medical Center, Rotterdam	2015	0.3
BROK (Basiscursus Regelgeving Klinisch Onderzoek), NFU BROK Academie	2016	1.5
Biomedical English Writing and Communication, Erasmus MC University Medical Center, Rotterdam	2017	3
Specific courses		
Advanced Immunology 4-day short course, Erasmus MC University Medical Center, Rotterdam, The Netherlands	2017	2
European School of Neuro-immunology course, Venice, Italy	2017	2
Boerhaave cursus epilepsie: de nieuwe classificatie, Heemstede, The Netherlands	2017	1
Epilepsy Guideline Development AIOS neurology, Heeze and Utrecht, The Netherlands	2017-2019	1
Presentations		
Children's Autoimmunity related to Neuropsychiatric symptoms, Chorea and Epilepsy: The CHANCE study. Spring meeting NVKN (Nederlandse Vereninging voor Kinderneurologie), Heemstede	2015	1
Autoimmune epilepsy: Effects and side-effects of antiepileptic drugs. International League against Epilepsy Congress, Barcelona	2017	1
Pediatric anti-NMDAR encephalitis: evaluation of long-term neuropsychological outcome. Dutch Association of Neurology (NVN), Nunspeet	2017	1
Pediatric anti-NMDAR encephalitis: evaluation of long-term neuropsychological outcome. Encephalitis Society Conference, London	2017	1
Neuronal antibodies in patients with chronic focal epilepsy of unknown origin: ACES a prospective cohort study. International League against Epilepsy Congress, Vienna	2018	1
Antibodies in Cryptogenic focal Epilepsy Signs and Symptoms (ACES) score. American Academy of Neurology Annual Meeting 2019, Philadelphia	9	1
Evaluation of seizure treatment in anti-LGI1, anti-NMDAR and anti-$GABA_BR$ encephalitis. American Academy of Neurology Annual Meeting 2019, Philadelphia	2019	1
Autoimmune encephalitis and epilepsy: implications for clinical practice. Dutch Association of Neurology (NVN), Nunspeet	2019	1
(Inter)national conferences		
The Lancet Neurology Autoimmune Disorders Conference, Barcelona (attendance)	2015	1
Scientific retreat, Department of Neuro-oncology Erasmus MC (attendance and oral presentation)	2015	0.5
European Association of Neurology congress, Copenhagen (attendance and poster presentation)	2016	1
Italian Association of Neuroimmunology congress, Venice (attendance and poster presentation)	2017	1
International League against Epilepsy Congress, Barcelona (attendance and oral presentation)	2017	1
Encephalitis Society Conference, London(attendance and oral presentation)	2017	1
International League against Epilepsy Congress, Vienna (attendance and oral presentation)	2018	1
The Lancet Summit: Inflammation and Immunity in Disorders of the Brain and Mind, Barcelona (attendance and poster presentation)	2018	1
American Academy of Neurology Annual Meeting 2019, Philadelphia (attendance and two oral presentations)	2019	1
Teaching		
Supervising psychology student, Erasmus MC University Medical Center, Rotterdam, the Netherlands	2016	1
Guideline Erasmus MC: diagnosis and treatment of autoimmune encephalitis	2016	1
Supervising HLO student, Erasmus MC University Medical Center, Rotterdam, the Netherlands	2017	1
Teaching minor pediatric neurology, Erasmus MC University Medical Center, Rotterdam, the Netherlands	2017 and 2018	1

	Year	ECTS
Other		
Neuro-immunology meeting (multidisciplinary patient consultation, monthly), Erasmus MC University Medical Center, Rotterdam, the Netherlands	2015-2019	0.5
Research meeting (weekly), Laboratory of neuro-oncology, Erasmus MC University Medical Center, Rotterdam, the Netherlands	2014-2019	1
Journal club neuro-immunology, Erasmus MC University Medical Center, Rotterdam, the Netherlands	2017	1
Outpatient clinic neuro-immunology (monthly)	2014-2018	1
Development of module immunology of the Dutch epilepsy guideline, Dutch Association of Neurology (NVN), Utrecht, The Netherlands	2017-2019	1

LIST OF ABBREVIATIONS

ACES	Antibodies Contributing to focal Epilepsy Signs and symptoms
ADEM	Acute disseminated encephalomyelitis
AEDs	Antiepileptic drugs
AES	Autoimmune etiology of seizures
AIE	Autoimmune encephalitis
AMPAR	α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid receptor
CA	Cerebellar ataxia
Caspr2	Contactin Associated Proteine-2
CBA	Cell-based assay
CJD	Creutzfeldt–Jakob disease
CNS	Central nervous system
CSF	Cerebrospinal fluid
DPPX	Dipeptidyl-Peptidase-like Protein-6
ELISA	Enzyme-linked immunosorbent
FLAIR	Fluid-Attenuated Inversion Recovery
$GABA_AR$	Gamma-Aminobutyric Acid $Type_A$ receptor
$GABA_BR$	Gamma-Aminobutyric Acid $Type_B$ receptor
GAD65	Glutamic-Acid-Decarboxylase 65
GlyR	Glycine receptor
IHC	Immunohistochemistry
KCTD	Potassium channel tetramerization domain-containing
LE	Limbic encephalitis
LEMS	Lambert-Eaton myasthenic syndrome
LGI1	Leucine-rich glioma inactivated 1
mGluR	Metabotropic Glutamate receptor
mRS	modified Ranking Scale
NMDAR	N-Methyl-D-Aspartate receptor
OCB	Oligoclonal bands
PERM	progressive encephalomyelitis with rigidity and myoclonus
PNS	Paraneoplastic syndromes
RIA	Radio-immuno-assay
RPD	Rapidly progressive dementia
SCLC	Small cell lung cancer
SE	Status epilepticus
SPS	Stiff person syndrome
VGKC	Voltage-gated potassium channel