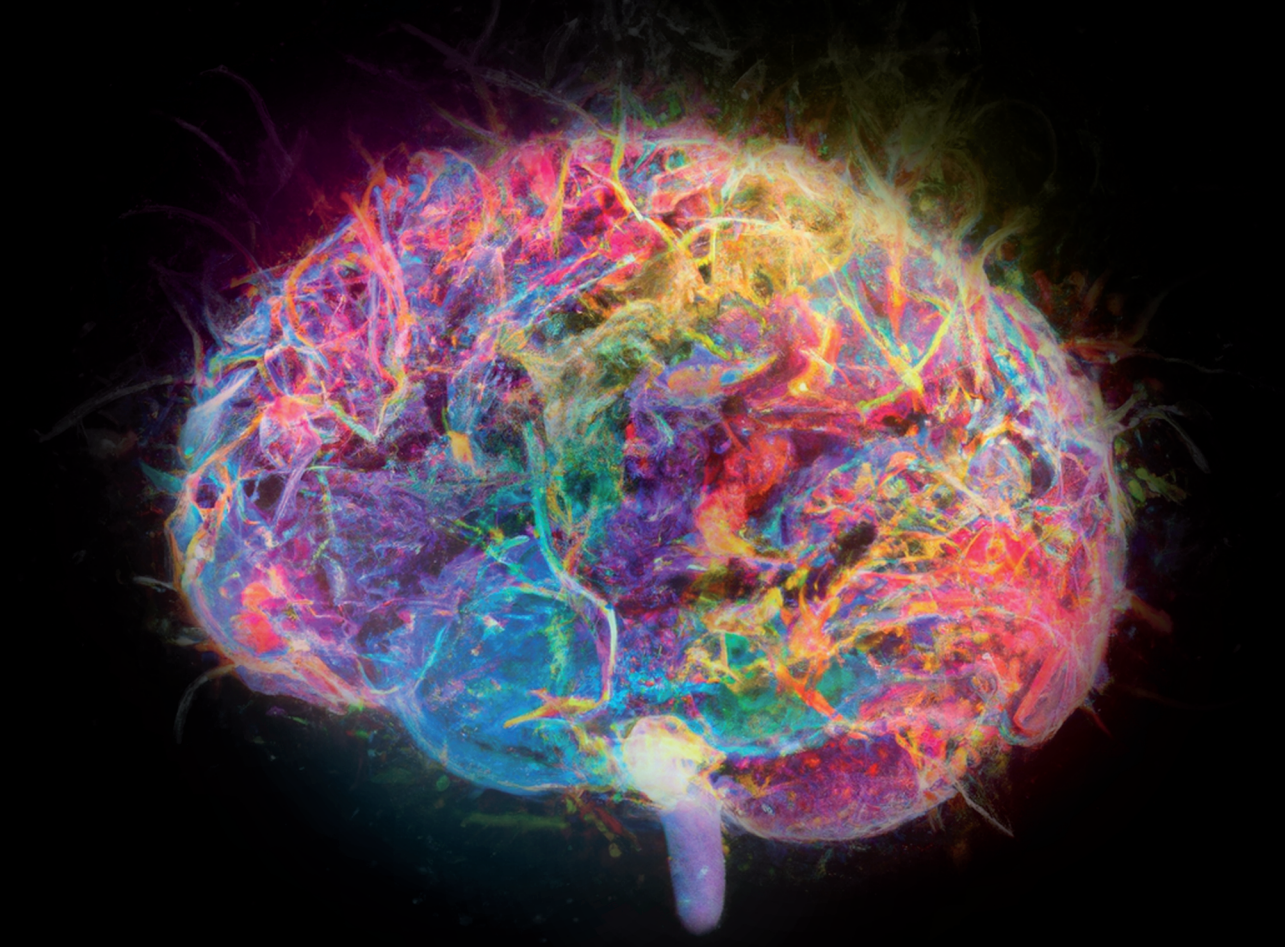
Recognition and treatment of autoimmune encephalitis

Focus on the elderly patient



Daniëlle Bastiaansen

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Focus op de oudere patiënt

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General introduction

Adapted from: Autoimmune encephalitis with anti-leucine-rich glioma-inactivated 1 or anti-contactin-associated protein-like 2 antibodies (formerly called voltage-gated potassium channel-complex antibodies).

A.E.M. Bastiaansen
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AUTOIMMUNE ENCEPHALITIS

Autoimmune diseases are characterized by a pathologic reaction directed to self-antigens leading to inflammation, cell injury, and functional deficits. In autoimmune encephalitis (AIE), antibodies recognize extracellular antigens in the nervous system. These antibodies are directly pathogenic and cause functional alterations of the target protein resulting in neurological symptoms. AIE is a rare but severe condition and diagnosis is essential as patients generally respond to immunotherapy. AIE can occur in association with tumors, although in a substantial number of patients no tumor is identified. The first association between malignancies, antineuronal antibodies and neurological symptoms, was in the 1960s long before the discovery of AIE.[1, 2] The difference with AIE is that these antibodies generally do not react to extracellular surface antigens as in AIE, but recognize intracellular proteins. These remote effects of cancer were called paraneoplastic neurological syndromes (PNS). Given that AIE too can occur in association with tumors, the differentiation between AIE and PNS is somewhat arbitrary. In the introduction, first AIE is discussed and the second part is dedicated to PNS.[3-5]

AIE is a relatively new disease entity since only in 2000 the first antibodies to extracellular neuronal proteins in the central nervous system were discovered, namely metabotropic glutamate receptor type 1 antibodies (mGluR1)[6] and voltage-gated potassium channel antibodies (VGKC).[7-9] A major breakthrough came when N-methyl-d-aspartate receptor (NMDAR) antibodies were discovered revealing a new clinical entity: anti-NMDAR encephalitis (2007).[10] Anti-NMDAR encephalitis is the most common subtype, although it is a rare disease with an incidence of approximately 0.7-2.2 per million annually.[11-14] After anti-NMDAR, many more antibodies were discovered including antibodies aimed at GlyR [15], $GABA_BR$ [16], AMPAR [17], mGluR5 [18], DPPX [19], $GABA_AR$ [20], and IgLON5 [21] (Figure 1). Twenty years after the discovery of VGKC-related autoimmunity, it is currently known that the antibodies are not directed at the VGKC itself but at two closely associated proteins, leucine-rich glioma-inactivated 1 (LGI1)[22] and contactin-associated protein-like 2 (Caspr2).[23, 24] The term VGKC-complex antibodies, lumping patients with anti-LGI1, anti-Caspr2 antibodies or lacking both, should be considered obsolete.[25, 26] The spectrum of AIE continues to rapidly expand with the growing discovery of neuronal autoantibodies. This thesis mainly focusses on AIE in patients with an older age, with special emphasis on cognitive symptoms and dementia syndromes. At the end of this thesis treatment in PNS is discussed.

Autoimmune encephalitis in elderly patients

AIE is characterized by a subacute onset of cognitive or psychiatric deterioration. Other common symptoms are seizures, movement disorders, sleep problems, speech dif-

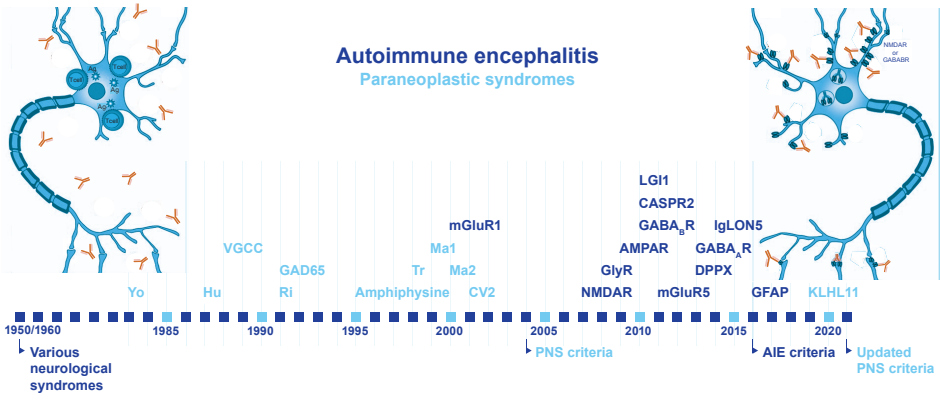

Figure 1. Growing spectrum of autoimmune encephalitis and paraneoplastic neurological syndromes.

ficulties, autonomic dysfunction, and an altered mental state.[3] Cognition is frequently affected in the most common AIE subtypes.[16, 27-30] The disease course can be slower than expected and mimic neurodegenerative dementia syndromes in the elderly patient. Contrary to neurodegenerative disease, patients with antibody-mediated encephalitis might benefit from immunotherapy and recover considerably or even completely. It is unknown how often AIE resembles dementia syndromes. In this thesis we search for red flags for AIE by studying patients with AIE who were initially suspected of dementia. Identifying these is essential for physicians to avoid misdiagnosis and inadvertently withholding treatment from patients (**Chapter 2**). In addition, we hypothesized that a small - but not insignificant - part of patients diagnosed with a neurodegenerative dementia syndromes, indeed suffer from AIE. To clarify this issue, we assessed the frequency of neuronal antibodies in a large cohort of patients with a presumed neurodegenerative dementia diagnosis in two memory clinics and describe the clinical characteristics of the patients with neuronal antibodies (**Chapter 3**).

Diagnosing AIE can be challenging, because AIE can present less fulminantly and with less notable encephalitis signs. Especially in elderly patients (e.g. NMDAR) the disease course is more protracted with a less outspoken clinical phenotype compared to younger patients.[27] In **Chapter 4** we try to confirm our suspicion that late-onset anti-NMDAR encephalitis is not as rare as initially thought and that malignancies are more frequently present.

Current diagnostic tools

MRI, CSF, and EEG

Physicians should be aware that inflammatory changes are not always present in AIE. The standard work-up when AIE is suspected involves brain MRI, CSF analysis and an EEG. All these ancillary tests can be normal, mildly abnormal or nonspecific.

MRI showing FLAIR/T2 hyperintensities of the mesiotemporal lobes (uni- or bilateral) is suggestive of AIE but other regions can be affected depending on antibody subtype.[3, 31, 32] On the other hand, extensive brain atrophy early in the disease course argues in favor of neurodegeneration. Abnormalities on diffusion weighted imaging MRI are more suggestive for Creutzfeldt-Jakob disease (CJD) in patients with rapidly progressive dementia.[33]

CSF analysis plays a central role in the diagnostic workup and can show: mild to moderate pleocytosis (usually <100 cells/μL), elevated protein concentration, elevated IgG index and oligoclonal bands.[3, 34] In neurodegenerative dementia syndromes, no abnormalities are expected in these routine CSF tests.

With exception of extreme delta brush in anti-NMDAR encephalitis (a pattern occurring in less than 10% of patients), alterations in EEG are rarely specific for AIE.[35] However, EEG can be useful in the differential diagnosis of other disorders e.g. CJD. Other reasons to perform EEG are to reveal subclinical seizures and non-convulsive status epilepticus. In many patients with AIE, EEG results only show minor encephalopathy similar to patterns seen in patients with neurodegenerative dementia.[36]

Depending on antibody subtype, a search for tumor presence is mandatory. In the elderly anti-NMDAR patient a thorough tumor workup, usually by FDG-PET/CT, is important to detect carcinomas. This is different form the workup in younger NMDAR patients as FDG-PET/CT is not sensitive to detect teratomas (**Chapter 4**).[27]

Antibody testing

The diagnosis of AIE strongly relies on the identification of neuronal antibodies. Both serum and CSF can be analyzed for antibodies. CSF is important because antibodies can sometimes be found only in CSF and not in serum. In addition, it is more rare to find false positive results in CSF.[37, 38] However, there are exceptions, e.g. anti-LGI1 and anti-GlyR antibodies are preferably tested in serum.

Most diagnostic laboratories use a commercial cell-based assay (CBA) when testing antineuronal cell surface antibodies. With a CBA, cells (usually human embryonic kidney [HEK] cells) are transfected with the suspected antigen, and these transfected cells are then incubated with patient's serum or CSF (Figure 2a). CBA's are only available for known antibodies, and especially serum CBA can yield false positive results or results that are unrelated to the syndrome. Research laboratories have additional techniques for antibody testing: immunohistochemistry (IHC) and immunocytochemistry of cultures of live rat hippocam-

pal neuron (LN). IHC can be used as a screening method whereby rat brain sections are incubated with patient's serum of CSF to find immunoreactivity (Figure 2b). An advantage of IHC is that it can provide a specific pathognomonic staining pattern (NMDAR, GAD65, and LGI1 antibodies), while a diffuse neuropil staining is seen in other neuronal cell surface antibodies. In LN, fluorescence is used for detection of bound antibodies to the outside of axons and dendrites of neurons (Figure 2c).[3, 38, 39] In the search for antibodies these three different techniques combined are very successful in identifying most antibodies.

As said, antibody testing may lead to misleading results when only serum is tested. Diagnosis might be missed as antibodies can be only detectable in CSF (15% of the patients with anti-NMDAR encephalitis). In addition, serum can yield positive but unconfirmed results (as reported in healthy controls, psychiatric conditions, and CJD).[40-47] Data involving false positive (unconfirmed) antibody test results in CSF are missing. In **Chapter 4** we describe the antibody test accuracy in both serum and CSF in our nationwide Dutch cohort of patients diagnosed with anti-NMDAR encephalitis. Although CSF testing is superior to serum, no test is perfect, and therefore it is important we challenge even this excellent test. This is particularly relevant when the clinical picture does not fit the identified antibody.

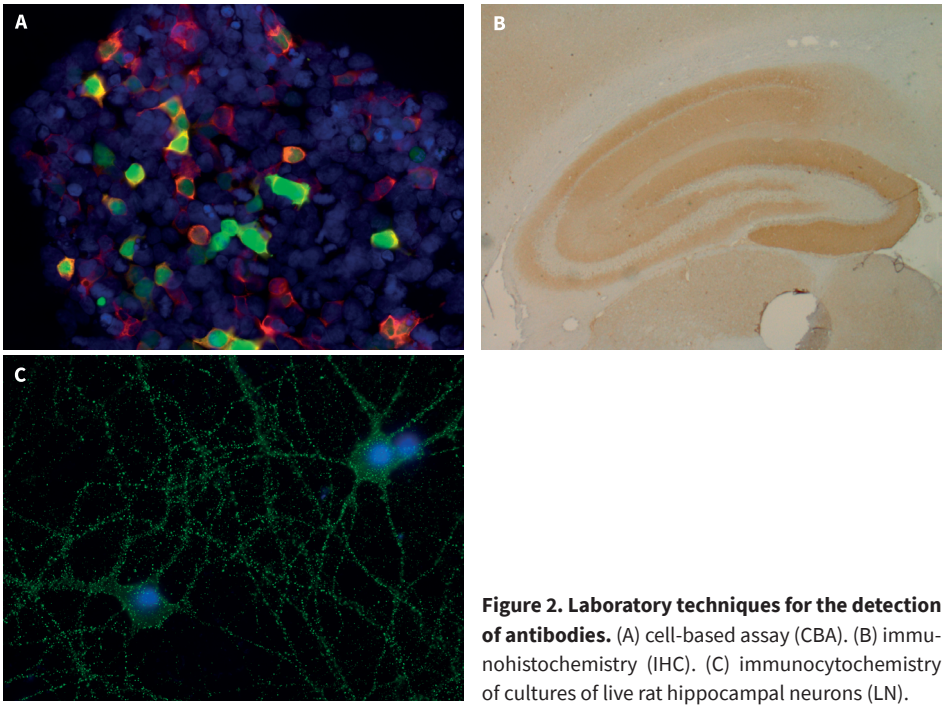

Figure 2. Laboratory techniques for the detection of antibodies. (A) cell-based assay (CBA). (B) immunohistochemistry (IHC). (C) immunocytochemistry of cultures of live rat hippocampal neurons (LN).

Graus criteria

Diagnosing AIE in the acute setting is challenging, because antibody test results are usually not immediately available. On the other hand, AIE is rare and has a complex differential diagnosis (e.g. structural lesions, infections, inflammation of other kind, metabolic or psychiatric disorders). In 2016 Graus et al. published guidelines to help physicians navigate through the differential diagnosis and select patients for antibody testing.[3] In addition, they provided diagnostic criteria assisting when to start empirical treatment with immunotherapy by establishment of an early diagnosis of probable or definite AIE awaiting neuronal antibody status. Furthermore, criteria for probable anti-NMDAR encephalitis were defined, and a novel diagnosis of antibody-negative but probable AIE was introduced since the absence of neuronal autoantibodies does not exclude AIE.
Generally the diagnostic criteria include subacute deterioration (rapid progression within months) of working memory deficits (short-term memory loss), altered mental status, or psychiatric symptoms, accompanied by seizures or abnormalities in ancillary testing suggestive of AIE.

Dementia biomarkers

In addition to the tests that are performed in the diagnostic workup for AIE, CSF dementia markers can be tested when dementia is suspected. These markers include total tau (t-tau), phosphorylated tau-181 (p-tau), and amyloid-beta-42 (Aβ42). T-tau is a sign of neuronal cell loss, p-tau is involved in aggregation of neurofibrillary tangles, and Aβ42 is a sign for cortical amyloid plaques. The last two are neurotoxic.[48] The various dementia subtypes have distinct marker profiles, for example the classic Alzheimer disease (AD) profile is a low Aβ42 and raised p-tau and t-tau, and in frontotemporal dementia (FTD) usually most markers are normal or there is only a (mildly) raised t-tau.[49] In dementia with Lewy bodies (DLB), half of the patients have a marker profile similar to AD. The sensitivity and specificity of these markers are limited and in the elderly healthy population, an abnormal marker profile can be found.[50, 51] Nevertheless, dementia markers can be helpful in the diagnostic process of a neurodegenerative dementia, as a normal marker profile can rule out AD with sufficient certainty. 14-3-3 is another marker and commonly attributed to CJD. However, this is known to be not highly specific (like t-tau), as it represents neuronal injury. Real-time quaking-induced conversion (RT-QuIC) was introduced more recently as a biomarker with higher specificity for CJD than 14-3-3.[52, 53] In AIE, dementia biomarkers (including 14-3-3) can show abnormal results. Differentiating between AIE and a neurodegenerative cause becomes more complex when CSF markers for dementia are abnormal. To clarify the frequency of these abnormal markers in AIE, we analyzed dementia biomarkers in AIE patients and give an overview in **Chapter 2**.

Treatment, prognosis and markers for prognosis

Prompt diagnosis is essential in AIE, since the earlier start of treatment improves outcome in the vast majority of patients. Treatment is aimed at neutralizing the overactive immune system.[3,54-56] There are several strategies for immunotherapy in the acute phase, depending on antibody subtype and mainly based on expert opinion. First-line therapy consist of corticosteroids (intravenous or oral), immunoglobulins, plasmapheresis, or frequently a combination of these first-line treatments. In non-responders or in case of a suboptimal response, second-line treatment can be administered consisting of rituximab, cyclophosphamide, or both. Chronic treatment, to prevent relapses after first-line therapy, consists mostly of azathioprine or mycophenolate mofetil. If present, anti-tumor therapy is of utmost importance to remove the trigger for antibody production and increase the chance for immunotherapy responsiveness.

Immunotherapy is effective in most patients (albeit antibody subtype dependent), although clinical improvement can take weeks to (many) months.[31] Treatment should be aggressive in many cases and should not be withheld in patients with a poor clinical condition, similar for anti-tumor therapy when it applies.

In the elderly population with AIE recovery is slower and less complete. Overall, 60% of the elderly patients had full or substantial recovery after 2 years, compared to 80% in younger patients (anti-NMDAR).[27] As it is known that brain plasticity and the capacity to recover diminish with age,[57] better chances for recovery necessitate early treatment. (**Chapter 4**)

In 2019, the anti-NMDAR encephalitis one-year functional status (NEOS) score was published. With this tool the functional status one year after disease onset can be predicted, helping clinicians to counsel families on expected disease course and recovery trajectory.[58] In addition, it could help to identify patients who could benefit from novel therapies. Table 1 shows the 5 items included in the score, wherein a higher NEOS score is strongly associated with progressively lower probability of good functional outcome. The score should not be used to predict final expected outcome since patients may still recover beyond one year after disease onset. A limitation of this score is that patients are already one month into treatment and that it does not predict response to immunotherapy, identifying patients needing aggressive treatment and avoid harmful side-effects in those with good outcome.

Biomarkers for disease severity and prognosis are limited in AIE. Neurofilament light chain (NfL) has been identified as a useful biomarker in several neuro-inflammatory and neurodegenerative disorders.[59] NfL is a marker of axonal damage. In **Chapter 5**, we investigated the relationship between NfL levels and disease severity and outcome in anti-NMDAR encephalitis.

Table 1. The anti-NMDAR encephalitis one-year functional status (NEOS) score

ICU admission required
No clinical improvement after 4 weeks of treatment
No treatment within 4 weeks of symptom onset
Abnormal MRI
CSF WBC count >20 cells/μL

Each item can be scored as 0 or 1, and an increasing NEOS score is associated with a lower chance of good recovery (mRS 0-2) at one year from onset.

PARANEOPLASTIC NEUROLOGICAL SYNDROMES

In PNS, antibodies generally do not react to extracellular surface antigens as in AIE, but recognize intracellular neuronal proteins. It is thought that the expression of proteins by the tumor provokes an autoimmune response. This response is not only directed against the tumor but also against nervous tissues explaining the neurologic features.

The immunopathogenesis is not fully understood, however it is most likely that the neuronal destruction is caused by cytotoxic T cells. The intracellular proteins cannot be reached by antibodies and many animal models failed to demonstrate antibody-induced disease. Contrary to AIE, the antibodies are not pathogenic but are considered a marker for PNS (and tumor presence). Furthermore, autopsy studies consistently showed T cell infiltrates surrounding neurons with associated neuronal loss. However, circulating antigen-specific T-cells were never consistently found.[60-63]

In 2004, diagnostic criteria for PNS were defined and the term “well characterized onconeuronal” antibody was introduced based on the frequent association with cancer and the associated neurological syndromes.[64] The criteria were updated in 2021 mostly because antibodies against extracellular antigens can also occur with cancer.[65] The term onconeural antibody was replaced by high-risk antibodies (>70% associated with cancer) consisting mostly of antibodies against intracellular antigens, for example anti-Hu, anti-Yo, and anti-Ri. In addition, the terms intermediated-risk (30-70%) and low-risk (<30%) antibodies were introduced. A PNS-Care score was introduced to obtain diagnostic certainty in diagnosing PNS (possible, probable, and definite PNS), combining clinical phenotype, antibody type, and cancer presence/absence. High-risk clinical phenotypes (formerly known as “classical PNS”) frequently have a paraneoplastic etiology and comprise: encephalomyelitis, limbic encephalitis, rapidly progressive cerebellar syndrome, opsoclonus-myoclonus, sensory neuronopathy, gastrointestinal pseudo-obstruction (enteric neuropathy), and Lambert-Eaton myasthenic syndrome.[65]

Anti-Hu is the most frequent high-risk antibody and in a high percentage associated with small cell lung cancer (SCLC). Patients having a PNS with anti-Hu antibodies (Hu-

PNS) predominantly present with a sensory neuronopathy caused by involvement of the sensory neurons of the dorsal root ganglia. Sometimes additional motor nerve roots are involved causing motor symptoms. Other presentations are gastrointestinal pseudo-obstruction due to myenteric plexus dysfunction, encephalomyelitis, or limbic encephalitis. Hu-PNS is a severe disease progressing rapidly over weeks to months leaving more than half of the patients bed- or wheelchair-bound. It has a poor prognosis, only fewer than 10% of patients improve and the median survival is less than one year. In over 70%, at the time of presentation, the patient is unaware of the cancer delaying the diagnosis.[20, 66-68] It is important to search for an underlying malignancy and start anti-tumor treatment as soon as possible. As is the case for AIE, start of (aggressive) treatment should be independent of the level of functioning in cancer patients with PNS. Previous trials with immunotherapy showed only improvement in a minority of patients (<10%). Since Hu-PNS is thought to be a T cell-mediated disease, we conducted a prospective trial with natalizumab (**Chapter 6**). Natalizumab is effective in the treatment of relapsing-remitting multiple sclerosis (MS).[69] It strongly inhibits migration of T cells into the nervous system and contributes to reduced activation of T cells that are already present in the CNS, lowering damage done to the nervous system in MS.[69-71]

HYPOTHESES

With increasing experience of AIE, it has become clear that often a neurodegenerative dementia has been part of the differential diagnosis. This has led us to form several hypotheses:

- AIE in elderly patients frequently can resemble neurodegenerative dementia syndromes, and we aim to identify red flags pointing towards AIE. (**Chapter 2**)
- Ancillary testing, including CSF white blood cell count and dementia biomarkers, can be different between AIE and neurodegenerative syndromes, but will it be sufficient to separate these entities? (**Chapter 2**)
- In patients diagnosed with a presumed neurodegenerative dementia, it is likely that a small, but clinically relevant proportion has neuronal antibodies, and in fact AIE. (**Chapter 3)**

Fifteen years after the discovery of anti-NMDAR encephalitis, there is more awareness and experience. This has several consequences:

- The age distribution and clinical phenotype has changed over this time period. It is expected that anti-NMDAR encephalitis is more diagnosed in elderly patients nowadays. (**Chapter 4**)
- More awareness leads to broader testing for NMDAR antibodies, and lower a priori chances demand for optimal testing characteristics. We hypothesize that there is still room for improvement, but it is unknown whether this even holds true for analysis of CSF (**Chapter 4**)
- NfL levels are reported to be increased in patients with an anti-NMDAR encephalitis. NfL is a marker for neuronal damage, and the NMDAR antibodies initially seem to cause functional deficits without structural damage. It is therefore unclear whether (serum) NfL, measured early in disease, has prognostic value for disease severity or prognosis. (**Chapter 5**)

There have been several attempts to treat anti-Hu associated paraneoplastic neurological syndromes, but studies showing benefit of specific treatments are still lacking. A new open-label study using natalizumab, comparing it to previous treatment studies, tried to fill this gap.

- Natalizumab might be beneficiary in the treatment of anti-Hu associated PNS. (**Chapter 6**)

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2

Autoimmune encephalitis resembling dementia syndromes

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ABSTRACT

Objective

Autoimmune encephalitis (AIE) can resemble neurodegenerative dementia syndromes, as patients do not always present as encephalitis. This study evaluates how frequently AIE mimics dementia, and provides red flags for AIE in middle-aged and older patients.

Methods

In this nationwide observational cohort study, patients with anti-LGI1, anti-NMDAR, anti-GABABR, or anti-CASPR2 encephalitis were included. They had to meet three additional criteria: age ≥45 years, fulfillment of dementia criteria, and no prominent seizures early in the disease course (≤4 weeks).

Results

Two-hundred-and-ninety patients had AIE, of whom 175 were ≥45 years. Sixty-seven patients (38%) fulfilled criteria for dementia without prominent seizures early in disease course. Of them, 42 had anti-LGI1 (48%), 13 anti-NMDAR (52%), 8 anti-$GABA_BR$ (22%), and 4 anti-CASPR2 (15%) encephalitis. Rapidly progressive cognitive deterioration was seen in 48 patients (76%), while a neurodegenerative dementia syndrome was suspected in half (n=33). In 17 patients (27%; 16/17 anti-LGI1) subtle seizures had been overlooked. Sixteen patients (25%) neither had inflammatory changes on brain MRI, nor CSF pleocytosis. At least one CSF biomarker, often requested when dementia was suspected, was abnormal in 27/44 tested patients (61%), while 8 had positive 14-3-3 results (19%). Most patients (84%) improved after immunotherapy.

Conclusion

Red flags for AIE in patients with suspected dementia are: 1) rapidly progressive cognitive decline, 2) subtle seizures, and 3) abnormalities in ancillary testing atypical for neurodegeneration. Physicians should be aware that inflammatory changes are not always present in AIE, and that biomarkers often requested when dementia was suspected (including 14-3-3) can show abnormal results. Diagnosis is essential as most patients profit from immunotherapy.

INTRODUCTION

Autoimmune encephalitis (AIE) comprise a group of antibody-mediated inflammatory brain diseases. Binding of these antibodies to extracellular epitopes of neuronal structures leads to cerebral dysfunction. Diagnostic criteria for AIE help to select patients for antibody testing. These criteria are characterized by a subacute deterioration of cognition, altered mental status, or psychiatric symptoms. These symptoms should be accompanied by seizures, new findings of focal involvement of the central nervous system, or inflammatory changes in cerebrospinal fluid (CSF; pleocytosis) or on brain MRI. [1] Anti-LGI1, anti-NMDAR, anti-$GABA_BR$, or anti-CASPR2 antibodies are the most common antibodies causing AIE and cognition is frequently affected in all these AIE subtypes. [2-5] Diagnosing AIE can be challenging since patients can present with less notable encephalitis signs. The disease course can mimic neurodegenerative dementia syndromes. Rapid progression is often expected, but slower progression has also been described, resulting in misdiagnosis or treatment delay leading to a worse outcome. [5-10] It is unknown how often AIE resembles dementia syndromes. [11, 12] In patients presenting with a possible dementia, clinical clues are essential for physicians to avoid misdiagnosis and inadvertently withhold patients from immunotherapy.

The study aim was to evaluate possible dementia diagnosis and to describe red flags for AIE in middle-aged and older patients with anti-LGI1, anti-NMDAR, anti-CASPR2, and anti-$GABA_BR$ encephalitis.

METHODS

Patients

We performed a nationwide observational cohort study in middle-aged and older patients with anti-LGI1, anti-NMDAR, anti-$GABA_BR$, and anti-CASPR2 encephalitis. The department of Neurology of the Erasmus University Medical Center is the national referral site for patients with suspected AIE, and the laboratory of Medical Immunology is the ISO 15189 accredited national referral site for anti-neuronal antibody testing. Patients were identified between August 1999 and September 2019, although 87% were identified after 2010. All Dutch patients with AIE with anti-LGI1, anti-NMDAR, anti-GABABR, or anti-CASPR2 antibodies were asked to participate. [3-5, 13] Antibodies were detected in serum, or in CSF using validated commercial cell based assays (CBA), and were confirmed with in house CBA, immunohistochemistry or live hippocampal neurons as described before. [3, 5, 14, 15] Only patients who were ≥45 years old at disease onset were included, as the main challenge to discriminate between AIE and neurodegenerative dementia is within this age group (Figure 1).

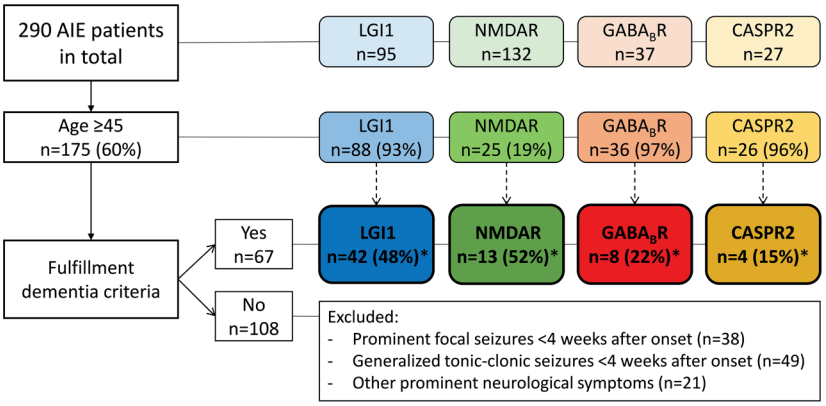

Figure 1. Patient inclusion.
In total, 290 patients with autoimmune encephalitis were identified. At disease onset, 175 of the patients had an age ≥45 year. Sixty-seven patients fulfilled the dementia criteria including the additional condition that no prominent seizures were present at early disease course (≤ 4 weeks). *percentage of the patients ≥45 years of age.

In addition to the tests that were performed in the diagnostic workup, CSF markers often requested when dementia was suspected (total tau (t-tau), phosphorylated tau-181 (p-tau), and 14-3-3) were determined in all patients with sufficient available CSF (n=12), in the ISO 15189 accredited lab at Radboud UMC. [16] Levels of t-tau and p-tau were measured using enzyme-linked immunosorbent assays (Fujirebio, Ghent, Belgium). From February 2019 a semi-automated version of the same ELISAs using Lumipulse (Fujirebio, Ghent, Belgium) was used. 14-3-3 was analyzed using Western blotting as previously described. [17] Furthermore, patients with a positive 14-3-3 and sufficient available CSF were post-hoc tested for RT-QuIC (Real-Time Quaking Induced Conversion). [18] All values were scored according to the reference values at the time of testing, and adjusted to current cut-off values in the figure for ease of comparison. Cut-off values to be considered abnormal were t-tau >400 pg/ml, p-tau >64 pg/ml, amyloid-beta-42 (Aβ42) <500 pg/ml, a t-tau/p-tau ratio >30, and a t-tau/Aβ42 ratio >0.52. A positive 14-3-3 or RT-QuIC was also abnormal. Based on these CSF markers, patients had a Creutzfeldt-Jakob disease (CJD) profile if the t-tau/p-tau ratio was abnormal, and an Alzheimer dementia (AD) profile was assigned when A β42 was lowered or the t-tau/Aβ42 ratio was abnormal. [19]
MRI images were reviewed at our site by neuroradiologists in most cases visiting our center, while in patients with LGI1 antibodies MRI images were scored by an independent neuroradiologist as published before. [3] In the remaining patients, radiographic outcomes were based on the radiology reports.

Clinical phenotype and dementia criteria

Clinical patient data were retrieved during a visit to our clinic in 48%, from telephone interviews with patients or relatives in 31%, and from medical files in 21%. The clini-

cal disease course was assessed for fulfillment of the 2011 NINCDS-ADRDA criteria for dementia. [20] These internationally accepted core clinical criteria can be used for the diagnosis of all-cause dementia. Dementia is diagnosed when there are cognitive or behavioral symptoms that: 1. Interfere with the ability to function at work or at usual activities; 2. Represent a decline from previous levels of functioning and performing; 3. Are not explained by delirium or major psychiatric disorder; 4. Cognitive impairment is detected and diagnosed through a combination of history-taking and a cognitive assessment; 5. The cognitive or behavioral impairment involves a minimum of two of the following domains: a. Impaired ability to acquire and remember new information; b. Impaired executive functions; c. Impaired visuospatial abilities; d. Impaired language functions; e. Changes in personality, behavior, or comportment. [20] Rapidly progressive dementia (RPD) was defined as fulfillment of the dementia criteria within 12 months or death within 2 years after the appearance of the first cognitive symptoms. [21]

In addition we excluded patients with prominent seizures early in the disease course (≤ 4 weeks), since this is less likely in neurodegenerative dementia syndromes, and physicians will already suspect inflammatory causes. Subtle seizures that remained unnoticed by the treating physician were not covered by this additional criteria.

Level of functioning was measured with the modified Rankin Scale (mRS), [22] and in most patients we had direct contact to obtain mRS scores. Cognitive domains were assessed by two persons independently reviewing all clinical charts, using neuropsychological assessments, Mini-Mental State Examinations, and Montreal Cognitive Assessments when available.

Statistics

Categorical data were compared using the Fisher-Freeman-Halton test. Continuous data were analysed using one-way analysis of variance with log-transformation because of skewed distribution (age at disease onset and delay until initiation of treatment after disease onset) and the Kruskall-Wallis test (days between onset and start seizures, days to cognitive decline after disease onset, duration of follow-up, mRS at follow-up). To assess multiple testing, p-values below 0.005 were considered significant. Values between 0.05 and 0.005 should be interpreted carefully and considered exploratory. Post-hoc analysis to evaluate differences between antibody types were assessed using the same statistical tests, corrected by Holm's method. We used SPSS 25.0 (SPSS Inc) for Windows for statistical analysis, as well as Prism 8.4.3 (GraphPad).

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by the Institutional Review Board of Erasmus MC. Written informed consent was obtained from all patients.

Data Availability Statement

Any data not published within this article are available at the Erasmus MC University Medical Center. Patient-related data will be shared upon reasonable request from any qualified investigator, maintaining anonymization of the individual patients.

RESULTS

Patient characteristics

In total, 290 patients with AIE were identified, of whom 95 patients harbored LGI1 antibodies, 132 NMDAR antibodies, 37 $GABA_BR$ antibodies, and 26 CASPR2 antibodies. At disease onset, 175 of the patients (60%) had an age ≥45 year, including 88 LGI1 (93%), 25 NMDAR (19%), 36 $GABA_BR$ (97%), and 26 CASPR2 (100%) encephalitis patients. These patients were assessed for fulfillment of the dementia criteria including the additional condition that no prominent seizures were present at early disease course. Sixty-seven patients fulfilled these criteria (39%): 42 LGI1 (48%), 13 NMDAR (52%), 8 $GABA_BR$ (22%), and 4 CASPR2 (15%) encephalitis patients (Figure 1). Patients who had no very rapid onset (only fulfilling dementia criteria beyond three months) and had neither MRI abnormalities nor CSF pleocytosis were highlighted in Supplementary Figure e-1 and Table e-1, as these pose the largest challenge. The CASPR2 encephalitis patients were excluded from statistical analysis (due to the small number) and described exploratively in Supplementary Text.

Of the remaining 63 patients with anti-LGI1, anti-NMDAR, and anti-$GABA_BR$ encephalitis, 37 were male (58%; Table 1). In anti-LGI1 encephalitis there was a trend towards a male predominance compared to the higher frequency of females in anti-NMDAR encephalitis ($p_{uncorrected} = 0.047$). The median age at onset was 64 years (IQR 58-72, range 48-85).

Almost all patients had cognitive deterioration (n = 62, 98%) and behavioral changes (n = 55, 87%).

Cognitive decline was the presenting symptom in most patients (n = 48, 76%; median time to cognitive decline 0 days). There was a rapidly progressive deterioration of cognitive symptoms in 48 patients (76%) and five patients were admitted to a closed psychogeriatric ward. In half of the patients (n = 33, 52%) a neurodegenerative dementia syndrome was suspected by the treating physician.

Cognitive domains were affected differently in the various AIE subtypes (Figure 2). Patients with anti-LGI1 or anti-$GABA_BR$ encephalitis had similarities with more prominent and more frequently severe impairment of visuospatial and executive functions (~70% in LGI1 and 55% in $GABA_BR$ encephalitis). In contrast, patients with anti-NMDAR encephalitis more frequently had impaired language functions (85%, $p < 0.0001$) and behavioral changes were more prominent.

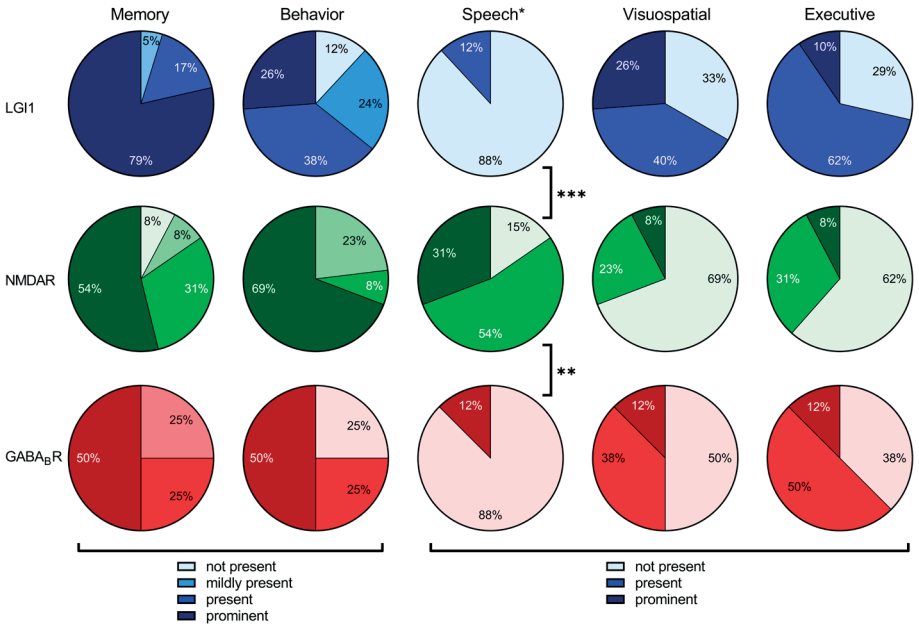

Figure 2. Cognitive domains in autoimmune encephalitis.
For patients with anti-LGI, anti-NMDAR, and anti-GABA$_B$R encephalitis cognitive symptoms were divided into 5 cognitive domains. The domains for memory and behavior were divided into 4 categories (not present, mildly present, present, prominent) and the speech, visuospatial, and executive domains were divided into 3 categories (not present, present, and prominent). ***p<0.0001 and **p=0.001 between anti-NMDAR and respectively anti-LGI1 and anti-GABA$_B$R.

Sleep related problems were most frequent in anti-LGI1 encephalitis (57%, p = 0.004). In anti-NMDAR encephalitis, patients experienced besides the speech problems, more movement disorders (46%, $p_{uncorrected}$ = 0.009; Table 1 and Supplementary Table e-2).
There were no prominent seizures early (≤4 weeks) in the disease course (exclusion criterion). If prominent seizures were present, these occurred after a median of 3 months (IQR 42-181 days). However, 40 patients (64%) developed seizures during the course of the disease. Looking scrutinously, actually 11 out of 40 patients with seizures (28%), had developed subtle seizures within 2 weeks after disease onset. However, in all patients these were initially missed faciobrachial dystonic seizures (FBDS) or non-motor subtle focal seizures. In total, subtle seizures were overlooked in a quarter of the patients (n = 17). Most subtle seizures were seen in anti-LGI1 encephalitis (n = 16) compared to the other AIE subtypes ($p_{uncorrected}$ = 0.011).

Table 1. Patient characteristics

	Total	LGI1 (n=42)	NMDAR (n=13)	$GABA_BR$ (n=8)	p-value[a]
Gender, male	37 (58%)	29 (69%)	4 (31%)	4 (50%)	0.047*
Age at onset	64 (58-72, 48-85)	66 (59-72, 49-82)	61 (57-68, 48-73)	73 (58-76, 55-85)	0.11
Cognition characteristics					
Cognitive symptoms	62 (98%)	41 (98%)	13 (100%)	1 (100%)	1.00
Median days to cognitive decline after disease onset	0 (0-0, 0-176)	0 (0-8, 0-176)	0 (0-0, 0-7)	0 (0-0, 0-0)	0.180
Cognitive decline presenting symptom	48 (76%)	30 (71%)	11 (85%)	7 (88%)	0.55
RPD	48 (76%)	33 (79%)	11 (85%)	4 (50%)	0.24
Dementia suspected by treating physician	33 (52%)	21 (50%)	7 (54%)	5 (63%)	0.87
Dementia markers tested	44 (65%)	27 (64%)	9 (69%)	5 (56%)	
Symptoms (during disease course)					
Behavioral changes	55 (87%)	35 (83%)	13 (100%)	7 (88%)	0.25
Speech problems	17 (27%)	5 (14%)	11 (85%)	1 (13%)	**<0.0001*** **
Movement disorders	12 (19%)	4 (10%)	6 (46%)	2 (25%)	0.009*
Awareness problems	4 (6%)	0	3 (23%)	1 (13%)	0.010*
Autonomic symptoms	15 (24%)	12 (29%)	3 (23%)	0	0.29
Sleep disorders	27 (43%)	24 (57%)	2 (15%)	1 (13%)	**0.004****
Epilepsy					
Seizures during disease course	40 (64%)	32 (76%)	3 (23%)	5 (63%)	**0.002****
Days between onset and start prominent seizures	95 (42-181, 30-1098)	117 (60-183, 30-1095)	221 (34-409, 34-409)	52 (38-85, 37-93)	0.44
Subtle seizures early in disease course	17 (27%)	16 (38%)	0	1 (13%)	0.011*
Ancillary testing					
Routine CSF normal[±]	31/58 (53%)	29/38 (76%)	2/13 (15%)	0/7	**<0.0001*** **
WBC elevated	21/58 (36%)	5/38 (13%)	11/13 (85%)	5/7 (71%)	
Total protein elevated	18/55 (33%)	6/38 (16%)	6/12 (50%)	3/5 (60%)	
IgG index elevated	9/18 (50%)	4/9 (44%)	2/5 (40%)	3/4 (75%)	
Oligoclonal bands present	5/9 (56%)	0/3	2/3 (67%)	3/3 (100%)	
MRI mesiotemporal hyperintensities	30/62 (48%)	24/41 (60%)	2/13 (15%)	4/8 (50%)	0.023*
EEG abnormal	31/56 (55%)	21/38 (55%)	6/12 (50%)	4/6 (67%)	0.84
Encephalopatic	28 (50%)	18 (49%)	6 (50%)	4 (67%)	

Table 1. Patient characteristics (*continued*)

	Total	LGI1 (n=42)	NMDAR (n=13)	$GABA_BR$ (n=8)	p-value[a]
Epileptic	13 (23%)	9 (24%)	3 (25%)	1 (17%)	
Encephalopatic and epileptic	10 (18%)	6 (16%)	3 (25%)	1 (17%)	
Tumor	10/60 (17%)	3/40 (8%)	3/13 (23%)	4/7 (57%)[#]	**0.004****

Data are n (%), n/n (%) or median (interquartile range; range).
*p-value <0.05, **p-value <0.005, ***p-value <0.0005
[a]Only p-values below 0.005 were considered relevant.
[#]3 small cell lung carcinoma and 1 unknown tumor
[±]In 8/31 patients OCB or IgG index was examined, and tested normal.
Abbreviations: LGI1 = leucine-rich glioma-inactivated 1; NMDAR = NMDA receptor; $GABA_BR$ = gamma-aminobutyric acid B-receptor; RPD = rapidly progressive dementia; CSF = cerebrospinal fluid; WBC = white blood cell count; MRI = magnetic resonance imaging; EEG = electroencephalogram.

Ancillary testing

Ancillary testing showed normal routine CSF results (white blood cell count, total protein; and if performed IgG index and oligoclonal bands) and no abnormalities related to AIE (hyperintensities of the mesial temporal lobe) on MRI T2/FLAIR in half of the patients (53% and 54%, respectively). In 16/61 (25%) neither CSF pleocytosis nor MRI inflammatory changes were found. In anti-LGI1, CSF was even more frequently normal (76%, p = <0.0001). In all patients, atrophy was rarely seen on initial MRI (n = 4) and no abnormalities on diffusion-weighed imaging (DWI) were reported. EEG showed epileptic discharges in 13 patients (23%) and in 25 patients (45%) the EEG was normal, similar between AIE subtypes. Tumor screening resulted in malignancies in 10 patients (17%) and as expected, in patients with $GABA_BR$ antibodies this was most frequent (57%; p = 0.004). Only two patients underwent ^{18}fluorodeoxyglucose positron emission tomography (FDG-PET) of the brain: one showed hypometabolism in the right caudate area, while the other was normal.

CSF biomarkers (total tau, phosphorylated tau, Aβ42) were tested in 44 patients (Aβ42 only in 29; Figure 3). High total tau was seen in 19 patients (45%), a high p-tau in 6 patients (16%) and a low Aβ42 in 12 patients (41%). A high tau/p-tau ratio (>30; suggestive for CJD) was present in 6/38 patients (16%) and 14-3-3 was (weakly) positive in 8 out of 42 patients (19%). Five patients with a positive 14-3-3 had been tested by RT-QuIC, and all tested negative. In anti-$GABA_BR$ encephalitis, the 14-3-3 test was most often found positive, but this was not significantly different compared to other AIE subtypes. The clinical profile of the AIE patients with a high total tau or high t-tau/Aβ42 is shown in Supplementary Table e-3. Based on the these CSF markers that are often requested when dementia was suspected, 14 patients were considered to have a CSF profile suitable for Alzheimer disease or CJD.

We could not identify significant differences between patient with and without RPD, except for the obvious time to dementia (data not shown).

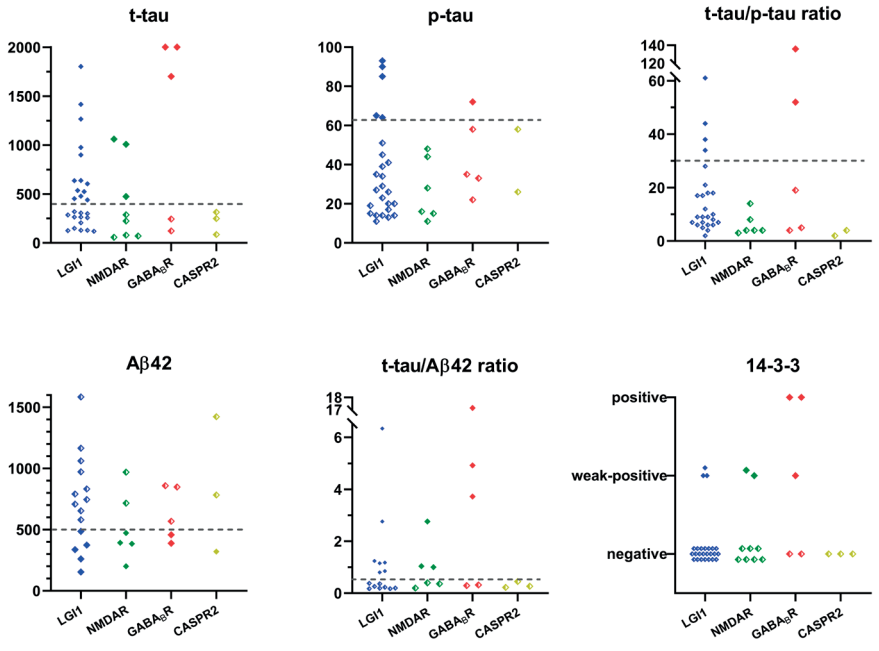

Figure 3. Dementia biomarkers in patients with autoimmune encephalitis.
Dementia CSF biomarkers in 44 patients with autoimmune encephalitis Cut-off values to be considered abnormal were t-tau >400 pg/ml, p-tau >64 pg/ml, amyloid-beta-42 (Aβ42) <500 pg/ml, a t-tau/p-tau ratio >30, and a t-tau/Aβ42 ratio >0.52. A positive 14-3-3 is abnormal. Two patients with t-tau values of 14720 and 2800 were maximized at 2001. Five patients with a positive 14-3-3 had been tested by RT-QuIC, all negative. Filled diamond symbols represents abnormal results and half-filled symbols represent normal results.

Treatment and outcome

Median mRS at onset was 3 (IQR 3-4; 3% ADL independent) and patients were admitted to the ICU in 16% of the total cohort (Table 2). Most patients (n = 59, 94%) were treated with first-line immunotherapy (combination of IV methylprednisolone or IV immunoglobulins). Nine patients (14%) received additional second-line immunotherapy (rituximab or cyclophosphamide). In 4/8 anti-GABA$_B$R encephalitis patients, no immunotherapy was administered. Two of these patients received chemotherapy for small cell lung carcinoma, and the remaining two were post-mortem diagnosed as anti-GABA$_B$R encephalitis. In anti-NMDAR encephalitis patients, second-line immunotherapy was administered more frequently (39%, p = 0.005).

The median delay until initiation of treatment after disease onset was 99 days (IQR 32-219).

To analyze the effects of treatment delay, without interference of antibody subtype, we assessed treatment in the largest AIE subtype (anti-LGI encephalitis). Patients with a longer delay until start of immunotherapy after disease onset (>60 days, n=28/41) had a

higher mRS at 6 and 12 months (mRS 3 [IQR 2-3] versus mRS 2 [IQR 1-2], p = 0.012; and mRS 2 [IQR 2-3] versus mRS 1 [IQR 1-2], p = 0.027 respectively). Similarly, more cognitive problems remained after 6 months in those treated later (96% versus 67%, p = 0.02), while a similar trend was seen at 12 months of follow-up (92% versus 67%, p = 0.10). Patients improved after therapy indicated by a lower mRS score after treatment (median mRS 2; 67% ADL independent). Only in anti-$GABA_BR$ encephalitis, patients tended to remain dependent more frequently, while in the other AIE subtypes the majority became independent ($p_{uncorrected}$ = 0.019). Cognitive deficits were still present after 12 months in the majority of patients (81%), and were similar between AIE subtypes. In total, encephalitis relapses were seen in 11 patients (17%) and 14 patients had died (22%).

Table 2. Treatment and outcome

	Total	LGI1 (n=42)	NMDAR (n=13)	$GABA_BR$ (n=8)	p-value[a]
Immune therapy					
Days to immunotherapy after disease onset	99 (32-219, 2-5080)	110 (38-258, 2-5080)	56 (18-148, 7-427)	29 (22-46, 15-63)	0.13
1st line immunotherapy	59 (94%)	42 (100%)	13 (100%)	4 (50%) [#]	**<0.0001*** **
IV methylprednisolone	52 (83%)	36 (86%)	12 (92%)	4 (50%)	0.055
IV immunoglobulins	43 (68%)	29 (69%)	12 (92%)	2 (25%)	0.006*
2nd line immunotherapy	9 (14%)	2 (5%)	5 (39%)	2 (25%)	**0.005****
Rituximab	7 (11%)	2 (5%)	3 (23%)	2 (25%)	0.057
Cyclophosphamide	4 (6%)	0 (0%)	3 (23%)	1 (13%)	0.010*
Evolution					
ICU	10 (16%)	3 (7%)	6 (46%)	1 (13%)	**0.004****
mRS at onset	3 (3-4, 2-5)	3 (3-4, 2-5)	4 (3-5, 3-5)	4 (3-5, 2-5)	0.086
0-2	2 (3%)	1 (2%)	0	1 (14%)	
3-5	61 (97%)	41 (98%)	13 (100%)	7 (86%)	
Best mRS after treatment (n=63)	2 (1-3, 0-5)	2 (1-3, 0-4)	2 (1-4, 0-5)	3 (2-4, 2-5)	0.019*
0-2	42 (67%)	31 (74%)	9 (69%)	2 (25%)	
3-5	21 (33%)	11 (26%)	4 (31%)	6 (75%)	
Cognitive complaints 6 months after onset	47/55 (86%)	35/40 (88%)	6/9 (67%)	6/6 (100%)	0.19
mRS 6 months after onset (n=59)	3 (2-3, 0-6)	2 (2-3, 1-4)	3 (2-6, 0-6)	4 (3-6, 2-6)	0.048*
0-2	28 (47%)	21 (53%)	6 (50%)	1 (13%)	
3-5	26 (44%)	19 (47%)	3 (25%)	4 (57%)	
6	5 (8%)	0	3 (25%)	2 (29%)	
Cognitive complaints 12 months after onset	38/47 (81%)	29/34 (85%)	4/8 (50%)	5/5 (100%)	0.058
mRS 12 months after onset (n=53)	2 (2-3, 0-6)	2 (1-3, 0-6)	2 (1-6, 0-6)	3 (3-6, 2-6)	0.057

Table 2. Treatment and outcome (*continued*)

	Total	LGI1 (n=42)	NMDAR (n=13)	$GABA_BR$ (n=8)	p-value[a]
0-2	27 (51%)	20 (57%)	6 (55%)	1 (13%)	
3-5	20 (38%)	14 (40%)	2 (18%)	4 (57%)	
6	6 (11%)	1 (3%)	3 (27%)	2 (29%)	
Cognitive complaints at last FU	44/57 (77%)	33/41 (81%)	4/9 (44%)	7/7 (100%)	0.020*
Months FU	16 (9-25, 1-164)	18 (11-25,3-164)	24 (10-32, 3-71)	12 (3-22, 1-39)	0.37
Relapse	11 (17%)	9 (21%)	2 (15%)	0	0.27
Death	14 (22%)	5 (12%)	4 (31%)	5 (63%)	0.042*

Data are n (%), n/n (%) or median (interquartile range; range)

*p-value <0.05, **p-value <0.005, ***p-value <0.0005

[a]Only p-values below 0.005 were considered relevant.

[#]2 patients who did not receive 1st line immunotherapy received chemotherapy

Abbreviations: LGI1 = leucine-rich glioma-inactivated 1; NMDAR = NMDA receptor; $GABA_BR$ = gamma-aminobutyric acid B-receptor; ICU = intensive care unit; mRS = modified Rankin Scale; FU = follow-up.

DISCUSSION

This nationwide observational cohort study evaluated cognitive characteristics in middle-aged or older patients with anti-LGI1, anti-NMDAR, anti-$GABA_BR$, and anti-CASPR2 encephalitis. We show that autoimmune encephalitis (AIE) can resemble dementia frequently, especially as rapidly progressive dementia (RPD). Ancillary testing can be misleading, lacking an inflammatory signature (in CSF or on brain MRI), while the CSF biomarker profile that is often requested for dementia workup might mimic a neurodegenerative syndrome. Seizures are often present both early and late in the disease course. These can be very subtle, and therefore easily overlooked.

Our study shows that a neurodegenerative dementia syndrome is frequently suspected initially in AIE patients. The cognitive deterioration has a rapidly progressive character in most patients, which is much larger than the prevalence of RPD in reported studies of dementia cohorts (4-30%)[23-25]. Literature on pure cognitive decline in patients with anti-neuronal autoantibodies is sparse, [26, 27] and in our experience many patients with RPD are not investigated for neuronal autoantibodies. Our results emphasize that part of the (older) patients with a possible dementia diagnosis should be tested for extracellular neuronal antibodies. In all AIE subtypes, we identified patients with cognitive deterioration fulfilling criteria for dementia. Encephalitis with anti-LGI1 antibodies is the most common subtype in this age category and the clinical picture mimics dementia most often. Fewer anti-NMDAR encephalitis patients were included in this study as this disease predominantly affects young adults. [28] Patients with anti-$GABA_BR$ encephalitis are characterized by severe seizures in many, [29] but can present as RPD. [5] Most anti-CASPR2 encephalitis patients had other symptoms, like (painful) polyneuropathy, cerebellar

dysfunction, or epilepsy. [4] Anti-IgLON5 encephalopathy has broad clinical phenotypes, including manifestations that can resemble dementia, [30] but as this disease is still evolving, we have not included these patients. Anti-AMPAR can occasionally present with cognitive decline without other symptoms, but is very rare. [31] Similarly, a recent publication also showed the even rarer AK5 antibodies to be associated frequently with cognitive decline, although MRI and CSF testing was very abnormal in almost all. [32]

Seizures are generally better known within AIE and less likely in dementia even though 10-22% of early-onset AD patients develop seizures, in all disease stages. [33] Our study shows that a high percentage (~two-third of the cohort) developed seizures, despite (arbitrarily) excluding patients with prominent seizures within the first four weeks. The seizures within this study appeared late in the disease course or were subtle seizures (FBDS or non-motor subtle focal seizures), often overlooked. Altogether, it indicates that seizures are an important red flag differentiating between a possible AIE when patients present with dementia symptoms. There should be more awareness for FBDS and non-motor focal seizures, since missing leads to a delay, incorrect diagnosis, and more important inadvertently withholding of immunotherapy resulting in worse outcomes, [3, 6] also seen in our cohort. These subtle seizures were almost exclusively seen in anti-LGI1 encephalitis. FBDS, one subtype, are known to be pathognomonic for anti-LGI1 encephalitis, and are defined as frequent attacks (>8 per day) lasting less than 30 seconds with a dystonic posture of the arm, often combined with a facial contraction. [34]

Frequently, ancillary testing showed no clues suggesting an autoimmune etiology: no abnormalities in CSF (e.g. pleocytosis) or no typical mesiotemporal hyperintensities on brain MRI, consistent with previous studies. [1, 26, 35] Patients with LGI1 or CASPR2 antibodies had more frequently normal CSF results, also in line with previous studies. [4, 36] In addition, EEG results were normal or only showing some encephalopathy in many AIE patients, similar to patterns seen in neurodegenerative dementia patients. Noteworthy, regular ictal EEG generally shows no abnormalities during FBDS. Similarly, EEG is unrevealing if patients have an epileptic focus deep in the temporal lobe. [37, 38] Lastly, tumors can be present in AIE but in general patients are only screened for tumors after antibody positivity. Therefore, in clinical practice this rarely points towards an autoimmune etiology in patients with cognitive deterioration. Differentiating between AIE and a neurodegenerative cause becomes more complex when CSF markers that are often requested when dementia is suspected, are abnormal. In almost half of our tested AIE patients (in whom Aβ42 was also tested), the combination of biomarkers were fitting a neurodegenerative dementia profile. Few cases had positive 14-3-3 results, sometimes attributed to Creutzfeldt-Jakob disease (CJD), but none had abnormalities on MRI-DWI. Unfortunately, we did not have data to evaluate the discriminatory value of FDG-PET. A selection of the 14-3-3 positive samples were analyzed by RT-QuIC, considered a more specific marker for CJD, and all had negative test results confirming the higher specific-

ity compared to 14-3-3.[39] Some of the CSF markers are known to be not highly specific for dementia (t-tau and 14-3-3) as these represent neuronal injury. The explanation for abnormal Aβ42 is currently unknown. Although we cannot exclude that patients were developing concomitant AD, the improvement to immunotherapy, and lack of cognitive deterioration over time, despite extended follow-up, make this highly improbable. Overall, physicians should be aware that ancillary testing can be deceivingly normal in many cases and dementia biomarkers can be 'falsely' positive. IgG index and oligoclonal bands in CSF can be helpful, and should be routinely tested to investigate an autoimmune etiology.

The dementia syndrome shows distinctive cognitive profiles in different AIE subtypes. Both anti-LGI1 and anti-$GABA_BR$ encephalitis are associated with visuospatial and executive dysfunction. This is consistent with cognitive dysfunction seen in dementia with Lewy bodies[40], and the regularly accompanied hallucinations and sleep problems are also known in AIE. Anti-NMDAR encephalitis is more reminiscent of frontotemporal dementia since language impairments and behavioral problems are more prominent in both diseases[41, 42]. Contrary to neurodegenerative dementia syndromes, AIE patients can be treated and generally respond well to immunotherapy. In this study looking at elderly AIE patients, in which most were initially suspected of having an untreatable dementia syndrome, many patients improved with immunotherapy. This improvement was seen despite the relatively long delay until treatment (median 99 days). This delay is witness to the difficulties in diagnosing AIE in older patients, as shown for anti-NMDAR encephalitis.[8] Nevertheless, patients became independent in their daily activities again (best mRS after treatment ≤ 2). However, better treatments and targeted guidance is necessary to reduce long-lasting cognitive dysfunction since a high percentage of patients in all subtypes of AIE still experience problems one year after disease onset. Research evaluating neuropsychological assessments is still sparse.[3, 13, 43] In patients with anti-LGI1 encephalitis long-term cognitive deficits were attributed to hippocampal damage,[43] and to reduced connectivity in anti-NMDAR encephalitis,[44] but direct links with poorer cognitive recovery are needed.

Although this study is nationwide, including four types of AIE, there are some limitations associated with the retrospective design of this study. First, detailed cognitive symptoms were not always accurately documented, especially during follow-up. Secondly, due to the low incidence of anti-$GABA_BR$ and anti-CASPR2 encephalitis and due to our restrictive selection criteria (mainly for anti-NMDAR and anti-CASPR2 encephalitis), we describe modest group sizes, especially compared to anti-LGI1 encephalitis. A large study examining antibodies in unselected patients with presumed dementia, without suspicion of autoimmunity, as well as patients with RPD would be most useful to consolidate our findings.

In conclusion, AIE can mimic dementia. Antibody testing should be considered more often and sooner in the disease course, especially if red flags are present. Red flags for AIE in patients ≥45 years are a rapidly progressive cognitive decline, abnormalities in ancillary testing (inflammatory changes in CSF or on MRI), easily missed subtle seizures early in the disease course, and prominent seizures later in the disease. Extensive brain atrophy early in disease course argue in favor of neurodegeneration, while abnormalities on MRI-DWI are more suggestive for CJD in RPD patients. CSF markers that are often requested when dementia is suspected (including tau, p-tau, amyloid-beta-42 and 14-3-3) can be positive in AIE. However, physicians should be aware that ancillary testing of CSF and brain MRI can be entirely normal in AIE, necessitating antibody testing when in doubt.

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SUPPLEMENTARY DATA

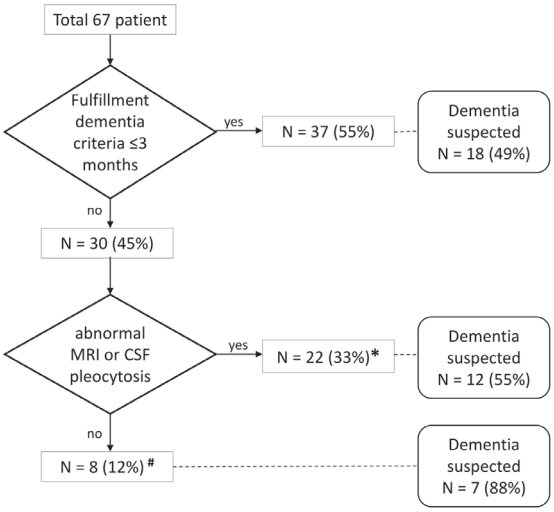
Supplementary Text. Anti-CASPR2 encephalitis patients (n = 4)

Four patients with anti-CASPR2 encephalitis were identified who were age ≥45 years and had fulfillment of the dementia criteria. The patients were all male with a median age of 77 years (range 67-86). Cognitive decline was present in all 4 patients and movement disorders (2/4) and sleep disorders (3/4) were seen. Rapidly progressive dementia was present in 3/4, and in those a neurodegenerative dementia syndrome was suspected by the treating physician. Two patients developed seizures 60 and 201 days after onset, respectively.

Ancillary testing showed that CSF was normal in 2/3 and MRI showed no signs of AIE in 3/4. Dementia biomarkers were abnormal in 1/3 (low amyloid-beta 42).

All patients were treated with 1st line immunotherapy and one with 2nd line immunotherapy. Two had a relapse and all experienced cognitive problems 12 months after onset. However, there was a good response to immunotherapy (median mRS after treatment was 2 [range 1-3]), with similar recovery after the relapses.

Supplementary Figure e-1. Flowchart showing patients who had no very rapid onset (only fulfilling dementia criteria beyond three months) and had neither MRI abnormalities nor CSF pleocytosis



*

- Mild pleocytosis in 2/6 who had abnormal WBC results
- MRI showed only retrospective very subtle AIE signs in 6/18 with AIE related abnormalities on mRI

#

If the six patients who had subtle MRI abnormalities in the mesiotemporal region initially not considered abnormal (out of 18), and two patients (out of six) with only mild pleocytosis (<10 WBC) were also considered, this group would contain 16 patients.

Supplementary Table e-1. Patients who had no very rapid onset (only fulfilling dementia criteria beyond three months) and had neither MRI abnormalities nor CSF pleocytosis.

AIE subtype	Age/sex	Disease course	RPD	Suspected for dementia	CSF markers	EEG
LGI1	71/M	Missed subtle seizures, after 1 month followed by cognitive decline, hallucinations. After 4 months tonic-clonic seizures.	no	Yes	t-tau 977, p-tau 65, Aβ42 154	Epileptic en encephalopathic
LGI1	57/M	Missed subtle seizures with cognitive decline, behavioral changes and sleep problems.	no	Yes	t-tau 299, p-tau 14, Aβ42 344	normal
LGI1	68/M	Subacute cognitive decline, after 2 months one tonic-clonic seizures followed with behavioral changes	no	No	normal	Encephalopathic
LGI1	80/M	Onset with behavioral changes, after 1 month followed by missed subtle seizures. 4 months after onset tonic-clonic seizures and progressive cognitive decline with ataxia, hallucinations, parkinsonism.	yes	Yes	Not performed	normal
LGI1	67/F	Presented with cognitive decline and missed subtle seizures.	yes	Yes	t-tau 440, p-tau 13, Aβ42 374	normal
GABAb	76/M	Acute cognitive decline after 2 months followed by seizures.	yes	Yes	t-tau 1702, p-tau 58, Aβ42 458	Encephalopathic
Caspr2	74/M	Memory and behavioral changes with disinhibited behavior, decorum loss, apraxia and later parkinsonism. 6 months after onset seizures.	yes	Yes	t-tau 86, p-tau 26, Aβ42 320	normal
Caspr2	86/M	Subacute progressive ataxia with cognitive decline suspected for CJD	no	Yes	normal	Encephalopathic

Supplementary Table e-2. Post-hoc analysis for comparisons between AIE subtypes.

	LGI1 – NMDAR	LGI1 - $GABA_BR$	NMDAR - $GABA_BR$
Gender, male	0.023	0.42	0.65
Speech problems	**<0.0001**	1.00	**0.002**
Movement disorders	**0.007**	0.24	0.40
Awareness problems	**0.011**	0.16	1.00
Sleep disorders	**0.011**	0.049	1.00
Seizures	**0.001**	0.41	0.16
Subtle seizures	**0.011**	0.24	0.38
Routine CSF abnormalities	**<0.0001**	**<0.0001**	0.52
MRI mesiotemporal hyperintesities	**0.010**	0.71	0.15
Tumor	0.15	**0.006**	0.17
1st line immunotherapy	-	**<0.0001**	**0.012**
IV immunoglobulins	0.15	0.041	**0.003**
2nd line immunotherapy	**0.006**	0.12	0.66
Cyclophosphamide	**0.011**	0.16	1.00
ICU	**0.003**	0.51	0.17
Death	0.19	**0.005**	0.20
Cognitive complaints at last FU	0.040	0.58	0.034
mRS 6 months after onset	0.53	**0.014**	0.092
Best mRS after treatment#	0.84	**0.005**	**0.024**

Only p-values below 0.017 were considered relevant, followed by p<0.025 and p<0.05 (Holm's method).

Abbreviations: AIE = autoimmune encephalitis; LGI1 = leucine-rich glioma-inactivated 1; NMDAR = NMDA receptor; $GABA_BR$ = gamma-aminobutyric acid B-receptor; ; CSF = cerebrospinal fluid; MRI = magnetic resonance imaging; ICU = intensive care unit; FU = follow-up; mRS = modified Rankin Scale.

Supplementary Table e-3. Clinical profile in AIE patients with a high total tau or high t-tau/Aβ42 ratio.

AIE subtype	Age/ sex	Dementia biomarkers						Dementia subtype*	RPD	Disease course	Ancillary tests		
		t-tau	p-tau	Aβ42	t-tau/ Aβ42	14-3-3	RT-QuIC				MRI	CSF	EEG
$GABA_BR$	56/M	14720	72	859	17.1	positive	negative	CJD	yes	Subacute cognitive decline, complete loss of memory and recognition, hallucinations, sleep disturbance, apraxia.	Typical AIE	105 WBC, elevated protein and IgG index, OCB	Encephalopathic
$GABA_BR$	72/M	2800	35	569	4.9	positive	negative	CJD	yes	Acute psychosis, within days followed by cognitive decline, only later on in disease course a few seizures and myoclonus.	Typical AIE	WBC 15	Normal
$GABA_BR$	76/M	1702	58	458	3.72	weak positive		AD	yes	Acute cognitive decline after 2 months followed by seizures.	Normal	Elevated protein	Encephalopathic
NMDAR	70/M	1062		385	2.75	negative		AD	yes	Progressive cognitive decline with behavioral changes, mutism and later mild autonomic dysregulation.	Normal	32 WBC	Normal
NMDAR	65/F	1008	48	970	1.04	negative		AD	yes	Progressive encephalopathy with apraxia, ataxia, tremors, walking difficulties. Later in disease course seizures.	Right parietal edema	158 WBC	Epileptic en encephalopathic
NMDAR	73/F	474		473	1.00	weak positive	negative	AD	yes	Subacute behavioral and cognitive decline with myoclonus. In retrospect, mild cognitive decline for 2 years.	Normal	54 WBC, elevated protein, OCB	Epileptic en encephalopathic
LGI1	66/M	1803	20	654	2.76	negative		CJD	yes	Missed FBDS, after 2 months cognitive decline, slow, walking difficulties, confabulations.	Atrophy		Normal

Supplementary Table e-3. Clinical profile in AIE patients with a high total tau or high t-tau/Aβ42 ratio. (*continued*)

AIE subtype	Age/ sex	Dementia biomarkers						Dementia subtype*	RPD	Disease course	Ancillary tests		
		t-tau	p-tau	Aβ42	t-tau/ Aβ42	14-3-3	RT-QuIC				MRI	CSF	EEG
LGI1	80/M	1417	85			negative			yes	Slowly progressive cognition and behavior problems, after months missed subtle seizures. After 6 months fast progression cognitive decline.	Typical AIE		Encephalopathic
LGI1	76/M	1266	29			weak positive	negative	CJD	yes	Missed FBDS, followed by cognitive decline, myoclonus and behavioral changes.	Normal	10 WBC, elevated protein	Epileptic en encephalopathic
LGI1	72/M	977	65	154	6.3	negative		AD	no	Missed subtle seizures, after 1 month followed by cognitive decline, hallucinations. After 4 months tonic-clonic seizures.	Normal	Elevated protein, elevated IgG index	Epileptic en encephalopathic
LGI1	82/F	899	90			negative			yes	Fast progressive cognitive decline with behavioral changes.	Typical AIE		encephalopathic
LGI1	74/F	638	51			negative			yes	Missed FBDS and subtle seizures. Later followed by cognitive decline, behavioral changes and hallucinations.	Basal ganglia hyperintensity		Normal
LGI1	71/M	636	64	791	0.80	negative		AD	yes	Cognitive decline, behavioral changes and nightly agitation. After 5 months followed by seizures.	Typical AIE	Elevated protein, elevated IgG index	Normal
LGI1	70/M	604	93	486	1.24			AD	no	Slow progressive cognitive decline with nightly agitation and behavioral changes.	Typical AIE		Encephalopathic

Supplementary Table e-3. Clinical profile in AIE patients with a high total tau or high t-tau/Aβ42 ratio. (*continued*)

AIE subtype	Age/ sex	Dementia biomarkers						Dementia subtype*	RPD	Disease course	Ancillary tests		
		t-tau	p-tau	Aβ42	t-tau/ Aβ42	14-3-3	RT-QuIC				MRI	CSF	EEG
LGI1	52/M	536	14			negative			yes	Cognitive decline, after weeks followed by missed subtle seizures.	Typical AIE		
LGI1	72/M	524	19			negative			yes	Progressive cognitive decline with apraxia and sleep problems.	Typical AIE		
LGI1	64/M	477	41			negative			yes	Subacute cognitive decline and behavioral changes, after 6 months followed by subtle seizures.	Typical AIE		normal
LGI1	66/M	452	26			negative			yes	Acute cognitive decline after 2 weeks followed by missed subtle seizures.	Typical AIE		Epileptic en encephalopathic
LGI1	67/F	440	13	374	1.18	negative		AD	yes	Presented with cognitive decline and missed subtle seizures.	Normal		Normal
LGI1	74/M	299	14	344	1.15	negative		AD	no	Missed subtle seizures with cognitive decline, behavioral changes and sleep problems.	Normal	Normal	Normaal
LGI1	76/V	286	11	377	0.85	weak positive		AD	yes	Missed subtle seizures, after 5 months followed by cognitive decline, behavioral changes and sleep problems.	Typical AIE	Normal	Epileptic en encephalopathic

*Dementia subtype was based on dementia biomarkers. Cut-off values to be considered abnormal were t-tau >400 pg/ml, p-tau >64 pg/ml, amyloid-beta-42 (Aβ42) <500 pg/ml, a t-tau/p-tau ratio >30, and a t-tau/Aβ42 ratio >0.52. A positive 14-3-3 or RT-QuIC was also abnormal. Based on these CSF markers, patients had a Creutzfeldt-Jakob disease (CJD) profile if the t-tau/p-tau ratio was abnormal, and an Alzheimer dementia (AD) profile was assigned when Aβ42 was lowered or the t-tau/Aβ42 ratio was abnormal.Abbreviations: AIE = autoimmune encephalitis; RPD = rapidly progressive dementia; MRI = magnetic resonance imaging; CSF = cerebrospinal fluid; EEG = electroencephalogram; LGI1 = leucine-rich glioma-inactivated 1; NMDAR = NMDA receptor; $GABA_BR$ = gamma-aminobutyric acid B-receptor; CJD = Creutzfeldt-Jakob disease; AD = Alzheimer disease; FBDS = faciobrachial dystonic seizures; WBC = white blood cell count; OCB = oligoclonal bands.

3

Antibodies associated with autoimmune encephalitis in patients with presumed neurodegenerative dementia

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ABSTRACT

Background

Autoimmune encephalitis (AIE) may present with prominent cognitive disturbances without overt inflammatory changes in MRI and cerebrospinal fluid (CSF). Identification of these neurodegenerative dementia diagnosis mimics is important, since patients generally respond to immunotherapy.

Objectives

To determine the frequency of neuronal antibodies in patients with presumed neurodegenerative dementia and describe the clinical characteristics of the patients with neuronal antibodies.

Methods

In this retrospective cohort study, 920 patient were included with neurodegenerative dementia diagnosis from established cohorts at two large Dutch academic memory clinics. In total, 1398 samples were tested (478 patients having both CSF and serum) using immunohistochemistry (IHC), cell-based assays (CBA) and live hippocampal cell cultures (LN). To ascertain specificity and prevent false positive results, samples had to test positive by at least two different research techniques. Clinical data was retrieved from patient files.

Results

Neuronal antibodies were detected in seven patients (0.8%), including anti-IgLON5 (n=3), anti-LGI1 (n=2), anti-DPPX, and anti-NMDAR. Clinical symptoms atypical for neurodegenerative diseases were identified in all seven, and included subacute deterioration (n=3), myoclonus (n=2), a history of autoimmune disease (n=2), a fluctuating disease course (n=1), and epileptic seizures (n=1). In this cohort, no patients with antibodies fulfilled the criteria for rapidly progressive dementia (RPD), yet a subacute deterioration was reported in three patients later in the disease course. Brain MRI of none of the patients demonstrated abnormalities suggestive for AIE. CSF pleocytosis was found in one patient, considered as an atypical sign for neurodegenerative diseases. Compared to patients without neuronal antibodies (4 per antibody-positive patient), atypical clinical signs for neurodegenerative diseases were seen more frequently among the patients with antibodies (100% vs 21%, p=0.0003), especially a subacute deterioration or fluctuating course (57% vs 7%, p=0.009).

Discussion

A small, but clinically relevant proportion of patients suspected to have neurodegenerative dementias have neuronal antibodies indicative of AIE and might benefit from immunotherapy. In patients with atypical signs for neurodegenerative diseases, clinicians should consider neuronal antibody-testing. Physicians should keep in mind the clinical phenotype and confirmation of positive test results to avoid false positive results and administration of potential harmful therapy for the wrong indication.

INTRODUCTION

Cognitive dysfunction can be the presenting and most prominent symptom in patients with autoimmune encephalitis (AIE).[1,2] Contrary to neurodegenerative diseases, patients with antibody-mediated encephalitis might benefit from immunotherapy and improve considerably.[3,4] The presence of neuronal antibodies has been reported predominantly in rapidly progressive dementia (RPD).[5,6] However, AIE can present less fulminantly, and is therefore potentially missed, resulting in diagnosis and treatment delay or even misdiagnosis.[7,8] We hypothesized that a small - but not insignificant - part of dementia syndromes is indeed caused by antibody-mediated encephalitis and underdiagnosed, withholding these patients available treatments. The wish to diagnose every single patient with autoimmune encephalitis is in opposition with the risk for false positive tests.[9] Therefore, we strictly adhere to confirmation of positive test results with two different test techniques. In this study we describe the frequency of neuronal antibodies in a cohort of patients diagnosed with various dementia syndromes in a memory clinic. In addition, we present clues to improve clinical recognition of AIE in dementia syndromes.

METHODS

Patients and laboratory studies

In this retrospective multicenter study, we tested for the presence of neuronal antibodies in serum and CSF samples from patients diagnosed with neurodegenerative dementia diagnosis, included earlier prospectively in established cohorts at two large Dutch academic memory clinics (Erasmus University Medical Center, Amsterdam University Medical Centers, location VUmc)[10] between 1998 and 2016 (84% last 10 years). All patients fulfilled the core clinical criteria for dementia, as defined by the National Institutes of Aging-Alzheimer Association workgroups.[11] Patients were classified in four subgroups (based on diagnostic criteria): Alzheimer's dementia (AD), frontotemporal dementia (FTD; both behavioral variant and primary progressive aphasia (PPA)), dementia with Lewy bodies (DLB) and other dementia syndromes.[11-14] Rapidly progressive dementia (RPD) was defined as dementia within 12 months or death within two years after the appearance of the first cognitive symptoms.[15] Patients with vascular dementia were not included. Clinic information was retrieved from the prospectively collected data. A subacute deterioration was defined as a marked progression of symptoms in 3 months and a fluctuating course as a disease course fluctuating over a longer period (e.g. weeks to months; different from the fluctuations within a day as seen in some DLB patients). Dementia markers were scored according to the reference values (per year and per center; included in Table 1).

All samples, stored in both cohorts' biobanks, were screened for immunoreactivity with immunohistochemistry (IHC), as previously described. [16] Preferably, paired serum and CSF were tested for optimal sensitivity and specificity. Samples that were showing a positive or questionable staining pattern, were tested more extensively using validated commercial cell-based assays (CBA) and in-house CBA (Supplementary eTable 1). In addition, these samples were tested with live hippocampal cell cultures (LN). [16, 17] To ascertain specificity, only samples that could be confirmed by CBA or LN were scored as positive, since there is a higher risk for false-positive test results in this population with a low a priori chance to have encephalitis. [9, 18] If IHC was suggestive for antibodies against intracellular (paraneoplastic) targets, this was explored by a different IHC technique. [19] Anti-thyroid peroxidase (TPO), voltage-gated calcium channel (VGCC) or low titer glutamic acid decarboxylase antibodies were not tested for as these are generally non-specific at these ages and are not associated with dementia syndromes.

Antibody-positive patients were described exploratory and compared to a randomly selected antibody-negative group (ratio 1:4) matched for memory clinic, dementia subtype, gender and age (+/-5 year). For these comparisons, medical records were additionally assessed for both the antibody positive and antibody-negative patients. All antibody-positive patients were reviewed by a panel consisting of neurologists specialized in neurodegenerative (FJ, HS, JS) or autoimmune diseases (JV, PSS, MT) and a consensus classification of AIE versus AIE with a neurodegenerative dementia comorbidity was reached.

Statistical analysis

We used IBM SPSS 25.0 (SPSS Inc) and Prism 8.4.3 (GraphPad) for statistical analysis. Baseline characteristics were analyzed using the Fisher exact test, the Fisher-Freeman-Halton test or the Kruskall-Wallis test, when appropriate. For group comparisons, encompassing categorical data, we used the Pearson Chi-Square test or the Fisher-Freeman-Halton test, when appropriate. Continuous data were analyzed using the Mann-Whitney U test. All p-values were two-sided and considered statistically significant when below 0.05. We applied no correction for multiple testing, and therefore p values between 0.05 and 0.005 should be interpreted carefully.

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by The Institutional Review Boards of Erasmus University Medical Center Rotterdam and Amsterdam University Medical Center, location VUmc. Written informed consent was obtained from all patients.

Data Availability Statement

Any data not published within this article are available at the Erasmus MC University Medical Center. Patient-related data will be shared upon reasonable request from any qualified investigator, maintaining anonymization of the individual patients.

RESULTS

In total, 1398 samples from 920 patients were tested (Figure 1; in 478 both CSF and serum [52%]). Three-hundred and fifty-eight patients were classified as AD (39%), 283 FTD (31%), and 161 DLB (17%). The fourth subgroup with other dementia syndromes consisted of 118 patients (13%), including progressive supranuclear palsy (n=48, 5%) and corticobasal syndrome (n=29, 3%). Median age at disease onset was 62 years (range 16 to 90 years). Male patients were overrepresented (n=542, 59%), and 60 patients (7%) fulfilled the criteria for rapidly progressive dementia (RPD; Supplementary eTable 2).

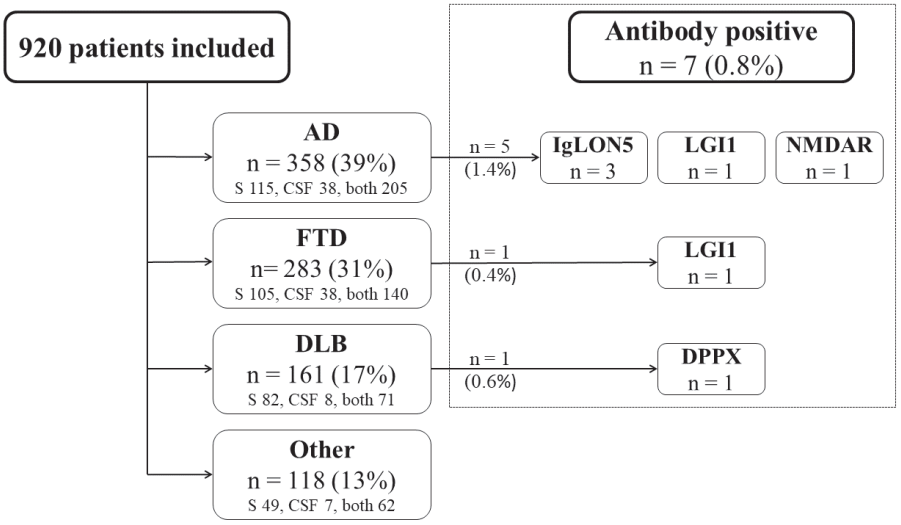

Figure 1. Flowchart of patient inclusion with antibody results.

In total, 920 patients (1398 samples) with a presumed neurodegenerative dementia syndrome were tested for the presence of neuronal antibodies in serum and CSF. Neuronal antibodies were detected in 7 patients (0.8%, 95% CI 0.2-1.3%); five among the 358 Alzheimer disease patients. Subclassification of the 'other' group is provided in supplementary table eTable 2.

Abbreviations: AD = Alzheimer disease, FTD = frontotemporal dementia, DLB = diffuse Lewy body dementia, S = serum, CSF = cerebrospinal fluid, IgLON5 = Ig-like Domain-Containing protein family member 5, LGI1 = Leucin-rich glioma inactivated protein 1, NMDAR = N-methyl-D-aspartate receptor, DPPX = dipeptidyl aminopeptidase-like protein 6.

Neuronal antibodies were detected in seven patients (0.8%; 5 in the AD group: 1.4%; Figure 1), including anti-IgLON5 (n=3), anti-LGI1 (n=2), anti-DPPX (n=1), and anti-NMDAR antibodies (n=1; Table 1). Among these seven, four patients were diagnosed retrospectively with an exclusive diagnosis of AIE, while three patients were classified to have AIE (anti-IgLON5 [n=2] and anti-NMDAR antibodies [n=1]) with a neurodegenerative dementia comorbidity. No patients with antibodies fulfilled the criteria for RPD, yet a subacute deterioration later in the disease was reported in three patients. Atypical clinical signs for neurodegenerative diseases were present in 7/7 antibody-positive patients (100% vs 21% in antibody-negative patients, p=0.0003; Table 2). These included a subacute deterioration (n=3), myoclonus (n=2), a fluctuating disease course over months (n=1), a history of autoimmune disease (n=2), and epileptic seizures (n=1; Table 1). Brain MRI of none of the patients demonstrated abnormalities suggestive for active AIE, in particular no hippocampal swelling nor increased T2-signal intensity. CSF pleocytosis was found in one patient. CSF biomarkers (t-tau, p-tau and Aβ42) were tested in 5/7 patients, and t-tau and p-tau were increased in four, while a low Aβ42 was seen in two. Of note, only one patient had the combination of reduced Aβ42 and increased p-tau/t-tau, and was diagnosed with a comorbid AD. No patient received immunotherapy. Two patients still alive (one anti-LG1, one anti-DPPX positive) were contacted, but refused to visit our clinic to try very delayed immunotherapy trials. Interestingly, the patient with anti-DPPX antibodies showed spontaneous improvement of cognitive disturbances, atypical for a pure neurodegenerative disease.

Compared to the patients without neuronal antibodies, subacute cognitive deterioration or fluctuating course was present more frequently (4/7 [57%] vs 2/28 [7%], p=0.009). While movement disorders (myoclonus) and autoimmune disorders were present in 2/7 patients each, this did not reach significance (Table 2).

Table 1. Patient characteristics of auto-antibody positive patients

	1	2	3	4	5	6	7
Antibody	**IgLON5**	**IgLON5**	**IgLON5**	**DPPX**	**LGI1**	**LGI1**	**NMDAR**
Sex	F	F	F	M	F	M	F
Age at onset, years	53	66	71	61	61	68	74
Clinical dementia diagnosis	AD	AD, primary progressive aphasia	AD, posterior cortical atrophy	DLB	AD	FTD with ALS	AD, primary progressive aphasia
Duration onset to dementia diagnosis, months	108	18	60	12	36	49	42
Presenting symptom	Memory disorders	Language disorders	Visual disorders	Memory disorders	Memory disorders	Behavioral disorders	Language disorders
Symptoms during disease course	Slow progressive memory disorders. **Subacute deterioration** in months, severe apraxia, aphasia, **myoclonus**, hallucinations, delusions and behavior problems. Admission to a closed psychiatric ward.	Word retrieval and phrase difficulties. Mild cognitive decline.	Progressive visuoperceptual and spatial disorders, apraxia, dyscalculia, mild behavioral disturbances, restless legs syndrome and **myoclonus**. No sleep disorders.	Memory disorders, altered gait, slurred speech, orthostatic hypotension, obstipation, axial rigidity, asymmetric hypokinetic-rigid syndrome, possible OSAS. **Fluctuating disease course.**	Slow progressive memory disorders. Five years after onset **subacute deterioration with progressive cognitive disorders**, behavior disturbances.	Slow progressive behavior problems Four years after onset right sided hand disability, fasciculations, cognitive decline and speech problems.	Slow progressive language disorder followed by behavior problems, right sided hand clumsiness and one **generalized seizure**.
Cognitive tests	MMSE 5/30 CDR 2	MMSE 13/27 CDR 0.5	MMSE 28/30 CDR 0.5	MMSE 22/30 CDR 0.5	MMSE 23/30 CDR 1	MMSE 27/30 CDR 1	MMSE 10/30 CDR 1

Table 1. Patient characteristics of auto-antibody positive patients (*continued*)

	1	2	3	4	5	6	7
Antibody	**IgLON5**	**IgLON5**	**IgLON5**	**DPPX**	**LGI1**	**LGI1**	**NMDAR**
NPA	Severe cognitive and language disorder. Problems with concentration, executive function, praxis.	Severe speech problems. No apparent other cognitive disorders.	Disturbances on visual tests	Learning disability and problems in speed, concentration, planning.	Memory disorder and mild speech problems.	Preoccupation hand function, severe speech problems, mild memory disorder.	Severe language disorders, decreased memory
History of autoimmune disease	No	**Rheumatoid arthritis**	**Ankylosing spondylitis**	No	No	No.	No
CSF	5 WBC, normal protein; tau and p-tau normal, AB42 ↓	Not performed	1 WBC, normal protein; tau ↑, p-tau ↑, AB42 normal.	**8 WBC**, normal protein; tau ↑, p-tau ↑, AB42 normal	2 WBC, normal protein; tau ↑↑, p-tau ↑, AB42 normal	Not performed	0 WBC, normal protein; tau ↑, p-tau ↑, AB42 ↓
MRI	Diffuse atrophy	Mild medial temporal atrophy. Old hemorrhage left parietal-occipital. Superficial siderosis, multiple lobair microbleeds	Extensive posterior atrophy	Post-traumatic atrophy and gliosis left temporal lobe	Diffuse and hippocampal atrophy.	Bilateral hippocampal temporal lobe atrophy	Not performed. CT severe atrophy left temporal lobe
EEG	Diffuse slowing, right parietal sharp waves	Multifocal slow wave activity predominantly left hemisphere	Mildly slow activity at temporal areas	Mild slowing background activity	Not performed	Not performed	Normal
Antibody tests serum	IHC positive CBA positive	IHC positive CBA positive	IHC positive CBA positive	IHC negative CBA negative LN negative	IHC positive CBA positive LN positive	IHC positive CBA positive LN positive	N.A.

Table 1. Patient characteristics of auto-antibody positive patients (*continued*)

	1	2	3	4	5	6	7
Antibody	**IgLON5**	**IgLON5**	**IgLON5**	**DPPX**	**LGI1**	**LGI1**	**NMDAR**
Antibody tests CSF	IHC positive CBA positive	N.A.	IHC negative CBA negative	IHC positive CBA weak positive LN positive	IHC negative, CBA negative LN negative	N.A.	IHC positive CBA positive LN weak positive
Clinical FU	Progressive cognitive decline. Died 1,5 years after diagnosis.	Deceased	Progression of visual disturbances. Died four years after diagnosis	Spontaneous gradual improvement and stabilization of cognitive disturbances	Gradual progression of cognitive disturbances. Unable to communicate. Requirement of help for personal care.	**Subacute deterioration,** died six months after diagnosis	Deceased
FU from onset (months)	127 †	120 †	108 †	87	158	55 †	54 †
Additional tests/ information	SPECT: bilateral parieto-temporal hypoperfusion			DAT-SPECT: inconclusive.	Mother rapidly progressive Alzheimer's disease. Genetic analysis (including APP, C9orf72, PRNP) no abnormalities	EMG: axonal damage, fasciculations. No fulfillment El Escorial criteria No C9orf72 repeat expansion.	
Final diagnosis	Anti-IgLON5 encephalitis	Anti-IgLON5 encephalitis and potentially comorbid neurodegenerative dementia (AD with vascular pathology)	Anti-IgLON5 encephalitis and PCA	Anti-DPPX encephalitis	Anti-LGI1 encephalitis	Anti-LGI1 encephalitis	Anti-NMDAR encephalitis and Alzheimer's disease

Abbreviations: IgLON5 = Ig-like Domain-Containing protein family member 5, LGI1 = Leucin-rich glioma inactivated protein 1, NMDAR = N-methyl-D-aspartate receptor, DPPX = dipeptidyl amino-peptidase-like protein 6, AD = Alzheimer disease; PCA = posterior cortical atrophy; FTD = frontotemporal dementia; DLB = diffuse Lewy body dementia, ALS = amyotrophic lateral sclerosis, M = male, F = female, MMSE = mini-mental state examination, CDR = clinical dementia rating, NPA = neuropsychological assessment, WBC = White Blood Cells, p-tau = phosphor tau, AB42 = amyloid beta 42, FU = follow-up, EEG = electroencephalography, MRI = magnetic resonance imaging, DAT-SPECT= dopamine receptor-single photon emission computed tomography, IHC = immunohistochemistry, CBA = cell based assay, LN = live neurons, N.A. = not applicable.
† = deceased.

Table 2. Comparisons between patients with neuronal auto-antibodies and antibody-negative patients.

	Antibody positive (n=7)	Antibody-negative (n=28)*	P value
Sex (male)	2 (29%)	8 (29%)	1.00
Ethnicity			0.75
Caucasian	6/6 (100%)	20/22 (91%)	
Asian	0	1/22 (5%)	
African	0	1/22 (5%)	
Age at onset, median in years (IQR, range)	66 (61-72, 53-74)	62 (58-66, 53-79)	0.24
Age at diagnosis (IQR, range)	68 (63-77, 63-78)	66 (61-67, 55-82)	0.19
Onset to diagnosis, median in years (IQR, range)	3 (2-5, 1-10)	3 (1-5, 1-10)	0.44
RPD	0	1 (4%)	1.00
Atypical symptoms †	7 (100%)	6 (21%)	**0.0003**
Subacute deterioration or fluctuation	4 (57%)	2 (7%)	**0.009**
History of autoimmune disease	2 (29%)	2 (7%)	0.17
Family history of AID	0	0	-
Symptom onset			0.51
Memory disorders	3 (43%)	16 (57%)	
Behavioral changes	1 (14%)	1 (4%)	
Other	3 (43%)	11 (39%)	
Symptoms			
Memory disorders	6 (86%)	27 (96%)	0.37
Behavioral changes	4 (57%)	10 (36%)	0.40
Seizures	1 (14%)	0	0.20
Speech problems	5 (71%)	17 (61%)	0.69
Movement disorders	3 (43%)	4 (14%)	0.12
Muscle stiffness	1 (14%)	4 (14%)	1.00
Sleep disorder	2 (29%)	3 (11%)	0.26
Autonomic symptoms	1 (14%)	1 (4%)	0.37
Ancillary testing			
Tumor screening	0	0	-
MMSE (IQR, range)	22 (10-27, 5-28)	24 (18-26, 7-30)	0.28
NPA performed	7	27	

Table 2. Comparisons between patients with neuronal auto-antibodies and antibody-negative patients. (*continued*)

	Antibody positive (n=7)	Antibody-negative (n=28)*	P value
CSF analyzed	5/7 (71%)	27/28 (96%)	0.10
Onset to CSF, median in years (IQR, range)	3.6 (2.2-7.6, 1.4-10.2)	2.6 (1.1-4.1, 0.7-7.6)	0.20
- WBC >5 cell/μL	1/5 (20%)	2/27 (7%)	0.41
- Total protein >0.58 g/L	1/5 (20%)	2/26 (8%)	0.42
- Total tau, high ‡	4/5 (80%)	22/27 (82%)	1.00
- Phospho tau , high ‡	4/5 (80%)	20/26 (77%)	1.00
- Aβ42, low ‡	2/5 (40%)	16/28 (57%)	0.64
MRI brain performed	6 (86%)	28 (100%)	0.20
- Atrophy	6/6 (100%)	25 (89%)	1.00
- Mesiotemporal hyperintensity	0	0	
EEG abnormal	4/6 (67%)	7/11 (64%)	1.00
- EEG epileptic	0	0	
- EEG slow	2/3 (67%)	6/10(60%)	1.00
mRS initial	2 (2-3, 1-3)	2 (2-2, 1-3)	0.77
CDR initial	1 (0.5-1, 0.5-2)	1 (0.5-1, 0.5-2)	0.75
Deceased	5 (71%)	20/27 (74%)	0.89
Follow-up, median months from onset (IQR, range)	68 (54-120, 32-120)	60 (44-84, 17-156)	0.39

*Antibody-negative patients were matched 4:1 for memory clinic of assessment, dementia subtype, gender and age (+/-5 year). †Atypical symptoms were RPD, pleocytosis, subacute deterioration, fluctuating disease course, myoclonus, history of autoimmune disease, epilepticus seizures. ‡ Dementia markers scored according to the reference values per year and per center.

Abbreviations: IQR = interquartile range, RPD: rapid progressive dementia, AID = autoimmuun disease, MMSE = mini-mental state examination, CDR = clinical dementia rating, NPA = neuropsychological assessment, CSF = cerebrospinal fluid, WBC = White Blood Cells, Aβ42 = amyloid beta 42, MRI = magnetic resonance imaging, EEG = electroencephalography, mRS = modified ranking scale.

DISCUSSION

In this large, multicenter, cohort study consisting of patients with a presumed neurodegenerative dementia diagnosis, we show that a small, but clinically relevant proportion (0.8%) has neuronal antibodies. In this particular group, four out of seven antibody-positive patients presented with an atypical clinical course (subacute deterioration

or fluctuating disease course), which is considered as a clinical clue ('red flag') for an antibody-mediated etiology of dementia.[4] Importantly, a fluctuating disease course was observed over a longer period (e.g. weeks or months) in AIE and should not be confused with shorter fluctuations of cognition or alertness (over the day) in DLB. Other known red flags, which we observed in these seven patients were myoclonus, epilepsy, pleocytosis or a history of autoimmune disorders, as described earlier. [1, 4-6] Compared to antibody-negative patients no significant difference was found related to these symptoms alone, probably due the low number of positive patients and related low power. However atypical clinical signs for neurodegenerative diseases together were seen significantly more frequently in the antibody-positve group. Within this cohort mostly devoid of RPD patients, none of the antibody-positive patients fulfilled the criteria for RPD, nor ancillary testing showed specific signs for AIE in most patients. This implicates AIE can resemble more protracted, progressive neurodegenerative dementia syndromes, as we reported earlier. [1]

Three antibody-positive patients had IgLON5 antibodies, which is a very rare and known to have heterogeneous (chronic) clinical manifestations including pronounced sleep problems, cognitive dysfunction, and movement disorders. [20, 21] Misdiagnosis with progressive supranuclear palsy (PSP) is reported, mainly associated with the preceding movement disorders. In addition, half of the patients have cognitive impairment of whom 20% fulfilled clinical criteria for dementia. [21] Interestingly, IgLON5 disease shares features with neurodegeneration as autopsy studies showed tau deposits. [22] However, there is a strong HLA association[20] and studies show antibodies directly bind to surface IgLON5 on neurons and directly alter neuronal function and structure, [23] suggesting a primary inflammatory disease.

In previous research, a notably higher frequency (14%) of neuronal antibodies in dementia patients was reported by Giannocaro et al. [24] The discrepancy with our test results is probably explained by differences in patient selection and antibody testing methodology. First, 30% of the patients in the cohort described by Giannocaro et al demonstrated CSF inflammatory abnormalities, indicating a relatively high pre-test probability of antibody-positivity compared to our study. [24] A lack of CSF pleocytosis probably better represents the population of memory clinics. Second, the previous study exclusively tested serum by cell-based assay without confirmatory tests nor testing antibodies in CSF. [24] We only considered antibody test results positive when confirmed by additional techniques to avoid suboptimal specificity and false-positive test results. [9]

Previous studies, including our own, suggested RPD as a relevant red flag for AIE, [1, 4, 9, 25] but we cannot determine this from our study based on the design of our study. We included patients at tertiary memory clinics without overt signs or symptoms suggestive for encephalitis. Therefore, the amount of RPD patients included was very limited (7%), comparable to other large dementia cohort studies, as was the amount of patients with

abnormal ancillary testing suggestive for AIE as this would have prompted a different approach than referral to a tertiary memory clinic. These patients with RPD and ancillary testing suggestive of AIE were not included in our study. Inclusion of those patients would have likely increased our rate of positivity.

The strength of our study is the large number of paired samples (serum and CSF combined) from a cohort with various presumed neurodegenerative diseases without AIE suspicion, representative for academic memory clinics. A limitation is the lack of neuropathological data to support our findings and make diagnoses of neurodegeneration or inflammation definite. If the symptoms are related to the presence of antibodies, we tried to overcome this concern in different ways. First, the presence of antibodies in serum and CSF was confirmed by different techniques (cell based assay, tissue immunohistochemistry, cultured live neurons), indicating optimal test-specificity. Second, afterwards patients were thoroughly reviewed by a panel of neurologists specialized in neurodegenerative or autoimmune disease to detect atypical signs and symptoms related to AIE. To our knowledge, this is the largest cohort of dementia patients examined for the presence of neuronal antibodies. An important limitation of this study is the small number of antibody-positive patients, underpowering the probability to identify significant differences between antibody-positive and -negative patients. The low number of RPD patients has probably added to this small number, and a prospective study including RPD patients is recommended. Nevertheless, several probable red flags could be identified.

Diagnosing AIE in dementia patients is highly relevant, since these patients might respond to immunotherapy. Therefore, clinicians should test for neuronal antibody in patients demonstrating red flags suggestive for an autoimmune etiology, if possible early in disease course. When profound temporal lobe atrophy already has developed, little effect is to be expected. Red flags identified in this study are subacute deterioration or fluctuating course. Other red flags described previously we also see reflected in our study, are autoimmune disorders, myoclonus, seizures and pleocytosis, [1, 4-6] Preferably, both serum and CSF should be tested and confirmed by additional techniques. Always consider the possibility of a false positive test result, especially when only using a single technique (like the commercial cell-based assay). If the clinical phenotype is atypical, confirmation in a research laboratory should be mandatory. The use of antibody panels is discouraged, especially including the paraneoplastic blots, as these are associated with higher risks of lack of clinical relevance. [26] This caution is even more warranted for tests not associated with neurodegenerative syndromes, but with a history of non-specificity, including VGKC (in the absence of LGI1 or CASPR2), VGCC, anti-TPO, and low titer anti-GAD65. [27-30] Further research should focus on improving clinical recognition of AIE in dementia patients determining the effect of immunotherapy in this specific patient category and assessing the frequency of AIE in RPD.

In conclusion, we have shown that a clinically relevant, albeit small proportion of patients with a suspected neurodegenerative disease and non-rapidly progressive course have neuronal antibodies indicative of AIE.

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SUPPLEMENTARY DATA

Supplementary eTable 1. Antibody tests

Test	antigen	Confirmation techniques
Commercial CBA (*Euroimmun, Lübeck, German)*	NMDAR	IHC, live neurons
	DPPX	IHC, live neurons
	CASPR2	IHC, live neurons
	LGI1	IHC, live neurons
	GABAbR	IHC, live neurons
	AMPAR	IHC, live neurons
Commercial ELISA (*Medizym anti-GAD 96, Medipan GMBH, Berlin, Germany*)	GAD65	IHC
In-house CBA	IgLON5 (live)	IHC, live neurons
	DPPX (fixed)	IHC, live neurons
	NMDAR (fixed)	IHC, live neurons
	LGI1 (live)	IHC, live neurons
	CTNT1 (fixed)	IHC, live neurons
	CASPR1 (fized)	IHC, live neurons
	AMPAR (live)	IHC, live neurons
	GABAbR (live)	IHC, live neurons
	GlyR (live)	

Supplementary eTable 2. Baseline characteristics of the total neurodegenerative dementia cohort based on the dementia subtypes.

	Total (n=920)	AD (n=358)	FTD (n=283)	DLB (n=161)	Other (n=118)	p-value
Sex (male)	542 (59%)	172 (48%)	169 (60%)	129 (80%)	65 (55%)	<0.0001
Age at onset, median in years (IQR, range; n=865)	62 (56-68, 16-90)	62 (56-69, 52-82)	60 (54-65, 40-90)	66 (60-71, 43-86)	63 (57-68, 16-83)	<0.0001
Age at memory clinic, median in years (IQR, range; n=846)	65 (59-71, 30-91)	66 (59-72, 33-85)	63 (58-68, 41-91)	68 (64-74, 45-85)	65 (60-71, 30-84)	<0.0001
Onset to memory clinic, median in years (IQR, range; n=862)	3 (1.8-4, 0-20)	2.6 (1.1-4, 0-15)	2.5 (1.7-4.8, 0-20)	3 (2-3, 0-15)	3 (2-3, 0-18)	0.093
RPD	60/862 (7%)	33/321 (10%)	18/275 (7%)	8/151 (5%)	1/115 (1%)	0.002

Supplementary eTable 3. Additional information on auto-antibody positive patients.

	1	2	3	4	5	6	7
Antibody	**IgLON5**	**IgLON5**	**IgLON5**	**DPPX**	**LGI1**	**LGI1**	**NMDAR**
Year outpatient clinic	2009	2012	2013	2016	2012	2011	2008
Clinical dementia diagnosis	AD	AD, primary progressive aphasia	AD, posterior cortical atrophy	DLB	AD	FTD with ALS	AD, primary progressive aphasia
Time onset to sample (months)	108	18	60	12	40	48	36
CSF	5 WBC, normal protein; tau and p-tau normal, AB42 ↓	Not performed	1 WBC, normal protein; tau ↑, p-tau ↑, AB42 normal.	8 WBC, normal protein; tau ↑, p-tau ↑, AB42 normal	2 WBC, normal protein; tau ↑↑, p-tau ↑, normal	Not performed	0 WBC, normal protein; tau ↑, p-tau ↑, AB42 ↓

First clinical description of antibodies: anti-IgLON5 2014, anti-DPPX 2013, anti-LGI1 2012, anti-NMDAR 2007.

4

Anti-NMDAR encephalitis in the Netherlands, focusing on late-onset patients and antibody test accuracy

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ABSTRACT

Objective

To describe the clinical features of anti-NMDAR encephalitis, emphasizing on late-onset patients and antibody test characteristics in serum and CSF.

Methods

Nationwide observational Dutch cohort study, in patients diagnosed with anti-NMDAR encephalitis between 2007 and 2019.

Results

One-hundred-and-twenty-six patients with anti-NMDAR encephalitis were included with a median age of 24 years (range 1-86). The mean annual incidence was 1.00/million (95%-CI 0.62-1.59). Patients ≥45 years of age at onset (19%) had fewer seizures (46% vs 71%, p=0.021), fewer symptoms during disease course (3 vs 6 symptoms, p=0.020), and more often undetectable serum antibodies compared to younger patients (p=0.031). In the late-onset group, outcome was worse and all tumors were carcinomas (both p<0.0001). CSF was more accurate than serum to detect anti-NMDAR encephalitis (sensitivity 99% vs 68%, p<0.0001). Using cell-based assay (CBA), CSF provided an unconfirmed positive test result in 11/2600 subjects (0.4%); 6/11 had a neuroinflammatory disease (other than anti-NMDAR encephalitis). Anti-NMDAR encephalitis patients, who tested positive in CSF only, had lower CSF antibody titers (p=0.003), but appeared to have an equally severe disease course.

Conclusion

Anti-NMDAR encephalitis occurs at all ages, and is less rare in the elderly patients than initially anticipated. In older patients the clinical phenotype is less outspoken, has different tumor association, and a less favorable recovery. Detection of antibodies in CSF is the gold standard, and although the CBA has very good validity, it is not perfect. The clinical phenotype should be leading and confirmation in a research laboratory is recommended, when in doubt.

INTRODUCTION

Anti-NMDA receptor (NMDAR) encephalitis is a severe immune-mediated disorder, and patients generally respond well to immunotherapy. [1] Fast initiation of immunotherapy is associated with a better clinical outcome. [1-3] Marking a timely diagnosis of anti-NMDAR encephalitis can be challenging, because patients can present with less notable encephalitis signs, as suggested in late-onset patients (over 45 years of age at onset). [4] NMDAR antibody testing may lead to misleading results, when only serum is tested. Diagnosis might be missed as antibodies can be only detectable in CSF in 15% of the patients. [5, 6] In addition, serum can yield positive but unconfirmed results, [7] as reported by earlier studies in healthy controls, patients with psychiatric conditions, or Creutzfeldt-Jakob disease (CJD). [8-14] However until today, data involving unconfirmed antibody test results in CSF is missing.

We report the pitfalls in the diagnosis of anti-NMDAR encephalitis, emphasizing on the clinical characteristics of late-onset patients, and antibody test accuracy in serum and CSF.

METHODS

Patients

We performed a nationwide, partly retrospective cohort study in Dutch patients with anti-NMDAR encephalitis. Patients were identified between March 2007 and December 2019. The department of Neurology of the Erasmus University Medical Center is a European Reference Network site and the national referral site for patients with suspected autoimmune encephalitis (AIE); the Laboratory Medical Immunology (department of Immunology) is the EN ISO 15189;2012 accredited national referral site for anti-neuronal antibody testing. Therefore, we could identify all patients with positive NMDAR antibodies and each patient was asked to participate. Part of the children with anti-NMDAR encephalitis were described before by De Bruijn et al. [15] Late-onset was defined as age of onset ≥45 years. [4] Antibodies were detected in serum, and/or in cerebrospinal fluid (CSF) using commercial cell-based assay (CBA, Euroimmun AG, Lübeck, Germany), and when in doubt by in-house CBA as well. Antibodies detected by CBA were confirmed with alternative antibody tests based on immunohistochemistry (IHC) and live hippocampal neurons (LN) as described before. [6, 16] If a sample was tested positive by CBA, but the positive result could not be confirmed by neither IHC nor LN, we defined that sample as 'unconfirmed'. Those unconfirmed CSF samples were sent for additional confirmation to the laboratory of Professor Dalmau (Hospital Clinic, University of Barcelona, Spain) and

in-house CBA, IHC and LN were performed.[17] Antibody titers were determined by IHC of pre-treatment serum and CSF samples.[6]

Clinical information

Clinical patient data about the disease course were obtained from detailed interviews with patients or relatives during a visit to our clinic in addition to the medical records (81%), or from medical records only (19%). Level of functioning was measured with the modified Rankin Scale (mRS).[18] Failure to first-line treatment was considered if no clinical improvement occurred within 2 weeks from start of immunotherapy.

In order to determine if final diagnoses were concordant with antibody results and assess whether our confirmatory tests were accurate we analyzed all patients with positive anti-NMDAR CBA, including those in whom we could not confirm CSF antibodies by additional tests.

Standard Protocol Approvals, Registrations, and Patient Consents

This retrospective study was waived and declared non-WMO complicit by the Institutional Review Board of Erasmus MC. Written informed consent was obtained from all patients.

Statistics

Statistical analysis was performed using IBM SPSS 25.0 (SPSS Inc) for Windows, as well as Prism 8.4.3. (GraphPad). For group comparisons, using categorical data, we used the Pearson Chi-Square test or the Fisher-Exact test when appropriate. Continuous data were analyzed using the Student's t-test or the Mann-Whitney U test, when appropriate. The annual incidence rate was calculated with 95%-CI assuming a Poisson distribution, and Dutch population data were used from StatLine (opendata.cbs.nl/#/CBS/nl/). Diagnostic test evaluation was compared using McNemar's paired test and Chi-square test for proportions. Seasonal patterns were analyzed using directional, circular statistics, and significance was determined by Rayleigh Z statistics.[19] We applied no correction for multiple testing, and therefore p values between 0.05 and 0.005 should be considered with care.

Data Availability Statement

Any data not published within this article are available at the Erasmus University Medical Center. Patient-related data will be shared upon reasonable request from any qualified investigator, maintaining anonymization of the individual patients.

RESULTS

Incidence and cohort description

The anti-NMDAR encephalitis cohort consisted of 133 patients, identified between 2007 to 2019, of whom 126 consented to study participation and were included in this study. In the period from May 2015 to December 2019, we identified 79 patients with a mean incidence rate of 1.00/million (95%-CI 0.62-1.59). The annual incidence rate of anti-NMDAR encephalitis in five consecutive years is shown in eTable 1, showing a peak in 2017. There was a predominance of onset in May and June (Figure 1B), although this did not reach statistical significance (circular direction would aim at May 24, $Z=1.80$, $p < 0.20$; eFigure 1); Exploring patients with anti-NMDAR encephalitis without known trigger (no HSV, nor paraneoplastic origin), a tendency towards seasonal variance was seen, pointing towards May (circular direction May 11, $Z=2.55$, $p < 0.10$).

The median age of onset was 24 years (IQR 17-38) and 39 patients (31%) were children. Twenty-four patients (19%) were 45 years of age or older at disease onset (Figure 1A). There was a known female predominance (76%), mainly for patients 12-45 years (73/86 [85%] vs 8/16 [50%] 0-12 years vs 15/24 [63%] ≥ 45 years, $p=0.002$). It took a median of 26 days (IQR 16-53) from disease onset to diagnosis. Details are provided in eTable 2. Almost all patients were treated with first-line immunotherapy (n=123, 98%) and 51 patients (41%) with second-line treatment. Patients were treated after a median of 21 days (IQR 11-45) from symptom onset, and it took a median of 46 days (IQR 29-89) to the first signs of clinical improvement (from symptom onset). Sixty-seven patients (55%) showed no response to first-line immunotherapy within 2 weeks. After the first signs of recovery, patients became independent in their daily activities (mRS ≤ 2) after a median of 5 months from disease onset (IQR 2-10). Three-quarter (n=87) showed good functional outcome (as measured by mRS) at 12 months.

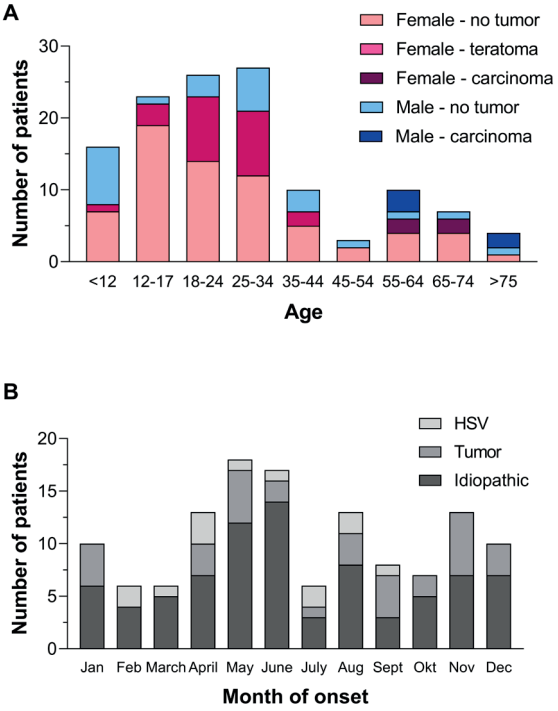

Figure 1. Distribution of age in combination with tumor presence and seasonal influences in anti-NMDAR encephalitis.

The figure involves the total cohort of 126 patients with anti-NMDAR encephalitis. (A) Age and tumor distribution. All tumors in patients ≥45 years were carcinomas, and in patients <45 years all were teratomas. The age categories <25 years have a different age distribution compared to other age categories.(B) Seasonal influences in anti-NMDAR encephalitis. The figure shows the month of onset divided by etiology (idiopathic, post-HSV, or tumor); Z=1.80 ($p < 0.20$) using Rayleigh Z statistics (data not shown). Figure is similar for the 2015-2019 sub-cohort (data not shown).

Late-onset patients

The median age of the twenty-four patients with disease onset ≥45 years of age was 64 years (range 46-86). Behavioral problems, seizures and sleep disorders occurred less frequently in patients ≥45 years ($p = 0.042$, $p = 0.021$, and $p = 0.003$, respectively, Table 1), as was the cumulative number of symptoms (median 3 vs 6, $p = 0.020$). The maximum severity of the disease (mRS) and the need for ICU support was similar in late-onset patients compared to younger patients (Table 2). Nine patients (38%) had an underlying tumor, all carcinomas, while in younger patients only teratomas were detected ($p < 0.0001$; Figure 1A). Abnormalities in ancillary testing typical for anti-NMDAR encephalitis showed no differences between both groups. In addition, median serum and CSF titers were similar between late-onset and younger patients. However, the patients ≥45 years of age had more often antibodies detectable only in CSF (seronegative; 30% vs 10% in younger adults, $p = 0.031$).

Table 1. Clinical features of anti-NMDAR encephalitis patients (defined by the age of onset).

	Age <45 (n=102)	Age ≥45 (n=24)	p value
Gender, female	81 (79%)	15 (63%)	0.080
Age of onset (mean, SD)	21 (10.2)	64 (9.5)	**<0.0001**
Onset to diagnosis, days (median, IQR, range)	26 (16-48, 5-5845)	25 (16-88, 5-210)	0.74
Symptoms			
Behavioral changes	98 (96%)	20 (83%)	**0.042**
Cognitive decline	88 (86%)	22 (92%)	0.74
Speech problems	66 (65%)	14 (58%)	0.56
Seizures	72 (71%)	11 (46%)	**0.021**
Movement disorders	67 (66%)	12 (50%)	0.15
Awareness problems	51 (50%)	8 (33%)	0.14
Autonomic symptoms	45 (44%)	7 (30%)	0.23
Hypoventilation	24 (24%)	7 (29%)	0.56
Sleep disorders	51 (51%)	4 (17%)	**0.003**
Number of symptoms (median, IQR, range)	6 (4-7, 0-9)	3 (2-7, 1-9)	**0.020**
Ancillary testing			
CSF abnormal	84 (86%)	19 (83%)	0.75
WBC elevated	74 (76%)	18 (78%)	0.78
WBC (median, IQR, range)	18 (5-47, 0-267)	23 (6-66, 2-235)	0.53
Total protein elevated	20 (21%)	8 (36%)	0.17
IgG index elevated	10/33 (30%)	2/6 (33%)	
Oligoclonal bands present	27/36 (75%)	3/4 (75%)	
Antibody titer CSF (n=106; median, IQR, range)	1:32 (1:16-1:128)	1:64 (1:8-1:512, neg-1:2048)	0.36
Antibody titer serum (n=104; median, IQR, range)	1:800 (1:200-1:1600, 1:100-1:6400)	1:200 (1:100-1:1600, 1:100-1:12800)	0.27
Seronegative NMDAR antibody [†]	9/89 (10%)	6/20 (30%)	**0.031**
MRI abnormalities AIE related	22/99 (31%)	7/23 (30%)	0.69
EEG abnormal	84/91 (92%)	15/19 (79%)	0.10
Posterior rhythm abnormal	27/77 (35%)	5/17 (29%)	0.78
Tumor	24 (24%)	9 (38%)	0.19
Teratomas	24/102	0/24	**0.007**
Carcinomas	0/102	9/24 (38%)	**< 0.0001**
Post-HSV	9 (9%)	4 (17%)	0.27

Abbreviations: SD = standard deviation, IQR = interquartile range, CSF = cerebrospinal fluid, WBC = white blood cells count, MRI = magnetic resonance imaging, AIE = autoimmune encephalitis, EEG = electroencephalogram, HSV = herpes simplex virus.

Data are n (%), n/n (%), mean (SD), or median (interquartile range, range).

[†] Serum was tested negative both by CBA and IHC.

First-line immunotherapy and second-line immunotherapy were evenly used in late-onset and younger patients, and there were no differences between both groups in the time until start of treatment and in failure to first-line immunotherapy (Table 2). Regarding outcome, patients ≥45 years had a worse outcome. Functional independence (mRS ≤2) was only achieved at a median of 12 months (IQR 5-13) for the patients ≥45 years, while this was 4 months for younger patients (IQR 2-7; p = <0.0001). After one year, 64% of the patients ≥45 years had a poor outcome (mRS ≥3) compared to 18% in the younger patients (p = <0.0001), and more patients died in the late-onset group (38% vs 2%, p = <0.0001; (eFigure 2)

Table 2. Treatment and outcome in anti-NMDAR encephalitis patients (defined by the age of onset).

	Age <45 (n=102)	**Age ≥45 (n=24)**	**p value**
Hospital admission	96 (94%)	24 (100%)	0.59
Hospital stay, days (median, IQR, range)	55 (24-83, 0-551)	46 (26-87, 7-262)	0.71
Onset to admission (median, IQR, range)	3 (0-13, 0-88)	7 (0-28, 0-170)	0.34
ICU admission	50 (49%)	11 (46%)	0.82
ICU stay, days (median, IQR, range)	28 (4-49, 1-307)	36 (5-71, 2-132)	0.39
Immunotherapy			
First-line immunotherapy	99 (97%)	24 (100%)	1.00
Onset to first-line IT, days (median, IQR, range)	21 (10-42, 2-510)	23 (13-81, 1-181)	0.33
First-line IT to improvement, days (median, IQR, range)	21 (8-41, -383-774) ∞	14 (7-37, 5-93)	0.79
Failure of first-line IT *	55/98 (56%)	12/23 (52%)	0.73
Second-line immunotherapy	44 (43%)	7 (29%)	0.21
Onset to second-line IT, days (median, IQR, range)	31 (23-61, 12-822)	38 (31-214, 26-310)	0.13
Outcome			
Onset to improvement, days (median, IQR, range)	44 (30-79, 1-974)	90 (25-195, 7-366)	0.20
Time to mRS 2, months (median, IQR, range)	4 (2-7, 0-not achieved)	12 (5-13, 1-not achieved)	**<0.0001**
mRS max (median, IQR, range)	4 (3-5, 2-5)	4 (3-5, 3-5)	0.99
mRS at start IT (median, IQR, range)	4 (3-5, 2-5)	4 (3-5, 3-5)	0.92
mRS at 12 months (median, IQR, range)	2 (1-2, 0-6)	3 (2-6, 0-6)	**<0.0001**
Poor outcome at last FU (mRS ≥3)	15/101 (15%)	13/24 (54%)	**<0.0001**
Relapse	18 (18%)	4 (17%)	1.00
Deceased	2 (2%)	9 (38%)	**<0.0001**

Abbreviations: IQR = interquartile range, ICU = intensive care unit, IT = immunotherapy, mRS = modified Rankin scale, FU = follow-up.

Data are n (%), n/n (%), mean (SD), or median (interquartile range, range).

∞ 9 patients showed clinical improvement before start of immunotherapy, 7 of whom within 26 days prior to treatment. All were not completely recovered for which immunotherapy was administered.

* Failure to first-line immunotherapy was defined as no clinical improvement within two weeks after start of treatment.

Antibody test accuracy

Antibody test accuracy was investigated from May 2015 to December 2019. Within this period, 79 patients with anti-NMDAR encephalitis were identified, while 21 patients had results that could not be confirmed (by other research techniques).

Accuracy of CSF was superior compared to serum reflected by higher sensitivity percentages, for both CBA and IHC ($p < 0.0001$), while specificity was at least similar (Table 3). One third of patients had been missed if only serum would have been tested (sensitivity 68%). Of 2600 CSF samples, an unconfirmed result was identified in eleven (0.4%).

Table 3. Anti-NMDAR antibody tests accuracy in serum and CSF samples

	SERUM			
	NMDARE	**No-NMDARE**		
CBA +	46	19	65	PPV 71% (60-80%)
CBA -	22	3141	3163	NPV 99.3% (99.0-99.5%)
	68	3160	3228	
	Sens 68% (55-78%)	Spec 99.4% (99.1-99.6%)		
IHC +	52	2	54	PPV 96% (87-99.1%)
IHC -	19	2215	2234	NPV 99.2% (98-99.4%)
	71	2217	2288	
	Sens 73% (61-83%)	Spec 99.9% (99.7-99.9%)		
	CSF			
	NMDARE	**No-NMDARE**		
CBA +	78	11 ±	89	PPV 88% (80-93%)
CBA -	1 ±	2589	2590	NPV 99.9% (99.7-99.9%)
	79	2600	2679	
	Sens 99% (93-99.9)	Spec 99.6% (99.2-99.8)		
IHC +	76	0	76	PPV 100% (-)
IHC -	1 ±	1306	1307	NPV 99.9% (99.5-99.9%)
	77*	1306	1383	
	Sens 99% (93-99.9%)	Spec 100% (99.7-100%)		

Abbreviations: CBA = cell based assay, IHC = immunohistochemistry, NMDARE = anti-NMDAR encephalitis, Sens = sensitivity, Spec = specificity, PPV = positive predictive value, NPV = negative predictive value, CI = confidence interval.

Sensitivity, specificity, PPV and NPV are depicted as % (95% CI).

From May 2015 to December 2019, in total 79 positive anti-NMDAR encephalitis patients were identified, and 21 clinical irrelevant positive patients.

*One patient with anti-NMDAR encephalitis could not be scored with IHC because of high background (live neurons were positive). Another patient's CSF could not be tested with IHC because no more CSF was available (this was not the patient with a negative CBA result).

± These patients are elucidated in Table 4.

Specificity was high (>99%) in all tests, although these small differences are relevant when testing high volumes. This is reflected by the CBA showing only a moderate positive predictive value (PPV) testing in serum, as 29% of the positive tests were unconfirmed. In CSF, PPV was seriously better (88%) as 11 patients had unconfirmed positivity ($p = 0.008$ compared to serum; Table 4). Confirmation with IHC added value to determine clinical relevance, mostly reflected in a lower number of unconfirmed positive results ($p = 0.008$ for serum and $p = 0.006$ for CSF). This resulted in a higher PPV in both serum (96%) and CSF (100%), favoring IHC over CBA.

Patients with unconfirmed positive NMDAR results in CSF

Eleven patients had an unconfirmed CBA result in CSF for NMDAR antibodies (between 2015 and 2019), considering negative results on both IHC and LN. Similarly, serum from 10 of these patients was tested and showed in nine of them the same combination of positive CBA unconfirmed by alternative research techniques (IHC and LN). Our results were re-confirmed in a reference laboratory. Individual data of all 11 patients are shown in Table 4. Four patients had a psychiatric or neurodegenerative etiology, all without evidence for autoimmunity. Six patients had an inflammatory etiology of disease different from anti-NMDAR encephalitis, and one patient had an infectious condition.

Undetectable serum antibodies

Fifteen out of the 109 patients (14%) with available serum had no detectable NMDAR antibodies, tested by both CBA and IHC (eTable 3). The other 94 patients had antibodies in both serum and CSF (seropositive) using IHC and/or CBA. Compared to seropositive patients, the seronegative patients were older at disease onset with a median age of 35 versus 23 years ($p = 0.016$). Seronegative patients tended to have less symptoms, although this did not reach statistical significance (4 compared to a number of 6 symptoms in seropositive patients; $p = 0.10$). ICU admission was evenly required in both groups. Time to diagnosis and time to start of immunotherapy were similar in both groups, as were functional outcome and relapse rates. The seronegative patients had significantly lower antibody titers in CSF compared to seropositive patients (median 1:8 [IQR 1:2-1:32] vs 1:64 [IQR 1:16-1:256]; $p = 0.003$), and more frequently CSF pleocytosis (100% vs 71%, $p=0.020$). Two patients (13%) in the seronegative group had a tumor, compared to 26 seropositive patients (29%, $p=0.34$).

Table 4. Characteristics of patients with discordant anti-NMDAR antibody results in CSF.

Gender, age onset	Symptoms	Blood analysis	CSF	MRI T2/FLAIR	Tumor	EEG	Final diagnosis
Patients with a clinically-irrelevant positive anti-NMDAR CBA in CSF							
F, 65 y	Psychosis	n.p.	Normal, OCB n.p.	n.p.	Not screened	n.p.	Non-inflammatory: Psychiatric
M, 23 y	Cogn deter, psychosis. Mother with Huntington disease	n.p.	Normal, OCB -	Less volume nucleus caudatus both sides	Not screened	n.p.	Non-inflammatory: Schizophrenia. Refused Huntington testing.
M, 65 y	Cogn deter, behav change, psych sympt, aphasia. History of gait disturbance	Anti-TPO neg, Tau ↑	Normal, OCB n.p.	Normal	No	Slow	Non-inflammatory: Korsakoff-like syndrome
M, 55 y	behav change, apraxia, aphasia, psychosis	Limited info	Limited info	Limited info	Limited info	Less info	Non-inflammatory: Korsakoff-like syndrome.
F, 54 y	behav change, seizures	ANCA pos (PR3)	Normal. OCB n.p.	Chron mastoiditis, intracranial breakthrough, Gd+ meninges	Not screened	Normal	Infectious: Mastoiditis resulting in meningitis. Refused referral for PR3
F, 57 y	Progressive muscle strength loss, resp failure	n.p.	WBC 3, Prot ↑, OCB n.p.	n.p.	Not screened	n.p.	Inflammatory: GBS-AMSAN (NF155 pos)
F, 37 y	Vision problems and migraine.	n.p.	WBC 60, IgG index ↑, OCB +	Multipele white matter lesions with Gd+	Not screened	n.p.	Inflammatory: RR-MS
F, 74 y	Seizures, cogn deter, behav change	ANA ↑ (320), anti-dsDNA ↑ en sIL-2R ↑	Normal, OCB n.p.	Atrophy, mostly temporoparietal left	No	Normal	Inflammatory: Antiphospholipid syndr, corticobasal degeneration
M, 55 y	Seizure, loss of consciousness, cogn deter, behav change (3 episodes)	sIL-2R nl. ANA TPO neg, tTG endomysium nl	Normal, OCB -	Normal	No	Normal	Inflammatory: Encephalitis

Table 4. Characteristics of patients with discordant anti-NMDAR antibody results in CSF. (*continued*)

Gender, age onset	Symptoms	Blood analysis	CSF	MRI T2/FLAIR	Tumor	EEG	Final diagnosis
M, 71 y	Cogn deter, behav change, insomnia, fever, myoclonia. Sudden death after 1 month		WBC 66, Prot ↑, OCB n.p.	Normal	Probable bladder carcinoma	Diffuse slow	Inflammatory: Encephalitis
F, 27 y	behav change, seizures, psych sympt, dyskinesias, Sjogren History	ANA,anti-SSA pos. sIL-2R mild raised. Anti-dsDNA nl	WBC 7, OCB n.p.	Normal	No	Diffuse slow	Inflammatory: Encephalitis or neuro-Sjogren's syndrome
Anti-NMDAR encephalitis patients with a negative IHC in CSF							
F, 74 y	Post-HSV. seizures, psych sympt, aphasia		WBC 10, OCB n.p. LN+	post-HSV increased MT hyperintensity	Not screened	Diffuse slow	Post-HSV NMDAR encephalitis
Anti-NMDAR encephalitis patients with a negative CBA in CSF							
F, 15 y	Seizures, cogn deter, behav change, psych sympt, aphasia, dystonia.		WBC 9, OCB -. FU sample NMDAR +	Normal	No	Epileptic and slow	Inflammatory: Anti-NMDAR encephalitis

Abbreviations: CSF = cerebrospinal fluid, MRI = magnetic resonance imaging, EEG = electroencephalogram, FU = follow-up, F = female, M = male, n.p. = not performed, ANCA = antineutrophil cytoplasmic antibody, PR3 = proteinase 3, Gd+ = gadolinium enhancement, ANA = antinuclear Antibody, anti-dsDNA = anti-double stranded DNA, sIL-2R = soluble interleukin-2 receptor, anti-TPO = anti-thyroid peroxidase, anti-tTG = anti-tissue transglutaminase, pos = positive, neg = negative, OCB = oligoclonal bands, WBC = white blood cells count, prot = protein, LN = live neurons, HSV = herpes simplex virus, MT = mesiotemporal, GBS-AMSAN = Guillain-Barre syndrome - acute motor-sensory axonal neuropathy, RR-MS = relapsing remitting multiple sclerosis.

DISCUSSION

This nationwide observational cohort study shows that the CBA to detect NMDAR antibodies performs very well, but not perfectly. This was demonstrated by the finding that patients can have unconfirmed positive results in CSF. Secondly, anti-NMDAR encephalitis is less rare at older age than previously thought, and these patients have other paraneoplastic associations and a worse outcome. At last, patients without detectable serum antibodies have lower CSF antibody titers, but disease course appeared not to be milder.

To the best of our knowledge we are the first to describe a small series of patients without anti-NMDAR encephalitis despite positive CBA results in CSF. Lang and Prüss reviewed the presence of surface antibodies in 1650 control subjects, and found 16 cases with NMDAR antibodies in CSF by using CBA, but all 16 cases were (retrospectively) assessed to truly have had anti-NMDAR encephalitis. [20] We identified 11 patients with 'unconfirmed' CSF antibody results: next to a positive CBA, a positive result by at least one additional, different technique was mandatory, and this was not met, despite all samples being tested with three different techniques in two independent laboratories. As all samples tested positive by both commercial CBA and in house CBA, it should not be considered simply a false positive result. Although we cannot exclude that the antibody result is relevant, we consider clinical irrelevance as the most likely explanation. Seven patients had an inflammatory etiology for their symptoms suggesting that a broader immune response or antibody formation secondary to neuronal damage was present leading to these confusing antibody results. None of the patients fulfilled criteria for 'probable anti-NMDAR encephalitis', according to the Graus criteria. [21] In most of these 11 patients, clinical assessment proved to be of great importance as the phenotype was atypical for encephalitis with NMDAR antibodies. Therefore, despite positive antibody results, the physician should remain vigilant and open minded. In case of doubt, CBA result confirmation by a research (reference) laboratory using alternative techniques (IHC and/or LN) is advocated.

While CSF results can occasionally be difficult to interpret, this is much more frequently an issue in serum, as one quarter of the positive serum results were considered clinically irrelevant, similar to previous reports. [7, 22] Especially in those with a low pre-test probability, the risk to encounter unconfirmed or clinically irrelevant results becomes unacceptably high as reported by earlier studies in healthy controls, psychiatric patients, or CJD patients. [8-14] Confirmation in CSF is therefore essential, as CSF was superior to serum in the diagnosis of anti-NMDAR encephalitis, which is in line with previous studies. [6, 23]

Except for the high frequency of patients in the age ≥45 years, our cohort showed no discrepancies compared to other NMDAR cohorts. [2, 4] A fifth of our patients would be considered late-onset anti-NMDAR encephalitis, compared to only 5% in earlier reports. [4]

As the differential diagnosis in patients at older age is broader, this probably reflects better awareness of anti-NMDAR encephalitis, and a lower threshold to send samples for testing nowadays. Within our cohort, 78% of the late-onset patients were diagnosed after 2015 compared to 58% in younger patients, supporting this theory. Earlier it was suggested that late-onset patients had a less severe disease course,[4] but this was not confirmed in our study. We did confirm a lower frequency of seizures,[3,4] more frequent antibody negative results in serum,[5] and a trend in less female predominance.[4] All these items culminate in a lower number of symptoms. A higher level of suspicion is therefore necessary to recognize late-onset anti-NMDAR encephalitis, despite the broad differential diagnosis, as the outcome is worse at that age.[4] Although the time initial immunotherapy was similar in late-onset patients, they tended to have less and later initiation of second-line immunotherapy (both not significant, probably related to the modest sample size). As it is known that brain plasticity and the capacity to recover diminish with age,[24] better chances for recovery necessitate early and aggressive treatment. Especially as this is the factor best amended by physicians. Remarkably, in the patients above 45 years only carcinomas were identified, similar to previous publications,[4] while only teratomas were found in younger patients. This emphasizes the importance for a thorough, but different tumor work-up as FDG-PET/CT is best to detect carcinomas, while this is not sensitive to detect teratomas.[25]

We found the same frequency of patients with anti-NMDAR encephalitis without detectable serum antibodies as earlier reports.[5,6] The lack of serum antibodies was associated with lower antibody titers in CSF. Similarly, these patients were older at disease onset and had fewer tumors.[5] Although tumor difference was not significant in our cohort, the size and direction were similar to previous study, suggesting a lack of statistical power by sample size. All these observations suggest a less robust immune response, and the absence of detectable serum antibodies might well be a threshold issue. As CSF is diluted less under normal test conditions, and almost all patients show intrathecal antibody synthesis, CSF might provide a better signal to noise ratio. Lack of antibodies in serum is difficult to imagine, especially in patients with a paraneoplastic disease. In our cohort, two malignancies were discovered by tumor screening. One was a small cell lung carcinoma, probably related, and one a metastatic esophagus carcinoma, likely a coincidental finding.

Incidence of anti-NMDAR encephalitis was 1 per million per year over the last five years. This is in line with the reported incidence in the literature, ranging between 0.7 and 2.2 per million per year,[15,26-28] although those studies analyzed only children. Over the years the incidence has increased, and this was likely reflecting increased awareness due to the novelty. However, over the last 5 years, incidence peaked in 2017, suggesting a currently unknown trigger specific for that year. Over the years, onset of disease seems to cluster in late spring, although this did not reach statistical significance. Only one small study

in children has looked at seasonal patterns, also suggesting a predominance of the early warm months. [29] This association seemed slightly stronger when looking only at idiopathic anti-NMDAR encephalitis, similar to the study in children. Neoplasms and herpes simplex encephalitis are the only known triggers of anti-NMDAR encephalitis, [17, 30, 31] but the seasonal pattern and yearly varying incidence might suggest environmental triggers, like specific infections. Until now, no studies have identified consistent infectious triggers. [32] Despite the intriguing peak in 2017, we were unable to identify a higher incidence of for example influenza or influenza-like diseases in the Dutch Institute for Health Services Research (NIVEL) report. Future studies are necessary to elucidate a role for other infectious agents triggering the immune response in anti-NMDAR encephalitis comparing seasonal patterns in larger cohorts from different countries.

Although this study is nationwide, there are some limitations associated with the retrospective design of this study. First, clinical data was sometimes difficult to assess from the documentation, especially follow-up data. However, we could overcome this issue as we saw or interviewed most patients in our clinic, or had contact with the treating physician or caregiver. Secondly, the cohort is relatively modest in size also reflecting the rarity of the disease, yet we could include almost all patients accomplishing nationwide coverage. Due to the retrospective design, the ability to extract detailed functional outcomes was difficult and we decided therefore to use the mRS, despite its limitations. However, as long-term outcome was not the primary scope of our study, the current amount of data was sufficient to achieve our goals.

In conclusion, physicians should be aware that anti-NMDAR encephalitis can occur at all ages, and might be less rare later in life than previously anticipated. Complicating factor is the less outspoken nature of the disease in late onset patients, but early treatment is even more important due to the link with malignancies and as recovery is already slower and less complete. Physicians requesting NMDAR antibody tests should be aware of the pitfalls of the test, including the lower sensitivity and specificity in serum, and the need for CSF confirmation. However, as no test is ever perfect, the physician should always link the clinical phenotype with the antibody results (even in CSF). In doubt, a reference laboratory should serve to confirm or refute the diagnosis.

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SUPPLEMENTARY DATA

eTable 1. Annual incidence of anti-NMDAR encephalitis.

Year	No. of anti-NMDAR encephalitis patients	Incidence/million (95% CI)	No. of Dutch inhabitants
2015 (May-Dec)	14	1.24 (0.77-1.89) *	16,900,726
2016	17	1.00 (0.58-1.60)	16,979,120
2017	26	1.52 (0.99-2.23)	17,081,507
2018	10	0.58 (0.28-1.07)	17,181,084
2019	12	0.69 (0.36-1.21)	17,282,163
2015 - 2019	79	1.00 (0.62-1.59) **	17,098,077

** The incidence rate of 2015 was extrapolated to a whole year for correct incidence numbers.*

*** Based on 4 years and 8 months, as 2015 numbers were available for 8 months only.*

eTable 2. Patient characteristics of all Dutch anti-NMDAR encephalitis patients (n=126)

Gender, female	96 (76%)
Age of onset (median, IQR, range)	24 (17-38, 1-86)
<12	16 (13%)
12-17	23 (18%)
18-24	26 (20%)
25-34	27 (21%)
35-44	10 (8%)
45-54	3 (2%)
55-64	10 (8%)
65-74	7 (6%)
>75	4 (3%)
Onset to diagnosis, days (median, IQR, range)	26 (16-53, 4-5845)
Symptoms	
Behavioral changes	118 (94%)
Cognitive decline	110 (87%)
Speech problems	80 (64%)
Seizures	83 (66%)
Movement disorders	79 (63%)
Awareness problems	59 (47%)
Autonomic symptoms	52/125 (41%)
Hypoventilation	31 (25%)
Sleep disorders	55/124 (44%)
Hospital admission	120 (95%)
Hospital stay, days (median, IQR, range)	56 (27-86, 2-551)
ICU admission	61 (48%)
ICU stay, days (n=56; median, IQR, range)	29 (4-51, 1-307)
Ancillary testing	
CSF abnormal	103/121 (85%)
WBC elevated	92/121 (76%)
WBC (median, IQR, range)	18 (5-53, 0-267)
Antibody titer serum (n=102; median, IQR, range)	1:400 (1:200-1:1600, negative-1:12800)
Antibody titer CSF (n=104; median, IQR, range)	1:32 (1:8-1:128, negative-1:2048)
Seronegative	15/109 (14%)
MRI AIE related abnormalities [±]	29 (23%)
EEG abnormal	98/110 (89%)
Posterior rhythm abnormal	32/95 (34%)
Tumor	33/123 (27%)
Teratomas	24 (73%)
Carcinomas	9 (27%)
Post-HSV	13 (10%)

Immunotherapy	
First-line immunotherapy	123 (98%)
IV methylprednisolone	115/125 (92%)
IV immunoglobulins	99/125 (79%)
Plasma exchange	13/124 (11%)
Second-line immunotherapy	51 (41%)
Rituximab	46 (37%)
Cyclophosphamide	20 (16%)
Onset to first-line IT, days (median, IQR, range)	21 (11-45, 1-510)
Failure to first-line immunotherapy *	67/121 (55%)
Onset to improvement, days (n=114; median, IQR, range)	46 (29-89, 1-974)
First-line IT to improvement, days (n=109; median, IQR, range)	20 (7-41, -383-774) ∞
Second-line IT to improvement, days (n=49; median, IQR, range)	14 (3-31, -420-344) ∞
Outcome	
mRS max (n=126; median, IQR, range)	4 (3-5, 2-5)
mRS start IT (n=123; median, IQR, range)	4 (3-5, 2-5)
mRS 6 weeks (n=126; median, IQR, range)	3 (3-5, 0-6)
mRS 4 months (n=125; median, IQR, range)	3 (2-3, 0-6)
mRS 6 months (n=121; median, IQR, range)	2 (1-3, 0-6)
mRS 8 months (n=116; median, IQR, range)	2 (1-3, 0-6)
mRS 12 months (n=114; median, IQR, range)	2 (1-3, 0-6)
mRS 18 months (n=102; median, IQR, range)	1 (1-2, 0-6)
mRS 24 months (n=92; median, IQR, range)	1 (1-2, 0-6)
mRS last FU (median, IQR, range)	1 (0-2, 0-6)
Time to mRS 2, months (median, IQR, range)	5 (2-10, 0- not achieved)
Good mRS at 12 months	87/118 (74%)
FU, months (median, IQR, range)	27 (15-45, 2-180)
Good mRS at last FU	97/125 (78%)
Relapse	22 (18%)
Deceased	11 (9%)

Abbreviations: IQR = interquartile range, ICU = intensive care unit, CSF = cerebrospinal fluid, WBC = white blood cells count, MRI = magnetic resonance imaging, EEG = electroencephalogram, HSV = herpes simplex virus, IT = immunotherapy, mRS = modified Rankin scale, FU = follow-up.

Data are n (%), n/n (%), or median (interquartile range, range).

ǂ AIE related abnormalities included T2/flair hyperintensy mesiotemporal or thalamus region.

* Failure to first-line immunotherapy was defined as no clinical improvement within two weeks after start of treatment. Not all patients with first-line failure were treated with second-line therapy (mostly patients with onset <2012, early dead or children).

∞ Nine patients showed clinical improvement before start of first-line immunotherapy, 7 of whom within 26 days prior to treatment. Six patients showed clinical improvement before start of second-line therapy. All were not completely recovered for which immunotherapy was administered.

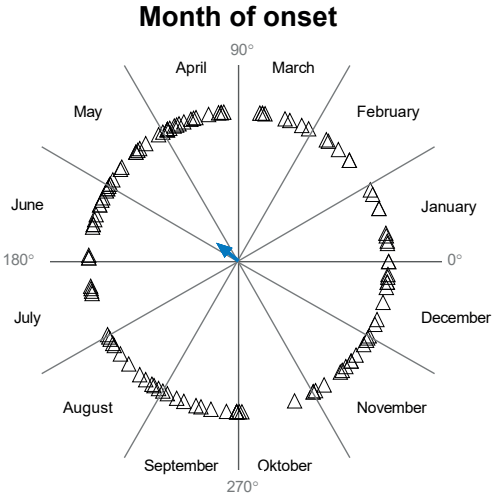
eTable 3. Serostatus in anti-NMDAR encephalitis (n=109).

	Seronegative (n = 15)	Seropositive (n = 94)	p value
Gender, female	11 (73%)	71 (76%)	1.00
Age of onset (mean, SD)	35 (22-67, 5-75)	23 (17-32, 1-86)	**0.016**
Onset to diagnosis, days (median, IQR, range)	23 (11-40, 5-179)	29 (16-54, 4-5845)	0.35
Symptoms			
Behavioral changes	13 (87%)	89 (95%)	0.25
Cognitive decline	12 (80%)	85 (90%)	0.37
Speech problems	7 (47%)	64 (68%)	0.11
Seizures	9 (60%)	63 (67%)	0.59
Movement disorders	6 (40%)	62 (66%)	0.054
Awareness problems	5 (33%)	47 (50%)	0.28
Autonomic symptoms	5 (33%)	44/93 (47%)	0.41
Hypoventilation	4 (27%)	21 (22%)	0.74
Sleep disorders	6 (43%)	44/92 (47%)	0.57
Number of symptoms (median, IQR, range)	4 (2-7, 1-7)	6 (4-7, 2-9)	0.10
Hospital admission	14 (93%)	91 (97%)	0.45
Hospital stay, days (median, IQR, range)	49 (18-93, 2-143)	57 (29-94, 3-551)	0.59
ICU admission	7 (47%)	45 (48%)	0.93
ICU stay, days (median, IQR, range)	19 (2-70, 1-71)	28 (4-50, 1-307)	0.47
Ancillary tests			
CSF abnormal	15 (100%)	75/90 (83%)	0.12
WBC elevated	15 (100%)	64/90 (71%)	**0.020**
WBC (median, IQR, range)	20 (10-54, 6-107)	14 (5-53, 0-267)	0.34
Total protein elevated	6 (40%)	16/86 (19%)	0.064
Antibody titer CSF (median, IQR, range)	1:8 (1:2-1:32, 1:2-1:128)	1:64 (1:16-1:256, negative-2048)	**0.0034**
MRI abnormalities AIE related	6 (40%)	15/90 (17%)	0.073
EEG abnormal	10/12 (83%)	77/85 (91%)	0.61
Posterior rhythm abnormal	1/9 (11%)	25/72 (34%)	0.26
Tumor	2 (13%)	26/91 (29%)	0.34
Teratomas	0	19	
Carcinomas	2 ±	7 ±±	
Post-HSV	2 (13%)	9 (10%)	0.65
Immunotherapy			

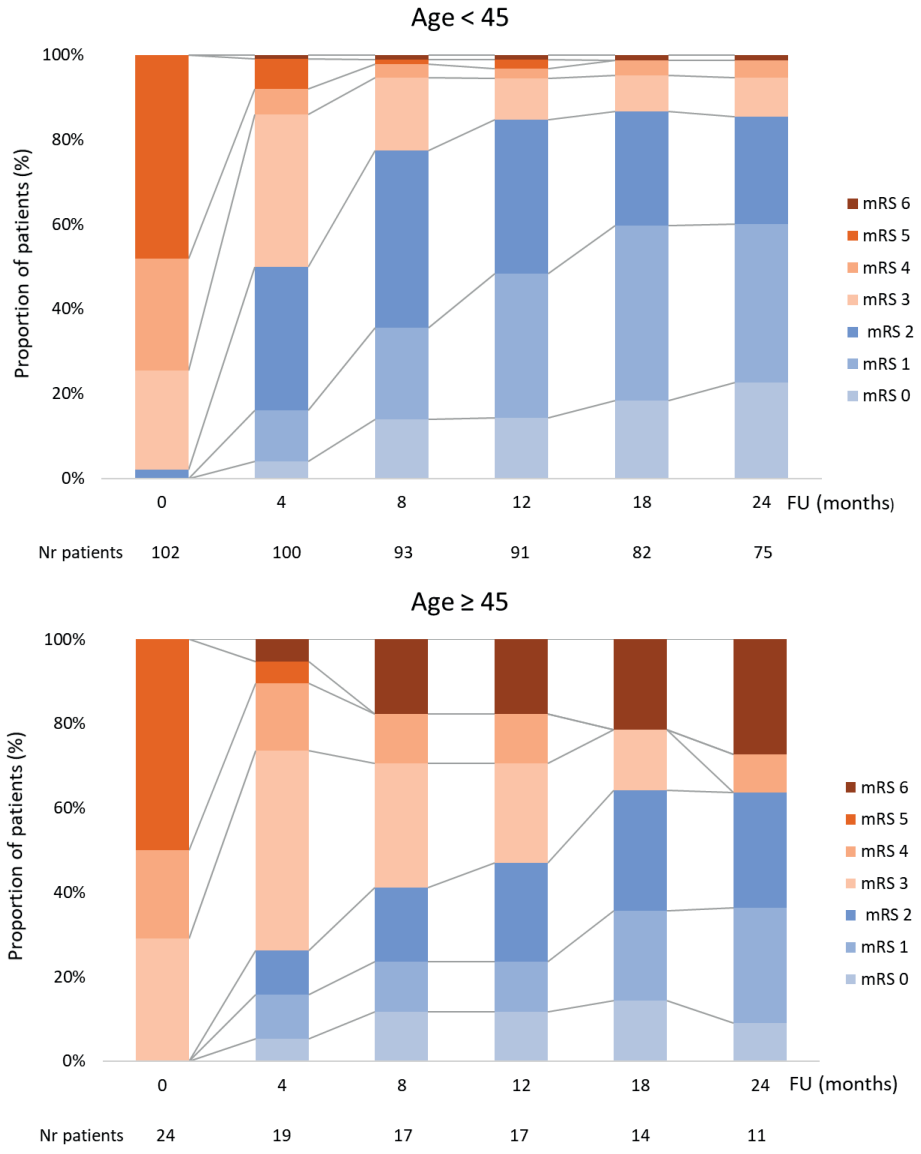
eTable 3. Serostatus in anti-NMDAR encephalitis (n=109). (*continued*)

	Seronegative (n = 15)	Seropositive (n = 94)	p value
First-line immunotherapy	14 (93%)	93 (99%)	0.26
Onset to first-line IT, days (median, IQR, range)	25 (15-36, 3-172)	21 (10-51, 2-307)	0.67
Failure of first-line IT *	7/14 (50%)	52/91 (57%)	0.62
Second-line immunotherapy	5 (33%)	42 (45%)	0.58
Outcome			
Onset to improvement, days (median, IQR, range)	37 (29-70, 1-366)	49 (33-90, 7-974)	0.26
mRS max (median, IQR, range)	4 (4-5, 2-5)	4 (3-5, 3-5)	0.73
mRS at 12 months (median, IQR, range)	2 (1-3, 0-6)	2 (1-2, 0-6)	0.56
Good mRS after 1 year	8/14 (57%)	66/88 (75%)	0.16
Good mRS at last FU	11/15 (73%)	72/93 (76%)	0.75
Relapse	3 (20%)	18 (19%)	1.00
Deceased	1 (7%)	8 (9%)	1.00

Abbreviations: SD = standard deviations, IQR = interquartile range, ICU = intensive care unit, CSF = cerebrospinal fluid, WBC = white blood cells count, MRI = magnetic resonance imaging, EEG = electroencephalogram, HSV = herpes simplex virus, IT = immunotherapy, mRS = modified Rankin scale, FU = follow-up.
Data are n (%), n/n (%), mean (SD), or median (interquartile range, range).
‡ one small cell lung carcinoma and one metastatic esophageal carcinoma.
‡‡ three small cell lung carcinoma, one colon carcinoma, one Merkel cell carcinoma, one endometrial carcinoma, one Hodgkin lymphoma.
* Failure to first-line immunotherapy was defined as no clinical improvement within two weeks after start of treatment.



eFigure 1. Seasonal pattern in anti-NMDAR encephalitis.
The figure represents the month of onset of all included anti-NMDAR encephalitis patients. Each square is one patient, showing a predominance of onset in May and June. Circular direction would aim at May 24 (Z=1.80, p < 0.20).



eFigure 2. Clinical outcome at follow-up.

Outcome was measured by modified Rankin scale (mRS). The top figure represent the patients <45 years of age and the bottom figure represent the patients ≥45 years of age. Patient who died in the acute phase of the disease with no recovery were excluded from this figure (1 patient in age group <45 and 5 patients in age group ≥45).

5

Predictive value of serum neurofilament light chain levels in anti-NMDA receptor encephalitis

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ABSTRACT

Background and objectives

Determinants of disease activity and prognosis are limited in anti-NMDAR encephalitis. Neurofilament light chains (NfL) are markers of axonal damage and have been identified as valuable biomarkers for neurodegenerative and other neuroinflammatory disorders. We aimed to investigate serum NfL levels in patients with anti-NMDAR encephalitis as a biomarker for disease severity and outcome.

Methods

In this retrospective study, NfL values were measured in all available pre-treatment serum and paired CSF samples of the nationwide anti-NMDAR encephalitis cohort. The values were analyzed in duplicate using Single Molecule Array (SIMOA) and compared to measurements in healthy references. Follow-up sera were tested to analyze longitudinal responsiveness, if at least available from two time points after diagnosis. Serum NfL levels were compared to data on disease activity (seizures, MRI and CSF findings), severity (mRS, admission days, ICU admission) and outcome (mRS and relapses), using regression analysis.

Results

We have included 71 patients (75% female; mean age 31.4, range 0-85 years). Paired CSF samples were available of 33 patients, follow-up samples of 20 patients. Serum NfL levels at diagnosis were higher in patients (mean 19.5 pg/mL, 95%-CI 13.7-27.7) than in references (mean 6.4 pg/mL, 95%-CI 5.8-7.2, $p<0.0001$). We observed a good correlation between serum and CSF NfL values (R=0.84, $p<0.0001$). Serum NfL levels and age correlated in patients (Pearson's R=0.57, $p<0.0001$) and references (R=0.62, $p<0.0001$). Increased NfL values were detected in patients post-HSV1 encephalitis (mean 248.8 vs 14.1 pg/mL, $p<0.0001$) and in patients with brain MRI lesions (mean 27.3 vs 11.1 pg/mL, $p=0.019$). NfL levels did relate to the long-term follow-up (mRS at 12 months; $\beta_{NfL}=0.55$, $p=0.013$), although largely explained by the effect of age on NfL levels and prognosis. In serial samples, NfL values did roughly follow clinical disease activity, albeit with delay.

Discussion/Conclusions

Increased serum NfL levels reflect neuro-axonal damage in anti-NMDAR encephalitis. No relationship was identified with disease severity, while the association with outcome was confounded by age. The implied role of sampling timing on NfL levels also limits the applicability of NfL as a prognostic marker.

INTRODUCTION

Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is a complex immune-mediated disorder characterized by antibodies in the cerebrospinal fluid (CSF) against the ionotropic glutamate receptor type 1 subunit of the NMDA receptor. Clinical features include behavioral changes, cognitive impairment, seizures, language disorders, movement disorders, and autonomic dysfunctions. It can occur as a paraneoplastic phenomenon, most often associated with ovarian teratomas, post-infectious after Herpes Simplex Virus encephalitis or sporadically.[1] The disease is treatable by removing the trigger (if paraneoplastic) and administering immunotherapy. Still, patients might require admission to the intensive care unit (ICU) during the acute stage. Many patients experience persisting neurological deficits and 12% of cases relapse within 2 years.[2] The outcome of anti-NMDAR encephalitis has previously been related to clinical factors like the requirement of ICU admission, treatment delay, and a lack of response to first line immunotherapy.[2,3] CSF leukocyte count and antibody titers correlate with outcome and clinical relapses.[3,4] However, titers do not consistently reflect disease activity.[5] Treatment decisions are currently based on clinical assessment since, despite several attempts, biomarkers for disease severity and prognosis are very limited.[6]

Neurofilaments, and in particular the light chain subunit, are released from axons after acute damage. Neurofilament light chain (NfL) levels have therewith been identified as a useful biomarker for disease activity and prognosis in different neuro-inflammatory and degenerative neurological disorders.[7] The strong correlation between CSF and serum NfL values and the high sensitivity of novel diagnostic techniques, allowing to quantify the lower levels detectable in serum, seem to expand the applicability of serum NfL as a biomarker.[8] The pre-analytical stability of NfL values (i.e. to delayed freezing and repeated thawing/freezing cycles) additionally raises the potential to investigate NfL as a biomarker.[9] In this study we investigate serum NfL levels at diagnosis and follow-up in patients with anti-NMDAR encephalitis to evaluate whether this biomarker of ongoing axonal damage correlates with disease severity and long-term outcome.

METHODS

Study subjects and sample selection

As the national referral center for autoimmune encephalitis of the Netherlands, accredited as European Reference Network site (ERN-RITA), we take note of all nationwide diagnoses of anti-NMDAR encephalitis. We have targeted all Dutch patients complying with the criteria for a definite anti-NMDAR encephalitis[10], based on (1) the availability of a sufficient amount of serum from the time of diagnosis, (2) serum drawn before start

of immunotherapy, and (3) relevant clinical data of at least four months after diagnosis (Supplementary figure 1). All eligible patients had previously consented to be in the nationwide anti-NMDAR encephalitis cohort and have been phenotyped clinically well (Suppl. table 1).[11] We compared the data to a healthy reference group (n=61; 70% female; mean age 41.9, range 25-67 years) and to previously suggested age-based cut-off values.[12-14] To correlate serum to CSF, we tested all available pre-treatment CSF samples drawn within 48 hours from the serum sample. To investigate NfL longitudinally, we selected those patients of whom we had sufficient amounts of sera from at least two different time points after diagnosis.

Clinical parameters

Extensive clinical data had been collected as part of our nationwide study.[11] Age of onset, preceding Herpes Simplex Virus encephalitis, concomitant tumors, the presence of seizures or movement disorders, cerebral MRI abnormalities and antibody titers were considered potentially relevant covariates for NfL levels. Maximum modified Rankin Scores (mRS), duration of hospital admission and the need for ICU admission were used as measures for disease severity. Short and long-term outcomes were quantified as the mRS score at 4 and 12 months after diagnosis, respectively. A relapse was defined as the (re-)emergence or worsening of clinical symptoms fitting the diagnostic criteria for anti-NMDAR encephalitis, after a period of at least two months of improvement or stabilization, combined with the confirmation of anti-NMDAR antibodies in CSF.[2,11]

Procedures for NMDAR antibody and NfL measurements

Anti-NMDAR antibodies were detected using cell-based assays (Euroimmun, AG, Lübeck, Germany) in CSF, and confirmed by immunohistochemistry, as described before.[11] All patients had antibodies in CSF. NfL concentration in serum and CSF was measured in duplicate using SIMOA NfL-light kit with SR-X immunoassay analyzer (Quanterix Corporation, Billerica, Massachusetts) as previously described,[15] by investigators blinded to clinical data. Comparison was made with sera from 61 healthy controls. The Mean intra-assay coefficient of variation (CV) of duplicates and inter-assay CV were 6.7% and 6.4%, respectively. Samples with CV above 20% were reanalyzed.

Standard Protocol Approvals, Registrations, and Patient Consents

This retrospective study was waived and declared non-WMO complicit by the Institutional Review Board of Erasmus MC. Written informed consent was obtained from all patients.

Statistics

The data on NfL values in serum and CSF were logarithmically transformed to adjust for skewness of the distribution. The descriptive statistics provided in this paper are centered around the geometric means. The correlation between NfL levels in serum and CSF was investigated by calculating Pearson's correlation coefficient. A good correlation allowed serum NfL to be used as a surrogate biomarker. The serum NfL levels of the patients were compared to healthy adult references, as well as to age-based cut-off values from literature, also including pediatric references.[12-14] The known influence of age on NfL levels was confirmed by fitting a linear regression model. The rest of the analyses were corrected for this effect by the addition of age as a covariate. As the less-extensively-investigated effect of age on NfL in children does not seem strictly linear in the lowest age range, and the included healthy references were adults, we also performed all analyses in the subgroup of adult patients.

The relationship between the independent variables tumor, preceding HSV1 infections and visible MRI abnormalities and the dependent variable serum NfL, and the relationship between serum NfL levels (independent variable) and duration of hospital admission were tested with variants of linear regression models, univariable and multivariable with age as a covariate. Because of the reported effect of a HSV1 encephalitis on both NfL levels and prognosis of anti-NMDAR encephalitis[16,17], we have left these patients out of the analyses to determine prognostic value of serum NfL in anti-NMDAR encephalitis (Suppl. figure 1). Logistic regression analysis was applied to investigate the relationship between serum NfL at diagnosis and the need for ICU admission, as measures of disease severity. The predictive value of early NfL levels for maximum disease severity (maximum mRS), outcome (mRS score at 4 and 12 months after disease onset) and time to recovery (improving to an mRS score ≤2) was explored with ordinal regression analysis. Patients with an mRS > 2 *before* disease onset were excluded from the latter analyses as we would not be able to determine the outcome specifically related to the anti-NMDAR encephalitis (Suppl. figure 1).

Data Availability

Any data not published within this article are available at the Erasmus University Medical Centre. Patient-related data will be shared on reasonable request from any qualified investigator, maintaining anonymity of the individual patients.

RESULTS

We included 71 anti-NMDAR encephalitis patients (75% female; mean age 31.4, range 0-85 years; Table 1), representative of the complete national cohort (Suppl. table 1).

Table 1 Patient characteristics of the included anti-NMDAR encephalitis patients.

Variable	Included patients (n=71)
Sex (female, %)	53 (75%)
Age (mean, IQR, range)	32 (18-41; 0.7-86)
Tumor (n, %)	20/69 (29%)
Preceding HSV infection (n, %)	8 (11%)
MRI abnormalities (n, %)	26 (38%)
Mesiotemporal hyperintensity #	15 (58%)
Mesiotemporal atrophy #	2 (8%)
Thalamic lesions #	4 (15%)
Multifocal white matter lesions #	3 (12%)
Brainstem lesions #	2 (8%)
Baseline mRS (median, IQR, range)*	0 (0-0; 0-4)
mRS at onset (median, IQR, range)	3 (2-3; 2-5)
Maximum mRS (median, IQR, range)	4 (3-5; 3-5)
Hospital admission days (mean, IQR, range)	80 (28-93; 3-632)
ICU admission (n, %)	32 (45%)
mRS after 12 months (median, IQR, range)	2 (1-2; 0-6)
Time to mRS2 (in months; mean, IQR, range)	5.6 (2-10; 1-not achieved)
Last mRS (median, IQR, range)	2 (1-3; 0-6)
Follow-up time in months (mean, IQR, range)	35 (14-45; 3-180)

* Six patients had an mRS > 2. # (n, % of patients with MRI abnormalities)

NfL levels and associated clinical factors

The serum NfL concentration at diagnosis was higher in anti-NMDAR encephalitis patients (mean 19.5 pg/mL, 95%-confidence interval (CI) 13.7-27.7) than in healthy controls (mean 6.4 pg/mL, 95%-CI 5.8-7.2, p<0.0001). A positive association was observed between serum NfL values and age at sampling, in both patients (Pearson's R=0.57, p<0.0001) and healthy controls (R=0.62, p<0.0001; Figure 1A). Serum and CSF NfL levels (n=33) showed a good correlation (Pearson's R=0.84, p<0.0001; Figure 1B). Patients with a post-HSV1 anti-NMDAR encephalitis had higher serum NfL values than those without a preceding infection (mean 248.8 vs 14.1 pg/mL, p <0.0001; Figure 2). Serum NfL levels were significantly higher in patients with cerebral MRI lesions compared to patients without (mean 27.3 vs 11.1 pg/mL, p=0.019, patients with post-HSV1 encephalitis were not included in this analysis; Figure 2). These effects were similar when age was added to the analysis as a co-variable (β_{HSV}=2.7, p<0.0001, β_{MRI}=0.70, p=0.012; Table 2). Analyzing these results in a slightly different way, using dichotomous age-based cut-off values,

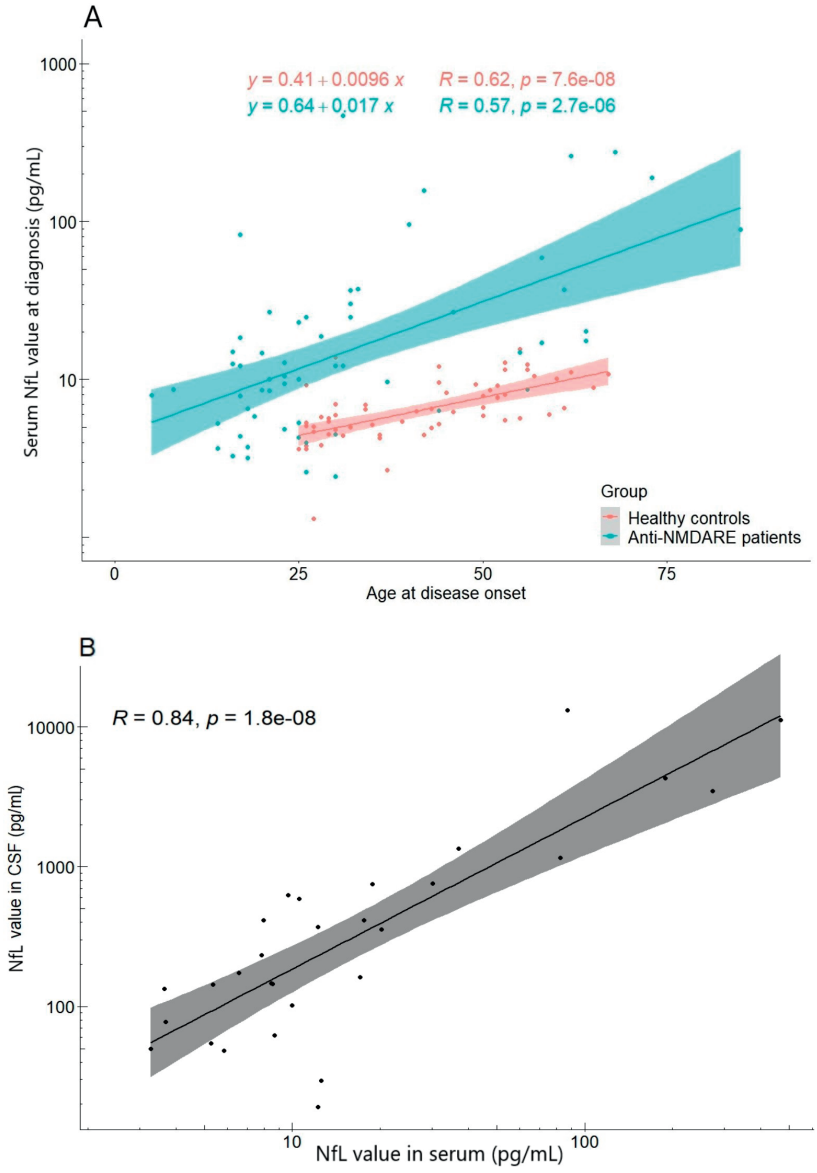

Figure 1. Serum NfL correlation with age and CSF.
NfL levels in serum correlate positively with age (A) and CSF (B).

confirmed these results: patients with increased serum NfL levels (n=39 [55%]) more frequently had a preceding HSV1 encephalitis (21% vs 0%, p=0.019) and more frequently had MRI abnormalities (54% vs 16%, p=0.002), compared to patients with serum NfL levels below the cut-off (Suppl. table 2).

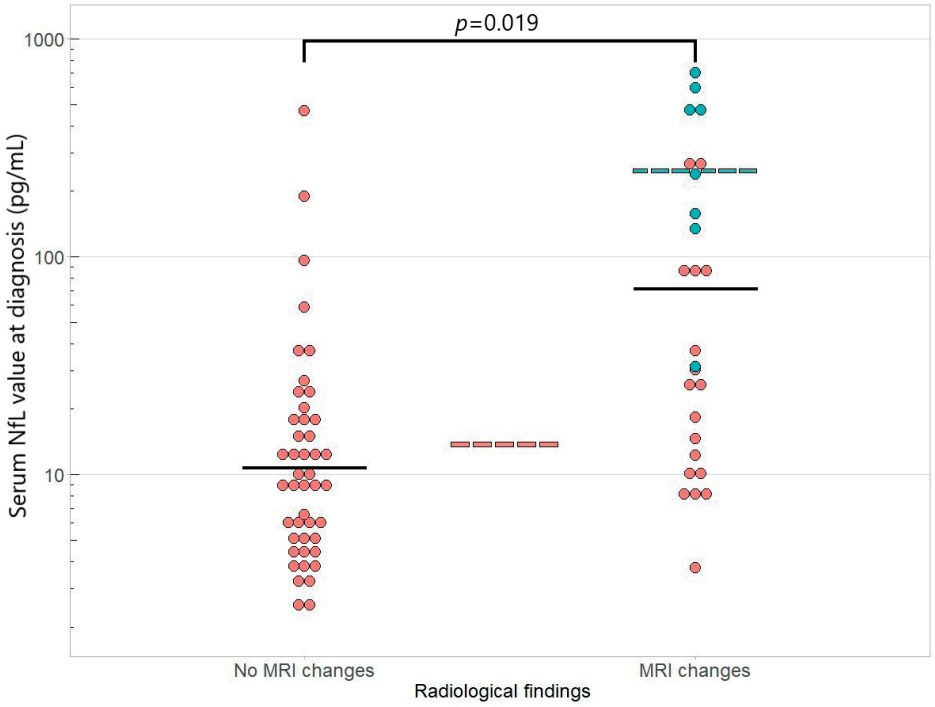

Figure 2. Serum NfL related to radiological findings.
Anti-NMDAR encephalitis patients with MRI abnormalities had higher NfL levels in serum (p=0.019; geographic means of patients with and without MRI abnormalities are represented by the black horizontal lines). Patients with a preceding HSV1 encephalitis (depicted in blue; all with MRI abnormalities) had even higher NfL levels in serum compared to patients without preceding a preceding HSV1 encephalitis (p<0.0001; the geographic means of patients with and without a preceding HSV1 encephalitis are represented by the blue and red dotted horizontal lines, respectively).

The presence of concomitant tumors, seizures and movement disorders, the delay between symptom onset and sample drawing, and serum and CSF antibody titers did not significantly relate to NfL levels, with or without age as co-variable (Table 2 & Suppl. figures 2-4).
A subgroup analysis of the adult patients (n=59) to account for different behavior of NfL as serum biomarker in children did not provide different results (Suppl. table 3).

The prognostic value of NfL for disease severity and outcome

NfL levels at diagnosis did not significantly differ between patients who needed ICU admission or not and did not relate to the maximum mRS score over the course of the disease (Suppl. figure 5) nor the duration of hospital admission (Suppl. figure 6), as markers for disease severity. Similarly, no relation was noted between NfL levels at diagnosis and disability (mRS) four months after disease onset (Suppl. figure 7).
In a univariable analysis, NfL serum levels at diagnosis were related to the outcome after 12 months (β_{NfL}=0.55, p=0.013) and the time until recovery (to an mRS≤2; β_{NfL}=0.31,

Table 2. Analyses with and without age correction.

Independent variables (NfL as a dependent variable)	Without age correction	With age as a covariate
Age[2]	β_{Age}= 0.037, ***p*<0.0001**	Not applicable
Tumor (with vs without)[1]	Mean 14.7 vs 22.0 pg/mL, *p*=0.24	β_{Tumor} = -0.57, *p*=0.12
Preceding HSV infection (with vs without)[1,*]	Mean 248.8 vs 14.1 pg/mL, ***p*<0.0001**	β_{HSV} = 2.7, ***p*<0.0001**
Seizures (with vs without)[1]	Mean 11.5 vs 20.2 pg/mL, *p*=0.097	$\beta_{Seizures}$ = -0.25, *p*=0.36
Movement disorders (with vs without)[1]	Mean 13.1 vs 16.6 pg/mL, *p*=0.47	$\beta_{Movement}$ = -0.12, *p*=0.66
MRI abnormalities (mean with vs without)[1]	Mean 27.3 vs 11.1 pg/mL, ***p*=0.019**	β_{MRI} = 0.70, ***p*=0.012**
Time from onset to sample drawing[2]	β_{Delay}= -0.0006, *p*=0.59	β_{Delay} = -0.0006, *p*=0.64
Serum antibody titre[2]	β_{Titer} = -0.044, *p*=0.56	β_{Titer} = -0.014, *p*=0.82
CSF antibody titre[2]	β_{Titer} = 0.037, *p*=0.51	β_{Titer} = 0.006, *p*=0.90
mRS at onset[5]	β_{mRS} = -0.044, *p*=0.87	β_{mRS} = 0.22, *p*=0.34
Dependent variables (NfL as an independent variable)[**]		
Maximum disease severity (mRS)[4]	β_{NfL}=0.18, *p*=0.38	β_{NfL} = 0.21, *p*=0.23
ICU admission (yes vs no)[3]	β_{NFL} = 0.10, *p*=0.65	β_{NFL} = 0.17, *p*=0.55
Duration of hospital admission (days)[5]	β_{NFL} = -0.086, *p*=0.44	β_{NFL} = -0.070, *p*=0.61
Disability (mRS) after 4 months[4]	β_{NfL}= 0.23, *p*=0.28	β_{NfL}= 0.10, *p*=0.69
Disability (mRS) after 12 months[4]	β_{NfL}= 0.55, ***p*=0.013**	β_{NfL}= 0.38, *p*=0.14 β_{Age}= 0.018, *p*=0.26
Time to recovery (mRS2)[5]	β_{NfL}= 0.31, ***p*=0.050**	β_{NfL}= 0.18, *p*=0.31 β_{Age}= 0.020, *p*=0.15

[1] Dichotomous independent variable, tested with a T-test.
[2] Continuous independent variable, tested with linear regression.
[3] Dichotomous dependent variable, tested by binomial logistic regression.
[4] Ordinal dependent variable, tested by ordinal logistic regression.
[5] Continuous dependent variable, tested by linear regression.
[*] Because of this known effect, we have excluded post-HSV encephalitis patients from the rest of the analyses.
[**] Patients with a premorbid mRS > 2 were excluded from these analyses.

p=0.050), although this seemed largely attributed to the effect of age at disease onset (β_{NfL}=0.38, *p*=0.14 and β_{Age}=0.018, *p*=0.26 for outcome after 12 months, Figure 3A; β_{NfL}=0.18, *p*=0.31 and β_{Age}=0.020, *p*=0.15 for recovery time, Figure 3B; Table 2). These findings were confirmed when applying dichotomous age-based cut-off values (*p*=0.069 for outcome after 12 months, *p*=0.14 for recovery time; Suppl. table 2), and a subgroup analysis of the adult patients showed no different results either (Suppl. table 3).

NfL in longitudinal follow-up sera

We included a total of 58 follow-up samples of 20 patients, of whom 10 had had at least one relapse of the encephalitis (Figure 4A) and 10 had a monophasic course.
When monitoring NfL levels over time, we noted that NfL values often increased considerably in the weeks after onset, especially while on ICU, and had a subsequent decrease

over time, more pronounced in patients discharged from ICU (Figure 4B & C, Suppl. figures 8 & 9). Interestingly, in an illustrative patient with a relapse, the main increase of NfL was seen only after the onset of symptoms (both in the initial episode and at relapse; Figure 4B). The suggestion of increase at the moment of onset of the relapse was similar to another patient who did not experience a relapse (Figure 4C). When focusing on the repeated serum measurements within the first months after disease onset, we see an increase of NfL levels up to 4-6 weeks (Figure 5A). This is in line with the observation that the majority of serum NfL measurements within the first weeks fall within the range of the healthy references, as opposed to the measurements after 2-4 weeks (Figure 5B).

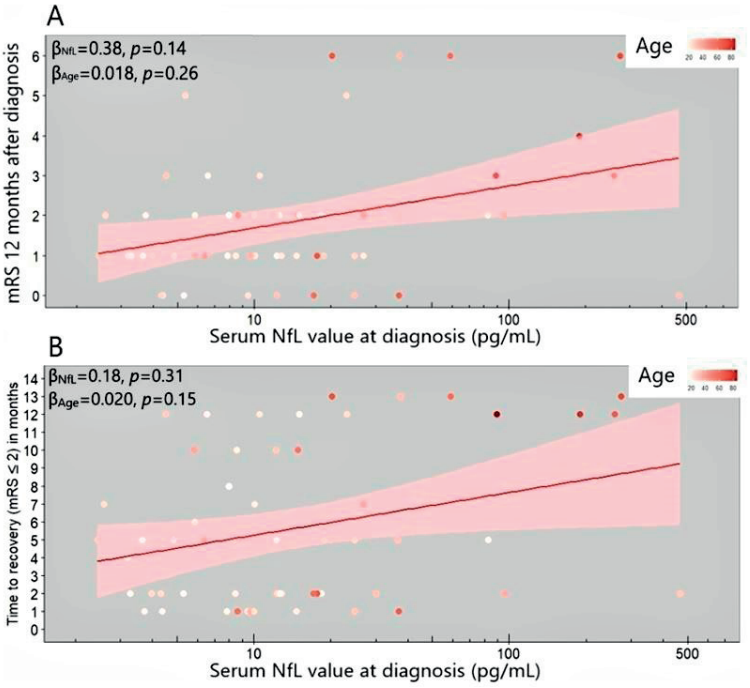

Figure 3. NfL, age and long-term outcome.
Higher NfL levels in serum were correlated to a worse outcome (higher mRS) after 12 months (A) and a longer time to recovery (B). As can be seen by the colored dots, this was largely influenced by the age of onset. Correction for age at onset negated the significant association.

DISCUSSION

In this study, we have investigated serum NfL as a biomarker in a large cohort of well characterized patients with anti-NMDAR encephalitis. We demonstrate several important aspects: 1) although serum NfL levels are increased in patients with an anti-NMDAR encephalitis, these do not provide independent prognostic value at diagnosis, neither

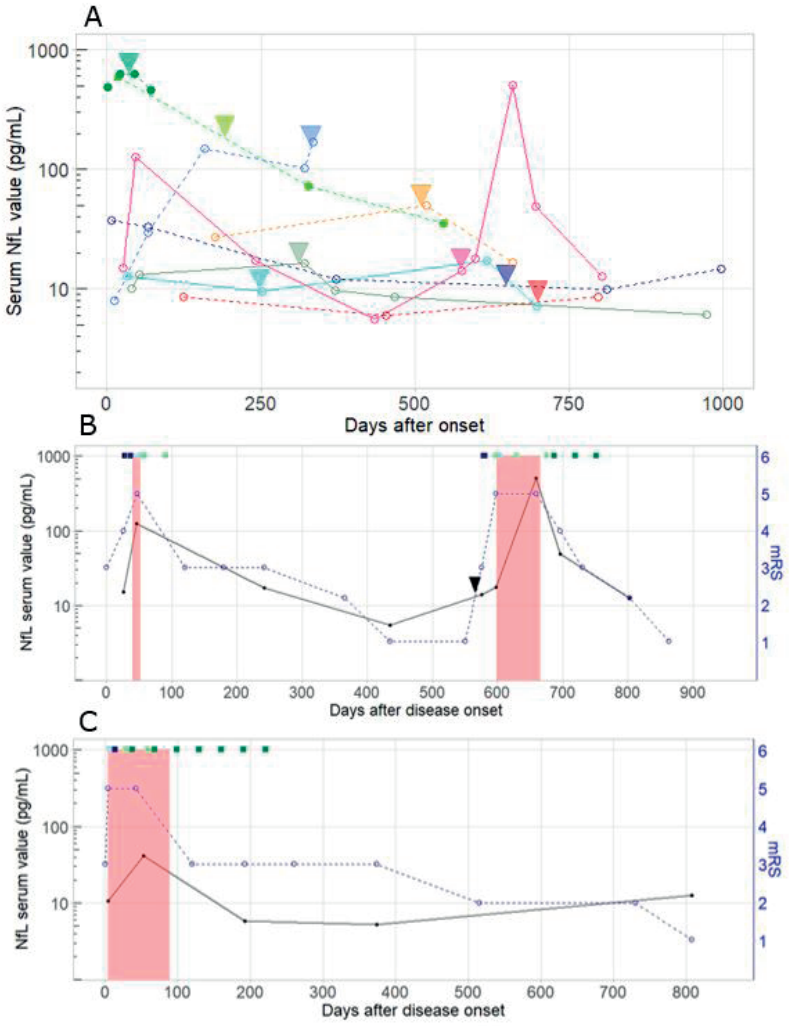

Figure 4. Longitudinal NfL levels in serum.
In all patients with a relapse (A), marked by the arrows. In two exemplary patients (B & C) we see an increase in NfL whilst admitted to the ICU (ICU admission annotated in red). The increase measured at the moment of relapse in patient B is similar to the one in the still improving patient (C), without a relapse. The considerable increase is only seen later during the relapse. The treatment regime is represented by the colored squares at the top of the figure; intravenous methylprednisolone courses in light blue, immunoglobulins in dark blue, Rituximab in light green and cyclophosphamide in dark green.

for maximum severity nor for long-term outcome; and 2) serum NfL can be used to monitor activity of disease in the chronic phase. However, timing of serum NfL sampling has an influence on the values found, complicating the use as biomarker to identify relapses early.

We have first established that serum NfL levels are increased in patients with anti-NMDAR encephalitis compared to the general population. Identified associations between NfL

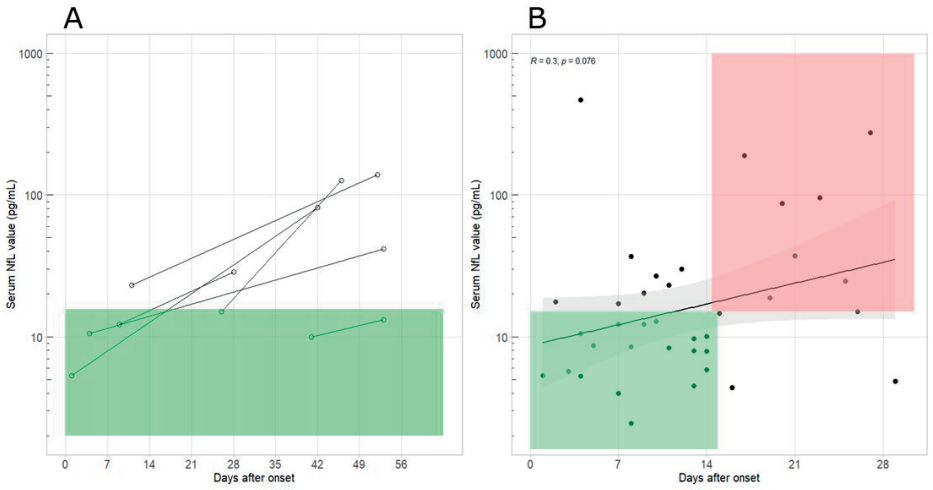

Figure 5. Details on timing of NfL measurements.
In all patients with multiple serum samples in the first two months after diagnosis, we see that the second measurements, starting at 28 days after diagnosis, exceed the normal range (A). The majority of all samples taken within the first two weeks after onset fall in the range of the healthy references (annotated with the green square; B).

levels and age, a preceding HSV1-encephalitis, and radiological signs of tissue damage are all in line with what we would expect, NfL being a marker of tissue injury associated with neuro-axonal damage.[8,17]

We identified no association between NfL levels at diagnosis and measures of maximum disease severity. In serial samples of patients admitted to the ICU, NfL levels increased within the first weeks, however the initial values at diagnosis had no predictive value for ICU admission. Using univariable analysis, an association between serum NfL values and outcome after a year seemed to be present. As we and others have identified age as a factor associated both with higher NfL levels and with longer time to recovery, correction for age at onset was warranted.[11] This explained at least the larger part of the difference in NfL levels, and no independent relationship between NfL and outcome at 12 months was identified.

These findings correspond partly with literature. Whereas other studies also negate the association between initial NfL levels, albeit in CSF, and disease severity[15,18], two studies do associate NfL levels with disease severity (i.e. ICU admission).[19,20] The referred samples in one were of the moment of determining severity and did not *precede* or *predict* disease severity (i.e. at diagnosis)[19]. Two of the mentioned studies, in homogeneous cohorts of anti-NMDAR encephalitis patients, also described no applicability of NfL levels in CSF or serum as a biomarker for outcome.[18,20] Two other studies found a correlation between NfL levels in diagnostic CSF samples and long-term outcome, even after (partial) correction for age, albeit in heterogeneous cohorts of patients with

autoimmune encephalitis or paraneoplastic syndromes with diverse pathophysiological mechanisms (not limited to anti-NMDAR encephalitis).[21,22]

The observed NfL increase in the weeks *after* symptom onset, was previously observed in a cohort of anti-NMDAR encephalitis patients.[19] This might suggest that axonal damage is not a hyperacute initial feature of the disease *causing* clinical symptoms, rather serum NfL levels likely reflect an integral measure of antecedent and ongoing neuronal damage. This additionally discourages the deployment of NfL as a biomarker, as the timing of sampling largely affects the values found. Although the longitudinal data is limited, we provide some data to suggest that the same delay in increase hampers the use of serum NfL as a marker to predict relapses. As serum levels do often increase, a delayed NfL measurement may be used as a marker to differentiate between a relapse, pseudorelapse (i.e. due to infection) or persisting neurological symptoms. As serum NMDAR antibodies are not very reliable,[4] and CSF NMDAR antibody titers at remission are often not available, this could still be very valuable to decide upon escalation of treatment or instalment of maintenance immunotherapy.

Our study has limitations, mainly related to the sample size and retrospective design. Although we have included all available pre-treatment samples of our nationwide cohort, anti-NMDAR encephalitis is a rare disease and the consequentially moderate sample size limits the power of our analyses. The retrospective study design did not allow to monitor NfL values at regulated time points and the longitudinal analysis is based on a limited subgroup only. In addition, follow-up was relatively short and we did not perform regular imaging at consistent intervals, so we were unable to correlate NfL levels with lesion load and brain volume loss over time. Last, we used the mRS to quantify disability and outcome, which, despite being the most commonly used scale, is crude and not specific for this condition. More sensitive (cognitive) measures might yield different results correlating NfL values and disability. Prospective, structured follow-up could solve the majority of these limitations in the future.

In conclusion, axonal damage is a feature of active anti-NMDAR encephalitis and measuring serum NfL might prove helpful in clinical practice to identify active disease, and monitor recovery. NfL levels are no independent predictors for disease severity or outcome. As timing of sampling seems to have a large impact on NfL values, the use of single values in prediction of disease severity, outcome or relapses is complicated.

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SUPPLEMENTARY DATA

Supplementary table 1. Characteristics of the included anti-NMDARE patients vs the whole nationwide cohort.

Variable	Included patients (n=71)	Whole national cohort (n=126)	p-value
Sex (female, %)	53 (75%)	96 (76%)	0.81[#]
Age (mean, IQR, range)	32 (18-41; 0.7-86)	29 (17-38; 0.7-86)	0.59[†]
Tumor (n, %)	20/69 (29%)	33/124 (27%)	0.72[#]
Preceding HSV infection (n, %)	8 (11%)	13 (10%)	0.82[#]
MRI abnormalities (n, %)	26 (38%)	41/122 (34%)	0.67[#]
mRS at onset (median, IQR, range)	3 (2-3; 2-5)	4 (2-3; 1-5)	0.49[‡]
Maximum mRS (median, IQR, range)	4 (3-5; 3-5)	4 (3-5; 2-5)	0.82[‡]
Hospital admission days (mean, IQR, range)	80 (28-93; 3-632)	56 (27-86, 2-632)	0.32[†]
ICU admission (n, %)	32 (45%)	61 (46%)	0.77[#]
mRS after 12 months (median, IQR, range)	2 (1-2; 0-6)	2 (1-3; 0-6)	0.83[‡]
Time to mRS2 (in months; mean, IQR, range)	5.6 (2-10; 1-not achieved)	5.5 (2-10; 0-not achieved)	0.94[†]
Last mRS (median, IQR, range)	2 (1-3; 0-6)	1 (0-2, 0-6)	0.61[‡]
Follow-up time in months (mean, IQR, range)	35 (14-45; 3-180)	35 (13-47, 2-180)	0.95[†]

[#] Chi-squared test, [†] T-test, [‡] Mann-Whitney U non-parametric test

Supplementary table 2 Characteristics of anti-NMDARE patients with or without increased NfL values in serum.

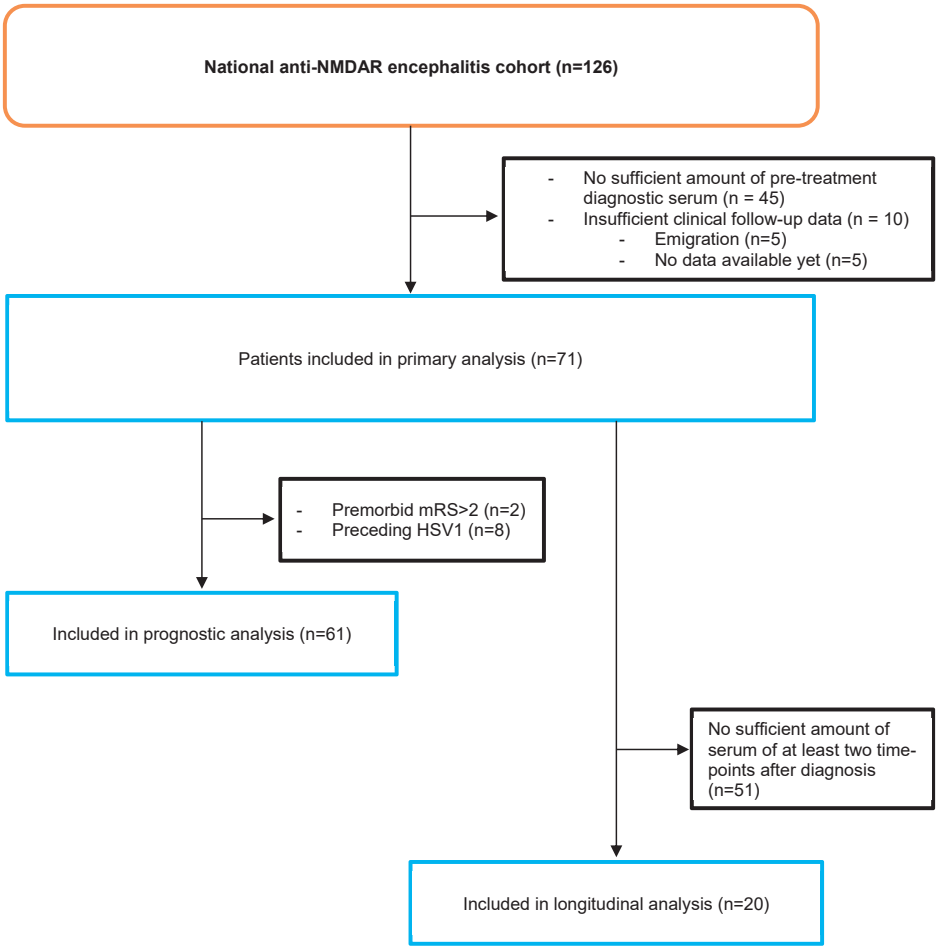
Variable	Patients with normal NfL (n=32)	NfL above cut-off value (n=39)	p-value
Sex (female, %)	24 (75%)	29 (74%)	1.00[#]
Age (mean, IQR, range)	23 (18-33; 4-65)	28 (21-45; 0.7-86)	0.30[†]
Tumour (n, %)	9/31 (29%)	11/38 (29%)	1.00[#]
Preceding HSV infection (n, %)	0 (0%)	8 (21%)	**0.019**[#]
MRI abnormalities (n, %) Without HSV patients	5 (16%) 5 (16%)	21 (54%) 13/31 (42%)	**0.002**[#] **0.042**[#]
mRS at onset (median, IQR, range)	3 (2-3; 2-5)	3 (2-3; 2-4)	0.59[‡]
Maximum mRS (median, IQR, range)	4.5 (3-5; 3-5)	4 (3.5-5; 3-5)	0.99[‡]
Hospital admission days (mean, IQR, range)	54 (33-89; 6-620)	43 (27-94; 3-551)	0.38[†]
ICU admission (n, %)	15 (47%)	17 (44%)	0.97[#]
mRS after 12 months (median, IQR, range)	1 (1-2; 0-6)	2 (1-3; 0-6)	0.069[‡]
Time to mRS2 (in months; mean, IQR, range)	4.7 (2-6.75; 1-13)	6.3 (2-12; 1-13)	0.14[†]
Last mRS (median, IQR, range)	1 (0.5-2; 0-6)	2 (1-3; 0-6)	0.44[‡]
Follow-up time in months (mean, IQR, range)	24 (14-53; 3-119)	24 (12-36; 3-180)	0.93[†]

[#] Chi-squared test, [†] T-test, [‡] Mann-Whitney U non-parametric test

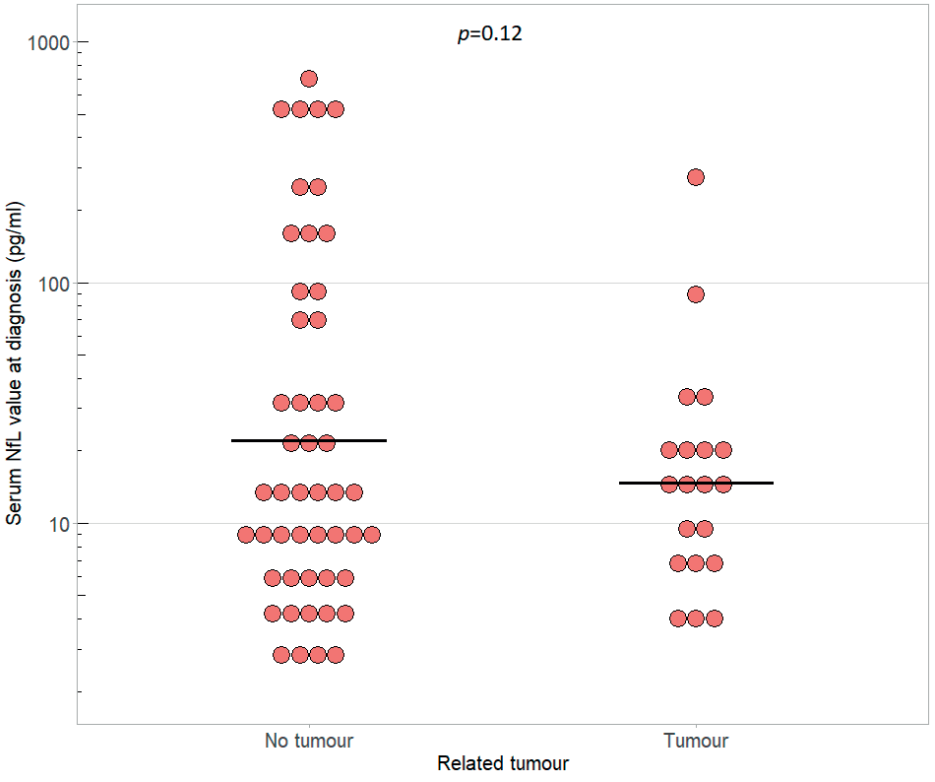
Supplementary table 3 Subgroup analysis of patients within the age range of the healthy references (n=59).

Independent variables (NfL as a dependent variable)	Without age correction	With age as a covariate
Age[2]	β_{Age}= 0.046, **p<0.0001**	Not applicable
Tumour (with vs without)[1]	Mean 14.7 vs 20.9 pg/mL, p=0.31	β_{Tumour}= -0.34, p=0.27
Preceding HSV infection (with vs without)[1,*]	Mean 234.0 vs 15.6 pg/mL, **p=0.001**	β_{HSV}= 1.55 **p=0.012**
Seizures (with vs without)[1]	Mean 16.2 vs 19.3 pg/mL, p=0.64	$\beta_{Seizures}$= 0.088, p= 0.75
Movement disorders (with vs without)[1]	Mean 14.2 vs 22.9 pg/mL, p=0.22	$\beta_{Movement}$= -0.32, p=0.23
MRI abnormalities (mean with vs without)[1]	Mean 43.1 vs 10.5 pg/mL, **p=0.0006**	β_{MRI}= 0.94, **p=0.0005**
Time from onset to sample drawing[2]	β_{Delay}= -0.0003, p=0.81	β_{Delay}= 0.0002, p=0.86
Serum antibody titre[2]	β_{Titre}= -0.10, p=0.25	β_{Titre}= -0.024, p=0.72
CSF antibody titre[2]	β_{Titre}= -0.042, p=0.52	β_{Titre}= -0.006, p=0.90
mRS at onset[5]	β_{mRS}= 0.44, p=0.17	β_{mRS}= 0.31, p=0.19
Dependent variables (NfL as an independent variable)[**]		
Maximum disease severity (mRS)[4]	β_{NfL}= 0.15, p=0.45	β_{NfL}= 0.10, p=0.66
ICU admission (yes vs no)[3]	β_{NFL}= -0.17, p=0.44	β_{NFL}= -0.23, p=0.32
Duration of hospital admission (days)[5]	β_{NFL}= -16.0, p=0.25	β_{NFL}= -11.7, p=0.55
Disability (mRS) after 4 months[4]	β_{NfL}= 0.20, p=0.36	β_{NfL}= 0.056, p=0.83
Disability (mRS) after 12 months[4]	β_{NfL}= 0.48, **p=0.034**	β_{NfL}= 0.30, p=0.25 β_{Age}= 0.022, p=0.20
Time to recovery (mRS2)[5]	β_{NfL}= 1.27, **p=0.005**	β_{NfL}= 0.95, p=0.12 β_{Age}= 0.032, p=0.46

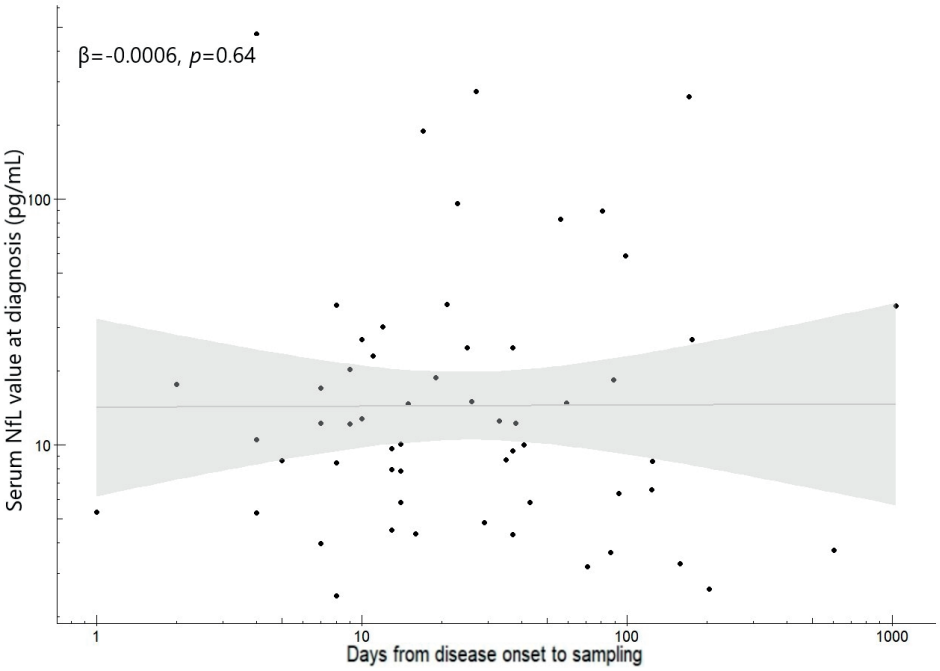
[1] Dichotomous independent variable, tested with a T-test.
[2] Continuous independent variable, tested with linear regression.
[3] Dichotomous dependent variable, tested by binomial logistic regression.
[4] Ordinal dependent variable, tested by ordinal logistic regression.
[5] Continuous dependent variable, tested by linear regression.
[*] Because of this known effect, we have excluded post-HSV encephalitis patients from the rest of the analyses.
[**] Patients with a premorbid mRS > 2 were excluded from these analyses.



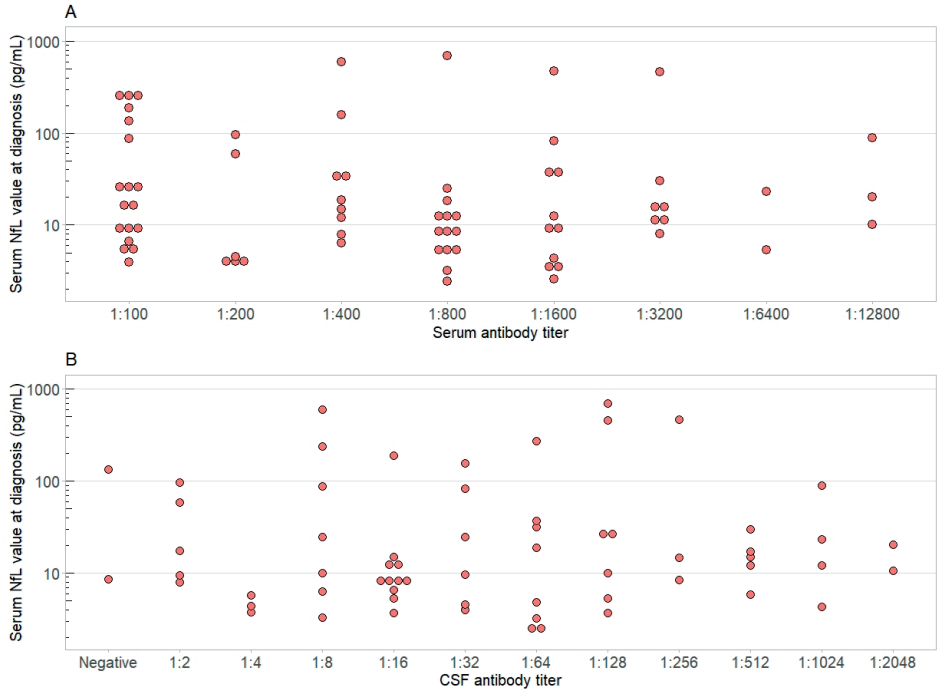
Supplementary figure 1. Flow-chart of in- and exclusion of patients from the national cohort in the primary analysis, prognostic calculations and longitudinal analysis.



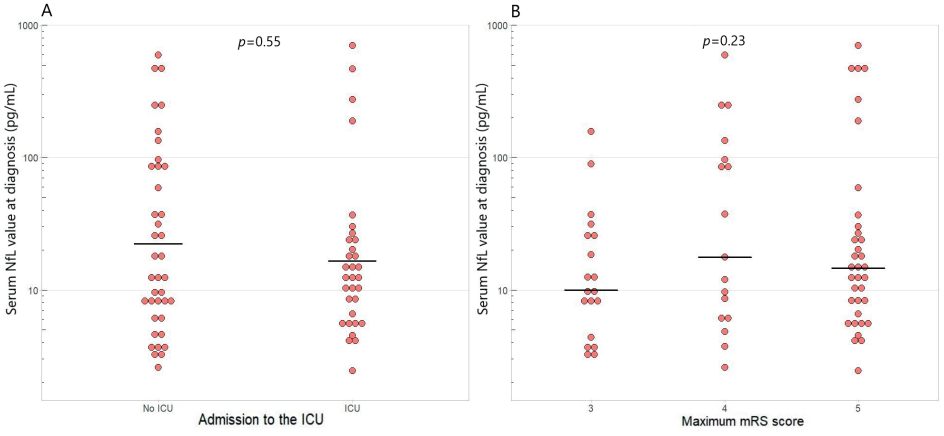
Supplementary figure 2. Serum NfL related to underlying tumors.
NfL levels in serum did not differ between patients with or without a tumor.



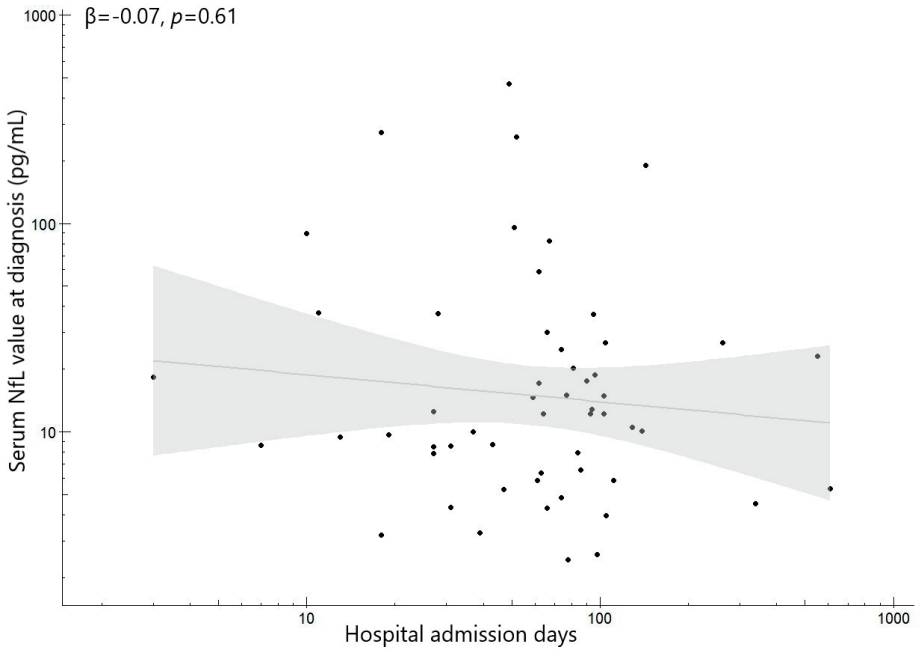
Supplementary figure 3. Serum NfL related to moment of sample drawing.
NfL levels at diagnosis did not relate to the time between onset and sampling.



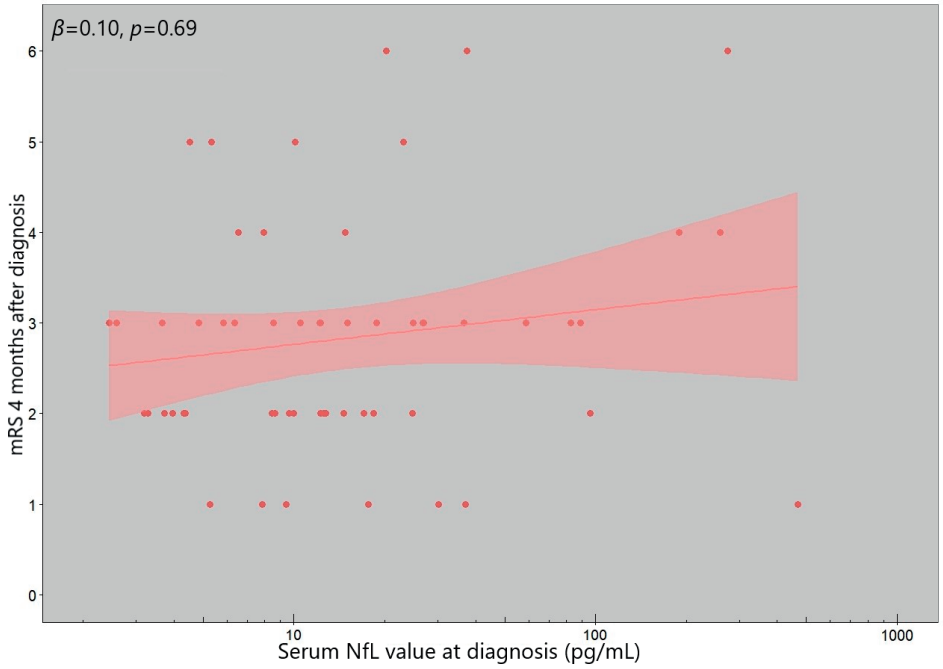
Supplementary figure 4. Serum NfL related to antibody titers in serum and CSF.
NfL levels in serum did not relate to the NMDAR antibody titers in serum (A) or CSF (B).



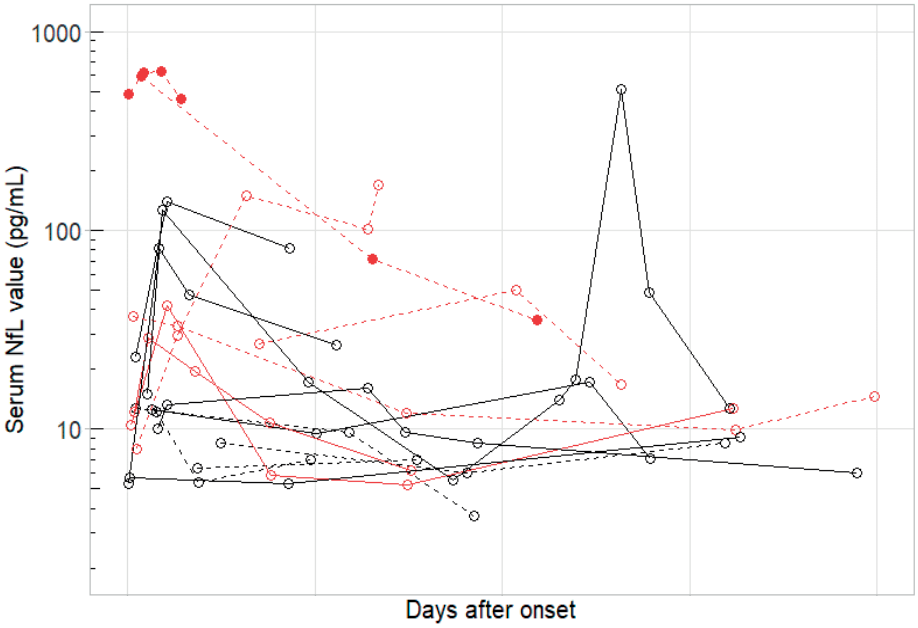
Supplementary figure 5. Serum NfL related to measures of disease severity.
NfL levels in serum did not differ between patients with or without the need for admission to the ICU (A), or with different levels of severity (maximum mRS scores) at nadir (B).



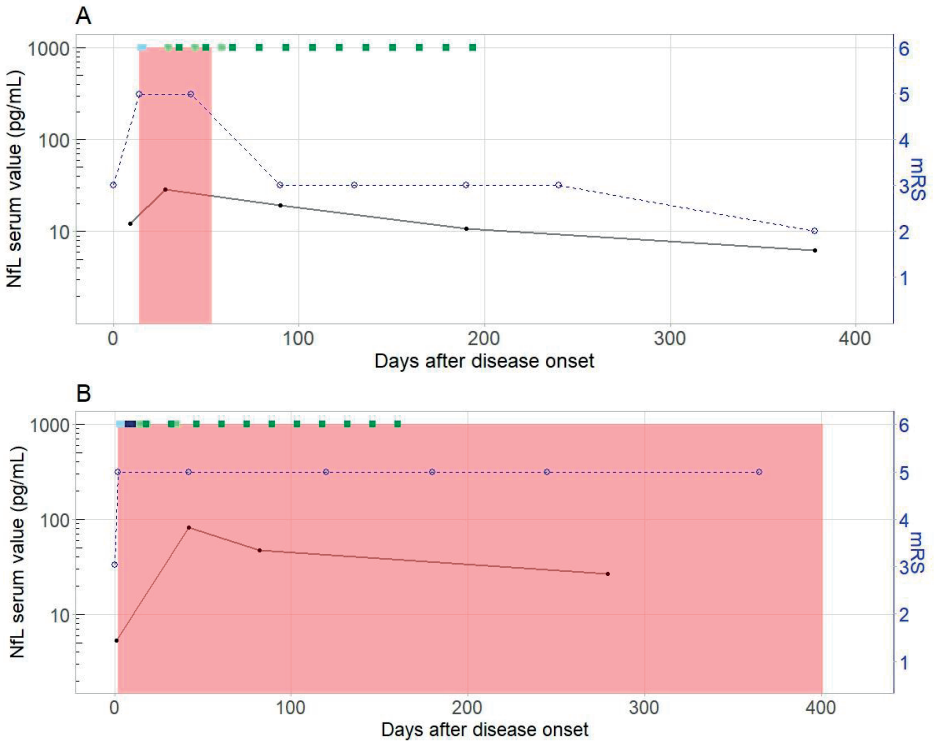
Supplementary figure 6. Serum NfL related to duration of hospital admission.
NfL levels in serum did not relate to the number of days admitted to the hospital.



Supplementary figure 7. Serum NfL related to short-term outcome. NfL levels in serum did not correlate to the mRS at 4 months.



Supplementary figure 8. Longitudinal NfL levels in serum in anti-NMDAR encephalitis.
Each line represents an individual patient and information on preceding HSV infections (closed circles, vs open without), disease severity (ICU admission; depicted with a regular line vs a dashed line for patients who did not require ICU admission) and radiological findings (patients with MRI abnormalities in red vs black without MRI abnormalities) are visually shown.



Supplementary figure 9. Longitudinal NfL measurements in two exemplary patiens.
NfL values increased considerably in the weeks after onset, especially while on ICU, and had a subsequent decrease over time, more pronounced in patients discharged from ICU.

6

Phase II trial of natalizumab for the treatment of anti-Hu associated paraneoplastic neurological syndromes

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ABSTRACT

Background

Paraneoplastic neurological syndromes with anti-Hu antibodies (Hu-PNS) have a very poor prognosis: more than half of the patients become bedridden and median survival is less than 12 months. Several lines of evidence suggest a pathogenic T cell-mediated immune response. Therefore, we conducted a prospective open-label phase II trial with natalizumab.

Methods

Twenty Hu-PNS patients with progressive disease were treated with a maximum of three monthly natalizumab cycles (300 mg). The primary outcome measure was functional improvement, this was defined as at least one point decrease in modified Rankin Scale (mRS) score at the last treatment visit. In addition, treatment response was assessed wherein a mRS score ≤3 after treatment was defined as treatment responsive.

Results

The median age at onset was 67.8 years (SD 8.4) with a female predominance (n=17, 85%). The median time from symptom onset to Hu-PNS diagnosis was 5 months (IQR 2-11). Most patients had subacute sensory neuronopathy (n=15, 75%), with a median mRS of 4 at baseline. Thirteen patients had a tumor, all small cell lung cancer. After natalizumab treatment, two patients (10%) showed functional improvement. Of the remaining patients, 60% had a stable functional outcome, while 30% showed further deterioration. Treatment response was classified as positive in nine patients (45%).

Conclusions

Natalizumab may ameliorate the disease course in Hu-PNS, but no superior effects above other reported immunosuppressive and immunomodulatory were observed. More effective treatment modalities are highly needed.

Trial registration: https://www.clinicaltrialsregister.eu/ctr-search/trial/2014-000675-13/NL

INTRODUCTION

Paraneoplastic neurological syndromes (PNS) are rare immune-mediated neurological disorders associated with malignancies. PNS with anti-Hu antibodies (Hu-PNS) are the most frequent among the PNS associated with well characterized onconeural antibodies. The underlying tumor in Hu-PNS is most often small cell lung carcer (SCLC). Hu-PNS is a severe disease progressing rapidly over weeks to months and has a poor prognosis: more than half of the patients become bed or wheelchair-bound, only 5 – 7% of patients improve and the median survival is less than one year. [1, 2] At the time of neurological presentation, the patient is not aware of the cancer in over 70%, delaying the diagnosis of Hu-PNS. [1-4] It is thought that the expression of Hu antigens by the tumor provokes an autoimmune response not only directed against the tumor but also against nervous tissues. [5] Although the anti-Hu antibodies (Hu-Ab) are present in high titers in serum and CSF, neuronal destruction in Hu-PNS is more likely caused by T cells than by Hu-Ab. Hu proteins are intracellular proteins that can not be reached by antibodies and many animal models failed to demonstrate Hu-Ab induced disease. Furthermore, autopsy studies consistently showed T cell infiltrates with cytotoxic T cells frequently surrounding neurons with associated neuronal loss. [6-8]

Natalizumab strongly inhibits the migration of activated T lymphocytes into the central nervous system (CNS) and is approved for the treatment of relapsing-remitting multiple sclerosis. [9] In addition, it may contribute to reduced activation of T cells already present in the CNS, leading to increased apoptosis of pathogenic T cells and lowering damage done to the nervous system. [10, 11]

We conducted a prospective open-label single-arm trial to evaluate the efficacy of off-label use of natalizumab in patients with progressive Hu-PNS. We monitored function and neurological impairment using well-defined clinical scales, as well as toxicity.

PATIENTS AND METHODS

Patients

At the Erasmus University Medical Center, the Departments of Neurology and Medical Immunology are the national referral centers for anti-neuronal antibody testing, diagnosis and treatment, accredited as European Reference Network site (ERN-RITA). Between March 2016 and June 2020, 80 patients were identified with increased serum titers of Hu-Ab (titer ≥400 by indirect immunofluorescence on monkey cerebellum, and confirmed by Euroimmun (Lübeck, Germany) and RAVO Diagnostika (Freiburg, Germany) blots). Inclusion criteria comprised a paraneoplastic neurological syndrome associated with increased Hu-Ab titer, progression of neurological symptoms over the last 4 weeks,

a modified Rankin Scale (mRS) [12] score ≥2, age ≥18 years, and an absolute CD4+ cell count ≥0.4x10^9 cells/liter. Exclusion criteria were unwillingness to undergo a lumbar puncture, known hypersensitivity to natalizumab or one of the additives, progressive multifocal leukoencephalopathy (PML), immune compromised patients (patients using immunosuppressive medications other than a short course (<2 weeks) of steroids), liver and renal failure, active infections, pregnancy, a history of active melanoma in the past 5 years, and T cell lymphoma or primary CNS lymphoma.

Of the 80 identified patients, 59 were excluded due to factors depicted in Figure 1. The remaining 21 patients were included in the trial and gave written informed consent. One of the patients died unexpectedly before administration of the first study medication and was excluded from the analysis.

We performed immunohistochemistry (IHC) to detect additional antibodies against extracellular neuronal proteins, on all sera and CSF samples of the included patients. [13, 14] When positive, confirmatory laboratory analyses were performed using validated commercial cell based assays (CBA) or live hippocampal neurons as described before. [13, 14]

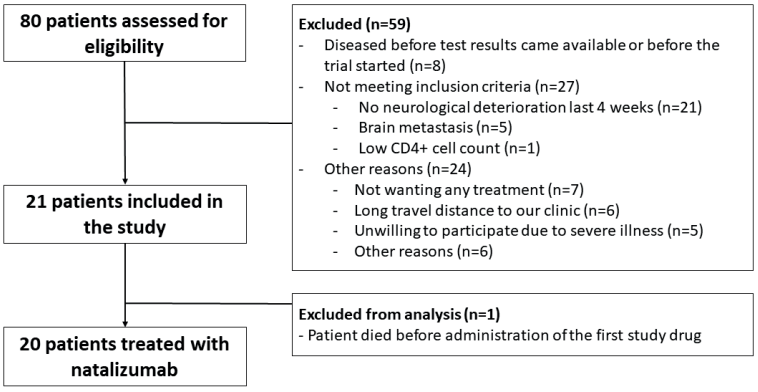

Figure 1. Patient inclusion.

In total, 80 patient with a high serum titer of Hu-Ab (≥400) were identified between March 2016 and June 2020. Twenty-one patients were included in this study, one of whom died before administration of study medication and was excluded from analysis. In total, 20 patients were treated with natalizumab and included for analysis.

Study design and treatment

We performed an open label single-arm, single center phase II study. The 20 treated patients were treated with a maximum of three monthly cycles of natalizumab (intravenous infusions of 300mg). Patients visited our clinic at least for every treatment cycle, four weeks after the third infusion and the last study visit occurred twenty weeks after the start of the trial (12 weeks after the last natalizumab cycle). Each study visit, patients were subjected to clinical evaluation, toxicity monitoring (according to the Common Terminology Criteria for Adverse Events (CTCAE)), and laboratory analysis. Natalizumab

was used as monotherapy and concomitant immunotherapy was not allowed. Treatment of an underlying malignancy, including chemotherapy, was allowed (PD-(L)1 checkpoint inhibition was not standard care for SCLC in the Netherlands). The study drug was discontinued when the mRS score increased ≥2 points or in case of intolerable toxicity. Use of natalizumab in multiple sclerosis has been associated with an increased risk of progressive multifocal leukoencephalopathy (PML), an opportunistic infection caused by JC virus, which may be fatal or result in severe disability. [9, 15] However, the intention to treat patients with only 3 cycles of natalizumab (12 weeks) renders the occurrence of PML very unlikely as most cases have occurred after >2 years of treatment. A data safety monitoring board was assigned to assess toxicity and review all serious adverse events. This study was approved by the Institutional Review Board of Erasmus MC (MEC-2015-607). Guidelines for neuro-oncology: Standards for investigational studies were followed (GNOSIS). [16]

Outcome

The primary outcome measure of this study was functional improvement, defined as at least one point decrease in mRS score after 12 weeks compared to baseline mRS score. We used a standardized mRS algorithm to achieve consistent scores. [17] In addition, we performed explorative analyses using the criteria for treatment response by Keime-Guibert et al. [18] in our cohort, and for comparison with previous studies. A positive treatment response was defined by these authors as improvement or stabilization in patients with an mRS score ≤3, and an improvement from mRS ≥4 to mRS ≤3. For both outcome scores, we additionally analysed mRS scores at 20 weeks compared to baseline.

The first secondary outcome measure was neurological improvement, assessed using the Edinburgh Functional Impairment Tests (EFIT). [19] The EFIT integrates upper and lower limb function, memory and a rating scale for dysphasia. Neurological improvement was defined as one point increase in overall EFIT score. Secondly, limitations in daily living activities were evaluated using the Barthel index (BI). [20]

The prospective open-label sirolimus trial, conducted in 17 patients with Hu-PNS in our institution from 2008 to 2012, was used as a historical control group. [21]

Statistical Analysis

Based on previous studies, the chance of improvement ≥1 point in mRS score in historical Hu-PNS controls was put at 10%. [1, 2, 18, 21, 22] We designed the study to detect improvement in 35% of the patients following natalizumab treatment. To achieve power of 80% with two sided a=0.05, we calculated a sample size of 18 patients. To allow for 10% drop-outs we intended to include 20 patients. Statistical analysis was performed using SPSS 25.0 (IBM, New York, NY) for Windows, as well as Prism 8.4.3 (GraphPad Prism Software Inc., San Diego, CA). All p-values were two-sided and were considered statistically significant

when below 0.05. Patientspecific baseline characteristics were evaluated using standard descriptive features: mean with standard deviation, median with interquartile range (IQR) and range for continuous variables, and frequency (proportions) for categorical variables. For group comparisons, encompassing categorical data, we used the Pearson Chi-Square test or the Fisher-Exact test if appropriate. Continuous data were analysed using the Student's t-test or the Mann-Whitney U test in case of skewed distribution. Wilcoxon matched pairs test was used to compare Hu-Ab titers in serum and CSF at baseline and after treatment (12 weeks after start trial).

RESULTS

Patients, treatment and toxicity

In total, 20 patients were treated with natalizumab (Table 1, and baseline cohort characteristics in Supplementary Table 1). The median age at onset was 67.8 (SD ±8.4) and there was a female predominance (n=17, 85%). Diagnosis of Hu-PNS took a median of 5 months (IQR 2-11) from symptom onset. In most patients, the dominant clinical syndrome was subacute sensory neuronopathy (SSN, n=6) or SSN combined with other symptoms (total n=15, 75%). Their median mRS at baseline was 4 (range 2-5).

Nine patients received a short course of immunotherapy (iv methylprednisolone (ivMP) or iv immunoglobulins (ivIg)), with a median of 28 days (IQR 18-64) prior to the start of natalizumab treatment. Eight out of nine patients subsequently progressed prior to study inclusion. All patients received a structured tumor work-up, including FDG-PET/CT imaging. Thirteen patients had a tumor, all SCLC, diagnosed median 5 months from onset of Hu-PNS (IQR 3-6, range 0,5-8). Ten patients received standard chemotherapy (a platinum-based drug plus etoposide) for SCLC concomitant with natalizumab. The remaining three patients received chemotherapy outside the study period, two patients before (-800 and -217 days) and one patient after (+155 days) the study. None of the patients received PD-(L)1checkpoint inhibitors for SCLC (extended disease), since this was not standard care in The Netherlands during the study period. No adverse effects were observed due to the combination of chemotherapy and natalizumab treatments. Thirteen patients (65%) completed the total of three natalizumab cycles, and the remaining seven patients received one or two cycles (Table 2). Reasons for discontinuing study treatment included: four patients died, two patients experienced too high a burden continuing the visits to our clinic, and one patient developed an uncontrollable anxiety for the study treatment. There were no serious adverse events (SAE) related to natalizumab treatment, and none of the patients withdrew because of natalizumab toxicity.

In total, ten patients had died at the last follow-up, and the cause of death was PNS in four patients, in another three patients it was due to the tumor, and three patients

requested euthanasia (Supplementary Table 2). Patients were followed from onset for a total of 19 months (IQR 13-27) and median overall survival was 13 months (Figure 2).

Table 1. Patient and tumor characteristics.

No.	Age/ sex	PNS - clinical phenotype	Onset to diagnosis Hu-PNS (months)	Tumor	Onset to diagnosistumor (months)	Tumor stage	Tumor treatment	Tumor response
1	64/F	SSN, MN, PLE	5	SCLC	5	LD	Chemo, RT	CR
2	57/F	SSN	62	SCLC	6	ED	Chemo, RT [a]	CR
3	66/F	SSN	3	SCLC	5	LD	Chemo, RT	CR
4	53/F	SSN, AN	2	SCLC	3	LD	Chemo, RT	CR
5	75/F	PLE, SSN	3	SCLC	3	LD	Chemo	Near CR
6	60/F	SSN	7	SCLC	8	LD	Chemo, RT	PR
7	75/F	SSN, PCD	1	SCLC	8	LD	Chemo, RT [b]	Unknown
8	73/M	SSN, PCD	1	SCLC	4	LD	Chemo, RT	CR
9	64/F	SSN	12	SCLC	5	ED	Chemo [c]	PR
10	61/F	PLE	0.2	SCLC	0.5	ED	Chemo, RT	PR
11	52/F	SSN	5	SCLC	5	LD	Chemo, RT	n.e.
12	76/F	MN	5	SCLC	6	LD	Chemo, RT	n.e.
13	65/F	PEM	0.3	SCLC	0.5	ED	Chemo	n.e.
14	72/F	SSN, MN	3	No	n.a.	n.a.	n.a.	n.a.
15	79/M	SSN, AN	36	No	n.a.	n.a.	n.a.	n.a.
16	80/F	PCD	11	No	n.a.	n.a.	n.a.	n.a.
17	72/F	SSN, AN	11	No	n.a.	n.a.	n.a.	n.a.
18	63/F	SSN	9	No	n.a.	n.a.	n.a.	n.a.
19	72/F	PLE, SSN	4	No	n.a.	n.a.	n.a.	n.a.
20	76/M	PCD	2	No	n.a.	n.a.	n.a.	n.a.

Abbreviations: PNS = paraneoplastic neurological syndrome, F = female, M = male, PLE = paraneoplastic limbic encephalitis, SSN = subacute sensory neuronopathy, AN = autonomic neuropathy, PCD = paraneoplastic cerebellar degeneration, MN = motor neuronopathy, PEM = paraneoplastic encephalomyelitis, SCLC = small-cell lung cancer, ED = extensive disease, LD = limited disease, Chemo = chemotherapy, RT = radiotherapy, PR = partial response, CR = complete response, n.a. = not applicable, n.e. = not evaluable.

[a-c] patients receiving treatment outside the study period; Time to start chemotherapy: -800 days (a), +155 days (b), and -217 days (c).

Outcome measures

Two patients (No. 1 and 14) reached the primary endpoint as they had a decrease of one point in mRS score compared to baseline (10%, Table 2). They had stable or improved scores on the secondary outcome measures. Both patients had a combined sensorimo-

Table 2. Natalizumab treatment and outcome.

No.	No. of Natalizumab cycles	Reason early treatment termination	mRS start	mRS 12 weeks	Functional Outcome	Positive treatment response	EFIT start	EFIT 12 weeks	Neurological Outcome	Onset to last FU (months)	Dead/alive at last FU
1	3	n.a.	4	3	**Improved**	Yes	2	2	Stable	16	Alive
2	3	n.a.	3	3	**Stable**	Yes	2	2	Stable	75	Alive
3	3	n.a.	3	3	**Stable**	Yes	2	2	Stable	28	Alive
4	2	Study burden	5	5	**Stable**	No	3	2[b]	Improved	22	Alive
5	3	n.a.	4	4	**Stable**	No	3	3	Stable	20	Dead
6	3	n.a.	3	3	**Stable**	Yes	1	1	Stable	33	Alive
7	3	n.a.	4	4	**Stable**	No	2	2	Stable	25	Dead
8	1	Study burden	4	5	**Worse**	No	3	-	n.a.	18	Alive
9	2	Died	3	6	**Worse**	No	1	2[b]	Worse	15	Dead
10	3	n.a.	3	4	**Worse**[a]	No	4	2	Improved[a]	7	Dead
11	1	Died	5	6	**Worse**	No	2	-	n.a.	6	Dead
12	1	Died	5	6	**Worse**	No	3	-	n.a.	7	Dead
13	1	Died	5	6	**Worse**	No	3	4[b]	Worse	2	Dead
14	3	n.a.	4	3	**Improved**	Yes	3	3	Stable	23	Dead
15	3	n.a.	2	2	**Stable**	Yes	2	2	Stable	41	Alive
16	3	n.a.	2	2	**Stable**	Yes	1	1	Stable	40	Dead
17	1	Study anxiety	3	3	**Stable**	Yes	1	-	n.a.	18	Alive
18	3	n.a.	3	3	**Stable**	Yes	1	1[c]	Stable	15	Alive
19	3	n.a.	4	4	**Stable**	No	3	3	Stable	13	Dead
20	3	n.a.	4	4	**Stable**	No	2	3	Worse	13	Alive

Abbreviations: mRS = modified Rankin Scale, EFIT = Edinburgh Functional Impairment Tests, FU = follow-up, n.a. = not applicable.

[a] Functional outcome was worse due to tumor progression while the neurological outcome remained improved.

[b] EFIT score 4 weeks after baseline.

[c] EFIT score 8 weeks after baseline.

tor neuronopathy (with accompanying limbic encephalitis symptoms in one). During treatment muscle strength improved and both regained the ability to walk without help. The mRS remained stable in twelve patients (60%), while six patients (30%) had further functional deterioration (Supplementary Figure 1). mRS scores per patient did not differ at timepoints 12 and 20 weeks. Nine patients (45%) were classified as treatment responders according to the Keime-Guibert criteria.[18] Both patients who improved by mRS had central nervous system involvement, while 9/18 patients who did not improve had only peripheral nervous system involvement (p = 0.48). Measuring a positive treat-

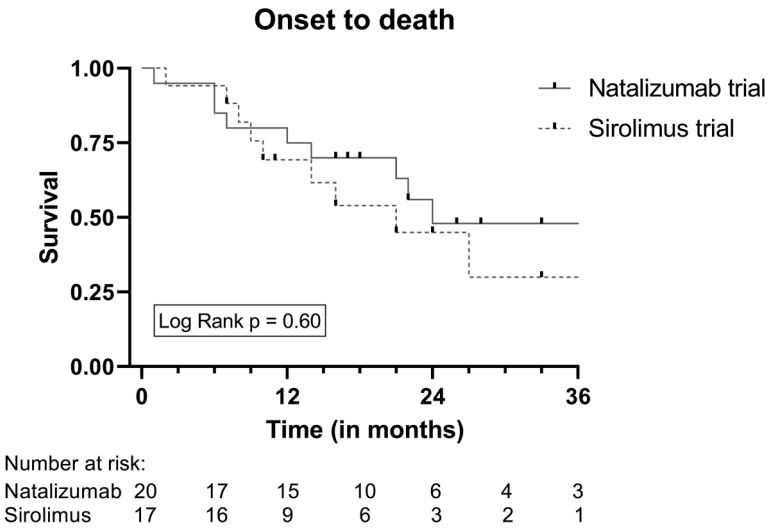

Figure 2. Kaplan-Meier estimates of survival for patients treated with natalizumab or sirolimus.
Kaplan-Meier estimates of survival in Hu-PNS patients. Patients were divided into two groups based on natalizumab or sirolimus treatment. Survival in the natalizumab trial is depicted with the continuous line and survival in the sirolimus trial is depicted with the dashed line.

ment response, 6/9 had only peripheral involvement, while 8/11 non-responders had central nervous system or combined involvement (p = 0.17).

At 12 weeks, the secondary endpoints were evaluable in thirteen patients (four patients died and three had other reasons for discontinuing study visits). Two patients improved on the EFIT scale, of whom one patient (No. 10) remained stable on the mRS scale until her functional status deteriorated due to tumor progression, and the other patient (No. 4) had stable mRS scores during the whole study period. Six out of sixteen patients improved on the BI (≥5 points), of whom five were treatment responders.

The patients classified as treatment responders had significantly better baseline mRS, EFIT and BI scores than the non-responders (Table 3). In addition, the time from onset of symptoms to Hu-PNS diagnosis was significantly longer in responders (9 vs 2 months, p = 0.008), probably reflecting the milder disease. First-line immunotherapy (ivMP or ivIg) was not associated with treatment response. Within the group of responders, fewer underlying tumors were detected and of the underlying tumors, more achieved complete remission. However, these changes were not significant. Patients with a tumor had a lower age at diagnosis and a worse mRS score at the end of the study, while all other characteristics did not differ significantly (Supplementary Table 3, and supplementary figure 2).

Table 3. Treatment response according to Keime-Guibert criteria [a]

	Treatment response (n=9)	No treatment response (n=11)	p value
Gender, female	8 (89%)	9 (82%)	1.00
Age at onset (median, IQR, range)	66 (61-75, 57-80)	72 (61-75, 52-76)	0.94
Onset to diagnosis, months (median, IQR, range)	9 (4-23, 3-62)	2 (1-5, 0,2-12)	**0.008**
PNS syndrome, only peripheral nervous system involvement	6/9 (67%)	3/11 (27%)	0.17
Tumor			
Tumor (all SCLC)	4 (44%)	9 (82%)	0.16
Onset to tumor, months	6.0 (1.1)	4.0 (2.6)	0.17
Tumor stage - ED	1 (25%)	3 (33%)	1.00
Tumor response – CR	3/4 (75%)	2/6 (33%)	0.52
Tumor response – PR and near CR	1/4 (25%)	3/6 (50%)	0.57
Ancillary testing			
Serum Hu titer, start (median, IQR, range)	1600 (1200-3200, 400-6400)	3200 (800-6400, 400->10000)	0.36
Serum Hu titer, 12 weeks (n=11) (median, IQR, range)	800 (400-1600, 0-6400)	3200 (2000-3200, 1600-3200)	0.082
CSF Hu titer, start (median, IQR, range)	24 (4-104, 0-512)	64 (26-160, 2-256)	0.24
CSF Hu titer, 12 weeks (n=9) (median, IQR, range)	2 (0-48, 0-64)	34 (4-112, 4-128)	0.17
WBC (mean, SD)	4.2 (2.3)	9.1 (7.6)	0.067
WBC elevated	2 (22%)	6 (55%)	0.20
Total protein elevated	4 (44%)	7 (64%)	0.65
IgG index elevated	1 (11%)	1 (9%)	1.00
Oligoclonal bands present	3/6 (50%)	3/4 (75%)	0.57
Treatment			
Immunotherapy before trial	2 (22%)	7 (64%)	0.092
No. Natalizumab cycles (median, IQR, range)	3 (3-3, 1-3)	2 (1-3, 1-3)	0.065
Outcome			
mRS, baseline (median, IQR, range)	3 (2-3, 2-4)	4 (4-5, 3-5)	**0.005**
mRS, follow-up (n=20) (median, IQR, range)	3 (2-3, 2-3)	4 (4-6, 4-6)	**<0.0001**
EFIT, baseline (median, IQR, range)	2 (1-2, 1-3)	3 (2-3, 1-4)	**0.015**
EFIT, follow-up (n=16) (median, IQR, range)	2 (1-2, 1-3)	3 (2-3, 2-4)	**0.036**
BI, baseline (median, IQR, range)	95 (57-97, 20-100)	40 (25-70, 5-885)	**0.012**
BI, follow-up (n=16) (median, IQR, range)	90 (81-98, 55-100)	40 (12-53, 10-75)	**0.001**
Follow-up			

Table 3. Treatment response according to Keime-Guibert criteria [a] (*continued*)

	Treatment response (n=9)	No treatment response (n=11)	p value
mRS last follow-up (median, IQR, range)	5 (3-6, 3-6)	6 (5-6, 3-6)	0.33
Dead at last follow-up	2 (22%)	8 (73%)	0.070
Onset to death, months (median, IQR, range)	30 (22-n.a., 22-39)	9 (6-19, 1-24)	0.07

Abbreviations: IQR = interquartile range, PNS = paraneoplastic neurological syndrome, SSN = subacute sensory neuronopathy, SCLC = small-cell lung cancer, ED = extensive disease, CR = complete response, PR = partial response, WBC = white blood cell count, SD = standard deviation, mRS = modified Rankin Scale, EFIT = Edinburgh Functional Impairment Tests, BI = Barthel Index.

Data are n (%), n/n (%), median (SD) or median (IQR, range).

[a] A positive treatment response was defined as improvement or stabilization in patients with an mRS score ≤3, and improvement from mRS ≥4 to mRS ≤3.[18]

Ancillary testing

In all patients, CSF was collected prior to the start of treatment. An elevated white blood cell count was present in eight patients (40%; maximum 26 WBC/µL), 11 (55%) had elevated total protein levels, two (10%) an increased IgG index, and 6/10 patients had oligoclonal bands. All these parameters were normal in five patients (25%). Nine patients consented to a second CSF evaluation after treatment which showed neither differences in WBC nor in total protein elevation (both p = 1.00). Hu-Ab median CSF titer at baseline was 32 (IQR 14-128) and 4 (IQR 1-64) after treatment (p = 0.67). In serum, median titer was 3200 before (IQR 1000-3200) and 1600 (IQR 800-3200) after treatment (p = 0.37; Supplementary Table 4). Hu-ab titers neither correlated with baseline mRS, nor with mRS change during follow-up.

IHC showed in all 20 patients' sera and CSF the typical Hu-staining pattern, and 18 were negative for additional antibodies. One patient's CSF showed a strong positive neuropil staining pattern, and antibody binding to membrane-bound proteins was confirmed using live hippocampal neurons. Results for anti-$GABA_BR$, anti-AMPAR, anti-VGKC, anti-CASPR2, anti-LGI1, anti-NMDAR, anti-GAD, anti-DPPX, anti-IgLON5, anti-VGCC, anti-CNTN1, anti-NF155 antibodies, all returned negative. This patient (No. 13) had encephalomyelitis, SCLC and high baseline Hu-Ab titers (serum 1:3200; CSF 1:64). Prior to diagnosis, she received ivIg without improvement. The patient died after one cycle of natalizumab. The CSF of another patient (No. 6) showed an atypical staining pattern on IHC, suitable with AQP4. This was confirmed by a CBA in serum. This patient presented with SSN, had SCLC and high Hu-Ab titers (serum 1:3200; CSF 1:16). SSN remained stable during the study period. Six months after natalizumab treatment, she developed optic neuritis attributed to the anti-AQP4 antibodies.

Comparison with treatment response from historical Hu-PNS patients

As we compared our data to the sirolimus trial[21] no difference in functional outcome was observed, 10% vs 6% showed improvement in mRS (p = 0.87; Supplementary Table 1). In addition, treatment response was similar between the two cohort (45% vs 41% responders, p = 0.82), as was neurological outcome (p = 0.53). The natalizumab cohort was comparable to the sirolimus cohort, but for a longer duration to tumor diagnosis (median 5 vs 2 months, p = 0.036). Baseline mRS appeared higher in the natalizumab cohort (median 4 vs 3, p = 0.18), without reaching statistical significance.

DISCUSSION

In this prospective open-label trial administering natalizumab in patients with Hu-PNS, we show that objective functional improvement is rare and achieved in 10%, while a stable situation was obtained in another 60%. Ascertained by the Keime-Guibert criteria, treatment response was classified as positive in 45%. As all patients had progressive neurological symptoms in the four weeks prior to inclusion, the high percentage of functional improvement and stabilization (70% together) suggests some efficacy of natalizumab. However, due to the non-randomized design of our study, it cannot be excluded that stabilization reflected the natural course of the disease as Hu-PNS ultimately reaches a plateau phase. [1-3]

Published studies of immunosuppression or immunomodulation in Hu-PNS using the same mRS based outcome criteria evaluated various treatments, including plasma exchange, ivMP, cyclophosphamide, ivIg, rituximab, and human chrionic gonadotropin. [18, 23-30] These studies found similar rates of objective functional improvement (0-40%, pooled 11%) and stabilization (20-71%, pooled 49%). Also, the treatment response was classified as positive in 0-65% (pooled 42%) of patients in these studies, similar to the positive response we found in 45%.[18, 23-30] In our institution, an earlier trial in patients with Hu-PNS was conducted by De Jongste et al. [21] treating patients with sirolimus (activated T cell suppressor). We used this cohort as a control group after showing that there were no relevant differences between the two cohorts. Treatment with sirolimus or natalizumab showed similar results in all outcome measures.

Previous studies in Hu-PNS found that in patients with a tumor, the functional outcome is better with antitumor treatment. [1, 22, 31] In our study, the outcome in the three patients with a tumor not receiving concomitant antitumor treatment was similar to the ten patients receiving concomitant antitumor therapy. As previously observed, we saw a trend in better functional outcome in patients without a tumor compared to patients with a tumor. [29] Five of nine patients with a positive outcome received only natalizumab without concomitant antitumor treatment indicating that immunosuppression may

ameliorate the disease course. [22] In patients receiving both chemotherapy and immunosuppressive or immunomodulatory therapy, it is unclear whether the immunotherapy has an additional effect.

As functional improvement is rare with the currently available treatment modalities, stabilization of the patient seems the most realistic treatment goal. Because of the rapidly progressive course of the disease, early diagnosis with the patient in a better condition is warranted. Indeed, moderate disability (mRS ≤3) at start of treatment associates with a more favorable outcome. [29]. Unfortunately, the median time from symptom onset to diagnosis was 5 months, which has not improved over the last 20 years. [1, 26, 27, 29] By this time, most patients already have severe symptoms, probably reflecting extensive and irreversible neuronal loss.

In Hu-PNS, patients can harbor other neuronal autoantibodies including those recognizing surface antigens. [2, 32] In these patients the neurological syndrome may be caused by the cell-surface antibodies while the Hu-Ab may be biomarkers of an underlying SCLC (15% of SCLC harbor Hu-Ab, most without PNS). [33] As their treatment strategies, response and outcome may be different, we screened for cell-surface antibodies. We identified a second antibody in two patients: one patient with a currently unidentified antibody and one with anti-AQP4 antibodies, a rare accompaniment. In both patients the clinical presentation and disease course was typical of Hu-PNS. The second patient developed optic neuritis six months after natalizumab treatment, most likely related to anti-AQP4 antibodies. We found no $GABA_BR$ antibodies, the most frequently described co-occurrence with Hu-Ab. [34, 35]

Limitations of our study are the small sample size and the open-label non-randomized design. A marginal positive effect of natalizumab cannot be excluded as the trial was not powered to detect a difference in effect <25% compared to historic studies. These limitations are directly related to the low incidence of Hu-PNS and the difficulty to accrue patients who are still in the progressive phase of the disease. Due to the severity of the disease, a high percentage of trial candidates were unwilling to participate in a trial outside their own region. This could have been a source for selection bias. However, our cohort still consisted of patients with a high mRS at baseline, similar to other studies in this field. Seven patients chose not to complete all three cycles of natalizumab. Some secondary or exploratory outcome measures could not be collected in these patients. However, as the mRS scores were always available, this did not change the primary outcome of our study. In our trial, almost half of the patients had received a form of first-line immunotherapy (ivMP or ivIg or both) in the referral hospital before the start of natalizumab treatment. As all but one of these patients had evident neurological progression prior to inclusion in the study, first-line immunotherapy is unlikely to have influenced the results. Finally, many of our patients had involvement of dorsal root ganglia (SSN) and there is very few data on the effect of natalizumab on the traffic of T cells

into dorsal root ganglia. [36] Natalizumab may theoretically be less effective in blocking T cell traffic into dorsal root ganglia than traffic into the central nervous system. However, we did in our study not observe better efficacy of natalizumab in patients with central or combined central and peripheral nervous system involvement than in patients with involvement of peripheral nervous system only.

To conclude, natalizumab may ameliorate the disease course in Hu-PNS. However, the efficacy of natalizumab seems not superior to other immunosuppressive and immunomodulatory treatment strategies. Rapid diagnosis of Hu-PNS followed by tumor identification and treatment are essential to stabilize the patient when still ambulatory. In patients without a tumor, or not receiving antitumor treatment for another reason, immunosuppressive or immunomodulatory therapies should be seriously considered. Until now, there is no preferred choice in the kind of immunotherapy. Better, more effective treatments are clearly still needed.

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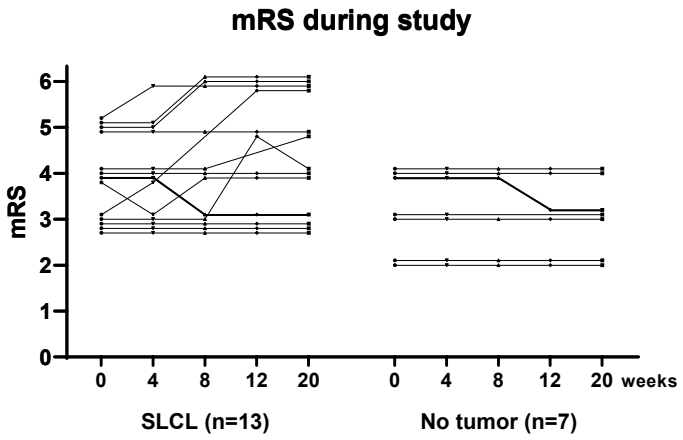
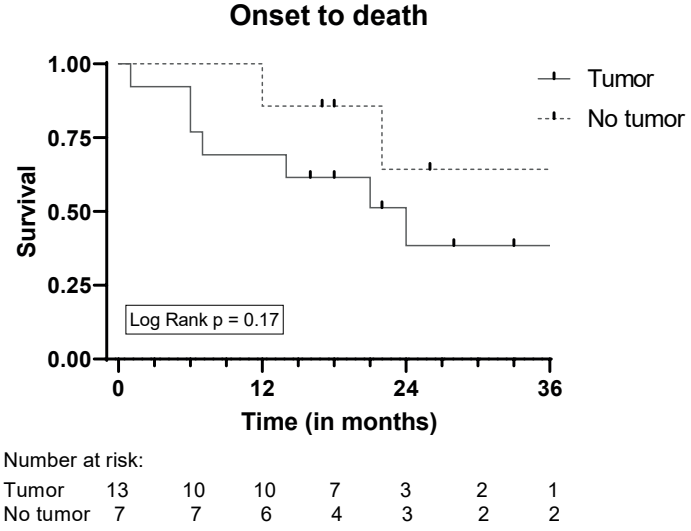
SUPPLEMENTARY DATA



Supplementary Figure 1. mRS scores during the total study period of 20 weeks. mRS scores per patient during the study period of 20 weeks. Patients were divided in two groups based on tumor presence. The two thick lines represents the two patients who showed improvement in mRS score.



Supplementary Figure 2. Kaplan-Meier estimates of survival for patients with and without a tumor. Kaplan-Meier estimates of survival in Hu-PNS patients. Patients were divided into two groups based on tumor presence. Tumor presence is depicted with the continuous line and no tumor is depicted with the dashed line.

Supplementary Table 1. Cohort characteristics and comparison between patients treated with natalizumab and a prior study with sirolimus treatment [21]

	Natalizumab trial (n=20)	Sirolimus trial (n=17)	p value
Gender, female	17 (85%)	11 (65%)	0.25
Age at onset (mean, SD)	67.8 (8.4)	65.2 (8.2)	0.35
Onset to diagnosis, months (median, IQR, range)	5 (2-11, 0.2-62)	4 (1-6, 0-11)	0.12
PNS syndrome			1.00[b]
SSN	6 (30%)	6 (35%)	
SSN with other peripheral nervous syndrome	3 (15%)	1 (6%)	
SSN with other central nervous syndrome	6 (30%)	2 (12%)	
PLE	1 (5%)	2 (12%)	
MN	1 (5%)	1 (6%)	
PCD	2 (10%)	3 (18%)	
PEM	1 (5%)	1 (6%)	
Tumor			
Tumor	13 (65%); all SCLC	15 (88%); 3 no biopsy; 10 SCLC	0.14
Onset to tumor diagnosis, months (median, IQR, range)	5 (3-6, 0.5-8)	2 (-4-5, -312-8)	**0.036**
Tumor stage			0.35
LD	9/13 (69%)	11/13 (85%)	
ED	4/13 (31%)	2/13 (15%)	
Chemotherapy	13/13 (100%)	10/12 (83%)	0.22
Tumor response			0.65
CR	5/9 (56%)	7/10 (70%)	
near CR	1/9 (11%)	0	
PR	3/9 (33%)	1/10 (10%)	
PD	0	2/10 (20%)	
N.E.	3	0	
Ancillary testing			
Serum Hu titer, baseline (median, IQR, range)	3200 (1000-3200, 400->10000)	3200 (1200-6400, 400-12800)	0.39
Serum Hu titer, 12 weeks (median, IQR, range)	1600 (800-3200, 0-6400); n=11	1600 (800-6400, 800-6400); n=11	0.52
CSF Hu titer, baseline (median, IQR, range)	32 (14-128, neg-512); n=18	32 (8-256, 8-2048); n=15	0.76

Supplementary Table 1. Cohort characteristics and comparison between patients treated with natalizumab and a prior study with sirolimus treatment [21] (*continued*)

	Natalizumab trial (n=20)	Sirolimus trial (n=17)	p value
CSF Hu titer, 12 weeks (median, IQR, range)	4 (1-64, 0-128); n=9	48 (10-224, 0-1024); n=12	0.11
Routine CSF normal	5 (25%)	5 (29%)	
WBC (median, IQR, range)	4 (3-9, 1-26)	5 (2-11, 1-49)	0.87
WBC elevated	8 (40%)	8 (47%)	0.67
Total protein elevated	11 (55%)	7 (41%)	0.40
IgG index elevated	2 (10%)	3 (18%)	0.64
Oligoclonal bands	6/10 (60%)	9/13 (69%)	0.69
Treatment			
Immunotherapy before trial	9 (45%)	3 (18%)	0.09
ivMP	2	3	
ivIg	4	3	
ivMP+ivIg	3	0	
Immunotherapy to start trial, days (median, IQR, range)	28 (18-64, 8-96)	n.e.	
No. Natalizumab cycles (median, IQR, range)	3 (1-3, 1-3)	n.a.	
Outcome			
Positive treatment response[a]	9 (45%)	7 (41%)	0.82
Functional outcome (mRS)			0.87
Improved	2 (10%)	1 (6%)	
Stable	12 (60%)	10 (59%)	
Worse	6 (30%)	6 (35%)	
mRS, baseline (median, IQR, range)	4 (3-4, 2-5)	3 (3-4, 2-5)	0.18
mRS, follow-up (median, IQR, range)	4 (3-5, 2-6)	4 (3-4, 1-6)	0.63
Neurological outcome (EFIT)			0.53
Improved	2 (13%)	2/14 (14%)	
Stable	11 (69%)	7/14 (50%)	
Worse	3 (20%)	5/14 (36%)	
EFIT, baseline (median, IQR, range)	2 (1-3, 1-4)	2 (2-2, 0-4)	0.78
EFIT, follow-up (median, IQR, range)	2 (2-3, 1-4)	2 (2-3, 1-4)	
BI, baseline (median, IQR, range)	65 (31-93, 5-100)	85 (47-100, 15-100)	0.17
BI, follow-up (median, IQR, range)	65 (37-90, 10-100)	n.e.	

Supplementary Table 1. Cohort characteristics and comparison between patients treated with natalizumab and a prior study with sirolimus treatment [21] (*continued*)

	Natalizumab trial (n=20)	Sirolimus trial (n=17)	p value
Follow-up			
Follow-up from onset, months (median, IQR, range)	19 (13-27, 2-75)	14 (8-22, 2-43)	0.24
mRS at last follow-up (median, IQR, range)	5 (3-6, 1-6)	n.e.	
Dead during study	4 (20%)	n.e.	
Dead, total at last follow-up	10 (50%)	9 (53%)	0.86
Onset to death, months (mean, SD)	15 (11.4)	12 (7.6)	0.49
Cause of death			0.21
PNS	4/10 (40%)	7/9 (78%)	
Tumor	3/10 (30%)	2/9 (22%)	
Euthanasia	3/10 (30%)	0	

Abbreviations: SD = standard deviation, IQR = interquartile range, PNS = paraneoplastic neurological syndrome, SSN = subacute sensory neuronopathy, PLE = paraneoplastic limbic encephalitis, PCD = paraneoplastic cerebellar degeneration, MN = motor neuronopathy, PEM = paraneoplastic encephalomyelitis, SCLC = small-cell lung cancer, LD = limited disease, ED = extensive disease, CR = complete response, PR = partial response, PD = progressive disease, n.e. = not evaluable, WBC = white blood cell count, ivMP = intravenous methylprednisolone, ivIg = intravenous immunoglobulins, mRS = modified Rankin Scale, EFIT = Edinburgh Functional Impairment Tests, BI = Barthel Index.

Data are n (%), n/n (%), median (SD) or median (IQR, range).

[a] A positive treatment response was defined as improvement or stabilization in patients with an mRS score ≤3, and improvement from mRS ≥4 to mRS ≤3.[18]

[b] Patients with only peripheral nervous system involvement compared with patients who had only central or combined peripheral and central nervous system involvement

Supplementary Table 2. Characteristics of the patients who requested euthanasia.

No. 9	Progressive sensory neuronopathy with hand function and walking difficulties. Seven months before start of the trial she was diagnosed with SCLC, extensive disease, and treated with chemotherapy with partial response. Four weeks after start of the trial there was neurological deterioration with cerebellar ataxia, hemiparesis and inability to walk. Imaging showed multiple (>15) brain metastases. Due to further neurological decline in combination with the poor prognosis, the patient withdrew from the trial.
No. 11	Progressive neurological dysfunction involving dysphagia resulting in tube feeding, inability to walk or sit due to severe sensory ataxia. Totally dependent. No abnormalities on extensive MRI imaging of brain and spinal cord No response on earlier ivIg before trial. Received one cycle of chemotherapy for SCLC, limited disease. After start of the trial she was diagnosed with SCLC and because of her extensive disabilities in combination with the poor prognosis the patient requested no further treatment and withdrew from the trial.
No. 13	Fast neurological deterioration with prominent loss of muscle strength in neck and arms, eye movement disorder, dysphagia, and respiratory failure. Bedridden. No behavior problems or cognitive decline. No abnormalities on MRI imaging of brain, spinal cord and plexus brachialis. ICU admittance due to progressive respiratory failure and need for ventilator (2,5 weeks). No response on earlier ivIg before trial. Received one cycle of chemotherapy for SCLC, extensive disease. After start of the trial she had further neurological deterioration and she was diagnosed with SCLC. The patient requested no further treatment due to her disabilities and poor prognosis and withdrew from the trial.

Supplementary Table 3. Characteristics of patients with and without a tumor.

	No tumor (n=7)	Tumor (n=13)	p value
Gender, female	5 (71%)	12 (92%)	0.27
Age at onset (mean, SD)	73.4 (5.7)	64.7 (8.2)	**0.022**
Onset to diagnosis, months (median, IQR, range)	9 (3-11, 2-36)	3 (1-6, 0.2-62)	0.18
PNS syndrome, only peripheral nervous system involvement	3 (43%)	6 (46%)	1.00
Ancillary testing			
Serum Hu titer, baseline (median, IQR, range)	1600 (400-3200, 400-3200)	3200 (1600-6400, 400->10000)	0.060
Serum Hu titer, 12 weeks (n=11) (median, IQR, range)	1800 (100-5600, 0-6400)	1600 (800-3200, 800-3200)	0.69
CSF Hu titer, baseline (median, IQR, range)	16 (0-48, 0-64)	64 (24-192, 2-512)	0.059
CSF Hu titer, 12 weeks (n=9) (median, IQR, range)	48 (8-64, 0-64)	4 (1-66, 0-128)	0.54
WBC (median, IQR, range)	4 (2-7, 2-9)	5 (3-13, 1-26)	0.28
WBC elevated	2 (29%)	6 (46%)	0.64
Total protein elevated	3 (43%)	8 (61%)	0.64
IgG index elevated	0	2 (15%)	0.52
Oligoclonal bands present	3/5 (60%)	3/5 (60%)	1.0
Treatment			
Immunotherapy before trial	1 (14%)	8 (62%)	0.07
No. Natalizumab cycles	3 (3-3, 1-3)	3 (1-3, 1-3)	0.20
Outcome			
Positive treatment response [a]	5 (71%)	4 (31%)	0.16
Functional outcome (mRS)			0.071
Improved	1 (14%)	1 (8%)	
Stable	6 (86%)	6 (46%)	
Worse	0	6 (46%)	
mRS, start (median, IQR, range)	3 (2-4, 2-4)	4 (3-4, 3-5)	0.11
mRS, follow-up (n=20) (median, IQR, range)	3 (2-4, 2-4)	4 (3-6, 3-6)	**0.019**
EFIT, start (median, IQR, range)	2 (1-3, 1-3)	2 (1-3, 1-3)	0.23
EFIT, follow-up (n=16) (median, IQR, range)	2 (2-3, 1-4)	2 (2-2, 1-4)	0.91
BI, start (median, IQR, range)	80 (40-100, 20-100)	55 (27-87, 5-95)	0.17
BI, follow-up (n=16) (median, IQR, range)	82 (65-92, 35-100)	52 (17-91, 10-100)	0.23
Follow-up			
Months follow-up (mean, SD)	25.7 (11.6)	21.1 (18.7)	0.56
mRS last follow-up (median, IQR, range)	6 (3-6, 3-6)	6 (4-6, 3-6)	1.00
Dead at last follow-up	3 (43%)	7 (54%)	1.00
Onset to death, months (mean, SD)	24.3 (13.7)	11.3 (8.6)	0.098

Abbreviations: SD = standard deviation, IQR = interquartile range, PNS = paraneoplastic neurological syndrome, SSN = subacute sensory neuronopathy, WBC = white blood cell count, mRS = modified Rankin Scale, EFIT = Edinburgh Functional Impairment Tests, BI = Barthel Index.

Data are n (%), n/n (%), median (SD), or median (IQR, range).

[a] A positive treatment response was defined as improvement or stabilization in patients with an mRS score ≤3, and improvement from mRS ≥4 to mRS ≤3.[18]

Supplementary Table 4. Anti-Hu antibody titer at baseline and after natalizumab treatment

	SERUM titer		CSF titer	
No.	**Baseline**	**Follow-up**	**Baseline**	**Follow-up**
1	6400	1600	32	n.a.
2	1600	800	128	2
3	3200	1600	512	n.a.
4	6400	n.a.	128	n.a.
5	3200	1600	256	4
6	3200	800	16	negative
7	400	3200	256	n.a.
8	6400	n.a.	128	n.a.
9	10000	n.a.	8	4
10	3200	3200	32	128
11	1600	n.a.	32	n.a.
12	800	n.a.	2	n.a.
13	3200	n.a.	64	n.a.
14	3200	6400	32	64
15	1600	negative	negative	negative
16	800	400	16	32
17	400	n.a.	negative	n.a.
18	1600	n.a.	n.a.	n.a.
19	3200	3200	n.a.	64
20	400	n.a.	64	n.a.

Abbreviations: n.a. = not applicable.

Anti-Hu antibody median CSF titer at baseline was 32 (IQR 14-128) and 4 (IQR 1-64) after treatment at 12 weeks (p = 0.67). In serum, median titer was 3200 before (IQR 1000-3200) and 1600 (IQR 800-3200) after treatment (p = 0.37). Wilcoxon matched pairs test was used for statistical analysis.

7

General discussion

Autoimmune encephalitis (AIE) is a relatively new disease entity and much remains to be discovered. It is known to be a severe but mostly treatable disorder in which cognition is frequently affected. The in 2016 published criteria provided a clinical guideline to diagnose AIE and were a significant improvement in AIE research. Among the main criteria, a subacute onset (meaning a rapid progression of less than 3 months) of neuropsychiatric symptoms is required, including cognitive impairment. However phenotypes vary widely, and a less fulminant and protracted disease course is seen as well.
This thesis focused on the recognition and treatment of the elderly patients with AIE and paraneoplastic neurological syndromes (PNS), drawing special attention to dementia syndromes.

DIAGNOSING AUTOIMMUNE ENCEPHALITIS IN ELDERLY PATIENTS

Despite that AIE has been around for almost 20 years, it is still a relatively new disease. Awareness and recognition are improving, as evidenced by increasing incidence and the growing number of antibody requests. Nevertheless, underrecognition of AIE remains a big problem. The Erasmus University Medical Center is the national referral site for patients with suspected AIE. It is our goal to identify every patient by raising awareness and increasing recognition. This is important as earlier recognition and treatment of AIE improves outcomes.[1, 2] One issue that leads to underrecognition is a more protracted disease course, as we noticed in our cohorts of patients with AIE. However, literature on a more slow disease course is sparse.[3, 4] In this respect, clinicians not specialized in AIE are not aided with the current criteria since a subacute onset (rapid progression of less than three months) is mandatory to enter the diagnostic flow chart as 'possible AIE', despite the warning for this slower disease course.[5, 6] In addition, it is known that cognitive dysfunction can be the presenting and most prominent symptom in patients with AIE. Yet again, literature is equally sparse on pure cognitive decline in patients with anti-neuronal antibodies.[7, 8] When patients have predominantly cognitive dysfunction in combination with a slower progression, the disease course can mimic neurodegenerative dementia syndromes. Especially since patients may present with less notable signs of encephalitis and without overt changes in brain MRI and cerebrospinal fluid (CSF).[9] In these patients, clinical clues are essential to avoid misdiagnosis and inadvertently withhold immunotherapy from patients. As few data exists, we first studied in **chapter 2** cognitive deterioration in several of our national cohorts of AIE subtypes to evaluate possible dementia diagnosis and to explore potential red flags for AIE (in middle-aged and older patients). We observed that many of our patients with AIE were initially suspected of having a neurodegenerative dementia syndrome. Similar to earlier (limited) research in patients with neuronal antibodies, cognitive deterioration predominantly had a

rapidly progressive character.[10-12] This percentage of rapidly progressive dementia (RPD; 76%) was much larger compared to the prevalence of RPD in dementia cohorts.[13-15] In our experience, patients with RPD are infrequently investigated for antibodies. Together these results underscore that in part of the (older) patients in whom the differential diagnosis comprises a possible dementia syndrome, anti-neuronal antibodies should be tested. As there are no extensive comprehensive trials investigating the frequency of anti-neuronal antibodies in neurodegenerative dementia syndromes, it remains unknown how big an issue it is exactly.

Giannocaro et al. showed in 2021 a prevalence rate of 14% in 93 patients with neurodegenerative disorders.[16] However this study was limited by using serum CBA only (as discussed later in this chapter), and there was a higher pre-test probability of antibody positivity due to a high percentage of CSF inflammatory abnormalities (selection bias). In a population with 'regular' neurodegenerative dementia, it is not common to find inflammatory changes in the CSF. Furthermore, by not using alternative research techniques that confirm antibody positivity, the results probably overreport antibody positivity due to false positive or clinically irrelevant test results. Overreporting due to the antibody testing method is a frequent problem in AIE research. In these earlier studies, it remains unclear whether the antibodies are responsible for the symptoms or whether they are a secondary epiphenomena. Therefore we performed a large, multicenter, cohort study consisting of patient with a presumed neurodegenerative dementia diagnosis in **chapter 3**. Up till now this is the largest cohort of dementia patients examined for the presence of neuronal antibodies, including 920 patients representative for academic memory clinics. The strength of this study is the use of a large number of paired samples (combined CSF and serum), and also the use of different laboratory techniques for positive test results (immunohistochemistry, cell-based assay, and cultured live neurons). Given the low a priori chance to find AIE, it is of high importance to have optimal test-specificity, and this is ensured using various techniques. We found a small proportion of 0.8% that had neuronal antibodies, which is a notably lower rate compared to earlier reports.[16] This discrepancy is probably explained by factors already discussed above. Furthermore, the fact that we had a low number of patients with RPD (7%) could have contributed to this low rate. Our studied patients were included in outpatient clinics in tertiary memory clinics, and those are mostly deprived of RPD patients and multiple atypical symptoms (since these patients are more frequently admitted to the ward).

Although 0.8% is a small proportion, it is still clinically relevant to identify patients in whom cognitive deterioration is caused by antibody-mediated encephalitis, to not withhold available treatments from them. Furthermore, since dementia is very common and prevalence increasing rapidly, a small proportion still represents significant numbers of patients. Physicians should remain vigilant for atypical signs pointing towards an autoimmune etiology. In relation to this, a goal of both of our dementia studies was to

examine whether there are clues to improve the clinical recognition of AIE. An important observation from our first study in AIE patients with suspected dementia was that red flags for AIE involved: 1) abnormalities in ancillary tests (inflammatory changes in the CSF or brain MRI), 2) (subtle) seizures, and 3) rapidly progressive cognitive decline as discussed before. Due to the design of the study involving only patients with AIE, the red flags are more geared towards AIE with seizures and inflammatory changes in the ancillary tests. The second discussed study in primarily neurodegenerative dementia showed the following red flags: 1) atypical clinical course (subacute deterioration or fluctuating disease course), 2) myoclonus, 3) seizures, 4) pleocytosis, and 5) other autoimmune disorders. None of the antibody-positive patients fulfilled the criteria for RPD, due to factors discussed above. A major drawback of this study was the small number of antibody-positive patients (n = 7), which underpowered the probability of identifying significant differences between antibody-positive and antibody-negative patients. Nevertheless, several probable red flags could be identified showing, among others, that patients with a rapidly and a non-rapidly progressive course can have neuronal antibodies indicative of AIE. In patients with atypical clinical signs of dementia, it is our opinion that clinicians should have a lower threshold for neuronal antibody testing.

The next step is to determine which antibodies should be tested when a dementia is suspected. Both studies show potential antibodies. The first mentioned study in AIE patients, is limited in that only a selection of antibody subtypes were included (NMDAR, LGI1, $GABA_BR$, CASPR2), although all four subtypes had patients that fulfilled the criteria for dementia. In the second study involving primarily neurodegenerative patients, we found anti-IgLON5, anti-LGI1, anti-DPPX, and anti-NMDAR antibodies. Antibodies should be requested on the basis of clinical characteristics. If no differentiating atypical symptoms are present, it seems reasonable to request the antibodies mentioned above, also based on prevalence. Anti-NMDAR is the main subtype that predominantly affects young adults, although more and more elderly patients are diagnosed with anti-NMDAR encephalitis (as discussed later in this chapter). Anti-LGI1 encephalitis is the second largest subtype, with more patients in the age category for dementia and a clinical profile that can mimic dementia, especially since subtle seizures are easily overlooked. Encephalitis with $GABA_BR$ or CASPR2 antibodies is less prone to mimic dementia, as most $GABA_B$ patients have severe seizures and CASPR2 patients mostly harbor other symptoms (painful polyneuropathy, cerebellar dysfunction, or epilepsy). However, patients with $GABA_BR$ antibodies can present with RPD without seizures during the disease course.[17] Also anti-DPPX is usually accompanied by other symptoms (CNS hyperexcitability and gastrointestinal symptoms). Misdiagnosis with progressive supranuclear palsy (PSP) is reported due to autonomic similarities.[18, 19] Similarly, several Dutch patients with anti-DPPX were mistaken for burnout, depression or other psychiatric diagnoses.[20] We did not find anti-AMPAR antibodies, although it can occasionally present with cognitive deterioration

without other symptoms, this is very rare. Anti-IgLON5 disease merits a special interest as we found three patients (out of seven positive patients) with this antibody in the dementia cohort. Antibodies to anti-IgLON5 are known to be associated with broad clinical phenotypes, including manifestations that can resemble dementia. Half of the patients have predominant cognitive impairment and together with a chronic development accompanied by frequent movement disorders, in some patients the diagnosis of Huntington disease or PSP was suspected in case of chorea or gait and oculomotor abnormalities.[21, 22] Intriguingly, IgLON5 disease shares features with neurodegeneration as autopsy studies showed tau deposits. However, precise pathophysiologic mechanisms remain unclear and also characteristics of autoimmune disease were reported. Namely, there is a strong HLA association suggesting a genetic predisposition for autoimmunity and in addition, studies showed surface IgLON5 alterations to antibodies. Together this could suggest a primary inflammatory disease and secondary neurodegeneration. Alternatively, anti-IgLON5 is a novel tauopathy and ongoing tau accumulation exacerbates an immunological response. The exact nature of this disease remains to be discovered.[23-25]

We discussed a more prolonged course of the disease as one reason for underrecognition. Another issue could be age. Of all currently known antibodies, encephalitis with anti-NMDAR antibodies is the most common and affects mainly younger patients. In **chapter 4** we describe that anti-NMDAR encephalitis is less rare later in life (≥ 45 years of age) than previously anticipated. A fifth of our patients would be considered late onset compared to only 5% in previous publications.[4] Even ~15% had an age of 60 or older. The higher percentage probably reflects the already improving awareness and a lower threshold for antibody testing nowadays. This is supported by the fact that most of our late-onset patients were diagnosed after 2015. A complicating factor is the less outspoken nature of the disease in late-onset patients and the broader differential diagnosis in this age category. A higher level of suspicion is therefore necessary to recognize late-onset anti-NMDAR encephalitis. Importantly, in our cohort all tumors were carcinomas, emphasizing the importance of correct diagnosis in these patients and subjecting them to a thorough (and different) tumor workup. Furthermore, elderly NMDAR patients had a worse outcome indicated by a longer interval to achieve functional independence (mRS score ≤2); 12 months instead of 4 months in younger patients. Furthermore, after one year, a significantly higher percentage had a poor outcome (mRS ≥3) compared to the younger group (respectively 65% and 18%). As it is known that brain plasticity and the capacity to recover diminish with age, better chances for recovery necessitate early treatment.

DIAGNOSTIC DILEMMAS

The first hurdle has been taken once AIE has become part of the differential diagnosis, but the next one will present itself immediately: how should you screen for AIE? Worldwide, it is most common to perform a serum cell based assay (CBA), one test per antibody subtype. Serum is preferred above CSF by clinicians (and patients) due to it is minimally invasive nature and it is less time consuming compared to performing a lumbar puncture. However, serum can provide false positive or clinically irrelevant results. This applies both to the clinic and to research, which can be potentially harmful. Research in AIE can provide high prevalence numbers based on false positive serum CBAs when no confirmative tests are used. Due to these inaccurate scientific reports, clinicians may be inclined to treat patients based on wrong assumptions, unaware of the wrong indication.

For this reason CSF is considered the gold standard for neuronal antibody testing. In **chapter 4**, we describe that the CBAs to detect NDMAR antibodies perform very well but not perfectly. We demonstrated that patients can even have unconfirmed positive results in CSF, meaning that the positive result could not be confirmed by alternative research techniques including immunohistochemistry and live neurons. This was the first report to describe patients without anti-NMDAR encephalitis despite a positive CBA result in CSF. One previous review described 16 cases with NMDAR antibodies in 1650 healthy controls, but all cases retrospectively truly had anti-NMDAR encephalitis.[26] Our results should not be considered false positive, as all samples were tested positive by both commercial CBA and in-house CBA in two independent laboratories. The outstanding question is whether the result is clinically relevant? We consider clinical irrelevance the most likely explanation because 1) none of the patients met the criteria for probable anti-NMDAR encephalitis according to Graus criteria,[5] and 2) part of the patients had a different etiology of their symptoms suggesting that there was a broader immune response or that there was antibody formation secondary to neuronal damage that led to these confusing antibody results. The clinical evaluation appeared of great importance since the phenotype in these patients was atypical for anti-NMDAR encephalitis. Physicians should always link the clinical phenotype with antibody results, even in CSF. When in doubt, a reference laboratory should be asked to confirm or refute the diagnosis!

Although CSF results can sometimes be difficult to interpret, this is much more of an issue in serum. In **chapter 4** we demonstrated that 25% of serum results were considered clinically irrelevant. When the differential diagnosis involves AIE, both serum and CSF should be analyzed for auto-antibodies. CSF should not only be tested for antibodies but also for IgG index and oligoclonal bands. These markers can help in the differential diagnosis pointing towards an autoimmune etiology and should be part of the standard workup when AIE is suspected.

Other diagnostic tools, including MRI and EEG, can show normal results or only minimal abnormalities (frequently overlooked). As a consequence, the results can be non-discriminative, for example an EEG with a slow background pattern. These modalities generally do not add conclusively in the acute phase for a fast and accurate diagnosis. Because antibody test results usually take 1-2 weeks depending on the antibody subtype, other diagnostic tools would be valuable. Alternatively, improvement of the current tools, including faster available antibody results, would be an improvement as well. In both **chapter 2 and 3** we show that CSF dementia biomarkers (including t-tau, p-tau, Aβ42, and 14-3-3 can be positive in AIE, making the difference between AIE and a neurodegenerative cause more complex. Most of these markers (14-3-3, tau and p-tau) are known not to be highly specific for a neurodegenerative cause, as they represent neuronal injury. Why Aβ42 can be abnormal in AIE is difficult to answer and currently unknown. It could be possible that those patients had concomitant Alzheimer's disease or would develop it within the next two decades. However, this remains unlikely as they did not develop cognitive decline over time, despite long follow-up, and because they improved with immunotherapy.

TREATMENT STRATEGIES

When patients are diagnosed with a probable or definite AIE, first-line immunotherapy usually consists of prednisone with or without immunoglobulins. Treatment regimens are generally based on expert opinion. In AIE there is no doubt about the effectiveness of immunotherapy,[1] and randomized controlled trials including a 'placebo-only' arm are considered non-ethical. Compared to AIE, treatment in paraneoplastic neurological syndromes (PNS) is less effective.[27] PNS with anti-Hu antibodies (Hu-PNS) are the most common within the PNS and earlier published treatment strategies mostly relate to this subtype. The main conclusions were that the immunosuppressive or immunomodulatory regimens studied had a poor response with only functional improvement in a minority of patients (around 10%).[28-36] Hu-PNS leaves patients severely debilitated, with more than half of patients bed or wheelchair-bound and a median survival of less than 12 months. Therefore new treatments are a highly unmet medical need. Since evidence suggests an underlying pathogenic T cell-mediated immune response responsible for damage to the nervous system, we conducted a prospective, one arm, open label trial with natalizumab in **chapter 6**. In this trial, patients with progressive Hu-PNS were treated with natalizumab, off-label. Most patients (13) also received chemotherapy for accompanying SCLC. The main finding was that natalizumab did not show superior efficacy over therapies studied earlier, as functional improvement was achieved in only 10%, similar to previous studies, using plasma exchange, ivMP, cyclophosphamide,

ivIg, rituximab, sirolimus, and human chorionic gonadotropin.[28-35] A high percentage of patients (60%) showed stabilization of their progressive neurological symptoms, suggesting some effect of natalizumab. Furthermore, five out of nine patients who received only natalizumab without concomitant antitumor therapy had a 'positive' outcome. This might suggest a hint towards natalizumab stabilizing or ameliorating the disease course. However, it is known that most patients with Hu-PNS reach a plateau phase,[37-39] so stabilization in our patients may reflect the 'natural' course of the disease rather than a treatment effect of natalizumab. Until now there is no preferred choice in the kind of immunotherapy for anti-Hu PNS. More effective and better treatments are clearly still needed.

Anti-Hu is the most common PNS but still very rare, with only 20-30 patients diagnosed each year in the Netherlands. Researching rare diseases presents several limitations, including difficulty in obtaining sufficient study participants due to the low incidence rate. Additionally, strict medical ethical regulations are necessary but can disproportionately impact trials with limited patient inclusions at every individual location. Every hospital will necessitate local approval, local agreements and local monitoring. Monitoring is performed at regular intervals and as inclusion rates are this low, basically every patients will be monitored even if the risk assessment has been low or moderate only. This creates a huge, non-efficient burden. The alternative is to include patients only in one center, as chosen in the natalizumab trial. This however has other disadvantages. For example in anti-Hu PNS, as patients often receive a concurrent cancer diagnosis, and may be unwilling to participate in a trial in another hospital. In addition, the severity of their condition is a big limitation to travel outside their region.

Early treatment is important, as patients have a more favorable outcome when they receive treatment in a better condition. Awareness is already discussed and is also relevant for PNS. Despite better awareness, the median time to diagnosis (5 months) has not improved over the past 20 years unfortunately. By this time, most patients already have severe symptoms, probably reflecting extensive and irreversible neuronal loss.

PROGNOSIS AND BIOMARKERS FOR PROGNOSIS

The patient's prognosis is largely dependent on the AIE subtype. For example, patients with anti-NMDAR encephalitis and anti-LGI1 encephalitis have frequently 'good functional recovery' two years after disease onset (~85% with a low mRS score). Patients with anti-$GABA_BR$ encephalitis respond to therapy, however, the survival rate is much lower due to the often accompanying SCLC. The most commonly used outcome scale in research on AIE and PNS is the modified Ranking Scale (mRS). Its appeal is in the simplicity as it addresses functional outcome related to a degree of disability, in which

patients with a score of 0-2 are independent and patients with a score of 3 or higher are dependent in their daily activities. A big limitation of this scale is that it is a broad, crude score. All aspects of physical and mental performance are combined in a single mRS grade, focusing on the functional part. The mRS scale is not at all specific for AIE. Residual symptoms of anxiety, fatigue, and (milder) cognitive or behavioral deficits are frequently present in patients after AIE and are not included in this score. However, these symptoms can have a huge impact on functioning and quality of life. In 2018, Lim et al. published a novel clinical scale named the Clinical Assessment Scale in Autoimmune Encephalitis (CASE).[40] This score consists of 9 items including seizure, memory dysfunction, psychiatric symptoms, consciousness, language problems, dyskinesia/dystonia, gait instability and ataxia. Each item could be valued between 0 to 3 points, accumulating to a total score ranging from 0 to 27 wherein the highest score correlates with higher clinical severity. It is the first specific score for AIE that rates clinical severity and could overcome the limitations of crude mRS scores by differentiating severity within the same mRS score. CASE is potentially a useful tool although it seems mostly applicable in the acute setting when patients have more severe symptoms (e.g. intractable seizures, low consciousness levels, brainstem dysfunction involving tube feeding and central hypoventilation). Apart from scoring clinical severity, it would be useful to investigate whether the score can be used to evaluate therapeutic responses.
A study examining all cognitive and emotional sequelae, including the ability to reintegrate into society (go back to school of work), quality of life after treatment, and the correlation between cognition and mRS or CASE scores, could be very informative. Patients could be subjected to neuropsychological tests and questionnaires at different time points in the disease course, to develop patient-reported outcome measures allowing better assessment of relevant functional outcomes.

As said, anti-NMDAR encephalitis is researched the most, and outcomes in patients comprise considerable and variable long-term disability. Previous research showed that intensive care admission, delay in treatment, and tumor presence are factors that are associated with a poorer outcome. Some of these elements were incorporated into a score that predicts neurological outcome one year after disease onset (NEOS score) in anti-NMDAR encephalitis. The main limitation of this score is that it does not predict response to immunotherapy, to distinguish patients who would benefit from more aggressive and prolonged immunotherapy administration. Another limitation is that the score cannot be used at acute presentation, but only in patients who are already one month into treatment. In general, earlier and more aggressive treatment is believed to result in better outcomes, so it would be most valuable to have a tool in the acute setting. However, ideally only in designated patients who do not or do not respond properly to first-line immunotherapy to avoid the potential harmful side-effects of more aggres-

sive treatment. We have collected a nationwide anti-NMDAR cohort that contains almost all Dutch patients with anti-NMDAR encephalitis. Preliminary assessment suggests that several factors associate with failure of first-line therapy, including diagnostic delay, admission to the intensive care unit, and elevated white blood cell count in the CSF. Although these preliminary data are based on a nationwide cohort, the small number of patients reflecting the rarity of the disease is a significant limitation that precludes a proper multivariable model. To develop a new scoring system that incorporates the effect of immunotherapy at presentation, we need to increase the number of patients, preferably by collaborating with international cohorts, and create both a development and a validation cohort. If successful, this model could aid in the decision to initiate more aggressive treatments immediately.

Currently, still little is known regarding biomarkers for disease activity and prognosis in AIE. Most biomarker studies are conducted in anti-NMDAR encephalitis, where candidates are selected based on existing knowledge in other inflammatory disorders. Most of the potential biomarkers remain in an exploratory phase due to the complex process of biomarker development together with the low incidence of the disease and its relatively recent discovery. Earlier attempts include antibody titers and CXCL13 (involved in recruitment of immune cells into the intrathecal compartment) in CSF.[41, 42] Both CXCL13 and antibody titers prove to be suitable biomarkers, although only moderately relevant early in disease course. Elevated levels can be found for months after the acute phase. Neurofilaments light chain (NfL) have been identified as a useful biomarker in different neuro-inflammatory and neurodegenerative disorders. Preferably, biomarkers are easily accessible with low impact on the patient, so rather serum instead of CSF. Based on the strong correlation between CSF and serum NfL values together with the higher sensitivity of new techniques, serum NfL was considered a potentially useful biomarker. In **chapter 5** we showed that although serum NfL levels are increased in anti-NMDAR encephalitis, these do not provide prognostic value at diagnosis, neither for maximum disease severity nor for prognosis. Secondly, serum NfL levels can help identify relapses retrospectively but not predict them in the acute setting. Deployment of NfL as a biomarker is further discouraged by the fact that the timing of sampling largely affects the values. Anti-NMDAR encephalitis generally has a subacute onset, with rapid progression of severity. NfL increases after diagnosis, following clinical symptoms. It suggests that axonal damage is not an acute initial feature of the disease causing clinical symptoms, rather serum NfL levels likely reflect an integral measure of antecedent and ongoing neuronal damage. This is more of an issue in anti-NMDAR encephalitis compared to other conditions like frontotemporal dementia or multiple sclerosis, in which direct cell toxicity or degeneration is an intrinsic part of the disease mechanism. Currently treatment decisions are based on the clinical assessment due to a lack of useful biomarkers. Future

research could involve broader (omics-based) screening for biomarkers, first hypothesis generating exploring new candidates, followed by proper validation. A disadvantage is the costly and complex analysis, where incidental findings are common given the small sample size in rare diseases.

FINAL REMARKS

In conclusion, this thesis provides several insights in diagnosing AIE and tries to raise awareness for this severe, but mostly treatable disease. We suggest that neuronal antibody testing is indicated in selected patients with a possible dementia when atypical signs are present. Patients with both a rapidly and non-rapidly progressive course can harbor neuronal antibodies. However, a prospective study in RPD patients with extensive antibody testing is lacking to evaluate the real world prevalence of AIE in this subcategory.

Diagnosing AIE is highly relevant and there should be a lower threshold to send samples for testing, preferably earlier in the disease course as outcome improves with fast initiation of immunotherapy. However clinicians should remain vigilant and regard the clinical phenotype cautiously, linking clinic and ancillary testing with the antibody results to distinguish from clinically irrelevant results (in both serum and CSF).

In the acute setting, it would be most relevant to have a tool that predicts failure to first-line therapy, allowing for escalation to more aggressive therapy at disease onset. This can only be achieved within international collaborative studies, as AIE is a rare disease and patient numbers should be increased to allow inclusion of both a development and validation cohort. This applies equally to extensive biomarker research.

For Hu-PNS until today no effective treatment exists. Our trial involving natalizumab infusions did not have the desired effect. Other treatment strategies should be studied, and could focus on other T-cell targeted therapies or combined modulation of B and T-cell activity (e.g. fingolimod, abatacept).

It is likely that the expanding use of immune checkpoint inhibitors will lead to increasing numbers of antibody mediated encephalitis, being one of the reasons why increasing awareness is an important and relevant topic. Recognition and treatment in AIE and PNS is still a work in progress.

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8

Summary / Samenvatting

SUMMARY

Antibody-mediated encephalitis, or autoimmune encephalitis (AIE), is a relative new disease entity since the first antibody was discovered in 2000. The antibodies recognize extracellular proteins in the nervous system resulting in neurological symptoms. The symptoms are characterized by a subacute onset, clarified as a rapid progression of less than 3 months, of neuropsychiatric symptoms. Cognitive deterioration is frequently present and can be predominantly affected. Nowadays it is known that the disease course can be less fulminant with a more protracted disease course. This seems mainly the case in the elderly population. Together this suggests that AIE should be able to mimic neurodegenerative dementia syndromes. Therefore, this thesis focuses on the recognition and treatment in the elderly patient with AIE, with special emphasis on cognitive symptoms and dementia syndromes. At the end of this thesis treatment in paraneoplastic neurological syndromes (PNS) is discussed. PNS have a strong association with malignancies, and the antibodies generally do not react to the extracellular surface of antigens, but recognize intracellular parts. Treatment in PNS generally is less effective compared to AIE and new therapies are an unmet medical need. In **chapter 1**, the general introduction, we provide an overview of the current knowledge of AIE and PNS, and at the end of this chapter our hypotheses are described.

In **chapter 2** we analyze how frequently AIE mimics dementia in elderly patients (≥45 years). Data from nationwide observational cohorts of patients with LGI1, NMDAR, $GABA_BR$, or CASPR2 encephalitis were assessed for fulfillment of dementia criteria. No prominent seizures early in disease course were allowed. This study showed that a neurodegenerative dementia was suspected by the treating physician in half of the patients. Furthermore, AIE can resemble dementia frequently, especially rapidly progressive dementia (RPD; 76%). Other red flags for AIE were seizures, wherein the subtle seizures had been overlooked early in disease course, and lastly abnormalities in ancillary testing atypical for neurodegeneration. Physicians should be aware that inflammatory changes are not always present in AIE (25% had neither inflammatory changes on brain MRI, nor CSF pleocytosis), and that dementia biomarkers (including t-tau, p-tau, Aβ42, and 14-3-3) can be positive in AIE.

In **chapter 3** a large cohort of patients with a (presumed) neurodegenerative dementia diagnosis were analyzed for neuronal antibodies. Patients had various dementia syndromes and were diagnosed in tertiary memory clinics. We found that a small but clinically relevant proportion (7/920 patients, 0.8%) had neuronal antibodies indicative of AIE, including anti-IgLON5 (n=3), anti-LGI1 (n=2), anti-DPPX (n=1), and anti-NMDAR antibodies (n=1). All patients had atypical signs for neurodegenerative dementia includ-

ing: subacute deterioration or fluctuating disease course (over a longer period), other autoimmune disorders, myoclonus, seizures, and pleocytosis. This cohort was mostly devoid of RPD patients, and all antibody-positive patients had a non-rapidly progressive course.

In **chapter 4** we describe the clinical features and pitfalls in the diagnosis of anti-NMDAR encephalitis, emphasizing on late-onset patients (≥45 years). In addition the antibody test characteristics are described in serum and CSF, as NMDAR antibody testing in serum can yield positive but unconfirmed results but data involving CSF are missing. We show that the commonly used test for NMDAR antibody testing (CBA) performs very well with good validity, but it is not perfect. Among others this was demonstrated by the finding that patients can have 'false positive' results in CSF. We want to highlight that anti-NMDAR encephalitis occurs at all ages and is less rare in the elderly patient (19%) than initially anticipated. Later in life the disease course is less outspoken albeit a worse outcome, and all tumors are carcinomas. It remains important to link the clinical phenotype with the antibody results (even in CSF). And in doubt, consult a reference laboratory.

In **chapter 5** we analyze the potential relevance of neurofilament light chain (NfL) as a prognostic biomarker in a large cohort of patient with anti-NMDAR encephalitis, since biomarkers are limited in AIE. NfL had been identified as a valuable marker in other disorders. Retrospectively, pre-treatment serum and paired CSF samples were measured and compared to healthy references. We found a very good correlation between serum and CSF. Serum NfL levels are increased in patients with anti-NMDAR encephalitis (reflecting neuro-axonal damage). However, it has no (or hardly any) prognostic value at diagnosis. Serum NfL can be used to monitor disease activity in the chronic phase, although timing of sampling seems to have a large impact on NfL values, complicating the use as biomarker to identify relapses early.

In **chapter 6** we describe a prospective trial with natalizumab in 20 patients with a progressive paraneoplastic neurological syndrome with anti-Hu antibodies (Hu-PNS). Hu-PNS has a very poor prognosis, and no effective treatment currently exists. We show that after natalizumab treatment functional improvement is rare and achieved in only 10% of the patients, while a stable situation was obtained in another 60%. Together, this suggests that natalizumab may ameliorate the disease course in Hu-PNS. However, this was not superior to other immunosuppressive and immunomodulatory treatment strategies.

Finally, **chapter 7** discusses the main findings of this thesis and provides recommendation for future research.

SAMENVATTING

Antilichaam-gemedieerde encefalitis, of auto-immuun encefalitis (AIE), is een relatief nieuwe ziekte-entiteit. Het eerste antilichaam werd ontdekt in 2000. De antilichamen herkennen extracellulaire eiwitten in het zenuwstelsel en dit zorgt voor de neurologische symptomen. De symptomen worden gekenmerkt door een subacuut begin (een snelle progressie van minder dan 3 maanden) van neuro-psychiatrische symptomen. Cognitieve achteruitgang is vaak aanwezig en kan sterk op de voorgrond staan van de klachten. Tegenwoordig is bekend dat het ziektebeloop minder heftig kan zijn en ook langzaam kan verlopen. Dit lijkt vooral het geval te zijn bij de oudere patiënt. Samen maakt dit dat AIE een neurodegeneratief dementie syndroom zou kunnen nabootsen. Daarom richt dit proefschrift zich op de herkenning en behandeling van de oudere patiënt met AIE, met nadruk op cognitieve symptomen en dementiesyndromen. Aan het eind van dit proefschrift wordt de behandeling van paraneoplastische neurologische syndromen (PNS) besproken. PNS hebben een sterke associatie met maligniteiten en de antilichamen reageren over het algemeen niet tegen extracellulaire antigenen maar herkennen intracellulaire eiwitten. Behandeling bij PNS is over het algemeen minder effectief in vergelijking met AIE en nieuwe therapieën zijn hard nodig. In **hoofdstuk 1**, de algemene introductie, geven we een overzicht van de huidige kennis van AIE en PNS, en aan het einde van dit hoofdstuk worden onze hypothesen beschreven.

In **hoofdstuk 2** analyseren we hoe vaak AIE dementie nabootst bij oudere patiënten (≥45 jaar). Data van landelijke observationele cohorten van patiënten met LGI1-, NMDAR-, $GABA_BR$- of CASPR2-encefalitis werden beoordeeld of er werd voldaan aan criteria voor dementie. Prominente aanvallen in het begin van het ziekteverloop waren niet toegestaan. Uit dit onderzoek bleek dat bij de helft van de patiënten een neurodegeneratieve dementie werd vermoed door de behandelend arts. Bovendien zagen we dat AIE vaak kan lijken op dementie, vooral in de vorm van een snel progressieve dementie (76%). Andere rode vlaggen voor AIE waren epileptische aanvallen, waarbij de subtiele aanvallen vroeg in het ziekteverloop over het hoofd waren gezien. En als laatste rode vlag: afwijkingen in aanvullende onderzoek die atypisch zijn voor neurodegeneratie. Artsen moeten zich ervan bewust zijn dat aanwijzingen voor inflammatie niet altijd aanwezig zijn bij AIE (25% had geen aanwijzingen voor inflammatie op MRI en ook geen CSF pleiocytose), en dat biomarkers voor dementie (waaronder t-tau, p-tau, Aβ42 en 14-3-3) positief kunnen zijn in AIE.

In **hoofdstuk 3** is een groot cohort patiënten met een (vermoedelijke) neurodegeneratieve dementie diagnose geanalyseerd op neuronale antistoffen. De patiënten hadden verschillende dementiesyndromen en werden gediagnosticeerd in tertiaire geheugen-

klinieken. We vonden dat een klein, maar klinisch relevante proportie (7/920 patiënten, 0,8%) neuronale antilichamen heeft passend bij AIE. Deze antistoffen waren anti-IgLON5 (n=3), anti-LGI1 (n=2), anti-DPPX (n=1), en anti-NMDAR (n=1). Alle patiënten hadden atypische kernmerken voor neurodegeneratieve dementie waaronder: subacute achteruitgang of een fluctuerend ziekteverloop (over een langere periode), andere autoimmuunziekten, myoclonus, epileptische aanvallen en pleiocytose. Dit cohort bevatte nauwelijks snel progressieve dementie patiënten en geen van de antilichaam-positieve patiënten had een snel progressief beloop.

In **hoofdstuk 4** beschrijven we de klinische kenmerken en valkuilen bij de diagnose van anti-NMDAR encefalitis, waarbij de nadruk ligt op patiënten met een laat ziektebegin (≥45 jaar). Bovendien worden de kenmerken van de antilichaamtest beschreven in serum en CSF. Het was bekend dat de test om NMDAR-antilichaam te detecteren in serum positieve, maar onbevestigde resultaten kunnen geven, maar data over CSF ontbreken vooralsnog. We laten zien dat de veelgebruikte test voor NMDAR-antilichaam (CBA) zeer goed presteert met een goede validiteit, maar dat het niet perfect is. Dit werd onder meer aangetoond doordat dat patiënten 'vals positieve' resultaten in CSF kunnen hebben. We willen benadrukken dat anti-NMDAR-encefalitis op alle leeftijden voorkomt en minder zeldzaam is bij oudere patiënten (19%) dan aanvankelijk werd gedacht. Later in het leven is het ziekteverloop minder uitgesproken, maar veelal hebben deze patiënten wel een slechtere uitkomst, en alle tumoren zijn carcinomen. Het blijft belangrijk om het klinische fenotype te koppelen aan de antistof resultaten (ook in CSF). Raadpleeg bij twijfel een referentielaboratorium.

In **hoofdstuk 5** analyseren we de mogelijkheid om neurofilament light chain (NfL) in te zetten als biomarker voor het voorspellen van uitkomst in een groot cohort patiënten met anti-NMDAR encefalitis. Er zijn beperkt biomarkers beschikbaar in AIE en NfL blijkt een waardevolle marker in andere aandoeningen. Retrospectief werden serum en gepaarde CSF-monsters (van voor behandeling met immuuntherapie) geanalyseerd en vergeleken met gezonde referenties. We vonden een zeer goede correlatie tussen serum en CSF. Serum NfL spiegels zijn verhoogd bij patiënten met anti-NMDAR-encefalitis (als gevolg van neuro-axonale schade), maar het heeft geen (of nauwelijks) prognostische waarde op het moment van diagnose. Serum NfL kan worden gebruikt om de ziekteactiviteit in de chronische fase te volgen, hoewel de timing van sample afname een grote invloed lijkt te hebben op de NfL-waarden. Dit maakt het gebruik als biomarker om vroegtijdig een terugval te identificeren moeilijk.

In **hoofdstuk 6** beschrijven we een prospectieve studie met natalizumab bij 20 patiënten met een progressief paraneoplastisch neurologisch syndroom met anti-Hu antilicha-

men (Hu-PNS). Hu-PNS heeft een zeer slechte prognose en er bestaat momenteel geen effectieve behandeling. We laten zien dat functionele verbetering na behandeling met natalizumab zeldzaam is en bij 10% van de patiënten wordt bereikt, terwijl bij nog eens 60% een stabiele situatie wordt bereikt. Tezamen suggereert dit dat natalizumab het ziekteverloop bij Hu-PNS kan beïnvloeden, maar dat het niet superieur is aan andere immunosuppressieve en immunomodulerende behandelingsstrategieën.

Tot slot worden in **hoofdstuk 7** de belangrijkste bevindingen van dit proefschrift bediscussieerd en worden er aanbevelingen gedaan voor toekomstig onderzoek.

Appendices

DANKWOORD

Aan al het mooie komt een eind... Wat een fantastische jaren heb ik mogen meemaken waarin zoveel is gebeurd en ik op vele manieren gegroeid ben tot de persoon die ik vandaag de dag ben. Heel wat mensen hebben bijgedragen aan dit proefschrift, veel dank aan allemaal! Een aantal wil ik hier graag persoonlijk bedanken.

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Mijn promotor prof. dr. Peter Sillevis Smitt. Beste Peter ook jou wil ik enorm bedanken voor de afgelopen leerzame jaren en de kans om dit promotietraject te mogen doen. Deze kans is in mijn ogen begonnen tijdens een polonaise op de Babinski skireis, maar of je deze herinnering deelt weet ik niet zeker (ik heb wel een foto als bewijs). Je hebt me alles geleerd over Hu-PNS en de patiënten die we samen gezien hebben, hebben een grote indruk op me achtergelaten. Je deur staat altijd open en het is ongelooflijk hoe snel je bent in het beoordelen/lezen van alles (wat ook voor Maarten geldt). Peter, bedankt voor alles.

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Dank ook aan alle co-auteurs voor het delen van de expertise en de meewerking aan projecten. Juna, je verdient een apart bedankje. Tijdens mijn onderzoekstijd werd jij mede autoimmuun staflid en jouw bijdrage is substantieel op vele vlakken!

Hierbij wil ik ook alle medewerkers op het AIE lab betrekken gezien de enorme berg samples die de afgelopen jaren zijn getest. Zonder deze resultaten zou er geen proefschrift zijn; mijn dank is groot. Ook de samenwerking met het Laboratorium Medische Immunologie was van grote waarde, met Marco Schreurs in het bijzonder. Irene, research verpleegkundige en mijn steun en toeverlaat voor de Hu studie. Je hebt me ontzettend geholpen met alle trial (rand)zaken waarvoor zeer veel dank.

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Twee jaar geleden (augustus 2021) ben ik met mijn opleiding tot neuroloog begonnen en het is genieten wat een hoop leuke (nieuwe en vooral ook jonge haha) collega's er zijn. Bedankt voor alle gezelligheid en collegialiteit. Afgelopen jaar ben ik op de KNF op een andere manier enthousiast geworden voor het vak. Veel dank voor de fijne werkplek, flexibiliteit en regelmaat zodat ik ruimte had om in de avond mijn proefschrift te kunnen schrijven. Robert, bedankt de introductie in AI waarmee ik de omslag deels heb geknutseld. Tessa, ik ben zo blij dat wij de hele stage samen hebben gedaan en we elkaar hierdoor hebben leren kennen. Dankjewel voor het hele fijne jaar! En dat er nog meer spelletjesavonden mogen volgen Maarten, Laurike, Yu Yi, Daan, Tessa en Joyce (KNF zwerver).

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Roel, mijn nummer 1, lieverd zolang jij bij me bent kan ik alles aan. We hebben zoveel hoogtepunten samen meegemaakt maar ook een ondenkbaar diep dal. Dit hebben we samen gedaan en heeft onze band alleen maar gesterkt. Ik ben trots op wat je bereikt in je carrière, je bent in de wieg gelegd voor je vak. Daarnaast ben je de allerbeste papa en geniet ik met volle teugen als je lekker met onze jongens in de weer bent. Ook ben jij degene die het meest met me te verduren heeft, ik waardeer je geduld. Bedankt voor de vele theetjes (en koekjes) als ik weer eens achter m'n laptop verstopt zat en ik de tijd niet kon vinden om dit zelf te doen. Bedankt dat je er bent en voor de mooie jaren die nog gaan komen.

Ties en Mats, mijn prachtige kleine draken. Ik geniet ervan dat jullie groter worden en we de wereld steeds verder gaan ontdekken samen. Jullie maken mijn leven compleet en ik ben ontzettend trots om jullie mama te zijn. Ik ben dolgelukkig dat jullie er zijn en ik kijk uit naar onze toekomst met z'n vieren.

Lieve kleine Julie, mijn laatste woorden voor jou. Ik denk aan je en zal je nooit vergeten.

ABOUT THE AUTHOR

Daniëlle (A.E.M.) Bastiaansen is geboren op 28 januari 1986 in Breda, in Nederland. Haar VWO diploma behaalde ze in 2004 (Mencia de Mendoza Lyceum te Breda). In dat jaar begon zij aan haar studie Biomedische wetenschappen aan de Universiteit van Utrecht waar ze in 2007 haar bachelor diploma behaald. In 2007 begon zijn aan haar Geneeskunde opleiding aan de Erasmus Universiteit in Rotterdam. 6 jaar later in november 2013 haalde ze haar diploma. Tijdens haar opleiding werkte ze jaren op de afdeling neurologie om de verpleging te ondersteunen als medisch student en tevens werd haar oudste co-schap gedaan op deze afdeling. Na het behalen van haar artsenexamen werkte ze als ANIOS neurologie in het Albert Schweitzerziekenhuis in Dordrecht tot ze begin 2015 terugkeerde naar het Erasmus Medisch centrum. Hier werkte ze eerst als ANIOS waarna ze aan haar promotietraject begon in 2016 tot en met 2021 onder begeleiding van Maarten Titulaer en Peter Sillevis Smitt. Tijdens haar promotietraject kreeg ze een opleidingsplek tot neuroloog en met deze opleiding startte ze vervolgens in 2021 en hier is ze op dit moment nog mee bezig. Daniëlle woont samen met Roel Bosman in Rotterdam en tijdens haar promotietraject kregen ze drie kinderen, Julie* (2017), Ties (2018), en Mats (2021).

LIST OF PUBLICATIONS

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*Both authors contributed equally to this work.

Other publications

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Muñoz-Lopetegi A, de Bruijn M, Boukhrissi A, **Bastiaansen AEM,** Nagtzaam MM, Hulsenboom ES, Boon AJ, Neuteboom RF, de Vries JM, Sillevis Smitt PA, Schreurs MW, Titulaer MJ. Neurological syndromes associated with anti-GAD65 Clinical and serological response to treatment. Neurol Neuroimmunol Neuroinflamm 2020;7:e696.

de Bruijn M, **Bastiaansen AEM,** Mojzisova H, van Sonderen A, Thijs RD, Majoie HJ, Rouhl RP, van Coevorden-Hameete MH, de Vries JM, Muñoz Lopetegi A, Roozenbeek B,. Sch-

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Bastiaansen AEM, Timmermans AM, Smid M, van Deurzen CHM, Hulsenboom ESP, Prager-van der Smissen WJC, Foekens R, Trapman-Jansen AMAC, Sillevis Smitt PAE, Luider TM, Martens JWM, vanDuijn MM. Metabotropic glutamate receptor 1 is associated with unfavorable prognosis in ER-negative and triple-negative breast cancer. Sci Rep. 2020 Dec 18;10(1):22292.

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PHD PORTFOLIO

	Year	ECTS
General courses		
BROK (Basiscursus Regelgeving Klinisch Onderzoek), NFU BROK Academie	2016	1.5
Course Patient Oriented Research: design, conduct and analysis	2016	0.3
Research Integrity Course	2017	0.3
Biomedical English Writing and Communication	2019	3
Specific courses		
Basic and translational oncology	2016	1.8
Biomedical Research Techniques	2016	1.5
Basic course on SPSS	2016	1
Basic and advanced course on Access	2016	0.3
Advanced Immunology 4-day short course	2017	2
Biostatistical methods I: basic principles A	2017	2
AIOS course Neuro-immunology and Infection	2020	0.5
Biemond Course Inflammation and Infection	2020	0.5
Presentations		
European School of Neuro-immunology course, Venice, Italy, poster presentation	2017	1
Encephalitis Society Conference, London, oral presentation	2019	0.6
European Association of Neurology congress, Oslo, Norway, oral presentation	2019	1.5
Encephalitis Society Conference, London, oral presentation	2020	0.6
Translation neuroscience network, Amsterdam, oral presentation	2020	0.6
Encephalitis Society Conference, London, poster presentation	2021	0.6
Referaat afdeling neurologie, Erasmus MC	2022	1.2
Attended (Inter)national conferences and workshops		
Wetenschapsdagen Nederlandse Vereniging Neurologie, Nunspeet	2016-2017	1
Italian Association of Neuroimmunology congress, Venice, Italy	2017	1
Translation neuroscience network, Amsterdam	2017	0.3
Teaching		
Supervising medical students, Erasmus MC University Medical Center	2016	2
Teaching physical examination to medicine students	2016-2021	0.2
Teaching course, Master Infection & Immunity	2018	0.2
Supervising master thesis, medical student	2020	3
Supervising master thesis, psychology student	2020	3
Alzheimer research group, Rotterdam, oral presentation	2020	0.5
Other		
Neuro-immunology meeting (multidisciplinary patient consultation, monthly)	2016-2021	0.5
Research meeting (weekly), Laboratory of neuro-oncology	2016-2021	1
Outpatient clinic neuro-immunology (and telephone-email consultations)	2016-2021	2
Journal club neuro-immunology	2017-2018	1