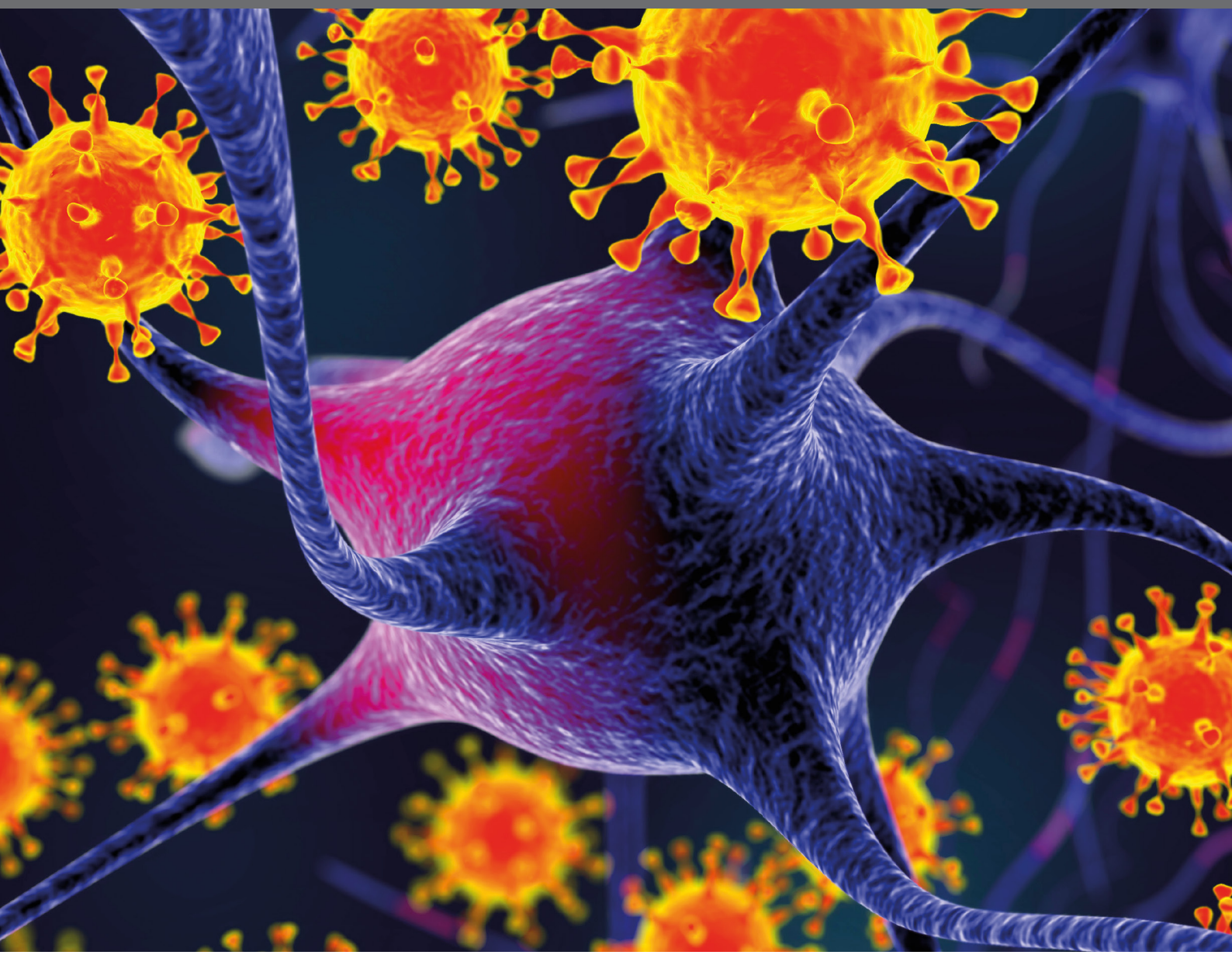
EMERGING CHALLENGES IN THE DIAGNOSIS AND TREATMENT OF AUTOIMMUNE ENCEPHALITIS

EDITED BY: Morten Blaabjerg, Thomas Seifert-Held and Johann Sellner
PUBLISHED IN: Frontiers in Neurology and Frontiers in Immunology



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88945-830-1
DOI 10.3389/978-2-88945-830-1

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

EMERGING CHALLENGES IN THE DIAGNOSIS AND TREATMENT OF AUTOIMMUNE ENCEPHALITIS

Topic Editors:
Morten Blaabjerg, Odense University Hospital and University of Southern Denmark, Denmark
Thomas Seifert-Held, Medical University of Graz, Austria
Johann Sellner, Paracelsus Medical University, Austria and Technische Universität München, Germany

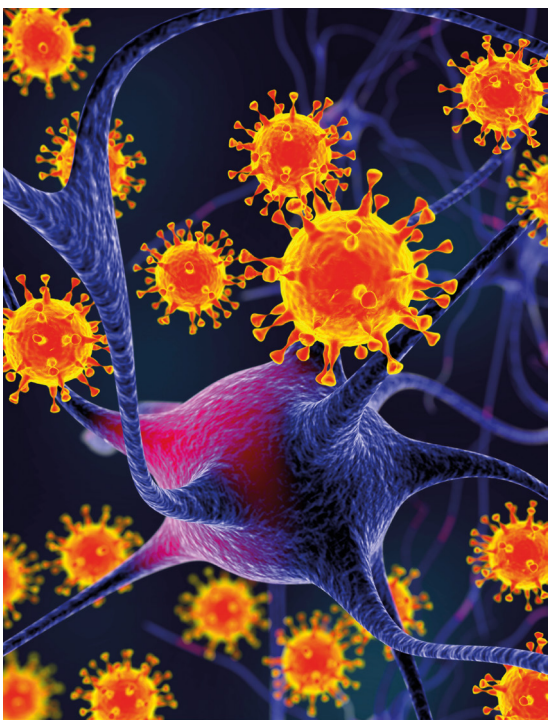


Image: Kateryna Kon/Shutterstock.com

The Research Topic entitled "Emerging Challenges in the Diagnosis and Treatment of Autoimmune Encephalitis" covers recent developments in an rapidly expanding field. We believe that the present Frontiers Research Topic eBook will provide the interested readers with updated knowledge on autoimmune encephalitis including real life clinical experience in diagnostic challenges, differential diagnosis and treatment of patients with autoimmune encephalitis.

Citation: Blaabjerg, M., Seifert-Held, T., Sellner, J., eds. (2019). Emerging Challenges in the Diagnosis and Treatment of Autoimmune Encephalitis. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-830-1

Table of Contents

frontiers in Neurology

EDITORIAL
published: 25 February 2019
doi: 10.3389/fneur.2019.00146

Editorial: Emerging Challenges in the Diagnosis and Treatment of Autoimmune Encephalitis

Morten Blaabjerg[1,2], *Thomas Seifert-Held*[3] *and Johann Sellner*[4,5]*

[1] Department of Neurology, Odense University Hospital, Odense, Denmark, [2] Department of Clinical Research, University of Southern Denmark, Odense, Denmark, [3] Department of Neurology, Medical University of Graz, Graz, Austria, [4] Department of Neurology, Christian Doppler Medical Center, Paracelsus Medical University, Salzburg, Austria, [5] Department of Neurology, Klinikum Rechts der Isar, Technische Universität München, München, Germany

Keywords: autoimmune, encephalitis, diagnosis, therapy, outcome, challenges

Editorial on the Research Topic

Emerging Challenges in the Diagnosis and Treatment of Autoimmune Encephalitis

Autoimmune encephalitis (AIE) is a group of antibody-mediated inflammatory CNS diseases with a variety of neurological and psychiatric symptoms. Patients often have deficits of subacute onset with affection of both limbic (e.g., amnesia, confusion, and epileptic seizures), and extra-limbic brain structures (1). Previously, AIE was considered a very rare paraneoplastic condition associated with intracellular (onconeural) antibodies and a very poor prognosis (e.g., anti-Hu syndrome) (2). However, during the last two decades it has become evident that AIE is much more frequently associated with antibodies directed against synaptic/cell surface proteins and often an underlying malignancy is absent. More than 10 synaptic antineuronal and glial antibodies associated with AIE have been identified, and new antibodies are described at an astonishing pace; many patients with so-called seronegative AIE will probably harbor antibodies that are yet to be isolated. Prognosis in AIE can be good, if aggressive immunomodulatory treatment is initiated early (3). Yet, chronic cognitive deficits, epilepsy and mood disorders are frequent, and this is particularly concerning in young patients who frequently are unable to join the workforce again (4). Thus, in addition to significant health-related concerns for the individual, AIE is also associated with substantial socioeconomic burden.

In the 11 articles that form this Frontiers Research Topic, eBook, the readers will find an update on key aspects including diagnostic challenges, pitfalls in antibody testing and clinical experience with management and treatment of AIE and related disorders from different expert centers.

Firstly, the current syndromes associated with antibodies against cell surface antigens, including the use of the current diagnostic criteria and treatment options are presented in a mini review paper by Hermetter et al. This is followed with real life clinical experience in a monocentric study of 38 consecutive patients with AIE presented by Macher et al. In this paper the important aspect of when to stop immunotherapy after the acute phase of encephalitis is also discussed based on their clinical experience.

One of the most important aspects in the diagnosis of AIE is identification of autoantibodies in CSF and/or serum. This is currently done using different antibody assays. The pitfalls of antibody detection and the use of different assays (e.g., cell or tissue based assays, radioimmunoassay) is elegantly reviewed by Ricken et al. This paper highlight the current state of the art in antibody testing dependent on antibody subtype and provides important consideration on assay choice and interpretation. The potential pitsfalls in antibody testing is further highlighted in a case description by Bien, presenting a case of overinterpretation and overtreatment of a patient with a low-titer contactin-associated protein-like 2 (Caspr2) antibody. Moreover,

OPEN ACCESS

Edited and reviewed by:
Hans-Peter Hartung,
Heinrich Heine Universität Düsseldorf, Germany

***Correspondence:**
Johann Sellner
j.sellner@salk.at

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *07 January 2019*
Accepted: *05 February 2019*
Published: *25 February 2019*

Citation:
Blaabjerg M, Seifert-Held T and Sellner J (2019) Editorial: Emerging Challenges in the Diagnosis and Treatment of Autoimmune Encephalitis. Front. Neurol. 10:146. doi: 10.3389/fneur.2019.00146

an attempt to optimize antibody detection is provided by Chiu et al. who present changes to the conventional anti-NMDAR antibody assay in order to increase detection rates.

Infectious encephalitis (IE) is one of the main differential diagnoses in the acute phase of AIE and due to the need for early treatment initiation, discrimination between these conditions are crucial. In their paper, Wagner et al. included all patients from their center with diagnosis of encephalitis over a 10-year period (33 AIE, 51 IE), and present some interesting distinctive clinical features (e.g., headache, fever, psychiatric symptoms). Applying the current diagnostic criteria for AIE to this cohort however, yielded very low diagnostic sensitivity and specificity. They moreover found this phenomenon to be clearly time dependent.

An association between demyelinating diseases and AIE has been established. The paper by Borisow et al. reviews these diseases and gives an overview on the diagnosis and treatment of neuromyelitis optica spectrum disorders (NMOSD) and the more recently described myelin-oligodendrocyte-glycoprotein (MOG)-associated encephalomyelitis. The paper highlights the differences in epidemiology, imaging and provides current knowledge on treatment strategy.

As the field of AIE expand, clinical descriptions of cases that differ from the original published case series are important to expand knowledge on the different antibody specific phenotypes. Two such cases are presented in this ebook. Montagna et al. describe a case of IgLON5-associated encephalitis with severe inflammatory lesions on brain MRI and no tau pathology on brain biopsy. The presented patient moreover had an atypical presentation with a rapid cognitive decline and a good response to immunotherapy. Interestingly, they found antibodies exclusively of the IgG1 subclass and not the prodominant IgG4 subclass initially described with IgLON5 encephalitis.

Similarly Bartels et al. describe three cases of Anti-ARCHGAP26 (RhoGTPase-activating protein 26) antibodies in whom, two were associated with isolated cognitive impairment and not with the cerebellar ataxia phenotype usually associated with this antibody. All three cases were found to have underlying malignancy.

Besides the general treatment recommendations described above, two further papers deals with this aspect. The paper by Mäkelä et al. focuses on the difficult clinical management of epilepsy associated with the Glutamic acid decarboxylase (GAD65) antibody. They present 6 cases from their center treated between 2013 and 2017, and highlight the importance of early immunotherapy in order to prevent tissue damage and refractory epilepsy in this patient group.

Finally, Zhu et al. performed an experimental interleukin-27 gene therapy using adeno-associated viral vector delivery to an experimental model of inflammatory disease. They were able to prevent the development of experimental autoimmun encephalomyelitis (EAE) in this model, but this approach was not effective in established inflammation likely due to expansion of myeloid cells.

In summary, although the field is rapidly expanding, we believe that the present Frontiers Research Topic eBook will provide the interested readers with updated knowledge on AIE including real life clinical experience in diagnostic challenges, differential diagnosis and treatment of patients with AIE.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Leypoldt F, Armangue T, Dalmau J. Autoimmune encephalopathies. *Ann N Y Acad Sci*. (2015) 1338:94–114. doi: 10.1111/nyas.12553
2. Berzero G, Psimaras D. Neurological paraneoplastic syndromes: an update. *Curr Opin Oncol*. (2018) 30:359–67. doi: 10.1097/CCO.0000000000000479
3. Titulaer MJ, McCracken L, Gabilondo I, Armangué T, Glaser C, Iizuka T, et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. *Lancet Neurol*. (2013) 12:157–65. doi: 10.1016/S1474-4422(12)70310-1
4. Yeshokumar AK, Gordon-Lipkin E, Arenivas A, Cohen J, Venkatesan A, Saylor D, et al. Neurobehavioraloutcomes in autoimmune encephalitis. *J Neuroimmunol*. (2017) 312:8–14. doi: 10.1016/j.jneuroim.2017.08.010

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 22 November 2018
doi: 10.3389/fimmu.2018.02708

Management of Autoimmune Encephalitis: An Observational Monocentric Study of 38 Patients

*Stefan Macher[1], Friedrich Zimprich[1], Desiree De Simoni[2], Romana Höftberger[2] and Paulus S. Rommer[1]**

[1] Department of Neurology, Medical University of Vienna, Vienna, Austria, [2] Institute of Neurology, Medical University of Vienna, Vienna, Austria

Over the last years the clinical picture of autoimmune encephalitis has gained importance in neurology. The broad field of symptoms and syndromes poses a great challenge in diagnosis for clinicians. Early diagnosis and the initiation of the appropriate treatment is the most relevant step in the management of the patients. Over the last years advances in neuroimmunology have elucidated pathophysiological basis and improved treatment concepts. In this monocentric study we compare demographics, diagnostics, treatment options and outcomes with knowledge from literature. We present 38 patients suffering from autoimmune encephalitis. Antibodies were detected against NMDAR and LGI1 in seven patients, against GAD in 6 patients) one patient had coexisting antibodies against $GABA_A$ and $GABA_B$), against CASPR2, IGLON5, YO, Glycine in 3 patients, against Ma-2 in 2 patients, against CV2 and AMPAR in 1 patient; two patients were diagnosed with hashimoto encephalitis with antibodies against TPO/TG. First, we compare baseline data of patients who were consecutively diagnosed with autoimmune encephalitis from a retrospective view. Further, we discuss when to stop immunosuppressive therapy since how long treatment should be performed after clinical stabilization or an acute relapse is still a matter of debate. Our experiences are comparable with data from literature. However, in contrary to other experts in the field we stop treatment and monitor patients very closely after tumor removal and after rehabilitation from first attack.

Keywords: autoimmune encephalitides, Iglon5, NMDAR, GAD 65, GAD67, autoantiboides

OPEN ACCESS

Edited by:
Johann Sellner, Universitätsklinikum Salzburg, Austria

Reviewed by:
Judith N. Wagner, Johannes Kepler University of Linz, Austria
Elisabeth Gulowsen Celius, Oslo University Hospital, Norway

***Correspondence:**
Paulus S. Rommer
paulus.rommer@meduniwien.ac.at

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology

Received: *30 June 2018*
Accepted: *01 November 2018*
Published: *22 November 2018*

Citation:
Macher S, Zimprich F, De Simoni D, Höftberger R and Rommer PS (2018) Management of Autoimmune Encephalitis: An Observational Monocentric Study of 38 Patients. Front. Immunol. 9:2708. doi: 10.3389/fimmu.2018.02708

INTRODUCTION

An association between malignancies and neurological symptoms not directly caused by the tumor itself has been described by Brouwer in 1919 and later on by Parker in 1933 (1). Thirty years ago antibodies targeting antigens Hu, Ri, Yo (anti-Hu, anti-Ri, anti-Yo) in patients with malignancies have been detected. Neurological symptoms in patients with malignancy has introduced the concept of paraneoplastic syndromes (PNS) in Neurology. Autoimmune mechanisms are hypothesized as pathopyhsiological background in PNS, as antibodies released in response to the underlying cancer are frequently found. The peripheral as well as the central nervous systems (CNS) can be affected. Encephalitis is often reported in cases with involvement of the CNS (2). Autoimmune encephalitis has to be differentiated from the PNS. It is defined as a disorder of the gray matter of the CNS that is caused by antibodies. These antibodies are targeting intracellular or surface antigens of neuronal cells in the CNS. Some of them are released

in response to an existing tumor, but not restricted to malignancies—as in the case of antibodies against aquaporin-4 and myelin oligodendrocyte glycoprotein (MOG). Studies on these antibodies have revolutionized our neuro-immunological concepts. Early and correct diagnosis is highly relevant, as treatment options are available. However, the clinical spectrum is broad and it is important to look beyond the borders of neurology and to integrate other medical disciplines in our concepts of disease management. Especially psychiatric symptoms are often associated with autoimmune mediated encephalitis. Limbic encephalitis (LE) is a frequent manifestation and is defined as inflammation of (but not restricted to) the limbic region in the brain. It has been described for the first time almost 60 years ago (3), and its association with cancer was reported in 1968 (2). Typical symptoms are subacute onset of seizures, short-term memory loss, confusion and other psychiatric symptoms (4). Over the last years, LE seems to be more common as previously assumed. It is often unrelated to an underlying malignancy (5). The incidence of encephalitis of any cause (not only autoimmune mediated) is about 2–3/100.000 (6). The leading causes are infections, but in about 20% of patients, an autoimmune genesis is suspected. In a major part of the patients no definitive cause is established (6). Prevalence of autoimmune mediated encephalitis is 13.7/100.000 per 2014. Retrospective analysis of patients below the age of 35 years admitted to a German intensive care unit (ICU) because of encephalitis of unknown origin showed that 1% of all patients suffered from N-methyl-D-aspartate-receptor-(NMDAR) encephalitis and a British prospective population based study revealed high numbers of patients suffering from acute demyelinating encephalomyelitis (ADEM) and or voltage-gated-potassium-channels (VGCC) or NMDAR-encephalitis. Autoimmune-mediated encephalitis is more common than previously assumed (7).

Increased awareness and testing over the last years has led to a more frequent diagnosis of autoimmune encephalitis. The diverse clinical symptoms hamper diagnosis and consequently the treatment, thereby influencing the outcome and prognosis of the patients.

The aim of our paper is to propose support in the management, diagnosis, and treatment of patients with immune mediated encephalitis based on pathophysiological concepts from the literature and the presentation of patients treated at our hospital. In our patients' cohort clinical symptoms, diagnostic approaches, pathophysiological considerations for treatment, treatment options and outcomes are presented.

METHODS

Current knowledge on the background and management of autoimmune mediated encephalitis diagnosed at our center is summarized. Subsequently, patients with encephalitis treated at our hospital are presented. Diagnostics, treatment and outcome are highlighted. Diagnostic and treatment algorithms will be compared with those in literature; differences in the management will be discussed. For this monocentric study all patients with a diagnosis of autoimmune mediated encephalitis who were treated at the department of Neurology at the Medical University of Vienna between 2015 and June 2018 are reported. Immunological assessment was performed by the clinical institute of Neurology. Serum and CSF samples were investigated with indirect immunohistochemistry for surface antibodies on post-fixed rat brains and for intracellular antibodies on fixed rat cerebellum using an avidin-biotin peroxidase technique. Samples showing specific tissue staining were further examined with a commercial immunoblot assay (Ravo Diagnostika, GmbH, Freiburg, Germany) for antibodies against classic paraneoplastic antigens (Hu, Yo, Ri, CV2, amphiphysin, Ma1/2, SOX1, and GAD65). Characterization of cell surface antibodies was established using a cell-based assay (commercial kit, Euroimmun, Lubeck; in-house; HEK293T cells expressing IgLON5, mGluR1, mGluR5, GABA(A)R, AMPAR, and glycin receptor). The treating physician proposed treatment. Outcome was assessed by specialists in neurology and categorized according the modified Rankin Scale (mrs) (8). The analysis gained by the local ethics committee (Medical University of Vienna, Vienna, Austria; 1773/2016).

REVIEW OF LITERATURE

The presentation of literature starts with the diagnostics procedure. Based on the diagnostics steps the various antibodies and their pathophysiological background causing encephalitis are will described in detail. The review ends with the proposed treatment strategies for the respective antibodies.

Diagnostics

Anamnesis

The medical history of patients and a detailed anamnesis on the evolvement of symptoms and the course of symptoms is the first step in the diagnosis of patients with immune mediated encephalitis. Medical history has to include previous or existing malignancies. The detection of antibodies, nevertheless, may precede the diagnosis of a malignancy for many years. Careful and repeated tumor screening as well as tumor surveillance have to be performed. Associated malignancies are gynecological cancers like ovarian and breast cancer (9), tumors of the lungs, i.e., small cell lung cancers (10), thymoma (11), but also testicular tumors, and Hodgkin's lymphoma (12). Some of the antibodies refer to certain malignancies and vice-versa being highly relevant in the diagnostic process. **Table 1** gives an overview of detected malignancies in our cohort of patients with encephalitis. Some of the patients are referred from other medical disciplines, thus an interdisciplinary management eases the appropriate tentative diagnosis. Especially referrals from psychiatrists are quite common in patients with autoimmune encephalitis. Patients are diagnosed with atypical psychosis

Abbreviations: ADEM, acute demyelinating encephalomyelitis; CSF, cerebrospinal fluid; CBA, cell based assay; DMT1, diabetes mellitus type I; ICU, intensive care unit; MRI, magnetic resonance imaging; NFL, neurofilament light chain; NMDA-R encephalitis, N-methyl-D-aspartat-receptor encephalitis; OCB, oligoclonal bands; SPS, stiff person syndrome; SPSD, stiff person spectrum disorder.

TABLE 1 | Initial findings in patients with autoimmune encephalitis at the time of first hospitalization.

Ab *n*	Positivity serum (s) liquor (csf)	Age in years (mean, range)	Sex (f in %)	Initial symptoms	MRI	EEG	CSF findings	Coexisting malignancy	Treatment	Symptoms to treatment (rounded; mean, range)	Hospitalization to 2nd line treatment (rounded; mean, range)	AED used
CASPR-2 *N* = 3	S = 3 Csf = 2	64 (56-68)	0	Psychiatric, mnestic, cognitive dysfunction, speech arrest	T2 abnormalities hippocampal (67%)	Regional slowing, generalized sharp waves (33%),	total protein elevation (33%)	Neuroendocrine tumor (33%)	Pulsed steroids, IVIG, PLEX, RTX, steroid maintenance	4 months	36 days (only one patient received 2nd line therapy)	LEV, LCM, PHT, VPA (66%)
LGI 1 *N* = 7	S = 6 Csf = 4	65 (47-77)	29	Psychiatric, cognitive, mnestic deterioration, vertigo, FBDS, pilomotor seizures, muscle cramps	T2 signal alterations mesiotemporal uni- or bilateral (80%)	Epileptiform activity (43%)	Mild pleocytosis (20%), protein elevation (80%)	None	Pulsed steroids, IVIG, PLEX, RTX, AZA, steroid maintenance	5 months (4 weeks-11 months)	6 months (3 weeks-24 months)	LEV, LTG, LCM, CBZ (86%)
NMDAR *N* = 7	S = 7 Csf = 7	28 (19-41)	71	Psychiatric, cognitive, mnestic dysfunction, myoclonus, seizures, focal dystonia, catatonia	T2 abnormalities hippocampal (29%) T2 abnormalities juxtacortical (14%)	Epileptiform activity (14%) Generalized slowing (14%)	Pleocytosis (86%) Protein elevation (71%) intrathecal IgG-synthesis (43%) OCB pos (71%)	Ovarian teratoma (43%) diffuse large B-cell lymphoma (14%)	Pulsed steroids, IVIG, PLEX, RTX, CYC, MTX, bortezomib, steroid maintenance	15 days (4 days–5 weeks)	4 weeks (3 weeks-6 weeks)*	LEV, LCM, VPA, PHT, FBM (71%)
AMPAR *N* = 1	S = 1	20	0	Cognitive dysfunction	Unilateral T2 signal alteration hippocampal	Normal	Mild protein elevation	None	Pulsed steroids, PLEX, RTX	7 days	3 weeks	None
IGLON5 *n* = 3	S+csf = 3	71 (64-76)	100	Cognitive dysfunction epileptic seizure bilateral vocal cord palsy vertigo ataxia	T2 abnormalities: hippocampal (33%), globus pallidus bilateral (33%)	Epileptiform activity (33%)	Protein elevation (100%)	None	Pulsed steroids, IVIG, immunoadsorption RTX, AZA, CYC, steroid maintenance	69 months(2 months–10 years)	26 months (10 weeks- 28 months), one patient did not receive second line therapy	LEV (66%)
Glycin *n* = 3	S = 3	52 (48-55)	66	Paraspasticity multiple cranial nerve palsies	Hemosiderin deposits in brainstem, rostral cervical myelon, cerebellum after cerebral hemorrhage (33%)	Unremarkable (only available from 2 patients)	Protein elevation (33%), no CSF available for one patient	None	IVIG, PLEX, RTX, steroid maintenance	32 months (2 weeks-60 months)	41 days (a single patient received second line therapy)	LTG (33%)
GAD (GAD-65 *n* = 4; GAD-67 *n* = 1; GAD, $GABA_A$ and $_B$ = 1)	S = 2 Csf = 3	50 (27-56)	83	Vertigo, stiffness, ataxia, dysarthria, epileptic seizures, catatonia	Multiple T2 lesions cortical, juxtacortical, infra- and supratentorial (17%) Atrophy and sclerosis of the hippocampal region (17%)	Only available for one patient (17%) with normal findings	Pleocytosis (20%) OCB pos (80%) Protein elevation (40%) Intrathecal IgG-synthesis (20%) n.a. (20%)	None	pulsed steroids, IVIG, RTX, PLEX MTX, CYC, AZA, mitoxantron, intrathecally TCA, dimethyl-fumarate	12 months (2weeks - 36 months); data only available from 4 patients	47 months (9 months; 7 years)**	GBN, PGN (33%)
Yo *N* = 3	S = 3	58 (52-68)	100	Vertigo, dysarthria, ataxia, mucle crampi	Normal findings (100%)	n.a.	Pleocytosis (67%) OCB pos (100%) Intrathecal IgG-synthesis (67%)	Ovarian cancer (67%) Breast cancer (33%)	pulsed steroids, IVIG, PLEX, RTX, CHT/Rx	14 months (12 months, 15 months), data not available from 1 patient***	15 months (13 months, 17 months)	None
Ma-2 *N* = 2	S+csf = 2	66 (60-71)	100	Psychiatric, DBN, ataxia, rigor	T2 signal alterations in hippocampal region, frontobasal and basal ganglia (50%), global atrophy, small vessel disease (50%)	Normal (50%)	Pleocytosis (50%) Protein elevation(100%) Intrathecal IgG-synthesis (50%) OCB pos (50%)	Lung cancer (50%), cervical carcinoma (50%)	pulsed steroids, IVIG, CYC, steroid maintenance, CHT/Rx	12 months (2weeks, 24 months)	6 months (only one patient received 2nd line therapy)	None

(Continued)

TABLE 1 | Continued

Ab n	Positivity serum (s) liquor (csf)	Age in years (mean, range)	Sex (f in %)	Initial symptoms	MRI	EEG	CSF findings	Coexisting malignancy	Treatment	Symptoms to treatment (rounded; mean, range)	Hospitalization to 2nd line treatment (rounded; mean, range)	AED used
CV-2 $N = 1$	S+csf = 1	51	0	Vertigo, ataxia, paraparesis	Unremarkable	n.a.	Pleocytosis, protein elevation, OCB pos.	Lung cancer	Steroid maintenance, CHT/Rx	7 months	None	None
TPO/TG $N = 2$	S = 2	31 (21-40)	50	Epileptic seizures cognitive dysfunction, psychiatric symptoms (paranoia, psychosis)	Unremarkable (100%)	Epileptiform activity (50%), generalized slowing (50%)	Pleocytosis (50%), protein elevation (50%)	None	Pulsed steroids, IVIG, AZA, steroid maintencance	42 months (24 months, 60 months)	30 months (only one patient received 2nd line therapy)	LEV, CBZ, LTG, LCM, TPM, PER (100%)

Azathioprine (AZA), Cyclophosphamide (CYC), Intravenous immunoglobulins (IVIG), Plasma-exchange (PLEX), Rituximab (RTX), Methotrexate (MTX), chemotherapy and/or radiotherapy (CHT/Rx), Levetiracetam (LEV), Carbamazepine (CBZ), Lamotrigine (LTG), Lacosamide (LCM), Topriamate (TPM), Perampanel (PER), Felbamate (FBM), Gabapentine (GBN), Pregabaline (PGN), Valproate (VPN).

**The patient suffering from DLBCL had chronic immunosuppression with MMF and received RTX 9 months after initial hospitalization due to NMDAR ab positivity.*

***3/6 patients received 2nd line therapy, medical record is only available from 2 patients.*

****Symptoms to treatment in PNS concerns either first or second line therapy as used for autoimmune encephalitis but not start of chemotherapy.*

showing clear psychotic symptoms, but diagnostic criteria for specific syndromes are not yet fulfilled (13).

Clinical Presentations

Patients suffering from encephalitis may present with manifold symptoms including ataxia, cerebellar syndromes, movement disorders and chorea, bulbar dysfunctions, stiff person syndrome (SPS) and progressive encephalomyelitis with rigidity and myoclonus (PERM), opsoclonus-myoclonus-ataxia, seizures, down beat nystagmus, autoimmune-related retinopathy and optic neuropathy, autonomic dysfunction, neuropathic pain, peripheral nerve hyperexcitability, (atypical) psychosis and confusion, cognitive decline, sleep disorders, insomnia, and weight loss. In patients with prior history of malignancy, a new onset of neurological symptoms is suspicious for paraneoplastic syndromes. In patients with no prior history of malignancy the diagnostic procedure is more challenging and has to take into account possible malignancies (14).

Magnetic Resonance Imaging (MRI)

Brain MRI has to be performed in all patients that raise suspicion of encephalitis. In a majority of patients with NMDAR encephalitis brain MRI does not show any abnormalities at onset of symptoms (15). When abnormalities are detected they are non-specific (16). In contrast, MRI abnormalities can usually be found in patients with LE and antibody against Leucine-rich glioma Inactivated 1 (LGI1) and α-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid receptor (AMPAR) (17). Imaging studies in anti-LGI1 patients frequently show abnormalities in the hippocampal region and the temporal lobe. Bilateral hippocampal volume reduction has been reported with exception of the cornu ammonis (CA 1) region (18). Hippocampus atrophy and mesial temporal sclerosis is often observed in patients with VGCC complex antibodies, brain atrophy may be reversible in anti-NMDAR encephalitis (19–22). Infectious encephalitis (especially herpes simplex virus, HSV) is an important differential diagnosis. In most cases, abnormalities in the hippocampal region do not show contrast enhancement, diffusion restriction, or necrosis in autoimmune encephalitis, which may be helpful to differentiate from infectious encephalitis. Absence of basal ganglia involvement in temporomesial lesions may be suggestive of HSV (23). Patients with anti-Contactin-associated protein-like 2 (CASPR2) antibody associated encephalitis show these abnormalities to a much lesser extent (6). Nevertheless, contrast enhancement has been reported in paraneoplastic encephalitis (24). In patients with Glycin-R antibodies, two out of three patients do not show abnormalities on brain and spinal cord MRI. Abnormal cMRI results included unspecific alterations like small vessel disease (SVD) and white matter lesions (WML) (25). Brain MRI is usually normal at onset of symptoms in patients with onconeural antibodies (anti-Yo). Cerebellar atrophy might be visible after paraneoplastic cerebellar degeneration (PCD) is established (1). In half of the patients with anti-Hu antibodies abnormalities on MRI can be found (26, 27). Further patients with epileptic seizures may show temporal diffusion restriction (low ADC value) which may not necessarily indicate limbic encephalitis (28). MRI is essential for

ruling out other causes; however, detected abnormalities in brain MRI might not be specific for the various antibodies.

Cerebrospinal Fluid (CSF) and Electroencephalogram (EEG)

As MRI in patients with HSV-encephalities often shows abnormalities in the temporal pole that are similar to those in patients with LE, it might be difficult to differentiate between both causalities. Thus, patients' CSF has to be analyzed. Whereas, the CSF in patients with infectious encephalitis shows pleocytosis with a moderately to highly elevated cell count and the infectious agent can frequently be detected by PCR, CSF findings are not specific for the various syndromes (paraneoplastic or not) and for respective antibodies. CSF findings can be normal, but also mild to moderate elevated cell count is seen in patients with autoimmune encephalitis (see **Table 1**). Over course of time, the cell count may normalize and intrathecal synthesis and oligoclonal bands (OCBs) may be present. Titres in the CSF for the various antibodies might differ or might—as seen for some cases of anti-NMDAR encephalitis—only be detectable in CSF and be more predictable for disease activity (13, 16, 29). In conclusion the CSF is helpful in differentiating between infectious and non-infectious disease (6), but can be normal and there are no distinct patterns for the various autoantibodies associated syndromes.

Similarly, the EEG may be helpful, although non-specific abnormalities are seen in infectious and immune-mediated encephalitis (6). Some EEG findings—the so called extreme delta brushes—have been reported in adults with anti-NMDAR encephalitis (30, 31).

The appropriate tentative diagnosis should take into account the results from lumbar puncture (and the correct interpretation of it) as well as the medical history, anamnesis, EEG findings, and the results from MRI. Based on the findings the suspected syndrome should be confirmed by testing for autoimmune encephalitis associated autoantibodies. Antibodies are targeting either intracellular antigens or surface antigen (neuropil antibodies). See **Figure 1**. Onconeural and anti-glutamic acid decarboxylase (GAD) antibodies have intracellular targets, whereas neuropil antibodies targeting surface antigens like channels e.g., VGKC—LGl1, CASPR2—or receptors e.g., NMDAR, AMPAR, GABAR, mGLuR.

Antibodies

Onconeural Antibodies (CV2, Ma2 and Hu, Ri, Yo)

Neuronal nuclear antibodies targeting Hu, Ri, Yo have been established for decades. They are associated with various symptoms and are various cancers. Anti-Hu antibodies (also called ANNA1—anti-neuronal nuclear antibody) were first described in 1985 (33). They are targeting intracellular antigens and are released in reply to an underlying cancer. Hu antigens (ag) can be found in malignant cells but also in neuronal cells. Currently the pathogenetic role of anti-Hu antibodies is not proven at certainty. Anti-Hu antibodies lead to a strong autoimmune response with involvement of autoreactive T cells (34).

Anti-Yo syndromes are responsible for half of all patients with PCD. Still their prevalence is low. PCD will evolve over time and may precede the detection of malignancy. Symptoms including ataxia and cerebellar dysfunction usually develop over weeks to months (1). Extracerebellar symptoms, i.e., LE, is rarer in anti-Yo patients than in anti-Hu mediated disorders. Anti-Yo antibodies target the cytoplasm of cerebellar Purkinje cells, but also other nerval structures and brain regions such as retina, dorsal root ganglia. They have the ability to fix complement and are typically IgG antibodies (35). Besides IgG also IgM and IgA antibodies have been reported. Inflamed Purkinje layer shows infiltrates of CD8+ lymphocytes, B-and T-cells and plasma cells as well as microglia activation (1). When disease progresses a massive atrophy of the cerebellum may evolve. At this stage, inflammatory cells are often missing in the Purkinje layer (36). The pathophysiological role of the antibodies is not elucidated at certainty (35). Consequently, they are not suited as marker for disease activity. Interplay of B-cells, cytotoxic T-cells and a mooted dysregulation of calcium homeostasis are pathophysiologically important.

The low number of patients limits treatment experience. Corticosteroids, plasmapheresis (PLEX) and immunoglobulin (IViG) have not resulted in convincing results. Experiences on treatments including cyclophosphamide and rituximab are anecdotal. One trial utilized tacrolimus to target cytotoxic T-cells. Whereas, the number of cytotoxic T-cells decreased dramatically, the effect was reversible when treatment was terminated. Additionally, no effects on neurological symptoms were observed (1).

Anti-Ri antibodies (also called ANNA-2) are detected primarily in patients with breast cancers and are directed against neuronal nuclear proteins. Typical manifestations are opsoclonus-myoclonus-ataxia (37).

As for other onconeural antibodies like anti-Hu, -Ri, -Yo, and CV2 does not seem to be responsible for neuronal destruction. Anti CV2 antibodies target collapsing response-mediator protein-5 (CRMP5) and are mostly associated with small cell lung cancer (11). Most frequently anti-CV2/ CRMP5 antibodies cause subacute cerebellar degeneration, followed by encephalomyelitis, limbic encephalitis, optic neuritis and retinopathy in about one in one hundred patients (38). Besides pharmacological treatment, the removal of the tumor has also only little impact on prognosis (39).

However, the survival and neurological symptoms with onconeural antibodies are associated with type of tumor and specific antibody. Although anti-Hu and CV2 antibodies lead to similar symptoms, disease outcome favors CV2 (40).

In anti-Ma2 associated encephalitis patients may present with symptoms suggestive for narcolepsy. Cataplexia and excessive daytime sleepiness result from diencephalic involvement and deficient hypocretin transmission. However, in patients with idiopathic narcolepsy anti-Ma2 antibodies have not been found (41, 42). In addition, patients presented with head drop and upper limb involvement were finally diagnosed as encephalitis with progressive muscular atrophy or as myeloradiculopathy associated with anti-Ma2 antibodies (43, 44). Brainstem, limbic and/or diencephalic involvement will lead to respective

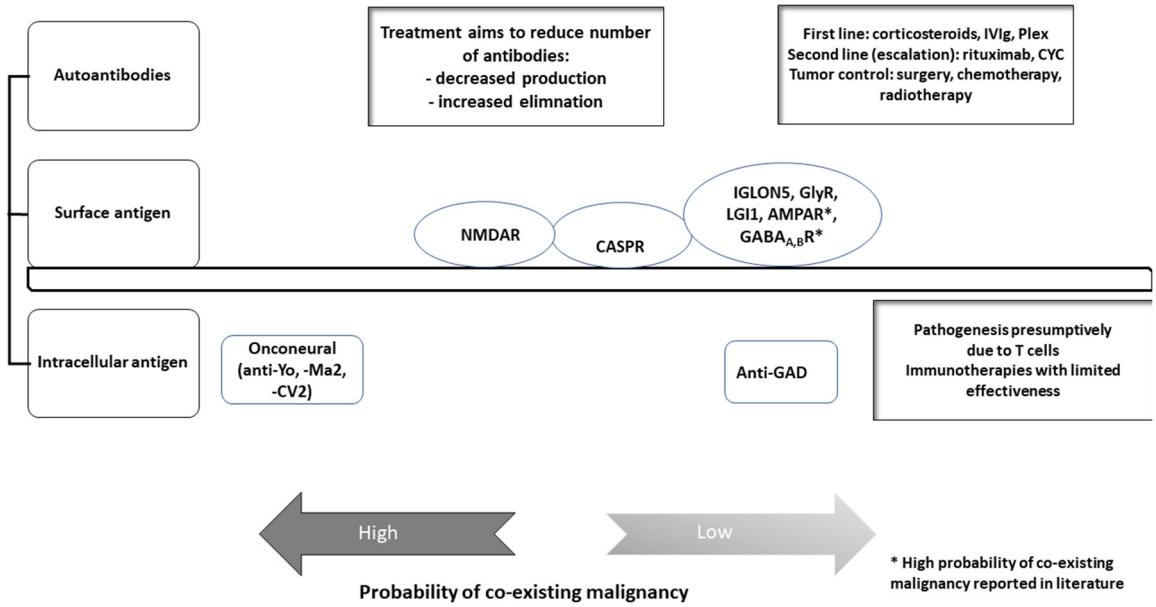


FIGURE 1 | Autoantibodies in autoimmune encephalitis. This figure gives an overview on the different autoantibodies and their antigens detected in our cohort. Treatment options and probability of co-existing malignancy differs for the various antibodies in our cohort. Modified to Prüß (32).

symptoms with ocular motoric disturbances, LE or symptoms suggestive for narcolepsy. CSF studies show increased protein concentration or pleocytosis, in some cases OCBs are positive. Lymphocytic infiltrates with predominantly T-cell infiltration are found in affected brain regions. Associated neoplasias are mainly testicular tumors or lung cancer. Clinical improvement was observed in patients that received a combination of tumor treatment (orchiectomy, chemotherapy, radiation) and immunotherapy (24). Stabilization or improvement has also been reported in another case series in patients receiving corticosteroids, IVIG and cyclophosphamide (45).

Whereas, the pathophysiological importance of onconeural antibodies is disputed and cytotoxic T cell may be responsible for the poor prognosis, other antibodies found in immune mediated encephalitis seem to be of great pathophysiological importance, especially for those targeting surface antigens.

Over the last years, reports of other antibodies causing encephalitis have increased tremendously. Three different targets of antibodies may be identified: 1. Receptors responsible for excitatory effects (NMDA-R, AMPA-R); 2. Receptor responsible for inhibitory effects (GAD, GABA-A, GABA-B, Gly-R); 3. Antibodies targeting channels and adhesion molecules (VGCC-, LGI1, Caspr2, IgLON5) (39).

Antibodies to Receptors Mediating Excitatory Effects

Antibodies against the N-methyl-D-aspartate receptor (NMDAR)

Antibodies against the receptor of NMDAR were described in 2005 in four female patients presenting with psychiatric symptoms for the first time. They responded to immunotherapy and/or ovarian teratoma resection. Incubation of patients' sera with rat hippocampal neuron cultures showed intense immunolabeling with ags localized in the molecular layer of the hippocampus (46). In 2007, the target auto-ags were identified as located in the extracellular domain of the NMDAR subtypes 1 (NR1) and 2B (NR2B), and to a lesser extent to the NR1 and NMDA-R subtype 2A(NR2A) as conformational epitope (47). The main cellular mechanisms accounting for the stereotypical course of anti-NMDAR encephalitis are: (1) Patients' CSF anti-NR1 antibodies or purified IgG reduce surface NMDAR protein and NMDAR cluster density in a titer dependent manner compared to healthy controls. (2) Additionally, patients' antibodies reversibly and specifically reduce NMDAR from excitatory synapses, and thereby not affecting the total number of excitatory synapses. (3) This process is mediated partly by capping, crosslinking and internalization of NMDAR independent from complement activation (48). Established treatment strategies comprise various forms of immunotherapy (corticosteroids, IViGs, plasmapheresis PLEX, rituximab, cyclophosphamide).

Antibodies against the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor: (AMPAR)

Anti-AMPAR antibodies have first been described in 2009 (49). AMPAR belongs to the glutamate (Glu) receptor, and is responsible for excitatory synaptic transmission in the brain. The antibodies target one of the subunits of the GluR: GluA1 or GluA2. GluA1 and GluA2 are surface ags. The binding to the receptors leads to an internalization of the receptors (50). Its importance for memory, learning and synaptic plasticity is well-characterized (51). Psychosis is quite often the initial symptom

and clinical presentation of patients is similar to those in anti-NMDAR encephalitis. An association with breast cancer, tumors of the lungs (e.g., small cell lung cancer) and the thymus has been observed (52, 53).

Antibodies to Receptors Mediating Inhibitory Effects

Antibodies against glutamic acid decarboxylase (GAD)

GAD is the enzyme needed in catalyzing the decarboxylation of glutamate to γ-aminobutyric acid (GABA). Anti-GAD antibodies are frequently detected concurrent with other antibodies—most frequently with antibodies against GABAR (54). The two receptors on which GABA acts as an inhibitory ligand in the CNS are $GABA_A$, an ionotropic receptor, and $GABA_B$ a metabotropic receptor. Of the two isoforms of the enzyme, GAD 65 and 67,the first is located mainly in synaptic vesicles and synthesizes GABA in an activity dependent manner, whereas GAD 67 is located in the cytosol; is constitutively active and accounts for a steady state of basal GABA level (55, 56). GAD antibodies are associated with various neurological diseases including stiff person spectrum disorders (SPSD), cerebellar ataxia, PERM, LE, epilepsy, down beat nystagmus, autoimmune-related retinopathy and optic neuropathy (ARRON syndrome) (57–59). Anti-amphipyhsin antibodies are commonly detected together with anti-GAD antibodies. Together these are the three auto-antigens for cerebellar ataxia, SPS and Batten's disease (60).

Anti-GAD titres in neurological diseases are usually substantially higher than in patients with diabetes mellitus type 1 (DMT1), though there is an overlapping range. Whether the ability to cross the blood brain barrier (BBB) is titer dependent is speculative (61), but might be an explanaition why low titres causing DMT1 and higher titres causing CNS symptoms. Vice-versa high titres in SPS can cause damage to the neuroendocrine beta islet cells, and over the course up to 30% of SPS patients develop autoimmune diabetes mellitus (62). GAD antibodies found in neurological diseases have a different epitope specificity than in patients with DMT1 (63, 64). If GAD antibodies are directly pathogenic or whether they are just an epiphenomenon for autoimmune disorders that are mediated by CD4+ T cells is still a matter of debate (65–67). Electrophysiological studies have led to SPS- like symptoms and cerebellar ataxia in rats after injection of sera from patients with antibodies against GAD into rat cerebellum and lumbar para-spinal region (64). It has also been shown that passive intrathecal transfer of IgG from SPS patients can cause SPS like motor symptoms in the rat model (68), elucidating pathophysiological relevant antibodies in SPS patients. Despite its intracellular location the intraperitoneally passive transfer of human IgG against synaptic amphiphysin in a rat model evoked symptoms analog to human SPS supporting a direct pathogenic role of the antibodies (69). A positive therapeutic effect after IVIG therapy in patients suffering from SPS has been reported before (70). Antibodies against GAD are usually not associated with tumors. However, patients with a concurrent antibodies to GAD directed against cell surface antigen seem to have a 7-fold higher risk of having an occult neoplasm (71, 72). Paraneoplastic SPS is mostly accompanied by anti-amphipysin antibodies and associated mainly with breast cancer (9). In some patients with endocrine autoimmunitiy the presence of GAD65 antibodies might precede the onset of a neurological disorder (73).

A randomized placebo-controlled trial of patients with SPS found no significant positive effect after the administration of rituximab over a period of 6 month though 4 patients improved markedly (74).

Antibodies against γ-aminobutyric acid (GABA)-receptors

Anti-GABAR antibody block the inihibitory effects mediated by GABA-R. There are two different forms of receptors: $GABA_A$ and $GABA_B$. They are usually associated with LE ($GABA_B$) or refractory seizures ($GABA_B$). Whereas, $GABA_B$-R antibodies are frequently associated with tumors, this association is less commonly seen in patients with antibodies against $GABA_A$-R. They usually respond to immunotherapy (75).

Antibodies against glycine receptors (GlyR)

In 2008, antibodies against GlyR were discovered in the serum of a patient diagnosed with PERM (76). GlyRs consist of alpha 1–3 and beta subunits (GLRA1-3). The alpha 1 and beta subunits of the GlyR are expressed abundantly in the pontine region, medulla oblongata and upper spinal cord (25, 38). The role of antibodies directed against GlyR A2 and GlyR A3 as intracellular epitope is unclear (25). The binding of Gly to its receptor leads to chloride influx and hyperpolarization of the postsynaptic cell. Whether the receptor internalization and the direct inhibition of the GlyR contributes to pathology remains unclear. In patients with paired serum-CSF samples the GlyR antibody titer was more prominent in the sera (25, 77). Patients may present mainly with muscle spasm, stiffness, rigidity, and myoclonus. In addition, cranial nerve involvement, excessive startle, walking problems, and cognitive deterioration are frequently associated symptoms. There is an association with neoplasms in up to one out of four patients. After treatment of cancer, neurological symptoms improved. Interestingly a major part of patients seem to improve with immunotherapy and became independent in daily activities (25, 77). PERM, a condition already described in the 1970s, can be distinguished from SPS by its progressive course, brainstem, cranial nerve and long tract involvement (78, 79).

Antibodies Targeting Channels and Adhesion Molecules

Antibodies against the voltage gated potassium channel-complex (VGCC-complex): Contactin-associated protein-like 2 (CASPR2), Leucine-rich, glioma Inactivated 1 (LGI1)

The discovery of CASPR2 and LGI1 as main auto-ags of the VGCC complex led to a better understanding of channelopathies. Clinical manifestation and responsiveness to steroids differs between the two antibodies (39, 40). CASPR2 is a cell adhesion molecule and can be found in the hippocampus, cerebellum, and in the juxtaparanodal area of myelinated nerves in the CNS and PNS. It is a transmembrane protein with a small intracellular and a large extracellular domain and belongs to the neurexin IV (Nrx-IV) superfamily. In the juxtaparanode region, CASPR2 together with TAG1 (a neuronal cell adhesion molecule) and protein 4.1B organize and localize Kv 1.1/Kv 1.2 channels (53, 80, 81).

Antibodies directed against CASPR2 are predominantly of the IgG4 subtype and importantly do not cause internalization of the protein and lack crosslinking as seen in other types of encephalitis (48, 82, 83). Antibodies to VGCC may be directly pathogenic and may disrupt the cell to cell interaction (84). The largest retrospective study of patients with CASPR2 antibodies showed that the majority of patients are males with a median age of 66 years. The most prominent symptoms are cognitive disturbance followed by seizures and peripheral nerve hyperexcitability. CSF was normal in more than two thirds of the patients and about 70% had a normal brain MRI. All patients had serum antibodies against CASPR2. Patients with a tumor might have low CSF titres or no antibodies detected in CSF by immunohistochemistry due to the primary peripheral involvement. Tumor prevalence may account to up to one fifth of patients and are in most cases thymomas or small cell lung cancers. In patients with a tumor surgery, the concomitant chemotherapy led to complete neurological remission. Relapses occurred in 25% of the patients, the earliest 8 months after the initial episode and symptoms were mostly similar than in the initial episode. Interestingly, Morvan Syndrome—characterized by peripheral nerve excitability, encephalopathy, autonomic dysfunction and sleeping disorder—was also associated with channelopathies (85). Response to treatment in patients with Morvan's Syndrome took longer than with other presenting symptoms but taken together 72% of patients became independent in daily activities at a median follow up of 36 months, whereas 21% of patients were treated with immunotherapy other than first line therapy (84, 86). Serum cut off titres of $\geq$1:200 showed good sensitivity and specificity for the diagnosis of CASPR 2 encephalitis especially when a brain MRI was performed in addition (87).

LGI1 stabilizes the compound between ADAM22 (a disintegrin and metalloproteinase domain) and ADAM23 close to VGCC in the presynaptically and to AMPA-R postsynaptically (88). There are about 300 reported patients with encephalitis associated with LGI1 antibodies resulting in an estimated incidence of 0.83/million (17, 86). The most common initial symptoms seem to be epileptic seizures and cognitive deterioration, though during the course of the disease more than 80% of the patients develop seizures (17, 89). Tumors are present in up to 20% of patients (17, 18, 90, 91). Further common associated symptoms are insomnia and dysautonomia. Faciobrachial dystonic seizures (FBDS) are reported in almost 50% of patients and are a characteristic (92–94). FBDS do not seem to respond to antiepileptic drugs but to immunotherapy (89, 95). Hyponatremia is found in 65% of the patients. Two out of three patients show hippocampal alterations in MRI at presentation, mostly unilateral and three out of four patients show normal CSF findings in the lumbar puncture.

First-line treatment response rate is effective in 80%, and improvement started with decrease in seizures and improvement of cognitive functions. Eighty-six perecnt had persistent amnesia for the initial disease and life events during the disease as well as retrograde amnesia representing as lack of memories for vacation. Relapses occurred in 35% (17). Imaging studies in LGI1 patients showed hippocampal volume reduction in all segments besides of the CA1 region. The duration of FBDS correlated inversely with the volume of the right pallidum (18). Rituximab seems to be safe and effective even in a later course of the disease in patients with LGI1 antibody encephalitis (96). Patients with LGI1 antibodies seems to have poor memory recovery probably because of structural damage due to hippocampal atrophy (97). Though as it has been observed in patients with FBDS the initiation of immunotherapy may prevent cognitive deterioration (95).

Antibodies against IglON5

Anti-IglON5 antibodies were first reported in eight patients with predominantly atypical sleep disorders in 2014 (98). IgLON5 is a neuronal cell adhesion molecule with unknown function. IgLON5 antibodies are accompanied by phospho-tau deposits in subcortical areas mainly in the hypothalamus, brainstem tegmentum and upper spinal cord (99). The hallmark of anti-IgLON5 associated encephalitis is parasomnia involving REM and non REM sleep with stridor, abnormal sleep behavior, e.g., patients mimic activities of daily living, and sleep apnoea (98, 100, 101). Four core symptoms have been reported in the largest published case series: (1) predominantly sleep disorder (2) bulbar dysfunction (3) progressive supranuclear palsy (PSP) like syndrome (4) cognitive deterioration or major neurocognitive disorder (102). The disease is strongly associated with the HLA-DRB1*1001 and DQB1*0501 alleles linking neurodegeneration with the immune system (101, 102). There is no ensured paraneoplastic origin though the presence or history of cancer which may not be causal has been described in patients (103). Different to NMDAR and AMPAR, anti-IgLON5 antibodies cause an irreversible downregulation of the surface protein. This is caused by IgG1 antibodies in a time dependent manner and may be a major reason why patients do not respond fully to immunotherapy (83). Antibodies to IgLON5 lead to irreversible internalization of the IgLON5 protein. Consequently, the long time period between symptom onset and start of immunotherapy may be responsible for the low effects of treatment. Besides IgG1 also IgG4 have been reported (103). It is still unclear if patients with predominantly IgG4 rather than IgG1 or the HLA-type is associated with better outcome.

Hashimoto encephalopathy (HE)/Steroid responsive encephalopathy associated with autoimmune thyroiditis (SREAT)

HE/SREAT was first described by Brain et al. (104). Under the aspect of unclear underlying pathophysiological mechanisms, there is discussion about the right terminus for the disease. As HE/SREAT is not necessarily associated with thyreoiditis, and not all patients respond to corticosteroids (105, 106). HE/SREAT is a diagnosis of exclusion and can be considered under certain conditions after alternative causes have been ruled out (14). Patients diagnosed with HE/SREAT frequently show elevated CSF protein or CSF pleocystosis. Cranial MRI is usually unremarkable. EEG may show unspecific abnormalities but does not show typical patterns like in patients with anti-NMDAR encephalitis. Generalized slowing is observed in patients, in some with lateralized slowing or intermittent rhythmic slowing in frontal or occipital regions with epileptic activity in some cases

and improvement of EEG pattern under therapy while follow up (106–108). HE/SREAT is not necessarily associated with hypo- or hyperthyroidism but serum anti-thyroid antibodies seem to present ubiquitary in these patients whereas CSF thyroperoxidase (TPO) and thyroglobulin (TG) antibodies are rarely positive (108). The thyroidea stimulating hormone (TSH) levels can be normal (108). Antibodies-titres and CSF protein seem to decrease concordantly to clinical improvement (106, 109), though the levels of CSF antibodies seems to be independent of the clinical stage of the disease (105). Patients with initial coma may have relapses more often than those without coma (108). Outcome is generally favorable and response to first line corticosteroids is good. Up to 18% of the disease-free population in an U.S. collective have TG or TPO antibodies. Antibodies are detected more often in older white females and the occurrence of TG antibodies in > 50% goes together with the appearance of TPO antibodies and vice versa (110). If anti-thyroid antibodies are causal to the disease or if they are just an epiphenomenon is not elucidated so far. Nevertheless, anti-TPO monoclonal antibodies bind to astrocytes (111).

Prognosis of Autoimmune Mediated Encephalitis

The various antibodies and the antigens they are targeting have major influence on prognosis. Patients with antibodies against intracellular antigens (see **Figure 1**) have worse prognosis (112). Pathomechanisms involve quite often cytotoxic T cells that are responsible for neuronal destruction (113). In patients with antibodies against surface antigens outcome may be better, as immunotherapies might be more effective in those patients. Antibodies titres as well as the epitope are of the utmost importance in those patients. Outcome in patients with NMDAR encephalitis might be better than in those with AMPA-R. In patients with antibodies against onconeural structures malignancies will be found, as these antibodies are well-established markers for PNS. Contrary, not all patients with antibodies against surface antigens will have neoplasias. However, a screening for neoplasias and malignancies is obligatory. Whereas, in patients with onconeuronal antibodies associated disorders outcome is generally poor even after removal of the tumor, the disease course in encephalitis might be improved in dependency of the various antibodies and underlying tumors after removal of them.

Treatment

The appropriate treatment aims to stop the immunological processes being causal for the disease and to treat sequelae of encephalitis. Existing malignancies should be removed and treated adequately as soon as possible to eliminate the causing "antigen." Additionally, immune-suppressive treatment should be started. Treatment options include corticosteroid, IViG treatment, plasmapheresis, rituximab, cyclophosphamide. For some patients a combination is needed (114).

Immunotherapy is of vital importance, however, also treatment of sequelae such as epileptic seizures is a major concern.

Conclusion

Some patients do not show immune mediated antibodies. In those patients the application of nuclear medicine diagnostics—especially in patients with unremarkable magnetic resonance imaging (MRI)—, and cerebrospinal fluid (CSF) analysis allows diagnosis of autoimmune encephalitis (14, 115, 116). Consequently, in patients with new onset of atypical psychosis and negative antibody-testing CSF analysis is recommended (117–121). Diagnosis of autoimmune encephalitis remains challenging not less than establishing an appropriate therapeutic concept for each patient. Hereto identification of prognostic factors as figured out in anti-NMDAR encephalitis may alter therapeutic strategy (15). Biomarkers like neurofilament light chains (NFL) and phospho-tau may offer future strategies for disease monitoring acting as a surrogate for disease activity (122). Still, there are unanswered questions regarding etiology. An infectious link was proposed in anti-NMDAR encephalitis, as patients with acute symptoms after HSV-encephalitis often show antibodies against NMDAR. In addition, an association between non-encepahlitic HSV-1 infection and NMDAR-encephalitis has been proposed based on results of a case-cohort study (123). CXCL 13 has been shown to be useful to identify acute neuroborreliosis and its utilization as biomarker for treatment response in patients with NMDA-R-encephalitis may offer future strategies (124, 125).

RESULTS

Thirty-eight patients diagnosed with autoimmune encephalitis were included in our analysis. Sixty-one percent were female. Mean age was 48 years (ranging from 19 to 77years), and was similar for sexes (females: 50 years [19–77] and males 53 years [21–77]). The youngest patients (mean age) were among the NMDAR, AMPAR and TPO/TG subgroup, and age was highest for patients with IGLON5 ($n = 3$, 71 years [64–76 y]), followed by Ma-2 with 66 years ($n = 2$, 60 and 71 years), LGI1 ($n = 7$, 65 years, [47–77 y]). Antibodies against NMDAR and LGI1 were detected in 7 patients and were the most common ones. Six patients had antibodies against GAD (5 patient's GAD-65, 1 patient GAD-67). One patient with anti-GAD-67 antibodies also showed antibodies against $GABA_A$ and $GABA_B$. Concomitant cancers were observed in 11 patients. All patients with anti-Yo, anti-Ma-2 and anti-CV2 antibodies and 57% of patients with NMDAR antibodies as well as one patient with CASPR2 antibodies had coexisting malignancies. In two patients with Ma-2 abs preceded tumor diagnosis, and in one patient with CV2 abs preceded tumor recurrence.Four patients with NMDA-R encephalitis had a malignancy (three women with teratoma, and one male with B cell lymphoma).

Patients with NMDAR encephalitis presented most frequently with neuropsychiatric symptoms (agitation, confusion and hallucinations). LE was the most common syndrome in patients with LGI1 and CASPR2 encephalitis. Mnestic and cognitive deficits but also seizures were common as initial symptoms in those patients. Spasticity and ataxia were the leading symptoms in patients with GAD-65 and GlyR antibodies. In one patient with

GlyR antibodies cranial nerve involvement was reported. Patients with ant-IgLON5 syndrome do not show distinct patterns of symptoms (see **Table 1**). All patients with anti-Yo antibodies were diagnosed with PCD and presented with ataxia. MRI abnormalities were detected in 47.4% of all patients and differed for the various antibody associated syndromes ranging from 0% for CV-2 ($n = 1$), SREAT ($n = 2$), Yo ($n = 3$) up to 100% for Ma-2 ($n = 2$) as well as the patient with antibodies against AMPAR. Whereas in LGI ($n = 7$) abnormalities were detected in 86% ($n = 6$), the rate was 43% ($n = 3$) for NMDAR ($n = 7$). Most prominent abnormalities were seen in the hippocampal and mesiotemporal region. These radiological findings correlated with symptoms (psychosis, cognitive, and mnestic deficits).

EEG abnormalities were either general slowing or epileptiform activity and were seen in 31.4% of the patients. Anticonvulsant drugs were used in 20 patients. Out of 25 patients who received second line therapy 7 patients (NMDAR: 2; LGI1: 1; AMPAR: 1; Ma-2: 1, Yo: 2) did not receive AED. Four of those patients (NMDAR, LGI1, AMPAR) recovered well (mRS $\leq$ 2). Most common AED used were Levetiracetam, Lacosamide and Lamotrigine. Ninety-two percent of patients with documented seizures in the subacute phase still had AED 12 months after start of immunosuppressive therapy. AED were most likely used in the subgroups with CASPR2, LGI1, NMDAR, IGLON 5, and TPO/TG antibodies ($\geq$66% of patients).

All patients with anti-NMDAR encephalitis and coexisting ovarian teratoma underwent surgery within 7 days after detection. Two out of these three patients had a favorable outcome (mRS 0). A patient with paraneoplastic anti-Ma2 brainstem-encephalitis was diagnosed with lung cancer several years before neurological deterioration indicated tumor recurrence. The patient received chemotherapy and radiation. About 12 months after onset of neurological symptoms antibody titres decreased. Clinical improvement was recognized though the patient is still not able to walk unassistedly. Similarly, a female patients with anti-Yo antibodies was treated for breast cancer (surgery, chemotherapy, radiation). Antibodies were detected after onset of ataxia. The other patient with Ma-2 suffering from limbic encephalitis received chemotherapy which started a few weeks after diagnosis. Immune-suppressive therapy with cyclophosophamid was started but was stopped after five cycles as the patients symptoms did not improve. A patient with CV-2 mediated brainstem encephalitis has recently been diagnosed and tumor management has been initiated. All patients with anti-Yo antibodies underwent surgery. As the time interval between surgery and start of immunosuppressive therapy is unknown we cannot report further details in this context. Outcome for patients with onconeural antibodies is worse than for those with surface antigens. None of the patients were independent in daily activities. *mrs was* $\geq$3 for all patients with onconeural antibodies.

Most administered treatment were IVIG (2 g/kg bodyweight over 5 days, up to three times) and pulsed steroids (1 g methylprednisolone for 5 consecutive days, followed by 75 mg oral dose tapered over 12–20 weeks) in 26 patients. Eleven patients received plasma exchange (cycles of up to 11 plasmapheresis and up to two cycles) or immunoadsorption. Twenty-four patients received $\geq$3 different immunotherapies and 12 patients were treated with $\geq$4 different immunomodulatory therapies. Only 2 patients responded well to first-line treatments (mRS score $\leq$2), and no escalation therapy was initiated. Twenty-five patients received second line therapy. Most common second lines treatments were rituximab in 22 cases (up to three times in the acute treatment, 375 mg/m^2 body surface), cyclophosphamide (750 mg) in 7 cases, methotrexate (10 mg weekly up to three times) and azathioprine (1.5 mg/kg bodyweight) in 3 cases. Twelve patients received an escalation treatment consisting of more than one second line treatment. Most commonly, rituximab and cyclophosphamide were given as add on (see **Table 1**).

Patients with at least 6 months of follow up were looked at in detail. Stratified by modified ranking scale patients that score 0 points had NMDAR (66%) and AMPAR antibodies (33%). We stopped treatment after initial application of rituximab (2 times, 14 day interval), after 13 and 20 months in those patients. Both patients with NMDAR antibodies underwent ovarian teratoma resection. Seven patients scored 1 point. All patient had treatment with rituximab initially (2 times, 14 day interval), and two did not receive further immunosuppression (both LGI1 antibodies). Two patients improved under first line therapy and are currently under observation (CASPR2 and LGI1). Two patients received chronic second line immunosuppression (non-paraneoplastic NMDAR and HE) and another patient is under chronic immunosuppression for 2 years now and will soon be reevaluated (LGI1). Of 5 patients scoring 2 points three received chronic second line immunosuppression (2 IgLON5, 1 LGI1), one patient with GlyR mediated SPS stabilized received IVIG with mild stabilizing effect but without significant improvement and refused second line therapy and another patient with GAD 67 antibodies improved distinctly under IVIG which was stopped after 4 cycles and is currently under observation.

DISCUSSION

Reports on autoimmune mediated encephalitis have increased tremendously over the last years. This development was mirrored in our institution by a large number of newly diagnosed and treated patients at our institution. The increased awareness may have led to more testing for autoantibodies and consequently to more diagnosed patients. Testing for antibodies in serum is easily available. Still sensitivity of antibody testing may be higher in CSF than in sera as reported for NMDAR encephalitis (29) and thereby diagnostic pathway is more invasive. Moreover, in consistency with literature (29) we observed that the clinical course correlates well with the NMDAR antibodies titres in *the* CSF of patients. This might be of importance especially in comatose patients when clinical neurological assessment is limited. For patients with suspicion for LGI1, CASPR2, IgLON5, GAD, and Glycine mediated disorder testing of serum may be sufficient, but as symptoms are unspecific we test antibodies in serum as well as in CSF.

Diagnosis is hampered by the presentation of very unspecific symptoms (118, 126), but early diagnosis and treatment initiation

are essential in the management of autoimmune encephalitis. We have observed wide disparity in the latency of diagnosis and treatment in our cohort. Patients diagnosed with anti-IGLON5 syndrome followed by HE and GlyR antibody associated encephalitis had the biggest latency in receiving a diagnosis and treatment with immunosuppressive agents. The highly variable interval in "first symptoms to immunosuppressive treatment" may be explained by the increasing awareness for NMDAR and LGI1 encephalities, whereas diagnosis might be challenging for HE/SREAT, or antibodies testing for IGLON5 or GAD-67 was not available until recently. One of our patients with antibodies to IGLON5 was diagnosed after 7 years of bilateral vocal cord palsy. Interestingly, he improved under immunotherapy. A case with CASPR2 encephalitis in our cohort had a very long disease course and similarly he improved under treatment. Both cases may suggest that even after a long period treatment should be initiated, and seems to be more effective than assumed (103, 127, 128).

First line therapy was initiated within the first few days after hospitalization for most of the patients (especially for those with NMDAR and LGI1 encephalitis). First-line therapies were pulsed steroids and IVIG. Treatment was escalated to PLEX in patients with antibodies against surface epitopes and who did not respond to high dose steroids or IVIG. Depending on the patient's clinical neurological condition during PLEX or after 6 cycles of PLEX second line therapy was initiated which is more or less in line with previous recommendations (16). Initiation of second line therapy has evolved earlier in the course of the disease over the last 4 years and may have improved the disease course. The first choice of second line immunotherapy was rituximab in most cases, in some cases simultaneously with cyclophosphamide. In patients with onconeural antibodies second line therapy was mostly cyclophosphamide and to a lesser extent rituximab. We figured out that the initiation of second line therapy is highly dependent on the notification of the antibodies status. None of our patients received second line therapy without diagnosis evidenced by respective antibodies. A prolonged disease course with no clear improvement was the basis for the decision to initiate escalation therapy in most of the patients. When escalation treatment should be started is a matter of discussion, as there are no clear guidelines. Maintenance therapy with rituximab has been established for varying duration. We re-evaluated immunosuppression with rituximab after 4 to 6 cycles (2–3 years after initiation). There is need to implement scales with sufficient sensitivity and other testing modalities (e.g., autonomic testing) to monitor patients in the acute phase but also in therapy surveillance.

If patients showed clinical improvement, regained autonomy in daily activities, CSF has normalized, MRI did not show new alterations and antibody status became negative than we usually discontinue chronic immunosuppression and arrange follow up controls in 6 months intervals. In cases with coexisting tumor we stop immunosuppression in our NMDAR patients 1 year after tumor removal, even if they had had second line therapy. This is not absolutely conform with the proposed management (16). Termination of immunosuppressive therapy needs to be discussed in each individual case and cannot be recommended without reservation for all patients with autoimmune encephalitis or even with the same distinct antibodies, although all of our patients had a monophasic course

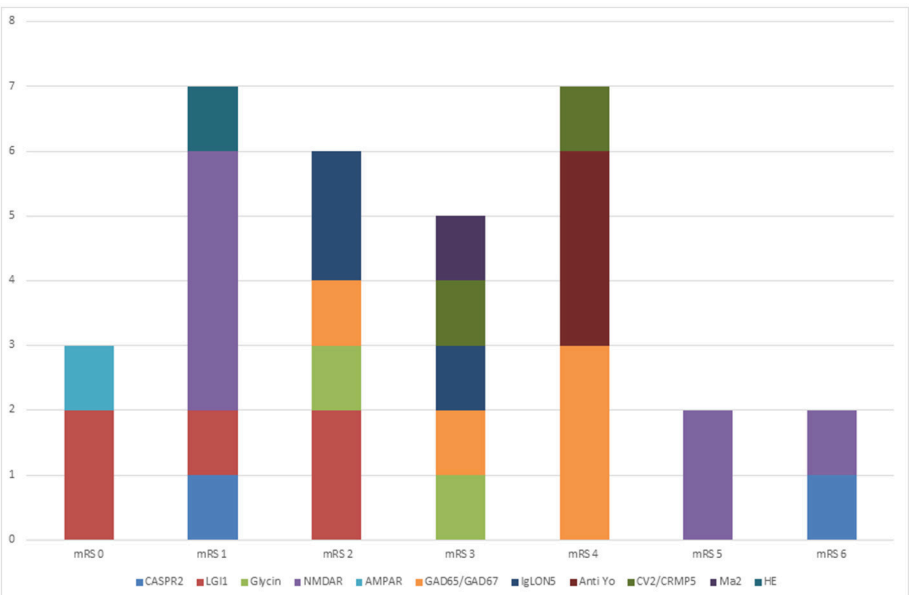


FIGURE 2 | Outcome of patients with autoimmune encephalitis. This figure shows the outcome of our patients (follow up at least six months) expressed. Thirty-nine percent of our patients show no or only mild deficits (mrs $\leq$ 2). Twenty-seven percent show severe disability and 7% died. Best prognosis had patients with anti-LGI1 encephalitis (75% mild disability, 25% moderate disability at last follow up). All patients with onconeural antibodies have severe disability at last follow up.

of the disease until now (even in those patients with second line treatment). In patients with stiffness and PERM besides immunotherapy also symptomatic treatment with high dose oral and intrathecal triamcinolone-acetonide that markedly reduced stimulus-evoked jerks, reduced rigidity and muscle spasms is of importance. Treatment response to immunotherapy in patients with cerebellar ataxia was markedly worse than in patients with SPS. On the other hand, patients with non-paraneoplastic ataxia and seropositivity for GAD65 antibodies respond better to immunotherapy than patients with coexisting malignancy. In those patients early treatment initiation is of the utmost importance (129, 130).

Despite the small number of patients almost all our patients with antibodies against LGI1 and NMDAR developed epileptic seizures which disappeared as they recovered. Seizures in our patients developed early in the course of the disease, although literature report manifestations in every stage of the disease (131). Seizures as first symptom of anti-NMDAR encephalitis are more common in men. Since we only have one male patient who did not develop epileptic seizures we cannot confirm that seizures manifest in men more frequently (132). Five patients with antibodies against LGI1 developed epileptic seizures. FBDS were observed in a single patient. Seizure control was achieved by early immunotherapy whereas cognitive deficits persisted in 80% of our patients, similarly to reports from literature (17, 133). All patients with seizures as initial symptom or in the subacute phase received AED and immunosuppressive therapy simultaneously. Most patients, especially those with LGI1 antibodies stabilized soon and did not suffer from further seizures. Patients with NMDAR encephalitis had seizures mostly as initial symptom or in the subacute phase often associated with autonomic dysregulation and need for intensive care treatment but not after clinical stabilization. Taken together we cannot link clinical improvement to AED. Some patients with limbic encephalitis did not suffer from seizures, maybe because of fast initiation of immunotherapy, did not require AED and improved markedly over the course of their disease.

Tumor screening was performed in all patients with anti-NMDAR encephalitis. In our female patients screening for teratoma was done either with computer tomography (CT), pelvic ultrasound or as recently reported by MRI (134) or a combination of these modalities. The removal of the ovaries in patients with teratoma is aimed to be conducted immediately after detection and diagnosis of NMDAR encephalitis as not only the severity of symptoms but also early initiation of immunotherapy and early teratoma resection predict good outcome (15). Tumor surveillance is of utmost importance as the relapse rate and prognosis depend on the tumor status (15), and tumor work up in yearly intervals should be performed (16). Besides teratoma, B cell lymphoma was diagnosed in one of our male patients. Detection of NMDAR antibodies preceded diagnosis in this patients. Whether there is a pathophysiological relation remains unclear. EBV is of importance in the pathophysiology of B cell lymphoma, and recently a case with anti-NMDA-R encephalitis associated with EBV was reported (135). Detection and removal of coexisting malignancy is important in the treatment of autoimmune encephalitis.

Interestingly, the age of patients at diagnosis differed for the various antibodies. Three cohorts were seen: age >65 years: LGI1, CASPR2, IGLON5, Ma2. Age between 50 and 60: Glycin, GAD, Yo, CV2. Age <30 years: NMDAR, AMPAR and HE. Whether there is a pathophysiological association is not clear.

Follow up data for at least 6 month is available for 29 patients. All patients with LGI1 encephalitis are independent in their daily activities and had mRS<2, whereas for NMDAR encephalitis patients mRS ranged from 0 till 6. Sixty-seven percent of our patients with antibodies against NMDAR had a favorable outcome which is comparable to previous data that showed 81% of patients had a favorable outcome after 24 months (15). Outcome in patients with onconeuronal antibodies had a worse outcome. None of those patients were independent in daily activities. Two deaths were reported: One patient suffering from NMDAR encephalitis and one patient with CASPR2 encephalitis. See **Figure 2**. One of our female patients has not recovered from encephalitis despite intense immunotherapy for over 2 year now. We administered bortezomib in this case which seems promising in patients with prolonged course (136–138). Interestingly, this patient shows improvement after 22 months of treatment with walking and participation in simple conversation. This shows due to the reversible and titer-dependent internalization of the NMDAR, symptoms are reversible (48), even after that long disease duration without full recovery. If the recovery of this patient refers to bortezomib is unclear as this patient received extensive immunosuppressive therapy before.

Over the last years other treatment options have been discussed including natalizumab, azathioprine, methotrexate, mycophenolate mofetil or tocilizumab (139, 140). However, natalizumab was considered ineffective in an atypical case with NMDAR antibodies. Positive effects on seizure control, but not on cognitive deterioration was seen, when used add-on (141, 142). Treatment with natalizumab may offer a therapeutic option in autoimmune encephalitis, but as we know from multiple sclerosis treatment it should take into account years of previous immunotherapies, anti JCV antibody index but also higher risk of PML under prolonged immunosuppressive therapy (143, 144).

CONCLUSION

We do show that real-life data gained in a single center is comparable with literature, although we do often stop maintenance treatment and introduce regular and close monitoring. The outcome is wide spread and depends mostly on time to diagnosis and to initiation of treatment as well as on the underlying autoantibodies and coexisting disorders, i.e., worse outcome in patients with onconeural antibodies. The frequency of autoimmune mediated encephalitis is increasing over time and more and more patients are referred from other disciplines—especially from psychiatry. This is of great importance as awareness of encephalitis mediated by autoantibodies in patients with manifold symptoms will lead to increasing numbers of

testing for autoantibodies and consecutively rising numbers of patients diagnosed with autoimmune encephalitis.

We identified anti-LGi1 and anti-NMDAR encephalitis as most common causes in our cohort. Finally there are pending questions:

1) How can the identification of patients with autoimmune encephalitis in view of mostly unspecific symptoms be made easier?
2) What are the best treatment options for the various antibody associated syndromes?
3) When should treatment be escalated and when can it be terminated (as seen in one of our patients with anti-NMDAR encephalitis who show improvement to treatment after 22 months)?

Ad (1) We suggest an interdisciplinary view. Testing for antibodies should be done for sera and CSF in patients with slightest suspicion (atypical or new onset psychosis at an older age with no explanation) or a history of co-existing malignancy. Testing should be performed at institutes with proven expertise.

Ad (2) For patients with antibodies against surface ags rituximab and plasmapheresis are promising agents. For patients with onconeural antibodies tumor control is by far the best treatment option. Steroids, cyclophosphamide, or IVIGs might have some effects. Trials on immunotherapeutics for those patients should be planned. More data on best treatment options is needed. International collaborations have to be initiated. Treatment should be performed in tertiary hospitals.

Ad (3) Studies and trials have to be implemented to test for scales, biomarkers. In individual cases treatment can be stopped, still close monitoring is needed (MRI, CSF, antibody titres, neuropsychological, and clinical evaluations).

Differential diagnosis is broad and essential to be taken into account. Anamnesis, correct interpretation of the CSF, radiological assessment are the clues to appropriate diagnosis. Whereas, we are testing on slightest suspect, other clinics with not that short way to diagnostics may have to set up diagnostic pathways. Autoimmune mediated encephalitis might still be underdiagnosed, thus awareness has to be increased and testing for antibodies should be performed in sera and CSF.

AUTHOR CONTRIBUTIONS

SM and PR are responsible for design, writing, intellectual content. All authors are responsible for intellectual content, critical review, and design.

REFERENCES

1. Venkatraman A, Opal P. Paraneoplastic cerebellar degeneration with anti-Yo antibodies – a review. *Ann Clin Transl Neurol*. (2016) 3:655–63. doi: 10.1002/acn3.328
2. Corsellis JA, Goldberg GJ, Norton AR. "Limbic encephalitis" and its association with carcinoma. *Brain* (1968) 91:481–96.
3. Brierley JB, Corsellis JAN, Hierons R, Nevin S. Subacute encephalitis of later adult life. Mainly affecting the limbic areas. *Brain* (1960) 83:357–68. doi: 10.1093/brain/83.3.357
4. Graus F, Saiz A. [Limbic encephalitis: a probably under-recognized syndrome]. *Neurol Barc Spain* (2005) 20:24–30.
5. Tüzün E, Dalmau J. Limbic encephalitis and variants: classification, diagnosis and treatment. *Neurologist* (2007) 13:261–71. doi: 10.1097/NRL.0b013e31813e34a5
6. Leypoldt F, Wandinger KP, Bien CG, Dalmau J. Autoimmune encephalitis. *Eur Neurol Rev*. (2013) 8:31–7. doi: 10.17925/ENR.2013.08.01.31
7. Granerod J, Ambrose HE, Davies NW, Clewley JP, Walsh AL, Morgan D, et al. Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study. *Lancet Infect Dis*. (2010) 10:835–44. doi: 10.1016/S1473-3099(10)70222-X
8. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke* (1988) 19:604–7. doi: 10.1161/01.STR.19.5.604
9. Murinson BB, Guarnaccia JB. Stiff-person syndrome with amphiphysin antibodies: distinctive features of a rare disease. *Neurology* (2008) 71:1955–8. doi: 10.1212/01.wnl.0000327342.58936.e0
10. Budde-Steffen C, Anderson NE, Rosenblum MK, Posner JB. Expression of an antigen in small cell lung carcinoma lines detected by antibodies from patients with paraneoplastic dorsal root ganglionpathy. *Cancer Res*. (1988) 48:430–4.
11. Sabater L, Saiz A, Dalmau J, Graus F. Pitfalls in the detection of CV2 (CRMP5) antibodies. *J Neuroimmunol*. (2016) 290:80–3. doi: 10.1016/j.jneuroim.2015.11.009
12. Titulaer MJ, Soffietti R, Dalmau J, Gilhus NE, Giometto B, Graus F, et al. Screening for tumours in paraneoplastic syndromes: report of an EFNS task force. *Eur J Neurol*. (2011) 18:19–e3. doi: 10.1111/j.1468-1331.2010.03220.x
13. Steiner J, Prüß H, Köhler S, Hasan A, Falkai P. [Autoimmune encephalitis with psychotic symptoms : diagnostics, warning signs and practical approach]. *Nervenarzt* (2018) 89:530–8. doi: 10.1007/s00115-018-0499-z
14. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol*. (2016) 15:391–404. doi: 10.1016/S1474-4422(15)00401-9
15. Titulaer MJ, McCracken L, Gabilondo I, Armangué T, Glaser C, Iizuka T, et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. *Lancet Neurol*. (2013) 12:157–65. doi: 10.1016/S1474-4422(12)70310-1
16. Dalmau J, Lancaster E, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R. Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *Lancet Neurol*. (2011) 10:63–74. doi: 10.1016/S1474-4422(10)70253-2
17. van Sonderen A, Thijs RD, Coenders EC, Jiskoot LC, Sanchez E, de Bruijn MAAM, et al. Anti-LGI1 encephalitis: clinical syndrome and long-term follow-up. *Neurology* (2016) 87:1449–56. doi: 10.1212/WNL.0000000000003173
18. Finke C, Prüss H, Heine J, Reuter S, Kopp UA, Wegner F, et al. Evaluation of cognitive deficits and structural hippocampal damage in encephalitis with leucine-rich, glioma-inactivated 1 antibodies. *JAMA Neurol*. (2017) 74:50–9. doi: 10.1001/jamaneurol.2016.4226
19. Zhang T, Duan Y, Ye J, Xu W, Shu N, Wang C, et al. Brain MRI characteristics of patients with anti-N-methyl-D-aspartate receptor encephalitis and their associations with 2-year clinical outcome. *AJNR Am J Neuroradiol*. (2018) 39:824–9. doi: 10.3174/ajnr.A5593
20. Kotsenas AL, Watson RE, Pittock SJ, Britton JW, Hoye SL, Quek AML, et al. MRI findings in autoimmune voltage-gated potassium channel complex encephalitis with seizures: one potential etiology for mesial temporal sclerosis. *AJNR Am J Neuroradiol*. (2014) 35:84–9. doi: 10.3174/ajnr.A3633
21. Miller TD, Chong TTJ, Aimola Davies AM, Ng TWC, Johnson MR, Irani SR, et al. Focal CA3 hippocampal subfield atrophy following LGI1 VGKC-complex antibody limbic encephalitis. *Brain* (2017) 140:1212–9. doi: 10.1093/brain/awx070
22. Iizuka T, Yoshii S, Kan S, Hamada J, Dalmau J, Sakai F, et al. Reversible brain atrophy in anti-NMDA receptor encephalitis: a long-term observational study. *J Neurol*. (2010) 257:1686–91. doi: 10.1007/s00415-010-5604-6

23. Oyanguren B, Sánchez V, González FJ, de Felipe A, Esteban L, López-Sendón JL, et al. Limbic encephalitis: a clinical-radiological comparison between herpetic and autoimmune etiologies. *Eur J Neurol.* (2013) 20:1566–70. doi: 10.1111/ene.12249
24. Dalmau J, Graus F, Villarejo A, Posner JB, Blumenthal D, Thiessen B, et al. Clinical analysis of anti-Ma2-associated encephalitis. *Brain* (2004) 127:1831–44. doi: 10.1093/brain/awh203
25. Carvajal-González A, Leite MI, Waters P, Woodhall M, Coutinho E, Balint B, et al. Glycine receptor antibodies in PERM and related syndromes: characteristics, clinical features and outcomes. *Brain* (2014) 137:2178–92. doi: 10.1093/brain/awu142
26. Rudzinski LA, Pittock SJ, McKeon A, Lennon VA, Britton JW. Extratemporal EEG and MRI findings in ANNA-1 (anti-Hu) encephalitis. *Epilepsy Res.* (2011) 95:255–62. doi: 10.1016/j.eplepsyres.2011.04.006
27. Honnorat J, Didelot A, Karantoni E, Ville D, Ducray F, Lambert L, et al. Autoimmune limbic encephalopathy and anti-Hu antibodies in children without cancer. *Neurology* (2013) 80:2226–32. doi: 10.1212/WNL.0b013e318296e9c3
28. Kim SE, Lee BI, Shin KJ, Ha SY, Park J, Park KM, et al. Characteristics of seizure-induced signal changes on MRI in patients with first seizures. *Seizure* (2017) 48:62–8. doi: 10.1016/j.seizure.2017.04.005
29. Gresa-Arribas N, Titulaer MJ, Torrents A, Aguilar E, McCracken L, Leypoldt F, et al. Antibody titres at diagnosis and during follow-up of anti-NMDA receptor encephalitis: a retrospective study. *Lancet Neurol.* (2014) 13:167–77. doi: 10.1016/S1474-4422(13)70282-5
30. Schmitt SE, Pargeon K, Frechette ES, Hirsch LJ, Dalmau J, Friedman D. Extreme delta brush: a unique EEG pattern in adults with anti-NMDA receptor encephalitis. *Neurology* (2012) 79:1094–100. doi: 10.1212/WNL.0b013e3182698cd8
31. Veciana M, Becerra JL, Fossas P, Muriana D, Sansa G, Santamarina E, et al. EEG extreme delta brush: an ictal pattern in patients with anti-NMDA receptor encephalitis. *Epilepsy Behav.* (2015) 49:280–5. doi: 10.1016/j.yebeh.2015.04.032
32. Prüß H. Neuroimmunologie: Neues zur limbischen Enzephalitis. *Akt Neurol.* (2013) 40:127–36. doi: 10.1055/s-0033-1337973
33. Graus F, Cordon-Cardo C, Posner JB. Neuronal antinuclear antibody in sensory neuronopathy from lung cancer. *Neurology* (1985) 35:538–43. doi: 10.1212/WNL.35.4.538
34. Albert ML, Austin LM, Darnell RB. Detection and treatment of activated T cells in the cerebrospinal fluid of patients with paraneoplastic cerebellar degeneration. *Ann Neurol.* (2000) 47:9–17. doi: 10.1002/1531-8249(200001)47:1<9::AID-ANA5>3.0.CO;2-I
35. Pignolet BS, Gebauer CM, Liblau RS. Immunopathogenesis of paraneoplastic neurological syndromes associated with anti-Hu antibodies: a beneficial antitumor immune response going awry. *Oncoimmunology* (2013) 2:e27384. doi: 10.4161/onci.27384
36. Jarius S, Wildemann B. 'Medusa head ataxia': the expanding spectrum of Purkinje cell antibodies in autoimmune cerebellar ataxia. Part 3: Anti-Yo/CDR2, anti-Nb/AP3B2, PCA-2, anti-Tr/DNER, other antibodies, diagnostic pitfalls, summary and outlook. *J. Neuroinflammation* (2015) 12:168. doi: 10.1186/s12974-015-0358-9
37. Kim H, Lim Y, Kim K-K. Anti-ri-antibody-associated paraneoplastic syndrome in a man with breast cancer showing a reversible pontine lesion on MRI. *J Clin Neurol Seoul Korea* (2009) 5:151–2. doi: 10.3988/jcn.2009.5.3.151
38. Waldvogel HJ, Baer K, Eady E, Allen KL, Gilbert RT, Mohler H, et al. Differential localization of γ-aminobutyric acid type a and glycine receptor subunits and gephyrin in the human pons, medulla oblongata and uppermost cervical segment of the spinal cord: an immunohistochemical study. *J Comp Neurol.* (2009) 518:305–28. doi: 10.1002/cne.22212
39. Lancaster E, Huijbers MGM, Bar V, Boronat A, Wong A, Martinez-Hernandez E, et al. Investigations of caspr2, an autoantigen of encephalitis and neuromyotonia. *Ann Neurol.* (2011) 69:303–11. doi: 10.1002/ana.22297
40. Lai M, Huijbers MGM, Lancaster E, Graus F, Bataller L, Balice-Gordon R, et al. Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. *Lancet Neurol.* (2010) 9:776–85. doi: 10.1016/S1474-4422(10)70137-X
41. Rojas-Marcos I, Graus F, Sanz G, Robledo A, Diaz-Espejo C. Hypersomnia as presenting symptom of anti-Ma2-associated encephalitis: case study. *Neuro Oncol.* (2007) 9:75–7. doi: 10.1215/15228517-2006-013
42. Overeem S, Dalmau J, Bataller L, Nishino S, Mignot E, Verschuuren J, et al. Hypocretin-1 CSF levels in anti-Ma2 associated encephalitis. *Neurology* (2004) 62:138–40. doi: 10.1212/01.WNL.0000101718.92619.67
43. Murphy SM, Khan U, Alifrangis C, Hazell S, Hrouda D, Blake J, et al. Anti Ma2-associated myeloradiculopathy: expanding the phenotype of anti-Ma2 associated paraneoplastic syndromes. *J Neurol Neurosurg Psychiatry* (2012) 83:232–3. doi: 10.1136/jnnp.2010.223271
44. Waragai M, Chiba A, Uchibori A, Fukushima T, Anno M, Tanaka K. Anti-Ma2 associated paraneoplastic neurological syndrome presenting as encephalitis and progressive muscular atrophy. *J Neurol Neurosurg Psychiatry* (2006) 77:111–3. doi: 10.1136/jnnp.2005.068775
45. Ortega Suero G, Sola-Valls N, Escudero D, Saiz A, Graus F. Anti-Ma and anti-Ma2-associated paraneoplastic neurological syndromes. *Neurol Barc Spain* (2018) 33:18–27. doi: 10.1016/j.nrl.2016.05.010
46. Vitaliani R, Mason W, Ances B, Zwerdling T, Jiang Z, Dalmau J. Paraneoplastic encephalitis, psychiatric symptoms, and hypoventilation in ovarian teratoma. *Ann Neurol.* (2005) 58:594–604. doi: 10.1002/ana.20614
47. Dalmau J, Tüzün E, Wu H, Masjuan J, Rossi JE, Voloschin A, et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann Neurol.* (2007) 61:25–36. doi: 10.1002/ana.21050
48. Hughes EG, Peng X, Gleichman AJ, Lai M, Zhou L, Tsou R, et al. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *J Neurosci.* (2010) 30:5866–75. doi: 10.1523/JNEUROSCI.0167-10.2010
49. Lai M, Hughes EG, Peng X, Zhou L, Gleichman AJ, Shu H, et al. AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. *Ann Neurol.* (2009) 65:424–34. doi: 10.1002/ana.21589
50. Bataller L, Galiano R, García-Escrig M, Martínez B, Sevilla T, Blasco R, et al. Reversible paraneoplastic limbic encephalitis associated with antibodies to the AMPA receptor. *Neurology* (2010) 74:265–7. doi: 10.1212/WNL.0b013e3181cb3e52
51. Huganir RL, Nicoll RA. AMPARs and synaptic plasticity: the last 25 years. *Neuron* (2013) 80:704–17. doi: 10.1016/j.neuron.2013.10.025
52. Höftberger R, van Sonderen A, Leypoldt F, Houghton D, Geschwind M, Gelfand J, et al. Encephalitis and AMPA receptor antibodies: novel findings in a case series of 22 patients. *Neurology* (2015) 84:2403-12. doi: 10.1212/WNL.0000000000001682
53. Lancaster E, Dalmau J. Neuronal autoantigens–pathogenesis, associated disorders and antibody testing. *Nat Rev Neurol.* (2012) 8:380–90. doi: 10.1038/nrneurol.2012.99
54. Lancaster E. The diagnosis and treatment of autoimmune encephalitis. *J Clin Neurol Seoul Korea* (2016) 12:1–13. doi: 10.3988/jcn.2016.12.1.1
55. Fenalti G, Law RHP, Buckle AM, Langendorf C, Tuck K, Rosado CJ, et al. GABA production by glutamic acid decarboxylase is regulated by a dynamic catalytic loop. *Nat Struct Mol Biol.* (2007) 14:280–6. doi: 10.1038/nsmb1228
56. Patel AB, de Graaf RA, Martin DL, Battaglioli G, Behar KL. Evidence that GAD65 mediates increased GABA synthesis during intense neuronal activity *in vivo*. *J Neurochem.* (2006) 97:385–96. doi: 10.1111/j.1471-4159.2006.03741.x
57. Saiz A, Blanco Y, Sabater L, González F, Bataller L, Casamitjana R, et al. Spectrum of neurological syndromes associated with glutamic acid decarboxylase antibodies: diagnostic clues for this association. *Brain* (2008) 131:2553–63. doi: 10.1093/brain/awn183
58. Almarzouqi SJ, Morgan ML, Carvounis PE, Lee AG. Autoimmune-related retinopathy and optic neuropathy accompanied by anti-GAD antibodies. *Neuro Ophthalmol.* (2015) 39:195–200. doi: 10.3109/01658107.2015.1050744
59. Fileccia E, Rinaldi R, Liguori R, Incensi A, D'Angelo R, Giannoccaro MP, et al. Post-ganglionic autonomic neuropathy associated with anti-glutamic acid decarboxylase antibodies. *Clin Auton Res.* (2017) 27:51–5. doi: 10.1007/s10286-016-0390-6
60. Platt MP, Agalliu D, Cutforth T. Hello from the other side: how autoantibodies circumvent the blood-brain barrier in autoimmune encephalitis. *Front Immunol.* (2017) 8:442. doi: 10.3389/fimmu.2017.00442
61. Mitoma H, Adhikari K, Aeschlimann D, Chattopadhyay P, Hadjivassiliou M, Hampe CS, et al. Consensus paper: neuroimmune mechanisms

of cerebellar ataxias. *Cerebellum Lond Engl.* (2016) 15:213–32. doi: 10.1007/s12311-015-0664-x
62. Ali F, Rowley M, Jayakrishnan B, Teuber S, Gershwin ME, Mackay IR. Stiff-person syndrome (SPS) and anti-GAD-related CNS degenerations: protean additions to the autoimmune central neuropathies. *J Autoimmun.* (2011) 37:79–87. doi: 10.1016/j.jaut.2011.05.005
63. Dinkel K, Meinck HM, Jury KM, Karges W, Richter W. Inhibition of gamma-aminobutyric acid synthesis by glutamic acid decarboxylase autoantibodies in stiff-man syndrome. *Ann Neurol.* (1998) 44:194–201. doi: 10.1002/ana.410440209
64. Manto MU, Laute MA, Aguera M, Rogemond V, Pandolfo M, Honnorat J. Effects of anti-glutamic acid decarboxylase antibodies associated with neurological diseases. *Ann Neurol.* (2007) 61:544–51. doi: 10.1002/ana.21123
65. Burton AR, Baquet Z, Eisenbarth GS, Tisch R, Smeyne R, Workman CJ, et al. Central nervous system destruction mediated by glutamic acid decarboxylase-specific CD4+ T cells. *J Immunol.* (2010) 184:4863–70. doi: 10.4049/jimmunol.0903728
66. Costa M, Saiz A, Casamitjana R, Castañer MF, SanmartÍ A, Graus F, et al. T-cell reactivity to glutamic acid decarboxylase in stiff-man syndrome and cerebellar ataxia associated with polyendocrine autoimmunity. *Clin Exp Immunol.* (2002) 129:471–8. doi: 10.1046/j.1365-2249.2002.01931.x
67. Holmøy T, Skorstad G, Røste LS, Scheie D, Alvik K. Stiff person syndrome associated with lower motor neuron disease and infiltration of cytotoxic T cells in the spinal cord. *Clin Neurol Neurosurg.* (2009) 111:708–12. doi: 10.1016/j.clineuro.2009.06.005
68. Hansen N, Grünewald B, Weishaupt A, Colaço MN, Toyka KV, Sommer C, et al. Human Stiff person syndrome IgG-containing high-titer anti-GAD65 autoantibodies induce motor dysfunction in rats. *Exp Neurol.* (2013) 239:202–9. doi: 10.1016/j.expneurol.2012.10.013
69. Sommer C, Weishaupt A, Brinkhoff J, Biko L, Wessig C, Gold R, et al. Paraneoplastic stiff-person syndrome: passive transfer to rats by means of IgG antibodies to amphiphysin. *Lancet Lond Engl.* (2005) 365:1406–11. doi: 10.1016/S0140-6736(05)66376-3
70. Dalakas MC, Fujii M, Li M, Lutfi B, Kyhos J, McElroy B. High-dose intravenous immune globulin for stiff-person syndrome. *N Engl J Med.* (2001) 345:1870–6. doi: 10.1056/NEJMoa01167
71. Graus F, Delattre JY, Antoine JC, Dalmau J, Giometto B, Grisold W, et al. Recommended diagnostic criteria for paraneoplastic neurological syndromes. *J Neurol Neurosurg Psychiatry* (2004) 75:1135–40. doi: 10.1136/jnnp.2003.034447
72. Ariño H, Höftberger R, Gresa-Arribas N, Martínez-Hernández E, Armangue T, Kruer MC, et al. Paraneoplastic neurological syndromes and glutamic acid decarboxylase antibodies. *JAMA Neurol.* (2015) 72:874–81. doi: 10.1001/jamaneurol.2015.0749
73. Fauser S, Uttner I, Ariño H, Scherbaum WA, Saiz A, Lewerenz J. Long latency between GAD-antibody detection and development of limbic encephalitis–a case report. *BMC Neurol.* (2015) 15:177. doi: 10.1186/s12883-015-0435-9
74. Dalakas MC, Rakocevic G, Dambrosia JM, Alexopoulos H, McElroy B. A double-blind, placebo-controlled study of rituximab in patients with stiff person syndrome. *Ann Neurol.* (2017) 82:271–7. doi: 10.1002/ana.25002
75. Guan HZ, Ren HT, Cui LY. Autoimmune encephalitis: an expanding frontier of neuroimmunology. *Chin Med J.* (2016) 129:1122–7. doi: 10.4103/0366-6999.180514
76. Hutchinson M, Waters P, McHugh J, Gorman G, O'Riordan S, Connolly S, et al. Progressive encephalomyelitis, rigidity, and myoclonus: a novel glycine receptor antibody. *Neurology* (2008) 71:1291–2. doi: 10.1212/01.wnl.0000327606.50322.f0
77. McKeon A, Martinez-Hernandez E, Lancaster E, Matsumoto JY, Harvey RJ, McEvoy KM, et al. Glycine receptor autoimmune spectrum with stiff-man syndrome phenotype. *JAMA Neurol.* (2013) 70:44–50. doi: 10.1001/jamaneurol.2013.574
78. Meinck HM, Thompson PD. Stiff man syndrome and related conditions. *Mov Disord.* (2002) 17:853–66. doi: 10.1002/mds.10279
79. Whiteley AM, Swash M, Urich H. Progressive encephalomyelitis with rigidity. *Brain* (1976) 99:27–42. doi: 10.1093/brain/99.1.27
80. Poliak S, Gollan L, Salomon D, Berglund EO, Ohara R, Ranscht B, et al. Localization of Caspr2 in myelinated nerves depends on axon-glia interactions and the generation of barriers along the axon. *J Neurosci.* (2001) 21:7568–75. doi: 10.1523/JNEUROSCI.21-19-07568.2001
81. Poliak S, Gollan L, Martinez R, Custer A, Einheber S, Salzer JL, et al. Caspr2, a new member of the neurexin superfamily, is localized at the juxtaparanodes of myelinated axons and associates with K+ channels. *Neuron* (1999) 24:1037–47. doi: 10.1016/S0896-6273(00)81049-1
82. Patterson KR, Dalmau J, Lancaster E. Mechanisms of Caspr2 antibodies in autoimmune encephalitis and neuromyotonia. *Ann Neurol.* (2017) 83:40–51. doi: 10.1002/ana.25120
83. Sabater L, Planagumà J, Dalmau J, Graus F. Cellular investigations with human antibodies associated with the anti-IgLON5 syndrome. *J Neuroinflammation* (2016) 13:226. doi: 10.1186/s12974-016-0689-1
84. van Sonderen A, Ariño H, Petit-Pedrol M, Leypoldt F, Körtvélyessy P, Wandinger KP, et al. The clinical spectrum of Caspr2 antibody-associated disease. *Neurology* (2016) 87:521–8. doi: 10.1212/WNL.0000000000002917
85. Barber PA, Anderson NE, Vincent A. Morvan's syndrome associated with voltage-gated K+ channel antibodies. *Neurology* (2000) 54:771–2. doi: 10.1212/wnl.54.3.771
86. Bastiaansen AEM, van Sonderen A, Titulaer MJ. 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). *Curr Opin Neurol.* (2017) 30:302–9. doi: 10.1097/WCO.0000000000000444
87. Bien CG, Mirzadjanova Z, Baumgartner C, Onugoren MD, Grunwald T, Holtkamp M, et al. Anti-contactin-associated protein-2 encephalitis: relevance of antibody titres, presentation and outcome. *Eur J Neurol.* (2017) 24:175–86. doi: 10.1111/ene.13180
88. Bien CG. [Principles of autoimmune and paraneoplastic encephalitis]. *Nervenarzt* (2018) 89:934–41. doi: 10.1007/s00115-018-0501-9
89. Li Z, Cui T, Shi W, Wang Q. Clinical analysis of leucine-rich glioma inactivated-1 protein antibody associated with limbic encephalitis onset with seizures. *Medicine* (2016) 95:e4244. doi: 10.1097/MD.0000000000004244
90. Pozo-Rosich P, Clover L, Saiz A, Vincent A, Graus F. Voltage-gated potassium channel antibodies in limbic encephalitis. *Ann Neurol.* (2003) 54:530–3. doi: 10.1002/ana.10713
91. Zuliani L, Saiz A, Tavolato B, Giometto B, Vincent A, Graus F. Paraneoplastic limbic encephalitis associated with potassium channel antibodies: value of anti-glial nuclear antibodies in identifying the tumour. *J Neurol Neurosurg Psychiatry* (2007) 78:204–5. doi: 10.1136/jnnp.2006.102467
92. van Sonderen A, Petit-Pedrol M, Dalmau J, Titulaer MJ. The value of LGI1, Caspr2 and voltage-gated potassium channel antibodies in encephalitis. *Nat Rev Neurol.* (2017) 13:290–301. doi: 10.1038/nrneurol.2017.43
93. Escudero D, Guasp M, Ariño H, Gaig C, Martínez-Hernández E, Dalmau J, et al. Antibody-associated CNS syndromes without signs of inflammation in the elderly. *Neurology* (2017) 89:1471–5. doi: 10.1212/WNL.0000000000004541
94. Irani SR, Michell AW, Lang B, Pettingill P, Waters P, Johnson MR, et al. (2011). Faciobrachial dystonic seizures precede Lgi1 antibody limbic encephalitis. *Ann. Neurol.* 69, 892–900. doi: 10.1002/ana.22307
95. Irani SR, Stagg CJ, Schott JM, Rosenthal CR, Schneider SA, Pettingill P, et al. Faciobrachial dystonic seizures: the influence of immunotherapy on seizure control and prevention of cognitive impairment in a broadening phenotype. *Brain* (2013) 136:3151–62. doi: 10.1093/brain/awt212
96. Irani SR, Gelfand JM, Bettcher BM, Singhal NS, Geschwind MD. Effect of rituximab in patients with leucine-rich, glioma-inactivated 1 antibody-associated encephalopathy. *JAMA Neurol.* (2014) 71:896–900. doi: 10.1001/jamaneurol.2014.463
97. Malter MP, Frisch C, Schoene-Bake JC, Helmstaedter C, Wandinger KP, Stoecker W, et al. Outcome of limbic encephalitis with VGKC-complex antibodies: relation to antigenic specificity. *J Neurol.* (2014) 261:1695–705. doi: 10.1007/s00415-014-7408-6
98. Sabater L, Gaig C, Gelpi E, Bataller L, Lewerenz J, Torres-Vega E, et al. A novel non-rapid-eye movement and rapid-eye-movement parasomnia with sleep breathing disorder associated with antibodies to IgLON5: a case series, characterisation of the antigen, and post-mortem study. *Lancet Neurol.* (2014) 13:575–86. doi: 10.1016/S1474-4422(14)70051-1

99. Gelpi E, Höftberger R, Graus F, Ling H, Holton JL, Dawson T, et al. Neuropathological criteria of anti-IgLON5-related tauopathy. *Acta Neuropathol.* (2016) 132:531–43. doi: 10.1007/s00401-016-1591-8
100. Gaig C, Iranzo A, Santamarí J, Dalmau J, Graus F. Sleep disorder associated with antibodies to IgLON5: parasomnia or agrypnia?-Authors' reply. *Lancet Neurol.* (2014) 13:864–5. doi: 10.1016/S1474-4422(14)70157-7
101. Graus F, Santamaría J. Understanding anti-IgLON5 disease. *Neurol Neuroimmunol Neuroinflammation* (2017) 4:e393. doi: 10.1212/NXI.0000000000000393
102. Gaig C, Graus F, Compta Y, Högl B, Bataller L, Brüggemann N, et al. Clinical manifestations of the anti-IgLON5 disease. *Neurology* (2017) 88:1736–43. doi: 10.1212/WNL.0000000000003887
103. Honorat JA, Komorowski L, Josephs KA, Fechner K, St Louis EK, Hinson SR, et al. IgLON5 antibody: Neurological accompaniments and outcomes in 20 patients. *Neurol Neuroimmunol Neuroinflammation* (2017) 4:e385. doi: 10.1212/NXI.0000000000000385
104. Brain L, Jellinek EH, Ball K. Hashimoto's disease and encephalopathy. *Lancet Lond Engl.* (1966) 2:512–4.
105. Ferracci F, Bertiato G, Moretto G. Hashimoto's encephalopathy: epidemiologic data and pathogenetic considerations. *J Neurol Sci.* (2004) 217:165–8. doi: 10.1016/j.jns.2003.09.007
106. Olmez I, Moses H, Sriram S, Kirshner H, Lagrange AH, Pawate S. Diagnostic and therapeutic aspects of Hashimoto's encephalopathy. *J Neurol Sci.* (2013) 331:67–71. doi: 10.1016/j.jns.2013.05.009
107. Castillo P, Woodruff B, Caselli R, Vernino S, Lucchinetti C, Swanson J, et al. Steroid-responsive encephalopathy associated with autoimmune thyroiditis. *Arch Neurol.* (2006) 63:197–202. doi: 10.1001/archneur.63.2.197
108. Laurent C, Capron J, Quillerou B, Thomas G, Alamowitch S, Fain O, et al. Steroid-responsive encephalopathy associated with autoimmune thyroiditis (SREAT): characteristics, treatment and outcome in 251 cases from the literature. *Autoimmun Rev.* (2016) 15:1129–33. doi: 10.1016/j.autrev.2016.09.008
109. Jamrozik Z, Janik P, Kiljanski J, Kwiecinski H. [Hashimoto's encephalopathy. Case report and literature review]. *Neurol Neurochir Pol.* (2004) 38:55–9.
110. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab.* (2002) 87:489–99. doi: 10.1210/jcem.87.2.8182
111. Blanchin S, Coffin C, Viader F, Ruf J, Carayon P, Potier F, et al. Anti-thyroperoxidase antibodies from patients with Hashimoto's encephalopathy bind to cerebellar astrocytes. *J Neuroimmunol.* (2007) 192:13–20. doi: 10.1016/j.jneuroim.2007.08.012
112. Leypoldt F, Wandinger KP. Paraneoplastic neurological syndromes. *Clin Exp Immunol.* (2014) 175:336–48. doi: 10.1111/cei.12185
113. Gebauer C, Pignolet B, Yshii L, Mauré E, Bauer J, Liblau R. CD4+ and CD8+ T cells are both needed to induce paraneoplastic neurological disease in a mouse model. *Oncoimmunology* (2016) 6:e1260212. doi: 10.1080/2162402X.2016.1260212
114. Hermetter C, Fazekas F, Hochmeister S. Systematic review: syndromes, early diagnosis, and treatment in autoimmune encephalitis. *Front Neurol.* (2018) 9:706. doi: 10.3389/fneur.2018.00706
115. Probasco JC, Solnes L, Nalluri A, Cohen J, Jones KM, Zan E, et al. Decreased occipital lobe metabolism by FDG-PET/CT: an anti-NMDA receptor encephalitis biomarker. *Neurol Neuroimmunol Neuroinflammation* (2018) 5:e413. doi: 10.1212/NXI.0000000000000413
116. Tripathi M, Tripathi M, Roy SG, Parida GK, Ihtisham K, Dash D, et al. Metabolic topography of autoimmune non-paraneoplastic encephalitis. *Neuroradiology* (2018) 60:189–98. doi: 10.1007/s00234-017-1956-2
117. Lang K, Prüss H. Frequencies of neuronal autoantibodies in healthy controls: estimation of disease specificity. *Neurol Neuroimmunol Neuroinflammation* (2017) 4:e386. doi: 10.1212/NXI.0000000000000386
118. Chen CH, Cheng MC, Liu CM, Liu CC, Lin KH, Hwu HG. Seroprevalence survey of selective anti-neuronal autoantibodies in patients with first-episode schizophrenia and chronic schizophrenia. *Schizophr Res.* (2017) 190:28–31. doi: 10.1016/j.schres.2017.03.012
119. Mantere O, Saarela M, Kieseppä T, Raij T, Mäntylä T, Lindgren M, et al. Anti-neuronal anti-bodies in patients with early psychosis. *Schizophr Res.* (2017) 192:404–7. doi: 10.1016/j.schres.2017.04.027
120. Kayser MS, Titulaer MJ, Gresa-Arribas N, Dalmau J. Frequency and characteristics of isolated psychiatric episodes in anti–N-methyl-d-aspartate receptor encephalitis. *JAMA Neurol.* (2013) 70:1133–9. doi: 10.1001/jamaneurol.2013.3216
121. Busse S, Busse M, Brix B, Probst C, Genz A, Bogerts B, et al. Seroprevalence of N-methyl-D-aspartate glutamate receptor (NMDA-R) autoantibodies in aging subjects without neuropsychiatric disorders and in dementia patients. *Eur Arch Psychiatry Clin Neurosci.* (2014) 264:545–50. doi: 10.1007/s00406-014-0493-9
122. Constantinescu R, Krýsl D, Bergquist F, Andrén K, Malmeström C, Asztély F, et al. Cerebrospinal fluid markers of neuronal and glial cell damage to monitor disease activity and predict long-term outcome in patients with autoimmune encephalitis. *Eur J Neurol.* (2016) 23:796–806. doi: 10.1111/ene.12942
123. Salovin A, Glanzman J, Roslin K, Armangue T, Lynch DR, Panzer JA. Anti-NMDA receptor encephalitis and nonencephalitic HSV-1 infection. *Neurol Neuroimmunol Neuroinflammation* (2018) 5:e458. doi: 10.1212/NXI.0000000000000458
124. Rupprecht TA, Manz KM, Fingerle V3, Lechner C1, Klein M4, Pfirrmann M, et al. Diagnostic value of cerebrospinal fluid CXCL13 for acute Lyme neuroborreliosis. A systemic review and meta-analysis. *Clin Microbiol Infect.* (2018) doi: 10.1016/j.cmi.2018.04.007. [Epub ahead of print].
125. Leypoldt F, Höftberger R, Titulaer MJ, Armangue T, Gresa-Arribas N, Jahn H, et al. Investigations on CXCL13 in anti-N-methyl-D-aspartate receptor encephalitis: a potential biomarker of treatment response. *JAMA Neurol.* (2015) 72:180–6. doi: 10.1001/jamaneurol.2014.2956
126. Herken J, Prüss H. Red flags: clinical signs for identifying autoimmune encephalitis in psychiatric patients. *Front Psychiatry* (2017) 8:25. doi: 10.3389/fpsyt.2017.00025
127. Bonello M, Jacob A, Ellul MA, Barker E, Parker R, Jefferson S, et al. IgLON5 disease responsive to immunotherapy. *Neurol Neuroimmunol Neuroinflammation* (2017) 4:e383. doi: 10.1212/NXI.0000000000000383
128. Haitao R, Yingmai Y, Yan H, Fei H, Xia L, Honglin H, et al. Chorea and parkinsonism associated with autoantibodies to IgLON5 and responsive to immunotherapy. *J Neuroimmunol.* (2016) 300:9–10. doi: 10.1016/j.jneuroim.2016.09.012
129. Rakocevic G, Raju R, Semino-Mora C, Dalakas MC. Stiff person syndrome with cerebellar disease and high-titer anti-GAD antibodies. *Neurology* (2006) 67:1068–70. doi: 10.1212/01.wnl.0000237558.83349.d0
130. Jones AL, Flanagan EP, Pittock SJ, Mandrekar JN, Eggers SD, Ahlskog JE, et al. Responses to and outcomes of treatment of autoimmune cerebellar ataxia in adults. *JAMA Neurol.* (2015) 72:1304–12. doi: 10.1001/jamaneurol.2015.2378
131. Leypoldt F, Armangue T, Dalmau J. Autoimmune encephalopathies. *Ann N Y Acad Sci.* (2015) 1338:94–114. doi: 10.1111/nyas.12553
132. Titulaer MJ, Dalmau J. Seizures as first symptom of anti-NMDA receptor encephalitis are more common in men. *Neurology* (2014) 82:550–1. doi: 10.1212/WNL.0000000000000131
133. Rocamora R, Becerra JL, Fossas P, Gomez M, Vivanco-Hidalgo RM, Mauri JA, et al. Pilomotor seizures: an autonomic semiology of limbic encephalitis? *Seizure* (2014) 23:670–3. doi: 10.1016/j.seizure.2014.04.013
134. Cleverly K, Gambadauro P, Navaratnarajah R. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis: have you checked the ovaries? *Acta Obstet Gynecol Scand.* (2014) 93:712–5. doi: 10.1111/aogs.12386
135. Derksen SJ, Goraj B, Molenaar JP, Van der Hoeven JG. Severe anti NMDA encephalitis and EBV infection. *Netherlands J Crit Care* (2013) 17:19–21.
136. Behrendt V, Krogias C, Reinacher-Schick A, Gold R, Kleiter I. Bortezomib treatment for patients with anti-N-methyl-d-aspartate receptor encephalitis. *JAMA Neurol.* (2016) 73:1251–3. doi: 10.1001/jamaneurol.2016.2588
137. Sveinsson O, Granqvist M, Forslin Y, Blennow K, Zetterberg H, Piehl F. Successful combined targeting of B- and plasma cells in treatment

refractory anti-NMDAR encephalitis. *J Neuroimmunol*. (2017) 312:15–8. doi: 10.1016/j.jneuroim.2017.08.011
138. Scheibe F, Prüss H, Mengel AM, Kohler S, Nümann A, Köhnlein M, et al. Bortezomib for treatment of therapy-refractory anti-NMDA receptor encephalitis. *Neurology* (2017) 88:366–70. doi: 10.1212/WNL.0000000000003536
139. Shin YW, Lee ST, Park KI, Jung KH, Jung KY, Lee SK, et al. Treatment strategies for autoimmune encephalitis. *Ther Adv Neurol Disord*. (2018) 11:1–19. doi: 10.1177/1756285617722347
140. Lee WJ, Lee ST, Moon J, Sunwoo JS, Byun JI, Lim JA, et al. Tocilizumab in autoimmune encephalitis refractory to rituximab: an institutional cohort study. *Neurother J Am Soc Exp Neurother*. (2016) 13:824–32. doi: 10.1007/s13311-016-0442-6
141. Malter MP, Frisch C, Zeitler H, Surges R, Urbach H, Helmstaedter C, et al. Treatment of immune-mediated temporal lobe epilepsy with GAD antibodies. *Seizure* (2015) 30:57–63. doi: 10.1016/j.seizure.2015.05.017
142. Fleischmann R, Prüss H, Rosche B, Bahnemann M, Gelderblom H, Deuschle K, et al. Severe cognitive impairment associated with intrathecal antibodies to the NR1 subunit of the N-methyl-D-aspartate receptor in a patient with multiple sclerosis. *JAMA Neurol*. (2015) 72:96–9. doi: 10.1001/jamaneurol.2014.1817
143. Bloomgren G, Richman S, Hotermans C, Subramanyam M, Goelz S, Natarajan A, et al. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. *N Engl J Med*. (2012) 366:1870–80. doi: 10.1056/NEJMoa1107829
144. Ho PR, Koendgen H, Campbell N, Haddock B, Richman S, Chang I. Risk of natalizumab-associated progressive multifocal leukoencephalopathy in patients with multiple sclerosis: a retrospective analysis of data from four clinical studies. *Lancet Neurol*. (2017) 16:925–33. doi: 10.1016/S1474-4422(17)30282-X

Conflict of Interest Statement: PR received speaker honoraries from Biogen, Roche, Sanofi Genzyme, Merck. PR received research support from Roche and Merck. PR served on advisory boards for Roche, Merck.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 23 October 2018
doi: 10.3389/fneur.2018.00888

Diagnosis and Treatment of NMO Spectrum Disorder and MOG-Encephalomyelitis

Nadja Borisow[1], Masahiro Mori[2], Satoshi Kuwabara[2], Michael Scheel[1,3] and Friedemann Paul[1,4]*

[1] NeuroCure Clinical Research Center, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany, [2] Department of Neurology, Graduate School of Medicine, Chiba University, Chiba, Japan, [3] Department of Neuroradiology, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany, [4] Experimental and Clinical Research Center, Max Delbrueck Center for Molecular Medicine and Charité – Universitätsmedizin Berlin, Berlin, Germany

Neuromyelitis optica spectrum disorders (NMOSD) are autoantibody mediated chronic inflammatory diseases. Serum antibodies (Abs) against the aquaporin-4 water channel lead to recurrent attacks of optic neuritis, myelitis and/or brainstem syndromes. In some patients with symptoms of NMOSD, no AQP4-Abs but Abs against myelin-oligodendrocyte-glycoprotein (MOG) are detectable. These clinical syndromes are now frequently referred to as "MOG-encephalomyelitis" (MOG-EM). Here we give an overview on current recommendations concerning diagnosis of NMOSD and MOG-EM. These include antibody and further laboratory testing, MR imaging and optical coherence tomography. We discuss therapeutic options of acute attacks as well as longterm immunosuppressive treatment, including azathioprine, rituximab, and immunoglobulins.

Keywords: neuromyelitis optica, aquaporin-4 antibodies, MOG-encephalomyelitis, diagnostic criteria, immunosuppressive treatment

OPEN ACCESS

Edited by:
Johann Sellner, Universitätsklinikum Salzburg, Austria

Reviewed by:
Barbara M. P. Willekens, Antwerp University Hospital, Belgium
Nicolas Collongues, Hôpitaux Universitaires de Strasbourg, France

****Correspondence:***
Nadja Borisow
nadja.borisow@charite.de

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *27 June 2018*
Accepted: *01 October 2018*
Published: *23 October 2018*

Citation:
Borisow N, Mori M, Kuwabara S, Scheel M and Paul F (2018) Diagnosis and Treatment of NMO Spectrum Disorder and MOG-Encephalomyelitis. Front. Neurol. 9:888. doi: 10.3389/fneur.2018.00888

INTRODUCTION

Neuromyelitis optica spectrum disorders (NMOSD) are rare chronic inflammatory central nervous system diseases distinct from multiple sclerosis (MS). The French term "neuro-myélite optique aiguë," which may be translated as "neuromyelitis optica acuta" was first used by Devic in 1894 (1, 2). In the majority of patients with NMOSD, autoantibodies (Abs) against the astrocyte aquaporin-4 (AQP4) water channel are detectable and patients typically suffer from recurrent attacks of severe optic neuritis or/and myelitis (3–7). In rarer cases, brainstem and brain involvement e.g., area postrema syndrome or diencephalic syndrome can occur (8, 9). Patients also frequently suffer from burdensome symptoms like pain, headache, depression, fatigue, and sleep disorders (10–14). Despite treatment, recovery from attacks is often incomplete and disease remission rarely occurs (15, 16). Thus, in relapsing NMOSD, which account for approximately 80–85% of cases, neurologic deficits frequently accumulate during the disease course. Patients without long-term immunosuppressive therapy have a worse prognosis with a higher mortality rate (17). Disease onset ranges between 4 and 88 years with a mean age at onset of 39 years (18–21). Women are disproportionately more often affected and, particularly in AQP4-seropositive patients, female to male-ratio can reach up to 10:1 (19, 22, 23). In 20–30% of patients, depending on the assay used, AQP4-Abs are not detectable (24, 25). Whether AQP4-Ab positive and AQP4-Ab negative diseases are varieties of the same disorder or rather reflect different disease entities is a topic of ongoing research (26–28).

Recently, various publications described the detection of serum-Abs against myelin-oligodendrocyte-glycoprotein (MOG) in AQP4-Ab negative NMOSD patients including pediatric cohorts and few patients with MS (29–41). In the past, MOG-Abs were particularly described in acute disseminated encephalomyelitis (ADEM), an inflammatory CNS disorder that, if it has an pediatric onset, is mostly monophasic and has a favorable outcome in the majority of cases (42, 43). MOG is a glycoprotein localized on the surface of the myelin sheath as well as of the cell body and processes of oligodendrocytes (44, 45). According to the revised 2015 NMOSD diagnostic criteria (46), diseases with or without evidence of AQP4-Abs as well as disorders with MOG-Abs can be assigned to the NMO spectrum. Although there are numerous overlaps in clinical presentation and imaging findings with NMOSD with and without AQP4-Ab, MOG-Ab-associated disease is more and more considered a disease entity in its own (47). Previous studies on NMOSD might have included patients with MOG-Abs and therefore overlapping features could have been reported in these studies. Various terms are used to describe the disease such as "MOG-antibody related disorder," "MOG-associated disease," "MOG antibody disease," "MONEM" or "MOG-encephalomyelitis (MOG-EM)"(40, 47–50). Hereafter, we use the term "MOG-EM," as it reflects the relevant symptoms of the disease and is used in several recent publications, e.g., (49). Although ADEM can also be accompanied by MOG-Abs (51), in this manuscript we do not regard MOG-Ab positive patients with ADEM-phenotype as part of the "MOG-EM" due to their distinct clinical characteristics. To date, the relevance of MOG-Abs and their nosologic categorization is a topic of current discussion and under further investigation (47, 52, 53).

To give an overview on diagnosis and treatment recommendations in NMOSD and MOG-EM, we here describe our own clinical experiences and give a review on the current literature using the Pubmed online database. We used the search terms "neuromyelitis optica," "neuromyelitis optica spectrum disorder," "MOG," aquaporin-4 antibodies," "MRI," "diagnostic criteria," "therapy," and combinations of these. To find all relevant publications, we did not restrict the year of publication; however, most reports originate from the last 5 years.

DIAGNOSIS

In NMOSD and MOG-EM, most common symptoms are optic neuritis and longitudinally extensive transverse myelitis (LETM). Signs of brainstem affection like persistent hiccup, nausea or vomiting should explicitly be asked for as they are often attributed to other reasons and are therefore not reported spontaneously by the patient. Rarer clinical manifestations of NMOSD comprise narcolepsy, acute diencephalic syndrome or muscle affection (54, 55), while in MOG-EM extraneural involvement such as reversible paraspinal muscle hyperintensity have been described, as well as MOG-Abs in combined central and peripheral demyelination syndromes (56, 57).

Like NMOSD, MOG-EM can affect optic nerve, spinal cord, and brainstem. However, some studies showed histopathological differences between NMOSD and MOG-EM (58, 59). AQP4-Abs bind to water channels located on astrocytes, whereas MOG-Abs target myelin-forming oligodendrocytes (53). Both types of antibodies may lead to disturbances of the integrity of blood brain barrier and to CNS inflammation (53, 60). However, while inflammation in MOG-EM primarily results in demyelination, demyelination in NMOSD seems to be a secondary phenomenon following astrocytic damage (61, 62).

In patients with AQP4-Abs, the most frequent symptoms at onset are optic neuritis in 37-54% of the patients, and LETM in 30–47% of the patients (26, 63, 64). In patients with MOG-Abs, optic neuritis was the first clinical manifestation in 33–64% whereas myelitis occurred in 18–33% of the patients as initial symptom (33, 48, 65). Also during the further course of the disease, optic neuritis seems to be more frequent in MOG-EM than in NMOSD with myelitis being less common (29, 66). However, in population-based ON studies and unselected cohorts of patients with ON, both the prevalence of AQP4-Abs and MOG-Abs is low (67–69). In MOG-EM, cases of encephalitis and seizures were described whereas these symptoms are rare in NMOSD (70–72). MOG-EM differs from NMOSD in further clinical characteristics e.g., in gender ratio and age at onset. In (relapsing) NMOSD, up to 90% of the patients are female, whereas the proportion of male patients in MOG-EM ranges from 43 to 63% (22, 26, 29–31, 73). The published mean age at onset ranges from 27 and 37 years in patients with MOG-EM (29–31, 73) and between 30 and 46 years for patients with NMOSD (19, 26, 29–31, 73). At onset, patients with MOG-Abs are more likely to suffer from simultaneous or rapidly sequential optic neuritis and LETM compared to patients with AQP4-Abs (31). In AQP4-Ab positive NMOSD, most patients (80–90%) have a relapsing disease course (26, 73, 74). In MOG-EM, monophasic disease course is considered to be more frequent, however, the duration of follow-up and a referral bias might have influenced these results (33, 73–76). Some studies showed lower disability outcomes, measured by the Expanded Disability Status Scale (EDSS), in MOG-EM than in NMOSD, suggesting a presumably more favorable prognosis (29–31, 73). However, long-term data from MOG-EM are scant. Whereas spinal cord lesions frequently affect cervicothoracic segments in NMOSD, they tend to be localized in thoracolumbar parts of the spinal cord including the conus in MOG-EM (29, 31). **Table 1** summarizes the epidemiological and clinical features in NMOSD and MOG-EM.

Antibody Diagnosis

A central component of diagnostics in NMOSD and MOG-EM is the detection of Abs in serum. AQP4-Abs were firstly described in 2004 and made it possible to differentiate NMOSD from MS (78). The best detection rates are provided by cell-based assays (CBA) (24, 32, 79, 80). In NMOSD, the sensitivity of these assays ranges between 80 and 100%, whereas specificity varies between 86 and 100% (24). Contrarily, enzyme-linked immunosorbent assays (ELISA) may lead to false-positive results and should not be used as sole method (81–83).

Specific antibodies against MOG are detectable in pediatric patients with acute disseminated encephalomyelitis (ADEM)

TABLE 1 | Epidemiological and clinical features in NMOSD and MOG-EM.

	AQP4-Ab positive NMOSD	MOG-EM
Mean age at onset [range]	40–46 years (26, 31, 73)	27–37 years (30, 73)
Female to male ratio [range]	7.2:1–10:1 (26, 29, 31, 73)	1:1.6–1.3:1 (29–31)
Median EDSS at last follow-up [range]	4.0–5.8 (29, 31, 73)	0–1.5 (29, 31, 73)
Frequency of coexisting autoimmune diseases	16–45% (31, 73)	6–11% (31, 73)
Localization of optic nerve lesions	orbital, chiasm (77)	orbital, canalicular, intracranial (77)
Features of optic neuritis	OCT: prominent RNFL thinning (77)	Severe optic nerve swelling at onset (77); frequently simultaneous or rapidly sequential optic neuritis and LETM (31)
Localization of spinal cord lesions	Cervical, thoracic (29)	Thoracic, lumbar (29), involving conus (31)
MRI brain lesions	More frequently lesions in medulla oblongata and area postrema (65)	More frequently ADEM-like brain lesions, deep gray matter lesions (31), lesions in pons, thalamus (65)

(84–86). In MS patients, MOG-Abs were described for the first time at the beginning of the 1990s (87). Further studies confirmed these findings (88–90). Later, MOG-Abs were found in AQP4-Ab negative patients with clinical symptoms of NMOSD (91, 92). Like in NMOSD, CBA are the current gold standard to detect MOG-Abs (39, 49). Formerly used assays had a low MOG specificity, which led to high rates of false positive results (39, 49). Therefore to date, cell-based assays targeting at full-length human MOG and the use of IgG1-specific secondary antibodies is highly recommended to avoid cross-reactivity with IgM and IgA antibodies (39, 49).

AQP4- and MOG-serostatus and Ab-level may change during the disease course. In patients with suspected NMOSD or MOG-EM without initial evidence for seropositivity, further Ab-testing may be required during the course of the disease, especially during acute attacks and intervals without treatment. AQP4-Abs usually stay detectable during remission, although the titer may be lower with immunosuppression (some patients even seroconvert to negative over time) and during acute attacks (93). In MOG-EM, approximately 80% of patients with evidence of MOG-Abs during acute attack remained seropositive during remission (33). However, the rate was lower with only 50% of patients remaining seropositive in a study from Korea (76). As in AQP4-Ab positive NMOSD, MOG-Ab serum titers are significantly higher during acute attack than during remission (33). In some studies, Ab-titers were associated with relapses and treatment status (32, 93, 94). However, the level of AQP4-titer does not seem to be predictive for long term disease course (95), and AQP4-Ab serostatus is not predictive of response to immunotherapy (96). Testing of patients with progressive MS for MOG antibodies is not warranted under most circumstances (97).

Testing CSF for AQP4- or MOG Abs is not routinely recommended as it does not seem to provide an additional benefit for diagnosing NMOSD or MOG-EM (98, 99). AQP4-Abs in CSF can be detected in only 70% of Aqp4-Ab seropositive patients and in none of the AQP4 seronegative patients (100). Like AQP4-Abs, MOG-Abs are produced mainly extrathecally and are therefore less frequent in CSF than in serum (32).

Comorbidity with other autoimmune disorders is frequent in NMOSD patients (101–105). Therefore, further tests for autoantibodies should comprise Abs associated with rheumatologic diseases e.g., ANA, ANCA, Anti-ds-DNA-Abs, and lupus anticoagulant. If there are clinical signs in anamnesis or examination, Ab testing for myasthenia gravis, coeliac disease, or paraneoplastic disorders should be performed (101, 106–110). In MOG-EM, the frequency of coexistent autoimmune diseases seems to be lower than reported for AQP4-Ab positive patients (33, 66).

Further Laboratory Diagnosis

Other laboratory tests are recommended to diagnose coexisting autoimmune disorders and to exclude other differential diagnoses. Next to routine laboratory tests, this includes differential blood count, blood sedimentation, folic acid, and vitamin B12 (111). To exclude sarcoidosis which is a relevant differential diagnosis as it can also manifest with optic neuropathy or myelopathy (112, 113), tests on hypercalcemia and hypercalciuria, interleukin-2-rezeptor (sIL-2 R), and angiotensin-converting enzyme (ACE) should be performed (112, 114).

Analysis of cerebrospinal fluid (CSF) might be helpful to exclude other diagnoses, especially to differentiate between NMOSD/MOG-EM and MS. In NMOSD, white cell counts were elevated in up to 50% of the patients, especially during acute attack, and in approximately 10% of the patients CSF-restricted oligoclonal IgG bands (OCB) can be detected (73, 100). Increased CSF/serum albumin ratio as a marker of dysfunction of blood brain barrier was found in 51% of NMOSD patients (100).

In MOG-EM, elevated white cell counts were found in 25–70% of the patients, whereas there was no differentiation between tests during acute attack and remission. (33, 66, 73). Like in NMOSD, OCB were detected in 10% of the MOG-EM patients and CSF/serum albumin ratio was elevated in 32% (33, 66, 73).

Magnetic Resonance Imaging

Next to the AQP4-Abs, MRI is an essential element to diagnose NMOSD. It helps to differentiate NMOSD from MS and other CNS disorders (115).

Spinal cord imaging was already included in the 2006 NMO diagnostic criteria (116). These criteria require MRI spinal cord lesion extending over $\geq$3 vertebral segments (116). However, in 15 percent of myelitis attacks, spinal cord lesions

do not extend over ≥3 vertebral segments which may lead to misdiagnosis or delayed diagnosis of NMOSD (117). Typical NMOSD lesions are located centrally in the spinal cord and involve more than the half of spinal cord cross-section area (118). It was suggested by Yonezu et al.(119) that "bright spotty lesions" are characteristic for NMOSD and might reflect microcystic defects of the spinal cord (113). The specificity of this sign however still needs to be confirmed in further studies. The interval between clinical symptoms and the MRI is influencing the MRI presentation of LETM lesions. They may not be present from relapse onset and may change into multiple short lesions or into spinal cord atrophy during the disease course (120, 121). Hence, there is the risk miss a typical MRI presentation of the LETM when the MRI is performed too early or too late (72). Other causes for longitudinally extensive spinal cord LETM lesions include sarcoidosis or spondylotic myelopathy or rarely MS and need to be considered (112, 122, 123). In addition longitudinally extensive myelitis lesions were recently described in patients with symptoms of meningitis, encephalitis and/or myelitis that were tested positive for glial fibrillary acidic protein (GFAP)-IgG (124–126).

Brain MRI at first presentation often shows no lesions which has been the reason to define normal brain MRI as one NMO diagnostic criterion in 2006 (116). However, more recent studies showed that the presence of cerebral lesions is not uncommon in the clinical course of NMOSD (127–129). Hence, the NMOSD 2015 diagnostic criteria have incorporated findings of cerebral MRI and define NMOSD-typical brain lesions (46). These lesions can be located at the periependymal surfaces of the third and fourth ventricle, in the area postrema, corpus callosum, hypothalamus or thalamus (130–132). In addition, subcortical or deep white matter lesions are possible. Meningeal enhancement has been reported in some cases, although this does not appear to be a very frequent imaging finding in NMOSD (133). Orbital MRI may show increased T2 signal and gadolinium enhancement of the optic nerve as signs of an optic neuritis. This can be helpful to diagnose MOG-EM or NMOSD in patients without AQP4-Abs (46, 77, 131, 134, 135). Chiasmal involvement is more common in AQP4-NMOSD than in MOG-EM (134).

A study by Ramanathan et al. showed no MRI brain lesions in a large proportion of MOG-EM patients (66). Conversely, other authors found supra- and infratentorial MRI abnormalities in 40–50% of the patients (33, 65). Brain imaging allows to distinguish MOG-EM from MS, but shows many overlaps with AQP4-Ab NMOSD (136–138). Moreover, a relevant number of patients show pathologic findings in MRI of optic nerve and spinal cord, comparable to NMOSD patients (33, 74). However, one study revealed a more frequent occurrence of optic nerve head swelling and retrobulbar affection of the optic nerve in MOG-EM compared to NMOSD (134).

Figure 1 shows MRI features of NMOSD and MOG-EM. Studies investigating non-conventional MR imaging in NMOSD will not be reviewed further as they currently lack implications for clinical management (139–141).

Optical Coherence Tomography

Optical coherence tomography is an interferometric technique using near infra-red backscattered light to generate high resolution images of the retina and its various layers, that is increasingly applied in various neuroimmunological disorders (142–148). OCT displays severe damage to the retinal nerve fiber layer and the ganglion cell layer following attacks of optic neuritis in both AQP4 NMOSD and MOG-EM that correlates with visual function and quality of life (149–158). It is currently a matter of debate if retinal damage following optic neuritis is equally severe in AQP4-NMOSD and MOG-EM (75, 77, 152, 159–162) and to which extent structural retinal alterations occur in NMOSD independently of optic neuritis attacks (143, 157, 163–167). Although the utility of OCT in patient management requires further investigation, it may help quantify the extent of structural retinal damage following optic neuritis attacks and thus hopefully inform treatment decisions (168–170), and support differential diagnosis in the near future.

Diagnostic Criteria

Current NMOSD diagnostic criteria were published by the International Panel for NMO Diagnosis in 2015 (46) and were aimed at taking recent advances in the field following the 2006 Wingerchuk criteria into consideration (116). They differentiate between NMOSD with AQP4-Abs and NMOSD without AQP4-Abs or unknown AQP4-Ab status.

In the case of positive AQP4-Ab status, one of the following clinical core symptoms is required:

1. Optic neuritis
2. Acute myelitis
3. Area postrema syndrome: episode of otherwise unexplained hiccups or nausea and vomiting
4. Acute brainstem syndrome
5. Symptomatic narcolepsy or acute diencephalic clinical syndrome with NMOSD-typical diencephalic MRI lesions
6. Symptomatic cerebral syndrome with NMOSD-typical brain lesions

NMOSD-typical brain lesions may involve the dorsal medulla, especially the area postrema, the periependymal surfaces of the third or fourth ventricle, the hypothalamus, thalamus, the corpus callosum, cerebral peduncles, and the internal capsule. Moreover, subcortical or deep white matter lesions and corticospinal tract lesions are possible. Alternative diagnoses e.g., MS, sarcoidosis, infectious or neoplastic diseases have to be excluded.

In patients without evidence of AQP4-Ab two of the above mentioned core clinical characteristics are necessary for NMOSD diagnosis. At least one of these core clinical characteristics has to be ON, LETM or area postrema syndrome. Moreover, supportive characteristics in cerebral, spinal cord or optic nerve MRI are required. These are

- normal brain MRI or long optic nerve lesions with increased T2 signal or gadolinium enhancement of the optic nerve or the chiasm in patients with ON,
- spinal cord MRI lesion or focal spinal cord atrophy extending over ≥3 segments in patients with myelitis and

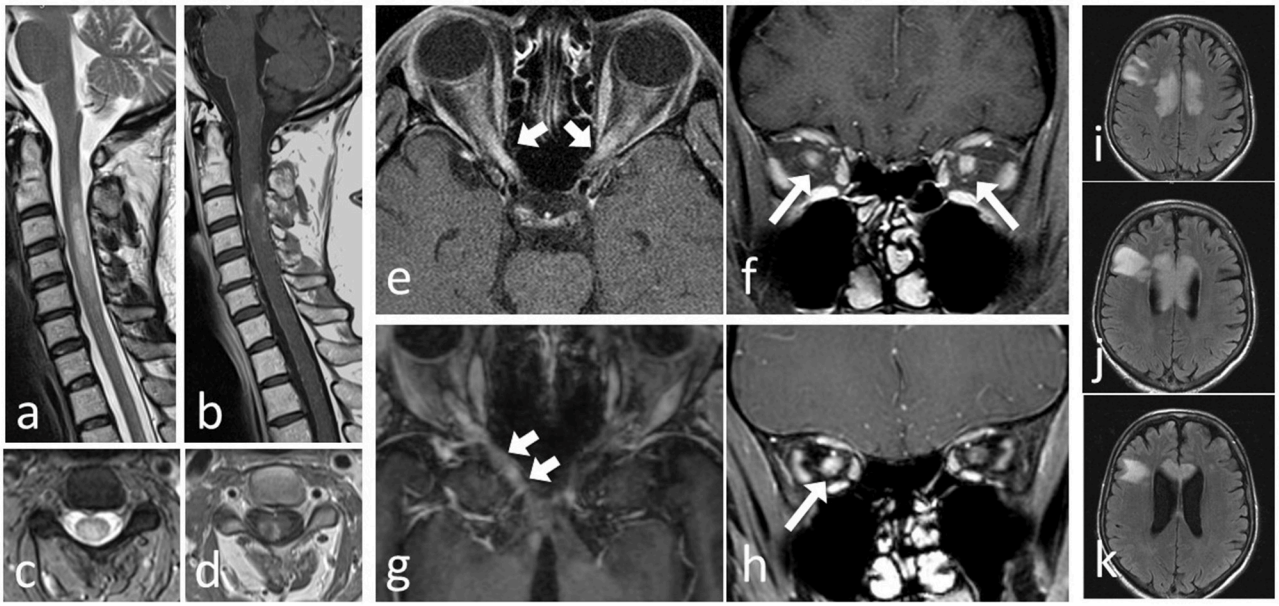


FIGURE 1 | MRI of patients with AQP4-Ab positive NMOSD and patients with MOG-EM. Severe cervical LETM in a NMOSD AQP+ patient: **(a)** T2 sagittal and **(b)** T2 axial of a cervical myelon lesion with ring Gd-Enhancement and T1 hypointense center in **(c)** T1+Gd sagittal and **(d)** T1+Gd axial. Bilateral opticusneuritis in a MOG-EM patient **(e)** T1+Gd axial and **(f)** T1+Gd coronar. Unilateral optic neuritis with chiasmal involvement in a NMOSD AQP4+ patient: **(g)** T1+Gd axial and **(h)** T1+Gd coronar. Tumefactive lesion involving the corpus callosum in a NMOSD AQP4+ patient **(i–k)** T2 axial.

- lesions involving dorsal medulla oblongata/area postrema in patients with area postrema syndrome
- periependymal brainstem lesions in patients with acute brainstem syndrome.

Using the 2015 instead of the 2006 criteria led to a significant increase in the number of patients diagnosed with NMOSD (138, 171, 172)

For MOG-EM, to date, no evidence based diagnostic criteria exist. However, NMOSD 2015 diagnostic criteria allow to include cases of NMOSD associated with other specific autoantibodies (46).

TREATMENT OF ACUTE ATTACKS

In NMOSD as well as in MOG-EM, acute attacks are usually treated with 1,000 mg intravenous methylprednisolone (IVMP) for 3–5 days. Jarius et al. showed complete or almost complete recovery in 50% of IVMP treated MOG-EM attacks (33). In NMOSD, IVMP led to complete recovery in 17–35% of the attacks (15, 173). In case of poor response, treatment escalation with 2,000 mg IVMP may improve outcome, for further therapy escalation plasma exchange (PLEX) or immunoadsorption are possible (15, 173–175). PLEX and immunoadsorption did not show a difference in their efficacy in the therapy of NMOSD attacks (176). They can also be used as first-line therapy (in particular in myelitis attacks) if response to methylprednisolone during previous attacks was poor. An early initiation of PLEX seems to improve the clinical outcome (176, 177).

PREVENTATIVE IMMUNOSUPPRESSIVE THERAPY

Attacks in NMOSD as well as in MOG-EM are often characterized by severe neurologic deficits with poor recovery. Frequently, a relevant disability persists after an attack. However, there are indications that MOG-EM has a less severe course than NMOSD and relapse risk depends on Ab status (30, 65, 66). In some patients with evidence of MOG-Abs, seroconversion to an Ab- negative status may occur during the disease course (30, 32, 76).

There is increasing evidence that immunosuppressive therapy is essential to reduce disease activity and to avoid further attacks. However, to date no placebo controlled trial has been published and only one open randomized clinical trial has been performed (178). Thus, the current treatment paradigm is based on case series, (retrospective) observational studies as well as expert opinion. Hereafter, we describe the to-date used treatments in NMOSD and MOG-EM (179).

Low Dose Prednisone/Prednisolone

Low dose oral corticosteroids are used in many neurologic diseases. Oral prednisone/prednisolone can be given subsequent to attack therapy with IVMP in decreasing dose levels and as comedication during the first months of azathioprine (AZA) or mycophenolate mofetil (MMF) treatment until these drugs exert their full efficacy. Possible side effects are weight gain, hypertension, thrombosis, osteoporosis, fungal and viral infections, hyperglycemia, gastritis and

peptic ulcer, psychiatric disturbances and a Cushing syndrome (180).

Data on long-term treatment with oral prednisone/prednisolone in NMOSD are limited. A few studies could show a decrease in ARR by low dose steroid therapy (181, 182). Moreover, it is known from treatment experiences with AZA that additional oral prednisone is effective to reduce disease activity during the first 3–6 months until AZA reaches its full efficacy.

In MOG-EM, low treatment failure rates were achieved with oral prednisone (66). The occurrence of relapses during tapering or after cessation of subsequent oral prednisone after IVMP attack treatment supports the beneficial effects of corticosteroid therapy in MOG-EM (33), at least in patients with persistence of MOG Abs (66). However, due to the known side effects and the existence of other treatment alternatives, a long-term therapy with low dose prednisone should be critically weighed.

Azathioprine

AZA is a purine analog, acts as antimetabolite and inhibits the differentiation of lymphocytes. Thereby it has antiproliferative and immunosuppressive effects. It is administered in a dose of 2–3 mg/kg body weight per day and reaches its full effectiveness after 3–6 months. During the initial period, additional oral prednisone [1 mg/kg/day] is necessary and can be slowly tapered when AZA becomes fully effective.

The most important side effect is a bone marrow depression with anemia, leuko- and/or thrombopenia. The risk of bacterial, viral or fungal infection is increased. Moreover, elevation of liver enzymes, nausea or emesis can appear. Rare side effects especially after long treatment duration include malignomas e.g., of the skin, and a progressive multifocal leukoencephalopathy (PML). Furthermore, add-on therapy with prednisone enhances the risk of side effects, like a diabetogenic metabolic state, thrombosis or psychiatric symptoms.

Patients with a congenital deficiency of thiopurinmethyltransferase (TPMT), an enzyme responsible for metabolisation of AZA, have a high risk of bone marrow depression. Therefore, it is recommended to test for TPMT-deficiency in patients with pronounced deterioration of blood count after initiation of AZA-therapy.

A recently published prospective randomized controlled trial compared the efficacy of AZA and rituximab (RTX) in NMOSD. It showed a significant decrease in mean ARR from 1 to 0.51 and a decrease in mean EDSS from 2.40 to 1.95 by AZA (178). 54% of the patient treated with AZA became relapse free after 1 year (178). A prospective study including 77 NMOSD patients (183) and other retrospective studies (181, 184–186) showed comparable results.

In a study by Jarius et al. 14 out of 17 MOG-patients (82%) suffered from at least one attack while treated with AZA (33). Attacks occurred mainly in patients that were not co-treated with oral prednisone and during the first 6 months. This highlights the need for co-treatment with oral prednisone until AZA reaches its full efficacy.

Rituximab

RTX is a monoclonal Ab directed against the surface molecule CD20 on B-lymphocytes. RTX leads to a depletion of CD20+B-lymphocytes, which act as precursor cells of antibody producing plasma cells (187). A thereby triggered reduction of antibody formation is presumably the RTX mechanism of action.

The most frequently used dose regimen is the intravenous administration of each 1,000mg with an interval of 2 weeks followed by 6-monthly dosages of 1,000mg (179, 188). Alternatively, initially 375 mg/m^2 body surface every week over a period of 4 weeks can be administered. As an alternative to a fixed dosage regimen every 6 months, monitoring of CD19+/CD20+ B-lymphocytes and administration of RTX in the case of reconstitution of these cells is possible (189, 190). Another option is the administration of RTX depending on monitoring of CD27+ memory B-cells which might in some cases allow to lower the cumulative RTX dose (191). An evidence that one of these regimens has therapeutic superiority over the other does not exist to date.

Before first administration, active infections like tuberculosis or hepatitis B have to be excluded (192). An update of vaccination status and anti-pneumococcal vaccination is recommended (192).

Side effects include infusion-related symptoms like pruritus, headache, rash or fever. To reduce the risk of these symptoms, a premedication with an analgesic/antipyretic and an antihistamine is recommended. The risk of infections and severe skin reactions like the Lyell-syndrome or the Stevens-Johnson-Syndrome is elevated. Cardiac symptoms e.g., arrhythmia or cardiac insufficiency were reported (193). Moreover, neurologists must be aware of hypogammaglobulinemia that may occur with long-term RTX treatment (194).

In 2005, an open label study described for the first time a significant reduction in disease activity in eight NMO patients treated with RTX (195). Since then, an increasing number of patients was treated with RTX. However, to date only a few prospective studies investigating the effect of RTX on NMOSD exist. The above mentioned study by Nikoo et al. showed a reduction of the ARR by 83 percent as mean ARR decreased from 1.30 to 0.21 (178). Mean EDSS decreased from 3.55 to 2.56. Other prospective and retrospective trials found significant reductions of ARR to values between 0.1 and 0.46 in adult and pediatric patients treated with RTX (181, 196–202). A further overview on efficacy and safety profile of RTX in NMOSD is given in the topical literature (203–205).

AZA and RTX are the most frequently used immunosuppressants in NMOSD. With regards to ARR and EDSS, comparison studies between both drugs seem to suggest a superiority of RTX compared to AZA (178, 184). Thus, currently RTX seems to be the most effective treatment in NMOSD, although some studies describe a rebound in disease activity shortly after RTX induction (199, 206). Treatment effect does not seem to depend on AQP4-serostatus (96).

In MOG-EM, treatment with RTX led to a decline in relapse rate in only 3 out of 9 patients (33). Most of the attacks occurred shortly after RTX infusion. Some authors recommend RTX as second-line-therapy if preventative treatment with

low-dose prednisone or monthly intravenous immunoglobulins (IVIG) is not effective (66). In patients with myelitis, RTX is recommended from an earlier stage as a myelitis often leads to severe residual deficits (66). Whether RTX is indeed less effective in MOG-EM than in NMOSD has to be analyzed in further studies (207).

Mycophenolate Mofetil

MMF is an immunosuppressant that inhibits the inosine monophosphate dehydrogenase. Thereby, the synthesis of guanosin nucleotide and subsequently, the proliferation of B- and T-lymphocytes is inhibited. The administered daily dose ranges between 750 and 3,000 mg/d (179, 208, 209).

The most common side effects are leucopenia, diarrhea, vomiting and sepsis. The risk of malignomas can be increased especially if MMF is combined with other immunosuppressants.

A retrospective observational study investigated the effect of MMF in NMOSD and MOG-EM. 33/67 (49%) of the patients were relapse-free, in 44/53 (83%) the EDSS improved or stabilized (208). Other observational studies showed similar results with proportions of relapse-free patients between 56 and 60% in NMOSD (210, 211) In comparison to AZA, MMF showed fewer side effects with equal efficacy (211, 212). As in treatment with RTX, response to MMF does not differ in dependence on AQP4-serostatus (96).

In MOG-EM patients, a combined therapy with MMF and steroids appeared to have a positive effect; however, this effect diminished after steroid tapering (66). As MMF may take several months to reach its full efficacy, add-on prednisone should be tapered only very slowly.

Intravenous Immunoglobulins

Even less data is available for treatment with IVIG in NMOSD. A small retrospective study including six patients with NMO/NMOSD treated with IVIG 2–3- monthly showed a decrease in ARR from 0.75 to 0.15 (213). One study investigated IVIG treatment of acute NMOSD relapses (214), however, further data on preventive IVIG therapy is lacking.

In a study by Ramanathan et al, 4 out of 7 MOG-EM patients treated with IVIG were relapse-free (66). The authors recommend prophylaxis with low-dose prednisone or monthly IVIG with MMF or RTX as a next step for treating MOG-EM. Jarius et al. reported data of one MOG-EM patient who was relapse-free during 11 months of IVIG treatment and 12 months after IVIG discontinuation (33).

Methotrexate

Methotrexate (MTX) is an analog of folate, acts as folate antagonist and inhibits the dihydrofolate reductase. Hereby it inhibits DNA and RNA synthesis and has an immunosuppressive and anti-inflammatory effect. Side effects include gastrointestinal symptoms like nausea or diarrhea, bone marrow depression and an increase of liver enzymes.

Retrospective studies in NMOSD showed a decrease of ARR between 64 and 87% (215–217). In MOG-EM, MTX led to disease stabilization in 5/6 patients (33). Therefore, MTX seems to be a treatment option in patients that do not respond to first-line-therapy or suffer from side effects of other treatments (216).

MS Immunomodulatory Medication and Rarer Treatment Options

Treatment with MS medications like interferon-beta, glatiramer acetate, fingolimod, alemtuzumab, natalizumab, and presumably also dimethyl fumarate is known to have no or even harmful effects in NMOSD (181, 218–230). Similar results were found in patients with MOG-EM which were treated with one of these drugs for suspected MS (33); however, studies on treatment effects of these drugs are even rarer than in NMOSD.

Mitoxantrone is able to significantly reduce ARR in NMOSD patients (231, 232), nevertheless, due to its cardio- and myelotoxic side effects and the availability of alternatives with fewer adverse events its use should be considered very critically (233–235). Cyclophosphamide does not seem to be effective in NMOSD (236). Data about the effects of mitoxantrone or cyclophosphamide in MOG-EM are missing.

Ongoing Studies

To date, various clinical trials are ongoing to investigate the effect of new drugs in NMOSD. A placebo-controlled clinical trial is testing the effect of inebelizumab (MEDI-551), a humanized monoclonal antibody against CD19+ B-cells on NMOSD relapse rate (237–239). The efficacy of B-cell-depleting therapy in NMOSD is well known from treatment with RTX. AQP4-Ab positive as well as AQP4-Ab negative patients with at least one relapse during the last year or with at least two relapses during the last 2 years before screening can be included in this study (237).

Another agent under investigation is eculizumab, a monoclonal antibody inhibiting the complement protein C5. There were encouraging findings from an open label study where 12 out of 14 highly active patients became relapse-free by eculizumab treatment (240, 241). A subsequent double-blind placebo-controlled phase 3 trial aiming to enroll approximately 130 patients is now in the open-label extension stage (242).

Tocilizumab, an inhibitor of the IL-6 signaling pathway, showed significant reduction of disease activity in two pilot studies including in total 15 patients with high-active NMOSD (243, 244). Moreover, it might be an option in NMOSD patients with concomitant cancer or paraneoplastic syndrome (245). To date, an open label randomized controlled trial comparing tocilizumab and AZA is recruiting patients (246). Satralizumab (SA237), a follow-on monoclonal antibody of tocilizumab, is under investigation in a placebo-controlled double-blind phase 3 study (247). Efforts to restore immune tolerance as novel therapeutic endeavor are in preparation, however, various technical and conceptual issues hamper prompt implementation in clinical trials and practice (248, 249).

Further information on ongoing or completed (pilot) studies as well as non-conventional treatment approaches, e.g., with cetirizine, regulatory dentritic cells or autologous bone marrow derived stem cells in NMOSD may be found at the website https://clinicaltrials.gov and in current literature (188, 250, 251).

SUMMARY

The diagnosis and treatment in NMOSD and MOG-EM require special clinical expertise. The 2015 NMOSD diagnostic criteria and the availability of antibody testing and MRI are the basis to diagnose and differentiate NMOSD or MOG-EM. Early diagnosis and initiation of adequate therapy are essential—at least in seropositive patients–to avoid disease attacks and persistent deficits. Long term immunosuppressive treatment, e.g., with RTX or AZA, has emerged to be the most effective therapies to reduce disease activity. Further therapeutic options, in particular various monoclonal antibodies are currently under clinical investigation in NMOSD.

AUTHOR CONTRIBUTIONS

NB performed literature research and drafted the manuscript. FP, MM, MS, and SK critically reviewed the manuscript.

REFERENCES

1. Devic E. Myélite aiguë compliquée de névrite optique. *Bull Médicale* (1894) 8:1033–4.
2. Jarius S, Wildemann B. The history of neuromyelitis optica. *J Neuroinflammation* (2013) 10:8. doi: 10.1186/1742-2094-10-8
3. Pache F, Wildemann B, Paul F, Jarius S. [Neuromyelitis optica]. *Fortschr Neurol Psychiatr.* (2017) 85:e1. doi: 10.1055/s-0035-1567186
4. Weinshenker BG, Wingerchuk DM. Neuromyelitis spectrum disorders. *Mayo Clin Proc.* (2017) 92:663–79. doi: 10.1016/j.mayocp.2016.12.014
5. Jarius S, Wildemann B, Paul F. Neuromyelitis optica: clinical features, immunopathogenesis and treatment. *Clin Exp Immunol.* (2014) 176:149–64. doi: 10.1111/cei.12271
6. Metz I, Beißbarth T, Ellenberger D, Pache F, Stork L, Ringelstein M, et al. Serum peptide reactivities may distinguish neuromyelitis optica subgroups and multiple sclerosis. *Neurol Neuroimmunol Neuroinflamm.* (2016) 3:e204. doi: 10.1212/NXI.0000000000000204
7. Mori M, Kuwabara S, Paul F. Worldwide prevalence of neuromyelitis optica spectrum disorders. *J Neurol Neurosurg Psychiatry* (2018) 89:555–6. doi: 10.1136/jnnp-2017-317566
8. Kremer L, Mealy M, Jacob A, Nakashima I, Cabre P, Bigi S, et al. Brainstem manifestations in neuromyelitis optica: a multicenter study of 258 patients. *Mult Scler.* (2014) 20:843–7. doi: 10.1177/1352458513507822
9. Wang KC, Lee CL, Chen SY, Lin KH, Tsai CP. Prominent brainstem symptoms/signs in patients with neuromyelitis optica in a Taiwanese population. *J Clin Neurosci.* (2011) 18:1197–200. doi: 10.1016/j.jocn.2010.12.052
10. Chavarro VS, Mealy MA, Simpson A, Lacheta A, Pache F, Ruprecht K, et al. Insufficient treatment of severe depression in neuromyelitis optica spectrum disorder. *Neurol Neuroimmunol Neuroinflamm.* (2016) 3:e286. doi: 10.1212/NXI.0000000000000286
11. Penner IK, Paul F. Fatigue as a symptom or comorbidity of neurological diseases. *Nat Rev Neurol.* (2017) 13:662–75. doi: 10.1038/nrneurol.2017.117
12. Song Y, Pan L, Fu Y, Sun N, Li YJ, Cai H, et al. Sleep abnormality in neuromyelitis optica spectrum disorder. *Neurol Neuroimmunol Neuroinflammation* (2015) 2:e94. doi: 10.1212/NXI.0000000000000094
13. Asseyer S, Schmidt F, Chien C, Scheel M, Ruprecht K, Bellmann-Strobl J, et al. Pain in AQP4-IgG-positive and MOG-IgG-positive neuromyelitis optica spectrum disorders. *Mult Scler J Exp Transl Clin.* (2018) 4:2055217318796684. doi: 10.1177/2055217318796684
14. Mizuno Y, Shinoda K, Watanabe M, Matsushita T, Yamasaki R, Kira JI. Short-lasting unilateral neuralgiform headache attacks with cranial autonomic symptoms in NMOSD. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e447. doi: 10.1212/NXI.0000000000000447
15. Kleiter I, Gahlen A, Borisow N, Fischer K, Wernecke KD, Wegner B, et al. Neuromyelitis optica: Evaluation of 871 attacks and 1,153 treatment courses. *Ann Neurol.* (2016) 79:206–16. doi: 10.1002/ana.24554
16. Pandit L, Mustafa S. Spontaneous remission lasting more than a decade in untreated AQP4 antibody-positive NMOSD. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e351. doi: 10.1212/NXI.0000000000000351
17. Mealy MA, Kessler RA, Rimler Z, Reid A, Totonis L, Cutter G, et al. Mortality in neuromyelitis optica is strongly associated with African ancestry. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e468. doi: 10.1212/NXI.0000000000000468
18. Collongues N, Marignier R, Zéphir H, Papeix C, Blanc F, Ritleng C, et al. Neuromyelitis optica in France: a multicenter study of 125 patients. *Neurology* (2010) 74:736–42. doi: 10.1212/WNL.0b013e3181d31e35
19. Pandit L, Asgari N, Apiwattanakul M, Palace J, Paul F, Leite MI, et al. Demographic and clinical features of neuromyelitis optica: A review. *Mult Scler.* (2015) 21:845–53. doi: 10.1177/1352458515572406
20. Krumbholz M, Hofstadt-van Oy U, Angstwurm K, Kleiter I, Jarius S, Paul F, et al. Very late-onset neuromyelitis optica spectrum disorder beyond the age of 75. *J Neurol.* (2015) 262:1379–84. doi: 10.1007/s00415-015-7766-8
21. Bove R, Elsone L, Alvarez E, Borisow N, Cortez MM, Mateen FJ, et al. Female hormonal exposures and neuromyelitis optica symptom onset in a multicenter study. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e339. doi: 10.1212/NXI.0000000000000339
22. Borisow N, Kleiter I, Gahlen A, Fischer K, Wernecke KD, Pache F, et al. Influence of female sex and fertile age on neuromyelitis optica spectrum disorders. *Mult Scler.* (2017) 23:1092–103. doi: 10.1177/1352458516671203
23. Wingerchuk DM. Neuromyelitis optica: effect of gender. *J Neurol Sci.* (2009) 286:18–23. doi: 10.1016/j.jns.2009.08.045
24. Waters P, Reindl M, Saiz A, Schanda K, Tuller F, Kral V, et al. Multicentre comparison of a diagnostic assay: aquaporin-4 antibodies in neuromyelitis optica. *J Neurol Neurosurg Psychiatry* (2016) 87:1005–15. doi: 10.1136/jnnp-2015-312601
25. Melamed E, Levy M, Waters PJ, Sato DK, Bennett JL, John GR, et al. Update on biomarkers in neuromyelitis optica. *Neurol Neuroimmunol Neuroinflamm.* (2015) 2:e134. doi: 10.1212/NXI.0000000000000134
26. Jarius S, Ruprecht K, Wildemann B, Kuempfel T, Ringelstein M, Geis C, et al. Contrasting disease patterns in seropositive and seronegative neuromyelitis optica: A multicentre study of 175 patients. *J Neuroinflammation* (2012) 9:14. doi: 10.1186/1742-2094-9-14
27. Jiao Y, Fryer JP, Lennon VA, Jenkins SM, Quek AML, Smith CY, et al. Updated estimate of AQP4-IgG serostatus and disability outcome in neuromyelitis optica. *Neurology* (2013) 81:1197–204. doi: 10.1212/WNL.0b013e3182a6cb5c
28. Kiyat-Atamer A, Ekizoglu E, Tüzün E, Kürtüncü M, Shugaiv E, Akman-Demir G, et al. Long-term MRI findings in neuromyelitis optica: seropositive versus seronegative patients. *Eur J Neurol.* (2013) 20:781–7. doi: 10.1111/ene.12058
29. Sato DK, Callegaro D, Lana-Peixoto MA, Waters PJ, de Haidar Jorge FM, Takahashi T, et al. Distinction between MOG antibody-positive and AQP4 antibody-positive NMO spectrum disorders. *Neurology* (2014) 82:474–81. doi: 10.1212/WNL.0000000000000101
30. Jurynczyk M, Messina S, Woodhall MR, Raza N, Everett R, Roca-Fernandez A, et al. Clinical presentation and prognosis in MOG-antibody disease: a UK study. *Brain J Neurol.* (2017) 140:3128–38. doi: 10.1093/brain/awx276
31. Kitley J, Waters P, Woodhall M, Leite MI, Murchison A, George J, et al. Neuromyelitis optica spectrum disorders with aquaporin-4 and myelin-oligodendrocyte glycoprotein antibodies: a comparative study. *JAMA Neurol.* (2014) 71:276–83. doi: 10.1001/jamaneurol.2013.5857
32. Jarius S, Ruprecht K, Kleiter I, Borisow N, Asgari N, Pitarokoili K, et al. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 1: Frequency, syndrome specificity, influence of disease activity, long-term course, association with AQP4-IgG, and origin. *J Neuroinflamm.* (2016) 13:279. doi: 10.1186/s12974-016-0717-1

33. Jarius S, Ruprecht K, Kleiter I, Borisow N, Asgari N, Pitarokoili K, et al. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 2: Epidemiology, clinical presentation, radiological and laboratory features, treatment responses, and long-term outcome. *J Neuroinflamm.* (2016) 13:280. doi: 10.1186/s12974-016-0718-0
34. Körtvélyessy P, Breu M, Pawlitzki M, Metz I, Heinze HJ, Matzke M, et al. ADEM-like presentation, anti-MOG antibodies, and MS pathology: TWO case reports. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e335. doi: 10.1212/NXI.0000000000000335
35. Spadaro M, Gerdes LA, Krumbholz M, Ertl-Wagner B, Thaler FS, Schuh E, et al. Autoantibodies to MOG in a distinct subgroup of adult multiple sclerosis. *Neurol Neuroimmunol Neuroinflamm.* (2016) 3:e257. doi: 10.1212/NXI.0000000000000257
36. Hamid SHM, Whittam D, Mutch K, Linaker S, Solomon T, Das K, et al. What proportion of AQP4-IgG-negative NMO spectrum disorder patients are MOG-IgG positive? A cross sectional study of 132 patients. *J Neurol.* (2017) 264:2088–94. doi: 10.1007/s00415-017-8596-7
37. Sepúlveda M, Armangué T, Sola-Valls N, Arrambide G, Meca-Lallana JE, Oreja-Guevara C, et al. Neuromyelitis optica spectrum disorders: Comparison according to the phenotype and serostatus. *Neurol Neuroimmunol Neuroinflamm.* (2016) 3:e225. doi: 10.1212/NXI.0000000000000225
38. Pandit L, Sato DK, Mustafa S, Takahashi T, D'Cunha A, Malli C, et al. Relapsing optic neuritis and isolated transverse myelitis are the predominant clinical phenotypes for patients with antibodies to myelin oligodendrocyte glycoprotein in India. *Mult Scler J Exp Transl Clin.* (2016) 2:2055217316675634. doi: 10.1177/2055217316675634
39. Waters P, Woodhall M, O'Connor KC, Reindl M, Lang B, Sato DK, et al. MOG cell-based assay detects non-MS patients with inflammatory neurologic disease. *Neurol Neuroimmunol Neuroinflamm.* (2015) 2:e89. doi: 10.1212/NXI.0000000000000089
40. Hacohen Y, Wong YY, Lechner C, Jurynczyk M, Wright S, Konuskan B, et al. Disease course and treatment responses in children with relapsing myelin oligodendrocyte glycoprotein antibody-associated disease. *JAMA Neurol.* (2018) 75:478–87. doi: 10.1001/jamaneurol.2017.4601
41. Hacohen Y, Absoud M, Deiva K, Hemingway C, Nytrova P, Woodhall M, et al. Myelin oligodendrocyte glycoprotein antibodies are associated with a non-MS course in children. *Neurol Neuroimmunol Neuroinflamm.* (2015) 2:e81. doi: 10.1212/NXI.0000000000000081
42. Pohl D, Alper G, Van Haren K, Kornberg AJ, Lucchinetti CF, Tenembaum S, et al. Acute disseminated encephalomyelitis: Updates on an inflammatory CNS syndrome. *Neurology* (2016) 87:S38–45. doi: 10.1212/WNL.0000000000002825
43. Berzero G, Cortese A, Ravaglia S, Marchioni E. Diagnosis and therapy of acute disseminated encephalomyelitis and its variants. *Expert Rev Neurother.* (2016) 16:83–101. doi: 10.1586/14737175.2015.1126510
44. Hjelmström P, Penzotti JE, Henne RM, Lybrand TP. A molecular model of myelin oligodendrocyte glycoprotein. *J Neurochem.* (1998) 71:1742–9.
45. Vourc'h P, Andres C. Oligodendrocyte myelin glycoprotein (OMgp): evolution, structure and function. *Brain Res Brain Res Rev* (2004) 45:115–24. doi: 10.1016/j.brainresrev.2004.01.003
46. Wingerchuk DM, Banwell B, Bennett JL, Cabre P, Carroll W, Chitnis T, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology* (2015) 85:177–89. doi: 10.1212/WNL.0000000000001729
47. Dos Passos GR, Oliveira LM, da Costa BK, Apostolos-Pereira SL, Callegaro D, Fujihara K, et al. MOG-IgG-associated optic neuritis, encephalitis, and myelitis: lessons learned from neuromyelitis optica spectrum disorder. *Front Neurol.* (2018) 9:217. doi: 10.3389/fneur.2018.00217
48. Cobo-Calvo Á, Ruiz A, D'Indy H, Poulat AL, Carneiro M, Philippe N, et al. MOG antibody-related disorders: common features and uncommon presentations. *J Neurol.* (2017) 264:1945–55. doi: 10.1007/s00415-017-8583-z
49. Jarius S, Paul F, Aktas O, Asgari N, Dale RC, de Seze J, et al. MOG encephalomyelitis: international recommendations on diagnosis and antibody testing. *J Neuroinflamm.* (2018) 15:134. doi: 10.1186/s12974-018-1144-2
50. Narayan R, Simpson A, Fritsche K, Salama S, Pardo S, Mealy M, et al. MOG antibody disease: a review of MOG antibody seropositive neuromyelitis optica spectrum disorder. *Mult Scler Relat Disord.* (2018) 25:66–72. doi: 10.1016/j.msard.2018.07.025
51. Dale RC, Tantsis EM, Merheb V, Kumaran RYA, Sinmaz N, Pathmanandavel K, et al. Antibodies to MOG have a demyelination phenotype and affect oligodendrocyte cytoskeleton. *Neurol Neuroimmunol Neuroinflamm.* (2014) 1:e12. doi: 10.1212/NXI.0000000000000012
52. Reindl M, Rostasy K. MOG antibody-associated diseases. *Neurol Neuroimmunol Neuroinflamm.* (2015) 2:e60. doi: 10.1212/NXI.0000000000000060
53. Zamvil SS, Slavin AJ. Does MOG Ig-positive AQP4-seronegative opticospinal inflammatory disease justify a diagnosis of NMO spectrum disorder? *Neurol Neuroimmunol Neuroinflamm.* (2015) 2:e62. doi: 10.1212/NXI.0000000000000062
54. Chen HX, Zhang Q, Lian ZY, Liu J, Shi ZY, Miao XH, et al. Muscle damage in patients with neuromyelitis optica spectrum disorder. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e400. doi: 10.1212/NXI.0000000000000400
55. Baba T, Nakashima I, Kanbayashi T, Konno M, Takahashi T, Fujihara K, et al. Narcolepsy as an initial manifestation of neuromyelitis optica with anti-aquaporin-4 antibody. *J Neurol.* (2009) 256:287–8. doi: 10.1007/s00415-009-0139-4
56. Pandit L, Mustafa S, Uppoor R, Nakashima I, Takahashi T, Kaneko K. Reversible paraspinal muscle hyperintensity in anti-MOG antibody-associated transverse myelitis. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e412. doi: 10.1212/NXI.0000000000000412
57. Vazquez Do Campo R, Stephens A, Marin Collazo IV, Rubin DI. MOG antibodies in combined central and peripheral demyelination syndromes. *Neurol Neuroimmunol Neuroinflamm.* (2018) doi: 10.1212/NXI.0000000000000503
58. Jarius S, Metz I, König FB, Ruprecht K, Reindl M, Paul F, et al. Screening for MOG-IgG and 27 other anti-glial and anti-neuronal autoantibodies in "pattern II multiple sclerosis" and brain biopsy findings in a MOG-IgG-positive case. *Mult Scler.* (2016) 22:1541–49. doi: 10.1177/1352458515622986
59. Spadaro M, Gerdes LA, Mayer MC, Ertl-Wagner B, Laurent S, Krumbholz M, et al. Histopathology and clinical course of MOG-antibody-associated encephalomyelitis. *Ann Clin Transl Neurol.* (2015) 2:295–301. doi: 10.1002/acn3.164
60. Takeshita Y, Obermeier B, Cotleur AC, Spampinato SF, Shimizu F, Yamamoto E, et al. Effects of neuromyelitis optica-IgG at the blood-brain barrier *in vitro*. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e311. doi: 10.1212/NXI.0000000000000311
61. Ratelade J, Zhang H, Saadoun S, Bennett JL, Papadopoulos MC, Verkman AS. Neuromyelitis optica IgG and natural killer cells produce NMO lesions in mice without myelin loss. *Acta Neuropathol.* (2012) 123:861–72. doi: 10.1007/s00401-012-0986-4
62. Misu T, Fujihara K, Kakita A, Konno H, Nakamura M, Watanabe S, et al. Loss of aquaporin 4 in lesions of neuromyelitis optica: distinction from multiple sclerosis. *Brain J Neurol.* (2007) 130:1224–34. doi: 10.1093/brain/awm047
63. Aboul-Enein F, Seifert-Held T, Mader S, Kuenz B, Lutterotti A, Rauschka H, et al. Neuromyelitis optica in Austria in 2011: to bridge the gap between neuroepidemiological research and practice in a study population of 8.4 million people. *PloS ONE* (2013) 8:e79649. doi: 10.1371/journal.pone.0079649
64. ZhangBao J, Zhou L, Li X, Cai T, Lu J, Lu C, et al. The clinical characteristics of AQP4 antibody positive NMO/SD in a large cohort of Chinese Han patients. *J Neuroimmunol.* (2017) 302:49–55. doi: 10.1016/j.jneuroim.2016.11.010
65. Cobo-Calvo A, Ruiz A, Maillart E, Audoin B, Zephir H, Bourre B, et al. Clinical spectrum and prognostic value of CNS MOG autoimmunity in adults: the MOGADOR study. *Neurology* (2018) 90:e1858–69. doi: 10.1212/WNL.0000000000005560
66. Ramanathan S, Mohammad S, Tantsis E, Nguyen TK, Merheb V, Fung VSC, et al. Clinical course, therapeutic responses and outcomes in relapsing MOG antibody-associated demyelination. *J Neurol Neurosurg Psychiatry* (2018) 89:127–37. doi: 10.1136/jnnp-2017-316880
67. Soelberg K, Jarius S, Skejoe H, Engberg H, Mehlsen JJ, Nilsson AC, et al. A population-based prospective study of optic neuritis. *Mult Scler.* (2017) 23:1893–901. doi: 10.1177/1352458517734070

68. Soelberg K, Specovius S, Zimmermann HG, Grauslund J, Mehlsen JJ, Olesen C, et al. Optical coherence tomography in acute optic neuritis: A population-based study. *Acta Neurol Scand.* (2018) doi: 10.1111/ane.13004 [Epub ahead of print].
69. Jarius S, Frederikson J, Waters P, Paul F, Akman-Demir G, Marignier R, et al. Frequency and prognostic impact of antibodies to aquaporin-4 in patients with optic neuritis. *J Neurol Sci.* (2010) 298:158–62. doi: 10.1016/j.jns.2010.07.011
70. Fujimori J, Takai Y, Nakashima I, Sato DK, Takahashi T, Kaneko K, et al. Bilateral frontal cortex encephalitis and paraparesis in a patient with anti-MOG antibodies. *J Neurol Neurosurg Psychiatry* (2017) 88:534–6. doi: 10.1136/jnnp-2016-315094
71. Ogawa R, Nakashima I, Takahashi T, Kaneko K, Akaishi T, Takai Y, et al. MOG antibody-positive, benign, unilateral, cerebral cortical encephalitis with epilepsy. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e322. doi: 10.1212/NXI.0000000000000322
72. Hamid SHM, Whittam D, Saviour M, Alorainy A, Mutch K, Linaker S, et al. Seizures and encephalitis in myelin oligodendrocyte glycoprotein IgG disease vs aquaporin 4 IgG disease. *JAMA Neurol.* (2018) 75:65–71. doi: 10.1001/jamaneurol.2017.3196
73. Höftberger R, Sepulveda M, Armangue T, Blanco Y, Rostásy K, Calvo AC, et al. Antibodies to MOG and AQP4 in adults with neuromyelitis optica and suspected limited forms of the disease. *Mult Scler.* (2015) 21:866–74. doi: 10.1177/1352458514555785
74. van Pelt ED, Wong YYM, Ketelslegers IA, Hamann D, Hintzen RQ. Neuromyelitis optica spectrum disorders: comparison of clinical and magnetic resonance imaging characteristics of AQP4-IgG versus MOG-IgG seropositive cases in the Netherlands. *Eur J Neurol.* (2016) 23:580–7. doi: 10.1111/ene.12898
75. Pandit L, Mustafa S, Nakashima I, Takahashi T, Kaneko K. MOG-IgG-associated disease has a stereotypical clinical course, asymptomatic visual impairment and good treatment response. *Mult Scler J Exp Transl Clin* (2018) 4:2055217318787829. doi: 10.1177/2055217318787829
76. Hyun JW, Woodhall MR, Kim SH, Jeong IH, Kong B, Kim G, et al. Longitudinal analysis of myelin oligodendrocyte glycoprotein antibodies in CNS inflammatory diseases. *J Neurol Neurosurg Psychiatry* (2017) 88:811–7. doi: 10.1136/jnnp-2017-315998
77. Akaishi T, Sato DK, Nakashima I, Takeshita T, Takahashi T, Doi H, et al. MRI and retinal abnormalities in isolated optic neuritis with myelin oligodendrocyte glycoprotein and aquaporin-4 antibodies: a comparative study. *J Neurol Neurosurg Psychiatry* (2016) 87:446–8. doi: 10.1136/jnnp-2014-310206
78. Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* (2004) 364:2106–12. doi: 10.1016/S0140-6736(04)17551-X
79. Waters PJ, Pittock SJ, Bennett JL, Jarius S, Weinshenker BG, Wingerchuk DM. Evaluation of aquaporin-4 antibody assays. *Clin Exp Neuroimmunol.* (2014) 5:290–303. doi: 10.1111/cen3.12107
80. Jarius S, Paul F, Franciotta D, Waters P, Zipp F, Hohlfeld R, et al. Mechanisms of disease: aquaporin-4 antibodies in neuromyelitis optica. *Nat Clin Pract Neurol.* (2008) 4:202–14. doi: 10.1038/ncpneuro0764
81. Lang K, Prüss H. Frequencies of neuronal autoantibodies in healthy controls: Estimation of disease specificity. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e386. doi: 10.1212/NXI.0000000000000386
82. Pittock SJ, Lennon VA, Bakshi N, Shen L, McKeon A, Quach H, et al. Seroprevalence of aquaporin-4-IgG in a northern California population representative cohort of multiple sclerosis. *JAMA Neurol.* (2014) 71:1433–6. doi: 10.1001/jamaneurol.2014.1581
83. Kister I, Paul F. Pushing the boundaries of neuromyelitis optica: does antibody make the disease? *Neurology* (2015) 85:118–9. doi: 10.1212/WNL.0000000000001749
84. Duignan S, Wright S, Rossor T, Cazabon J, Gilmour K, Ciccarelli O, et al. Myelin oligodendrocyte glycoprotein and aquaporin-4 antibodies are highly specific in children with acquired demyelinating syndromes. *Dev Med Child Neurol.* (2018) 60:958–62. doi: 10.1111/dmcn.13703
85. Brilot F, Dale RC, Selter RC, Grummel V, Kalluri SR, Aslam M, et al. Antibodies to native myelin oligodendrocyte glycoprotein in children with inflammatory demyelinating central nervous system disease. *Ann Neurol.* (2009) 66:833–42. doi: 10.1002/ana.21916
86. Lalive PH, Häusler MG, Maurey H, Mikaeloff Y, Tardieu M, Wiendl H, et al. Highly reactive anti-myelin oligodendrocyte glycoprotein antibodies differentiate demyelinating diseases from viral encephalitis in children. *Mult Scler.* (2011) 17:297–302. doi: 10.1177/1352458510389220
87. Xiao BG, Linington C, Link H. Antibodies to myelin-oligodendrocyte glycoprotein in cerebrospinal fluid from patients with multiple sclerosis and controls. *J Neuroimmunol.* (1991) 31:91–6.
88. Genain CP, Cannella B, Hauser SL, Raine CS. Identification of autoantibodies associated with myelin damage in multiple sclerosis. *Nat Med.* (1999) 5:170–5. doi: 10.1038/5532
89. Reindl M, Linington C, Brehm U, Egg R, Dilitz E, Deisenhammer F, et al. Antibodies against the myelin oligodendrocyte glycoprotein and the myelin basic protein in multiple sclerosis and other neurological diseases: a comparative study. *Brain J Neurol.* (1999) 122 (Pt 11):2047–56.
90. Klawiter EC, Piccio L, Lyons JA, Mikesell R, O'Connor KC, Cross AH. Elevated intrathecal myelin oligodendrocyte glycoprotein antibodies in multiple sclerosis. *Arch Neurol.* (2010) 67:1102–8. doi: 10.1001/archneurol.2010.197
91. Haase CG, Schmidt S. Detection of brain-specific autoantibodies to myelin oligodendrocyte glycoprotein, S100beta and myelin basic protein in patients with Devic's neuromyelitis optica. *Neurosci Lett.* (2001) 307:131–3. doi: 10.1016/S0304-3940(01)01949-8
92. Kitley J, Woodhall M, Waters P, Leite MI, Devenney E, Craig J, et al. Myelin-oligodendrocyte glycoprotein antibodies in adults with a neuromyelitis optica phenotype. *Neurology* (2012) 79:1273–7. doi: 10.1212/WNL.0b013e31826aac4e
93. Jarius S, Aboul-Enein F, Waters P, Kuenz B, Hauser A, Berger T, et al. Antibody to aquaporin-4 in the long-term course of neuromyelitis optica. *Brain* (2008) 131:3072–80. doi: 10.1093/brain/awn240
94. Valentino P, Marnetto F, Granieri L, Capobianco M, Bertolotto A. Aquaporin-4 antibody titration in NMO patients treated with rituximab: a retrospective study. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e317. doi: 10.1212/NXI.0000000000000317
95. Kessler RA, Mealy MA, Jimenez-Arango JA, Quan C, Paul F, López R, et al. Anti-aquaporin-4 titer is not predictive of disease course in neuromyelitis optica spectrum disorder: a multicenter cohort study. *Mult Scler Relat Disord.* (2017) 17:198–201. doi: 10.1016/j.msard.2017.08.005
96. Mealy MA, Kim SH, Schmidt F, López R, Jimenez Arango JA, Paul F, et al. Aquaporin-4 serostatus does not predict response to immunotherapy in neuromyelitis optica spectrum disorders. *Mult Scler.* (2017). doi: 10.1177/1352458517730131 [Epub ahead of print].
97. Jarius S, Ruprecht K, Stellmann JP, Huss A, Ayzenberg I, Willing A, et al. MOG-IgG in primary and secondary chronic progressive multiple sclerosis: a multicenter study of 200 patients and review of the literature. *J Neuroinflamm.* (2018) 15:88. doi: 10.1186/s12974-018-1108-6
98. Jarius S, Franciotta D, Paul F, Ruprecht K, Bergamaschi R, Rommer PS, et al. Cerebrospinal fluid antibodies to aquaporin-4 in neuromyelitis optica and related disorders: frequency, origin, and diagnostic relevance. *J Neuroinflamm.* (2010) 7:52. doi: 10.1186/1742-2094-7-52
99. Majed M, Fryer JP, McKeon A, Lennon VA, Pittock SJ. Clinical utility of testing AQP4-IgG in CSF: guidance for physicians. *Neurol Neuroimmunol Neuroinflamm.* (2016) 3:e231. doi: 10.1212/NXI.0000000000000231
100. Jarius S, Paul F, Franciotta D, Ruprecht K, Ringelstein M, Bergamaschi R, et al. Cerebrospinal fluid findings in aquaporin-4 antibody positive neuromyelitis optica: results from 211 lumbar punctures. *J Neurol Sci.* (2011) 306:82–90. doi: 10.1016/j.jns.2011.03.038
101. Iyer A, Elsone L, Appleton R, Jacob A. A review of the current literature and a guide to the early diagnosis of autoimmune disorders associated with neuromyelitis optica. *Autoimmunity* (2014) 47:154–61. doi: 10.3109/08916934.2014.883501
102. Pittock SJ, Lennon VA, de Seze J, Vermersch P, Homburger HA, Wingerchuk DM, et al. Neuromyelitis optica and non organ-specific autoimmunity. *Arch Neurol.* (2008) 65:78–83. doi: 10.1001/archneurol.2007.17
103. Sergio P, Mariana B, Alberto O, Claudia U, Oscar R, Pablo M, et al. Association of neuromyelitis optic (NMO) with autoimmune disorders:

report of two cases and review of the literature. *Clin Rheumatol.* (2010) 29:1335–8. doi: 10.1007/s10067-010-1502-7
104. Zekeridou A, Lennon VA. Aquaporin-4 autoimmunity. *Neurol Neuroimmunol Neuroinflamm.* (2015) 2:e110. doi: 10.1212/NXI.0000000000000110
105. Jarius S, Jacobi C, de Seze J, Zephir H, Paul F, Franciotta D, et al. Frequency and syndrome specificity of antibodies to aquaporin-4 in neurological patients with rheumatic disorders. *Mult Scler.* (2011) 17:1067–73. doi: 10.1177/1352458511403958
106. Jarius S, Paul F, Franciotta D, de Seze J, Münch C, Salvetti M, Ruprecht K, Liebetrau M, Wandinger KP, Akman-Demir G, et al. Neuromyelitis optica spectrum disorders in patients with myasthenia gravis: ten new aquaporin-4 antibody positive cases and a review of the literature. *Mult Scler.* (2012) 18:1135–43. doi: 10.1177/1352458511431728
107. Uzawa A, Mori M, Iwai Y, Kobayashi M, Hayakawa S, Kawaguchi N, et al. Association of anti-aquaporin-4 antibody-positive neuromyelitis optica with myasthenia gravis. *J Neurol Sci.* (2009) 287:105–7. doi: 10.1016/j.jns.2009.08.040
108. Beauchemin P, Iorio R, Traboulsee AL, Field T, Tinker AV, Carruthers RL. Paraneoplastic neuromyelitis optica spectrum disorder: a single center cohort description with two cases of histological validation. *Mult Scler Relat Disord.* (2018) 20:37–42. doi: 10.1016/j.msard.2017.12.012
109. Soelberg K, Larsen SR, Moerch MT, Thomassen M, Brusgaard K, Paul F, et al. Aquaporin-4 IgG autoimmune syndrome and immunoreactivity associated with thyroid cancer. *Neurol Neuroimmunol Neuroinflamm.* (2016) 3:e252. doi: 10.1212/NXI.0000000000000252
110. Sudo A, Chihara N, Takenaka Y, Nakamura T, Ueda T, Sekiguchi K, et al. Paraneoplastic NMOSD associated with EG junction adenocarcinoma expressing unprotected AQP4. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e482. doi: 10.1212/NXI.0000000000000482
111. Jarius S, Paul F, Ruprecht K, Wildemann B. Low vitamin B12 levels and gastric parietal cell antibodies in patients with aquaporin-4 antibody-positive neuromyelitis optica spectrum disorders. *J Neurol.* (2012) 259:2743–5. doi: 10.1007/s00415-012-6677-1
112. Flanagan EP, Kaufmann TJ, Krecke KN, Aksamit AJ, Pittock SJ, Keegan BM, et al. Discriminating long myelitis of neuromyelitis optica from sarcoidosis. *Ann Neurol.* (2016) 79:437–47. doi: 10.1002/ana.24582
113. Kidd DP, Burton BJ, Graham EM, Plant GT. Optic neuropathy associated with systemic sarcoidosis. *Neurol Neuroimmunol Neuroinflamm.* (2016) 3:e270. doi: 10.1212/NXI.0000000000000270
114. Sawaya R, Radwan W. Sarcoidosis associated with neuromyelitis optica. *J Clin Neurosci.* (2013) 20:1156–8. doi: 10.1016/j.jocn.2012.09.030
115. Geraldes R, Ciccarelli O, Barkhof F, De Stefano N, Enzinger C, Filippi M, et al. The current role of MRI in differentiating multiple sclerosis from its imaging mimics. *Nat Rev Neurol.* (2018) 14:199–213. doi: 10.1038/nrneurol.2018.14
116. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. *Neurology* (2006) 66:1485–9. doi: 10.1212/01.wnl.0000216139.44259.74
117. Flanagan EP, Weinshenker BG, Krecke KN, Lennon VA, Lucchinetti CF, McKeon A, et al. Short myelitis lesions in aquaporin-4-IgG-positive neuromyelitis optica spectrum disorders. *JAMA Neurol.* (2015) 72:81–7. doi: 10.1001/jamaneurol.2014.2137
118. Pekcevik Y, Mitchell CH, Mealy MA, Orman G, Lee IH, Newsome SD, et al. Differentiating neuromyelitis optica from other causes of longitudinally extensive transverse myelitis on spinal magnetic resonance imaging. *Mult Scler.* (2016) 22:302–11. doi: 10.1177/1352458515591069
119. Yonezu T, Ito S, Mori M, Ogawa Y, Makino T, Uzawa A, et al. "Bright spotty lesions" on spinal magnetic resonance imaging differentiate neuromyelitis optica from multiple sclerosis. *Mult Scler.* (2014) 20:331–7. doi: 10.1177/1352458513495581
120. Asgari N, Skejoe HPB, Lillevang ST, Steenstrup T, Stenager E, Kyvik KO. Modifications of longitudinally extensive transverse myelitis and brainstem lesions in the course of neuromyelitis optica (NMO): a population-based, descriptive study. *BMC Neurol.* (2013) 13:33. doi: 10.1186/1471-2377-13-33
121. Asgari N, Skejoe HPB, Lennon VA. Evolution of longitudinally extensive transverse myelitis in an aquaporin-4 IgG-positive patient. *Neurology* (2013) 81:95–6. doi: 10.1212/WNL.0b013e318297ef07
122. Flanagan EP, Krecke KN, Marsh RW, Giannini C, Keegan BM, Weinshenker BG. Specific pattern of gadolinium enhancement in spondylotic myelopathy. *Ann Neurol.* (2014) 76:54–65. doi: 10.1002/ana.24184
123. Whittam D, Bhojak M, Das K, Jacob A. Longitudinally extensive myelitis in MS mimicking neuromyelitis optica. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e333. doi: 10.1212/NXI.0000000000000333
124. Flanagan EP, Hinson SR, Lennon VA, Fang B, Aksamit AJ, Morris PP, et al. Glial fibrillary acidic protein immunoglobulin G as biomarker of autoimmune astrocytopathy: analysis of 102 patients. *Ann Neurol.* (2017) 81:298–309. doi: 10.1002/ana.24881
125. Fang B, McKeon A, Hinson SR, Kryzer TJ, Pittock SJ, Aksamit AJ, Lennon VA. Autoimmune glial fibrillary acidic protein astrocytopathy: a novel meningoencephalomyelitis. *JAMA Neurol.* (2016) 73:1297–307. doi: 10.1001/jamaneurol.2016.2549
126. Luessi F, Engel S, Spreer A, Bittner S, Zipp F. GFAPα IgG-associated encephalitis upon daclizumab treatment of MS. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e481. doi: 10.1212/NXI.0000000000000481
127. Pittock SJ, Lennon VA, Krecke K, Wingerchuk DM, Lucchinetti CF, Weinshenker BG. Brain abnormalities in neuromyelitis optica. *Arch Neurol.* (2006) 63:390–6. doi: 10.1001/archneur.63.3.390
128. Cabrera-Gómez JA, Quevedo-Sotolongo L, González-Quevedo A, Lima S, Real-González Y, Cristófol-Corominas M, et al. Brain magnetic resonance imaging findings in relapsing neuromyelitis optica. *Mult Scler.* (2007) 13:186–92. doi: 10.1177/1352458506070725
129. Kim HJ, Paul F, Lana-Peixoto MA, Tenembaum S, Asgari N, Palace J, Klawiter EC, et al. MRI characteristics of neuromyelitis optica spectrum disorder: an international update. *Neurology* (2015) 84:1165–73. doi: 10.1212/WNL.0000000000001367
130. Wang KY, Chetta J, Bains P, Balzer A, Lincoln J, Uribe T, et al. Spectrum of MRI brain lesion patterns in neuromyelitis optica spectrum disorder: a pictorial review. *Br J Radiol.* (2018) 91:20170690. doi: 10.1259/bjr.20170690
131. Carnero Contentti E, Daccach Marques V, Soto de Castillo I, Tkachuk V, Antunes Barreira A, Armas E, et al. Frequency of brain MRI abnormalities in neuromyelitis optica spectrum disorder at presentation: A cohort of Latin American patients. *Mult Scler Relat Disord.* (2018) 19:73–8. doi: 10.1016/j.msard.2017.11.004
132. Fan M, Fu Y, Su L, Shen Y, Wood K, Yang L, et al. Comparison of brain and spinal cord magnetic resonance imaging features in neuromyelitis optica spectrum disorders patients with or without aquaporin-4 antibody. *Mult Scler Relat Disord.* (2017) 13:58–66. doi: 10.1016/j.msard.2017.02.003
133. Asgari N, Flanagan EP, Fujihara K, Kim HJ, Skejoe HP, Wuerfel J, et al. Disruption of the leptomeningeal blood barrier in neuromyelitis optica spectrum disorder. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e343. doi: 10.1212/NXI.0000000000000343
134. Ramanathan S, Prelog K, Barnes EH, Tantsis EM, Reddel SW, Henderson APD, et al. Radiological differentiation of optic neuritis with myelin oligodendrocyte glycoprotein antibodies, aquaporin-4 antibodies, and multiple sclerosis. *Mult Scler.* (2016) 22:470–82. doi: 10.1177/1352458515593406
135. Akaishi T, Nakashima I, Takeshita T, Mugikura S, Sato DK, Takahashi T, et al. Lesion length of optic neuritis impacts visual prognosis in neuromyelitis optica. *J Neuroimmunol.* (2016) 293:28–33. doi: 10.1016/j.jneuroim.2016.02.004
136. Jurynczyk M, Geraldes R, Probert F, Woodhall MR, Waters P, Tackley G, et al. Distinct brain imaging characteristics of autoantibody-mediated CNS conditions and multiple sclerosis. *Brain J Neurol.* (2017) 140:617–27. doi: 10.1093/brain/aww350
137. Jurynczyk M, Tackley G, Kong Y, Geraldes R, Matthews L, Woodhall M, et al. Brain lesion distribution criteria distinguish MS from AQP4-antibody NMOSD and MOG-antibody disease. *J Neurol Neurosurg Psychiatry* (2017) 88:132–6. doi: 10.1136/jnnp-2016-314005
138. Hyun JW, Jeong IH, Joung A, Kim SH, Kim HJ. Evaluation of the 2015 diagnostic criteria for neuromyelitis optica spectrum disorder. *Neurology* (2016) 86:1772–9. doi: 10.1212/WNL.0000000000002655

139. Kremer S, Renard F, Achard S, Lana-Peixoto MA, Palace J, Asgari N, et al. Use of advanced magnetic resonance imaging techniques in neuromyelitis optica spectrum disorder. *JAMA Neurol.* (2015) 72:815–22. doi: 10.1001/jamaneurol.2015.0248
140. Finke C, Heine J, Pache F, Lacheta A, Borisow N, Kuchling J, et al. Normal volumes and microstructural integrity of deep gray matter structures in AQP4+ NMOSD. *Neurol Neuroimmunol Neuroinflamm.* (2016) 3:e229. doi: 10.1212/NXI.0000000000000229
141. Pache F, Zimmermann H, Finke C, Lacheta A, Papazoglou S, Kuchling J, et al. Brain parenchymal damage in neuromyelitis optica spectrum disorder - A multimodal MRI study. *Eur Radiol.* (2016) 26:4413–22. doi: 10.1007/s00330-016-4282-x
142. Oberwahrenbrock T, Traber GL, Lukas S, Gabilondo I, Nolan R, Songster C, et al. Multicenter reliability of semiautomatic retinal layer segmentation using OCT. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e449. doi: 10.1212/NXI.0000000000000449
143. Oertel FC, Zimmermann H, Paul F, Brandt AU. Optical coherence tomography in neuromyelitis optica spectrum disorders: potential advantages for individualized monitoring of progression and therapy. *EPMA J.* (2018) 9:21–33. doi: 10.1007/s13167-017-0123-5
144. Zimmermann H, Oberwahrenbrock T, Brandt AU, Paul F, Dörr J. Optical coherence tomography for retinal imaging in multiple sclerosis. *Degener Neurol Neuromuscul Dis.* (2014) 2014:4:153-62. doi: 10.2147/DNND.S73506
145. Oertel FC, Zimmermann H, Brandt AU, Paul F. [Optical coherence tomography in neuromyelitis optica spectrum disorders]. *Nervenarzt* (2017) 88:1411–20. doi: 10.1007/s00115-017-0444-6
146. Ayadi N, Dörr J, Motamedi S, Gawlik K, Bellmann-Strobl J, Mikolajczak J, et al. Temporal visual resolution and disease severity in MS. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e492. doi: 10.1212/NXI.0000000000000492
147. You Y, Graham EC, Shen T, Yiannikas C, Parratt J, Gupta V, et al. Progressive inner nuclear layer dysfunction in non-optic neuritis eyes in MS. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e427. doi: 10.1212/NXI.0000000000000427
148. Waldman AT, Liu GT, Lavery AM, Liu G, Gaetz W, Aleman TS, et al. Optical coherence tomography and visual evoked potentials in pediatric MS. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e356. doi: 10.1212/NXI.0000000000000356
149. Bennett JL, de Seze J, Lana-Peixoto M, Palace J, Waldman A, Schippling S, et al. Neuromyelitis optica and multiple sclerosis: seeing differences through optical coherence tomography. *Mult Scler.* (2015) 21:678–88. doi: 10.1177/1352458514567216
150. Petzold A, Wattjes MP, Costello F, Flores-Rivera J, Fraser CL, Fujihara K, et al. The investigation of acute optic neuritis: a review and proposed protocol. *Nat Rev Neurol.* (2014) 10:447–58. doi: 10.1038/nrneurol.2014.108
151. Finke C, Zimmermann H, Pache F, Oertel FC, Chavarro VS, Kramarenko Y, et al. Association of visual impairment in neuromyelitis optica spectrum disorder with visual network reorganization. *JAMA Neurol.* (2018) 75:296–303. doi: 10.1001/jamaneurol.2017.3890
152. Pache F, Zimmermann H, Mikolajczak J, Schumacher S, Lacheta A, Oertel FC, et al. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 4: afferent visual system damage after optic neuritis in MOG-IgG-seropositive versus AQP4-IgG-seropositive patients. *J Neuroinflammation* (2016) 13:282. doi: 10.1186/s12974-016-0720-6
153. Schneider E, Zimmermann H, Oberwahrenbrock T, Kaufhold F, Kadas EM, Petzold A, et al. Optical coherence tomography reveals distinct patterns of retinal damage in neuromyelitis optica and multiple sclerosis. *PloS ONE* (2013) 8:e66151. doi: 10.1371/journal.pone.0066151
154. Havla J, Kümpfel T, Schinner R, Spadaro M, Schuh E, Meinl E, et al. Myelin-oligodendrocyte-glycoprotein (MOG) autoantibodies as potential markers of severe optic neuritis and subclinical retinal axonal degeneration. *J Neurol.* (2017) 264:139–51. doi: 10.1007/s00415-016-8333-7
155. Schmidt F, Zimmermann H, Mikolajczak J, Oertel FC, Pache F, Weinhold M, et al. Severe structural and functional visual system damage leads to profound loss of vision-related quality of life in patients with neuromyelitis optica spectrum disorders. *Mult Scler Relat Disord.* (2017) 11:45–50. doi: 10.1016/j.msard.2016.11.008
156. Bouyon M, Collongues N, Zéphir H, Ballonzoli L, Jeanjean L, Lebrun C, et al. Longitudinal follow-up of vision in a neuromyelitis optica cohort. *Mult Scler.* (2013) 19:1320–2. doi: 10.1177/1352458513476562
157. Oertel FC, Zimmermann H, Mikolajczak J, Weinhold M, Kadas EM, Oberwahrenbrock T, et al. Contribution of blood vessels to retinal nerve fiber layer thickness in NMOSD. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e338. doi: 10.1212/NXI.0000000000000338
158. Gelfand JM, Cree BA, Nolan R, Arnow S, Green AJ. Microcystic inner nuclear layer abnormalities and neuromyelitis optica. *JAMA Neurol.* (2013) 70:629–33. doi: 10.1001/jamaneurol.2013.1832
159. Jelcic I, Hanson JVM, Lukas S, Weber KP, Landau K, Pless M, et al. Unfavorable structural and functional outcomes in myelin oligodendrocyte glycoprotein antibody-associated optic neuritis. *J Neuro-Ophthalmol.* (2018) doi: 10.1097/WNO.0000000000000669
160. Akaishi T, Kaneko K, Himori N, Takeshita T, Takahashi T, Nakazawa T, et al. Subclinical retinal atrophy in the unaffected fellow eyes of multiple sclerosis and neuromyelitis optica. *J Neuroimmunol.* (2017) 313:10–5. doi: 10.1016/j.jneuroim.2017.10.001
161. Stiebel-Kalish H, Lotan I, Brody J, Chodick G, Bialer O, Marignier R, et al. Retinal nerve fiber layer may be better preserved in MOG-IgG versus AQP4-IgG optic neuritis: a cohort study. *PloS ONE* (2017) 12:e0170847. doi: 10.1371/journal.pone.0170847
162. Martinez-Lapiscina EH, Sepulveda M, Torres-Torres R, Alba-Arbalat S, Llufriu S, Blanco Y, et al. Usefulness of optical coherence tomography to distinguish optic neuritis associated with AQP4 or MOG in neuromyelitis optica spectrum disorders. *Ther Adv Neurol Disord.* (2016) 9:436–40. doi: 10.1177/1756285616655264
163. Yamamura T, Nakashima I. Foveal thinning in neuromyelitis optica: A sign of retinal astrocytopathy? *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e347. doi: 10.1212/NXI.0000000000000347
164. Dalmau J. Precision in neuroimmunology. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e345. doi: 10.1212/NXI.0000000000000345
165. Jeong IH, Kim HJ, Kim N-H, Jeong KS, Park CY. Subclinical primary retinal pathology in neuromyelitis optica spectrum disorder. *J Neurol.* (2016) 263:1343–8. doi: 10.1007/s00415-016-8138-8
166. Oertel FC, Kuchling J, Zimmermann H, Chien C, Schmidt F, Knier B, et al. Microstructural visual system changes in AQP4-antibody-seropositive NMOSD. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e334. doi: 10.1212/NXI.0000000000000334
167. Oertel FC, Havla J, Roca-Fernández AI, Lizak N1, Zimmermann H, Motamedi S, et al. Retinal ganglion cell loss in neuromyelitis optica: a longitudinal study. *J Neurol Neurosurg Psychiatry* (2018) doi: 10.1136/jnnp-2018-318382[Epub ahead of print].
168. Nakamura M, Nakazawa T, Doi H, Hariya T, Omodaka K, Misu T, et al. Early high-dose intravenous methylprednisolone is effective in preserving retinal nerve fiber layer thickness in patients with neuromyelitis optica. *Graefes Arch Clin Exp Ophthalmol.* (2010) 248:1777–85. doi: 10.1007/s00417-010-1344-7
169. Merle H, Olindo S, Jeannin S, Valentino R, Mehdaoui H, Cabot F, Donnio A, et al. Treatment of optic neuritis by plasma exchange (add-on) in neuromyelitis optica. *Arch Ophthalmol.* (2012) 130:858–62. doi: 10.1001/archophthalmol.2012.1126
170. Brandt AU, Specovius S, Oberwahrenbrock T, Zimmermann HG, Paul F, Costello F. Frequent retinal ganglion cell damage after acute optic neuritis. *Mult Scler Relat Disord.* (2018) 22:141–7. doi: 10.1016/j.msard.2018.04.006
171. Hamid SH, Elsone L, Mutch K, Solomon T, Jacob A. The impact of 2015 neuromyelitis optica spectrum disorders criteria on diagnostic rates. *Mult Scler.* (2017) 23:228–33. doi: 10.1177/1352458516663853
172. Sepúlveda M, Aldea M, Escudero D, Llufriu S, Arrambide G, Otero-Romero S, et al. Epidemiology of NMOSD in Catalonia: Influence of the new 2015 criteria in incidence and prevalence estimates. *Mult Scler.* (2017) doi: 10.1177/1352458517735191 [Epub ahead of print].
173. Abboud H, Petrak A, Mealy M, Sasidharan S, Siddique L, Levy M. Treatment of acute relapses in neuromyelitis optica: Steroids alone versus steroids plus plasma exchange. *Mult Scler.* (2016) 22:185–92. doi: 10.1177/1352458515581438
174. Aungsumart S, Apiwattanakul M. Clinical outcomes and predictive factors related to good outcomes in plasma exchange in severe attack

of NMOSD and long extensive transverse myelitis: Case series and review of the literature. *Mult Scler Relat Disord.* (2017) 13:93–7. doi: 10.1016/j.msard.2017.02.015

175. Faissner S, Nikolayczik J, Chan A, Gold R, Yoon M-S, Haghikia A. Immunoadsorption in patients with neuromyelitis optica spectrum disorder. *Ther Adv Neurol Disord.* (2016) 9:281–6. doi: 10.1177/1756285616646332
176. Kleiter I, Gahlen A, Borisow N, Fischer K, Wernecke KD, Hellwig K, et al. Apheresis therapies for NMOSD attacks: a retrospective study of 207 therapeutic interventions. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e504. doi: 10.1212/NXI.0000000000000504
177. Bonnan M, Valentino R, Debeugny S, Merle H, Fergé JL, Mehdaoui H, Cabre P. Short delay to initiate plasma exchange is the strongest predictor of outcome in severe attacks of NMO spectrum disorders. *J Neurol Neurosurg Psychiatry* (2018) 89:346–51. doi: 10.1136/jnnp-2017-316286
178. Nikoo Z, Badihian S, Shaygannejad V, Asgari N, Ashtari F. Comparison of the efficacy of azathioprine and rituximab in neuromyelitis optica spectrum disorder: a randomized clinical trial. *J Neurol.* (2017) 264:2003–9. doi: 10.1007/s00415-017-8590-0
179. Trebst C, Jarius S, Berthele A, Paul F, Schippling S, Wildemann B, et al. Update on the diagnosis and treatment of neuromyelitis optica: recommendations of the Neuromyelitis Optica Study Group (NEMOS). *J Neurol.* (2014) 261:1–16. doi: 10.1007/s00415-013-7169-7
180. Patt H, Bandgar T, Lila A, Shah N. Management issues with exogenous steroid therapy. *Indian J Endocrinol Metab.* (2013) 17:S612–7. doi: 10.4103/2230-8210.123548
181. Stellmann JP, Krumbholz M, Friede T, Gahlen A, Borisow N, Fischer K, et al. Immunotherapies in neuromyelitis optica spectrum disorder: efficacy and predictors of response. *J Neurol Neurosurg Psychiatry* (2017) 88:639–47. doi: 10.1136/jnnp-2017-315603
182. Watanabe S, Misu T, Miyazawa I, Nakashima I, Shiga Y, Fujihara K, et al. Low-dose corticosteroids reduce relapses in neuromyelitis optica: a retrospective analysis. *Mult Scler.* (2007) 13:968–74. doi: 10.1177/1352458507077189
183. Qiu W, Kermode AG, Li R, Dai Y, Wang Y, Wang J, et al. Azathioprine plus corticosteroid treatment in Chinese patients with neuromyelitis optica. *J Clin Neurosci.* (2015) 22:1178–82. doi: 10.1016/j.jocn.2015.01.028
184. Zhang M, Zhang C, Bai P, Xue H, Wang G. Effectiveness of low dose of rituximab compared with azathioprine in Chinese patients with neuromyelitis optica: an over 2-year follow-up study. *Acta Neurol Belg.* (2017) 117:695–702. doi: 10.1007/s13760-017-0795-6
185. Elsone L, Kitley J, Luppe S, Lythgoe D, Mutch K, Jacob S, et al. Long-term efficacy, tolerability and retention rate of azathioprine in 103 aquaporin-4 antibody-positive neuromyelitis optica spectrum disorder patients: a multicentre retrospective observational study from the UK. *Mult Scler.* (2014) 20:1533–40. doi: 10.1177/1352458514525870
186. Costanzi C, Matiello M, Lucchinetti CF, Weinshenker BG, Pittock SJ, Mandrekar J, et al. Azathioprine: tolerability, efficacy, and predictors of benefit in neuromyelitis optica. *Neurology* (2011) 77:659–66. doi: 10.1212/WNL.0b013e31822a2780
187. Bennett JL, O'Connor KC, Bar-Or A, Zamvil SS, Hemmer B, Tedder TF, et al. B lymphocytes in neuromyelitis optica. *Neurol Neuroimmunol Neuroinflamm.* (2015) 2:e104. doi: 10.1212/NXI.0000000000000104
188. Kleiter I, Gold R. Present and future therapies in neuromyelitis optica spectrum disorders. *Neurother J Am Soc Exp Neurother.* (2016) 13:70–83. doi: 10.1007/s13311-015-0400-8
189. Ellwardt E, Ellwardt L, Bittner S, Zipp F. Monitoring B-cell repopulation after depletion therapy in neurologic patients. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e463. doi: 10.1212/NXI.0000000000000463
190. Evangelopoulos ME, Andreadou E, Koutsis G, Koutoulidis V, Anagnostouli M, Katsika P, et al. Treatment of neuromyelitis optica and neuromyelitis optica spectrum disorders with rituximab using a maintenance treatment regimen and close CD19 B cell monitoring. A six-year follow-up. *J Neurol Sci.* (2017) 372:92–6. doi: 10.1016/j.jns.2016.11.016
191. Cohen M, Romero G, Bas J, Ticchioni M, Rosenthal M, Lacroix R, Brunet C, et al. Monitoring CD27+ memory B-cells in neuromyelitis optica spectrum disorders patients treated with rituximab: results from a bicentric study. *J Neurol Sci.* (2017) 373:335–8. doi: 10.1016/j.jns.2017.01.025
192. Ciron J, Audoin B, Bourre B, Brassat D, Durand-Dubief F, Laplaud D, et al. Recommendations for the use of Rituximab in neuromyelitis optica spectrum disorders. *Rev Neurol.* (2018) 174:255–64. doi: 10.1016/j.neurol.2017.11.005
193. Rommer PS, Dörner T, Freivogel K, Haas J, Kieseier BC, Kümpfel T, et al. Safety and clinical outcomes of rituximab treatment in patients with multiple sclerosis and neuromyelitis optica: experience from a national online registry (GRAID). *J Neuroimmune Pharmacol.* (2016) 11:1–8. doi: 10.1007/s11481-015-9646-5
194. Marcinnò A, Marnetto F, Valentino P, Martire S, Balbo A, Drago A. Rituximab-induced hypogammaglobulinemia in patients with neuromyelitis optica spectrum disorders. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e498. doi: 10.1212/NXI.0000000000000498
195. Cree BAC, Lamb S, Morgan K, Chen A, Waubant E, Genain C. An open label study of the effects of rituximab in neuromyelitis optica. *Neurology* (2005) 64:1270–2. doi: 10.1212/01.WNL.0000159399.81861.D5
196. Zéphir H, Bernard-Valnet R, Lebrun C, Outteryck O, Audoin B, Bourre B, et al. Rituximab as first-line therapy in neuromyelitis optica: efficiency and tolerability. *J Neurol.* (2015) 262:2329–35. doi: 10.1007/s00415-015-7852-y
197. Radaelli M, Moiola L, Sangalli F, Esposito F, Barcella V, Ferrè L, et al. Neuromyelitis optica spectrum disorders: long-term safety and efficacy of rituximab in Caucasian patients. *Mult Scler.* (2016) 22:511–9. doi: 10.1177/1352458515594042
198. Torres J, Pruitt A, Balcer L, Galetta S, Markowitz C, Dahodwala N. Analysis of the treatment of neuromyelitis optica. *J Neurol Sci.* (2015) 351:31–5. doi: 10.1016/j.jns.2015.02.012
199. Kim SH, Huh SY, Lee SJ, Joung A, Kim HJ. A 5-year follow-up of rituximab treatment in patients with neuromyelitis optica spectrum disorder. *JAMA Neurol.* (2013) 70:1110–7. doi: 10.1001/jamaneurol.2013.3071
200. Cabre P, Mejdoubi M, Jeannin S, Merle H, Plumelle Y, Cavillon G, et al. Treatment of neuromyelitis optica with rituximab: a 2-year prospective multicenter study. *J Neurol.* (2018) 265:917–25. doi: 10.1007/s00415-018-8771-5
201. Kim SH, Jeong IH, Hyun JW, Joung A, Jo HJ, Hwang SH, et al. Treatment outcomes with rituximab in 100 patients with neuromyelitis optica: influence of FCGR3A polymorphisms on the therapeutic response to rituximab. *JAMA Neurol.* (2015) 72:989–95. doi: 10.1001/jamaneurol.2015.1276
202. Nosadini M, Alper G, Riney CJ, Benson LA, Mohammad SS, Ramanathan S, et al. Rituximab monitoring and redosing in pediatric neuromyelitis optica spectrum disorder. *Neurol Neuroimmunol Neuroinflamm.* (2016) 3:e188. doi: 10.1212/NXI.0000000000000188
203. Damato V, Evoli A, Iorio R. Efficacy and safety of rituximab therapy in neuromyelitis optica spectrum disorders: a systematic review and meta-analysis. *JAMA Neurol.* (2016) 73:1342–8. doi: 10.1001/jamaneurol.2016.1637
204. Kim SH, Kim HJ. Rituximab in neuromyelitis optica spectrum disorders: why not as first-line therapy. *JAMA Neurol.* (2017) 74:482. doi: 10.1001/jamaneurol.2016.5933
205. Das G, Damotte V, Gelfand JM, Bevan C, Cree BAC, Do L, et al. Rituximab before and during pregnancy: A systematic review, and a case series in MS and NMOSD. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e453. doi: 10.1212/NXI.0000000000000453
206. Perumal JS, Kister I, Howard J, Herbert J. Disease exacerbation after rituximab induction in neuromyelitis optica. *Neurol Neuroimmunol Neuroinflamm.* (2015) 2:e61. doi: 10.1212/NXI.0000000000000061
207. Whittam D, Cobo-Calvo A, Lopez-Chiriboga AS, Pardo S, Dodd J, Brandt A, et al. Treatment of MOG-IgG-associated demyelination with Rituximab: a multinational study of 98 patients (S13.003). *Neurology* (2018) 90.
208. Montcuquet A, Collongues N, Papeix C, Zephir H, Audoin B, Laplaud D, et al. Effectiveness of mycophenolate mofetil as first-line therapy in AQP4-IgG, MOG-IgG, and seronegative neuromyelitis optica spectrum disorders. *Mult Scler.* (2017) 23:1377–84. doi: 10.1177/1352458516678474

209. Jacob A, Matiello M, Weinshenker BG, Wingerchuk DM, Lucchinetti C, Shuster E, et al. Treatment of neuromyelitis optica with mycophenolate mofetil: retrospective analysis of 24 patients. *Arch Neurol.* (2009) 66:1128–33. doi: 10.1001/archneurol.2009.175
210. Chen H, Zhang Y, Shi Z, Feng H, Yao S, Xie J, Zhou H. The Efficacy and tolerability of mycophenolate mofetil in treating neuromyelitis optica and neuromyelitis optica spectrum disorder in Western China. *Clin Neuropharmacol.* (2016) 39:81–7. doi: 10.1097/WNF.0000000000000131
211. Yang Y, Wang CJ, Wang BJ, Zeng ZL, Guo SG. Comparison of efficacy and tolerability of azathioprine, mycophenolate mofetil, and lower dosages of rituximab among patients with neuromyelitis optica spectrum disorder. *J Neurol Sci.* (2018) 385:192–7. doi: 10.1016/j.jns.2017.12.034
212. Chen H, Qiu W, Zhang Q, Wang J, Shi Z, Liu J, et al. Comparisons of the efficacy and tolerability of mycophenolate mofetil and azathioprine as treatments for neuromyelitis optica and neuromyelitis optica spectrum disorder. *Eur J Neurol.* (2017) 24:219–26. doi: 10.1111/ene.13186
213. Viswanathan S, Wong AHY, Quek AML, Yuki N. Intravenous immunoglobulin may reduce relapse frequency in neuromyelitis optica. *J Neuroimmunol.* (2015) 282:92–6. doi: 10.1016/j.jneuroim.2015.03.021
214. Elsone L, Panicker J, Mutch K, Boggild M, Appleton R, Jacob A. Role of intravenous immunoglobulin in the treatment of acute relapses of neuromyelitis optica: experience in 10 patients. *Mult Scler.* (2014) 20:501–4. doi: 10.1177/1352458513495938
215. Tackley G, O'Brien F, Rocha J, Woodhall M, Waters P, Chandratre S, et al. Neuromyelitis optica relapses: Race and rate, immunosuppression and impairment. *Mult Scler Relat Disord.* (2016) 7:21–5. doi: 10.1016/j.msard.2016.02.014
216. Kitley J, Elsone L, George J, Waters P, Woodhall M, Vincent A, et al. Methotrexate is an alternative to azathioprine in neuromyelitis optica spectrum disorders with aquaporin-4 antibodies. *J Neurol Neurosurg Psychiatry* (2013) 84:918–21. doi: 10.1136/jnnp-2012-304774
217. Ramanathan RS, Malhotra K, Scott T. Treatment of neuromyelitis optica/neuromyelitis optica spectrum disorders with methotrexate. *BMC Neurol.* (2014) 14:51. doi: 10.1186/1471-2377-14-51
218. Papeix C, Vidal JS, de Seze J, Pierrot-Deseilligny C, Tourbah A, Stankoff B, et al. Immunosuppressive therapy is more effective than interferon in neuromyelitis optica. *Mult Scler.* (2007) 13:256–9. doi: 10.1177/1352458506070732
219. Palace J, Leite MI, Nairne A, Vincent A. Interferon Beta treatment in neuromyelitis optica: increase in relapses and aquaporin 4 antibody titers. *Arch Neurol.* (2010) 67:1016–7. doi: 10.1001/archneurol.2010.188
220. Ayzenberg I, Schöllhammer J, Hoepner R, Hellwig K, Ringelstein M, Aktas O, et al. Efficacy of glatiramer acetate in neuromyelitis optica spectrum disorder: a multicenter retrospective study. *J Neurol.* (2016) 263:575–82. doi: 10.1007/s00415-015-7991-1
221. Kleiter I, Hellwig K, Berthele A, Kümpfel T, Linker RA, Hartung HP, et al. Failure of natalizumab to prevent relapses in neuromyelitis optica. *Arch Neurol.* (2012) 69:239–45. doi: 10.1001/archneurol.2011.216
222. Kowarik MC, Hoshi M, Hemmer B, Berthele A. Failure of alemtuzumab as a rescue in a NMOSD patient treated with rituximab. *Neurol Neuroimmunol Neuroinflamm.* (2016) 3:e208. doi: 10.1212/NXI.0000000000000208
223. Gelfand JM, Cotter J, Klingman J, Huang EJ, Cree BAC. Massive CNS monocytic infiltration at autopsy in an alemtuzumab-treated patient with NMO. *Neurol Neuroimmunol Neuroinflamm.* (2014) 1:e34. doi: 10.1212/NXI.0000000000000034
224. Fujihara K, Nakashima I. Secondary progression and innate immunity in NMO: A possible link to alemtuzumab therapy? *Neurol Neuroimmunol Neuroinflamm.* (2014) 1:e38. doi: 10.1212/NXI.0000000000000038
225. Azzopardi L, Cox AL, McCarthy CL, Jones JL, Coles AJ. Alemtuzumab use in neuromyelitis optica spectrum disorders: a brief case series. *J Neurol.* (2016) 263:25–9. doi: 10.1007/s00415-015-7925-y
226. Kira JI. Unexpected exacerbations following initiation of disease-modifying drugs in neuromyelitis optica spectrum disorder: Which factor is responsible, anti-aquaporin 4 antibodies, B cells, Th1 cells, Th2 cells, Th17 cells, or others? *Mult Scler.* (2017) 23:1300–2. doi: 10.1177/1352458517703803
227. Yoshii F, Moriya Y, Ohnuki T, Ryo M, Takahashi W. Fingolimod-induced leukoencephalopathy in a patient with neuromyelitis optica spectrum disorder. *Mult Scler Relat Disord.* (2016) 7:53–7. doi: 10.1016/j.msard.2016.03.004
228. Min JH, Kim BJ, Lee KH. Development of extensive brain lesions following fingolimod (FTY720) treatment in a patient with neuromyelitis optica spectrum disorder. *Mult Scler.* (2012) 18:113–5. doi: 10.1177/1352458511431973
229. Yamout BI, Beaini S, Zeineddine MM, Akkawi N. Catastrophic relapses following initiation of dimethyl fumarate in two patients with neuromyelitis optica spectrum disorder. *Mult Scler.* (2017) 23:1297–300. doi: 10.1177/1352458517694086
230. Gahlen A, Trampe AK, Haupeltshofer S, Ringelstein M, Aktas O, Berthele A, et al. Aquaporin-4 antibodies in patients treated with natalizumab for suspected MS. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e363. doi: 10.1212/NXI.0000000000000363
231. Cabre P, Olindo S, Marignier R, Jeannin S, Merle H, Smadja D, et al. Efficacy of mitoxantrone in neuromyelitis optica spectrum: clinical and neuroradiological study. *J Neurol Neurosurg Psychiatry* (2013) 84:511–6. doi: 10.1136/jnnp-2012-303121
232. Kim SH, Kim W, Park MS, Sohn EH, Li XF, Kim HJ. Efficacy and safety of mitoxantrone in patients with highly relapsing neuromyelitis optica. *Arch Neurol.* (2011) 68:473–9. doi: 10.1001/archneurol.2010.322
233. Stroet A, Hemmelmann C, Starck M, Zettl U, Dörr J, Friedemann P, et al. Incidence of therapy-related acute leukaemia in mitoxantrone-treated multiple sclerosis patients in Germany. *Ther Adv Neurol Disord.* (2012) 5:75–9. doi: 10.1177/1756285611433318
234. Dörr J, Bitsch A, Schmailzl KJG, Chan A, von Ahsen N, Hummel M, et al. Severe cardiac failure in a patient with multiple sclerosis following low-dose mitoxantrone treatment. *Neurology* (2009) 73:991–3. doi: 10.1212/WNL.0b013e3181b878f6
235. Paul F, Dörr J, Würfel J, Vogel HP, Zipp F. Early mitoxantrone-induced cardiotoxicity in secondary progressive multiple sclerosis. *J Neurol Neurosurg Psychiatry* (2007) 78:198–200. doi: 10.1136/jnnp.2006.091033
236. Bichuetti DB, Oliveira EML, Boulos Fde C, Gabbai AA. Lack of response to pulse cyclophosphamide in neuromyelitis optica: evaluation of 7 patients. *Arch Neurol.* (2012) 69:938–9. doi: 10.1001/archneurol.2012.545
237. *A Double-masked, Placebo-controlled Study With Open Label Period to Evaluate MEDI-551 in Neuromyelitis Optica and Neuromyelitis Optica Spectrum Disorders - Full Text View - ClinicalTrials.gov*. Available online at: https://clinicaltrials.gov/ct2/show/NCT02200770 (Accessed April 18, 2018).
238. Chen D, Gallagher S, Monson NL, Herbst R, Wang Y. Inebilizumab, a B Cell-Depleting anti-CD19 antibody for the treatment of autoimmune neurological diseases: insights from preclinical studies. *J Clin Med.* (2016) 5:E107. doi: 10.3390/jcm5120107
239. Cree BA, Bennett JL, Sheehan M, Cohen J, Hartung HP, Aktas O, et al. Placebo-controlled study in neuromyelitis optica-Ethical and design considerations. *Mult Scler.* (2016) 22:862–72. doi: 10.1177/1352458515620934
240. Pittock SJ, Lennon VA, McKeon A, Mandrekar J, Weinshenker BG, Lucchinetti CF, et al. Eculizumab in AQP4-IgG-positive relapsing neuromyelitis optica spectrum disorders: an open-label pilot study. *Lancet Neurol.* (2013) 12:554–62. doi: 10.1016/S1474-4422(13)70076-0
241. Paul F. Hope for a rare disease: eculizumab in neuromyelitis optica. *Lancet Neurol.* (2013) 12:529–31. doi: 10.1016/S1474-4422(13)70089-9
242. *An Open Label Extension Trial of Eculizumab in Relapsing NMO Patients - Full Text View - ClinicalTrials.gov*. Available online at: https://clinicaltrials.gov/ct2/show/NCT02003144 (Accessed April 17, 2018).
243. Araki M, Matsuoka T, Miyamoto K, Kusunoki S, Okamoto T, Murata M, et al. Efficacy of the anti-IL-6 receptor antibody tocilizumab in neuromyelitis optica: a pilot study. *Neurology* (2014) 82:1302–6. doi: 10.1212/WNL.0000000000000317
244. Ringelstein M, Ayzenberg I, Harmel J, Lauenstein AS, Lensch E, Stögbauer F, et al. Long-term therapy with interleukin 6 receptor blockade in highly active neuromyelitis optica spectrum disorder. *JAMA Neurol.* (2015) 72:756–63. doi: 10.1001/jamaneurol.2015.0533
245. Tanaka M, Yanagida H, Suzumura A. Treatment for paraneoplastic neuromyelitis optica spectrum disorder (NMOsd): Probable effects of

tocilizumab for both cancer and NMOsd. *Mult Scler Relat Disord.* (2018) 20:82–3. doi: 10.1016/j.msard.2017.12.002

246. *Tocilizumab vs Azathioprine in Neuromyelitis Optica Spectrum Disorders - Full Text View - ClinicalTrials.gov*. Available online at: https://clinicaltrials.gov/ct2/show/NCT03350633 (Accessed August 14, 2018)
247. *Efficacy and Safety Study as Add-on Therapy of SA237 to Treat NMO and NMOSD - Full Text View - ClinicalTrials.gov*. Available online at: https://clinicaltrials.gov/ct2/show/NCT02028884 (Accessed April 18, 2018).
248. Steinman L, Bar-Or A, Behne JM, Benitez-Ribas D, Chin PS, Clare-Salzler M, et al. Restoring immune tolerance in neuromyelitis optica: part I. *Neurol Neuroimmunol Neuroinflamm.* (2016) 3:e276. doi: 10.1212/NXI.0000000000000276
249. Bar-Or A, Steinman L, Behne JM, Benitez-Ribas D, Chin PS, Clare-Salzler M, et al. Restoring immune tolerance in neuromyelitis optica: Part II. *Neurol Neuroimmunol Neuroinflamm.* (2016) 3:e277. doi: 10.1212/NXI.0000000000000277
250. Paul F, Murphy O, Pardo S, Levy M. Investigational drugs in development to prevent neuromyelitis optica relapses. *Expert Opin Investig Drugs* (2018) 27:265–71. doi: 10.1080/13543784.2018.1443077
251. Hodecker SC, Stellmann JP, Rosenkranz SC, Young K, Holst B, Friese MA, et al. Ruxolitinib treatment in a patient with neuromyelitis optica: a case report. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e328. doi: 10.1212/NXI.0000000000000328

Conflict of Interest Statement: FP serves on the scientific advisory board for Novartis; received speaker honoraria and travel funding from Bayer, Novartis, Biogen Idec, Teva, Sanofi-Aventis/Genzyme, Merck Serono, Alexion, Chugai, MedImmune, and Shire; is an academic editor for *PLoS One*, is an associate editor for *Neurology® Neuroimmunology & Neuroinflammation*; consulted for Sanofi-Genzyme, Biogen Idec, MedImmune, Shire, and Alexion; received research support from Bayer, Novartis, Biogen Idec, Teva, Sanofi-Aventis/Genzyme, Alexion, Merck Serono, German Research Council, Werth Stiftung of the City of Cologne, German Ministry of Education and Research, Arthur Arnstein Stiftung Berlin, EU FP7 Framework Program, Arthur Arnstein Foundation Berlin, Guthy Jackson Charitable Foundation, and National Multiple Sclerosis of the USA. SK serves as a Deputy Editor of Journal of Neurology, Neurosurgery, and Psychiatry and an Editorial Board member of Journal of the Neurological Sciences.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 10 October 2018
doi: 10.3389/fneur.2018.00841

Detection Methods for Autoantibodies in Suspected Autoimmune Encephalitis

*Gerda Ricken[1], Carmen Schwaiger[1], Desiree De Simoni[1], Valerie Pichler[1], Julia Lang[1], Sarah Glatter[1], Stefan Macher[2], Paulus S. Rommer[2], Petra Scholze[3], Helmut Kubista[4], Inga Koneczny[1] and Romana Höftberger[1]**

[1] Institute of Neurology, Medical University of Vienna, Vienna, Austria, [2] Department of Neurology, Medical University of Vienna, Vienna, Austria, [3] Department of Pathobiology of the Nervous System, Center for Brain Research, Medical University of Vienna, Vienna, Austria, [4] Center for Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria

OPEN ACCESS

Edited by:
Johann Sellner, Universitätsklinikum Salzburg, Austria

Reviewed by:
Andrea Harrer, Paracelsus Medizinische Privatuniversität, Austria
Patrick Joseph Waters, University of Oxford, United Kingdom

***Correspondence:**
Romana Höftberger
romana.hoeftberger@meduniwien.ac.at

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *12 July 2018*
Accepted: *18 September 2018*
Published: *10 October 2018*

Citation:
Ricken G, Schwaiger C, De Simoni D, Pichler V, Lang J, Glatter S, Macher S, Rommer PS, Scholze P, Kubista H, Koneczny I and Höftberger R (2018) Detection Methods for Autoantibodies in Suspected Autoimmune Encephalitis. Front. Neurol. 9:841. doi: 10.3389/fneur.2018.00841

This review provides an overview on different antibody test methods that can be applied in cases of suspected paraneoplastic neurological syndromes (PNS) and anti-neuronal autoimmune encephalitis (AIE) in order to explain their diagnostic value, describe potential pitfalls and limitations, and discuss novel approaches aimed at discovering further autoantibodies. Onconeuronal antibodies are well-established biomarkers for PNS and may serve as specific tumor markers. The recommended procedure to detect onconeuronal antibodies is a combination of indirect immunohistochemistry on fixed rodent cerebellum and confirmation of the specificity by line assays. Simplification of this approach by only using line assays with recombinant proteins bears the risk to miss antibody-positive samples. Anti-neuronal surface antibodies are sensitive and specific biomarkers for AIE. Their identification requires the use of test methods that allow the recognition of conformation dependent epitopes. These commonly include cell-based assays and tissue based assays with unfixed rodent brain tissue. Tissue based assays can detect most of the currently known neuronal surface antibodies and thus enable broad screening of biological samples. A complementary testing on live neuronal cell cultures may confirm that the antibody recognizes a surface epitope. In patients with peripheral neuropathy, the screening may be expanded to teased nerve fibers to identify antibodies against the node of Ranvier. This method helps to identify a novel subgroup of peripheral autoimmune neuropathies, resulting in improved immunotherapy of these patients. Tissue based assays are useful to discover additional autoantibody targets that play a role in diverse autoimmune neurological syndromes. Antibody screening assays represent promising avenues of research to improve the diagnostic yield of current assays for antibody-associated autoimmune encephalitis.

Keywords: paraneoplastic neurological syndrome, autoimmune encephalitis, onconeuronal antibodies, test methods, cell-based assay, tissue-based assay, anti-neuronal antibodies

INTRODUCTION

Autoimmune diseases in the brain may affect different parts of the nervous system including neurons, glial cells or components of the blood-brain barrier. The pathobiology can be predominantly driven by T-cells or B-cells that recognize cerebral antigens. The field of autoantibody mediated autoimmune diseases of the nervous system has been expanding in the recent years, propelled by the discovery of autoantibodies against synaptic or extrasynaptic membrane antigens that lead to a new approach in diagnosing and treating patients with suspected autoimmune neurological diseases (1). While autoimmune responses against intracellular antigens are mainly associated with paraneoplastic or idiopathic neurological syndromes with poor neurological outcome, patients with surface autoimmunity show substantial response to immunotherapy (1). Cell-mediated immune attack by T-cells resulting in progressive destruction of cells is a hallmark of paraneoplastic neurological syndromes (PNS) and may explain the limited response to immunotherapy (2). Although some pathogenic impact has been described for anti-amphiphysin antibodies (3), the mechanisms and functions of other autoantibodies that evolve in the context of classical paraneoplastic syndromes (so called onconeuronal antibodies) are still poorly understood and they are rather considered as an epiphenomenon. However, the antibodies indicate the paraneoplastic etiology of the associated neurological syndrome and may serve as biomarkers for recognizing an underlying malignancy (**Table 1**) (4). In contrast, autoantibodies against surface antigens may directly mediate the disease (e.g., by antigenic modulation or by recruitment of immune cells or components of the complement system), among the antibodies against neuronal membrane antigens, these effects are often reversible and explain the good response to immunotherapy. Autoantibodies against cell surface antigens on neurons and glial cells can be tumor associated but derive more frequently from an idiopathic origin (1). To date, more than 16 such autoimmune syndromes are known and are summarized in **Table 2**. These diseases occur worldwide in diverse ethnicities and cultures. Among the anti-neuronal surface antibodies, anti-NMDAR are probably the most common ones, followed by anti-LGI1 with a reported annual incidence of 0.83 per million in one Dutch study (7). Other antibodies seem to be less frequent or their incidence has to be defined in prospective experience. Many autoimmune neurological or demyelinating syndromes are currently considered as antibody negative despite some evidence that they are antibody-mediated. Among these are patients with suspected but yet unknown antigenic targets, and further studies are required to discover these. Nevertheless, a substantial fraction of seronegative patients may harbor known autoantibodies that could be detected with a more thorough testing strategy. The following review gives an overview of the most widely used test methods and their limitations in the detection of autoantibodies and provides an outlook on possible novel approaches that are able to broaden the spectrum of identifyable antibodies.

Abbreviations: AMPAR, amino-3-hydroxy-5-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CASPR1/2, contactin-associated protein-like 1/2; CNTN1, contactin1; D2R, dopamine-2 receptor; DPPX, dipeptidyl-peptidase-like protein-6; GABA A/B R, gamma-aminobutyric acid A/B receptor; GAD, glutamic acid decarboxylase; GlyR, Glycine receptor; LGI1, leucine-rich glioma-inactivated 1; mGluR1/5, metabotropic glutamate receptor type 1/5; NF155, neurofascin155; NMDAR, N-methyl-D-aspartate receptor; P/Q-type VGCC, P/Q-type voltage-gated calcium channel; VGKC, voltage-gated potassium channel.

AUTOANTIBODIES IN CLASSICAL PARANEOPLASTIC AND NON-PARANEOPLASTIC NEUROLOGICAL SYNDROMES

Background

Since the 1980s, detailed clinical and immunological studies revealed several autoantibodies against intracellular antigens that are associated with specific paraneoplastic or idiopathic neurological syndromes (8–15). Antibodies directed against intracellular antigens usually recognize linear epitopes that can be detected by methods such as western blot analysis, line assays, enzyme-linked immunosorbent assay (ELISA), fixed tissue- (fixed TBA) or cell-based assays (CBA), or radioimmunoassay (RIA). In clinical laboratories, line assays and fixed TBAs are used most frequently. For line assays, purified recombinant proteins (e.g., paraneoplastic antigens such as Yo, Hu, Ri, CV2/CRMP5, and others) are applied on blot strips and incubated with the patient's serum or CSF. Line assays are commercially available and include most of the currently known well-characterized autoantibodies that are screened within one test run. The fixed TBAs use paraformaldehyde-fixed rodent (mouse or rat) or monkey tissue (cerebellum and enteric nervous system). The fixation is necessary for the intracellular antigen retrieval. Autoantibodies are defined as well-characterized if the serum or CSF produces a recognizable staining pattern in the fixed TBA (e.g., selective staining of Purkinje cells with Yo-positive patient's serum) (**Figures 1A–J**) and the antibody specificity is confirmed with the recombinant line assay (16).

Challenges in Antibody Detection

Well-Characterized Onconeuronal Antibodies

To provide highest sensitivity and specificity for onconeuronal antibody testing, it is recommended to combine a fixed TBA and a line assay (16). Line assays may be more sensitive in some patients than indirect immunohistochemistry (17), in addition they can help to specify the onconeuronal antibody. Using the TBA alone has the disadvantage that concomitant antibodies such as anti-nuclear antibodies may mask the immunohistochemical staining pattern. On the other hand, commercial line assays may sometimes produce reactivity in control sera without reported cancer (18) and the clinical significance is unclear. Moreover, a recent study reported that the use of commercial line assays with recombinant protein harbors the risk to miss autoantibodies as it has been shown in 4 out of 53 patients with CV2/CRMP5-antibodies (19). It was hypothesized that the epitope repertoire of the CV2 antibodies that were

TABLE 1 | Antibodies targeting intracellular antigens.

Intracellular antigen	Associated tumor	Main syndrome	Most widely used test methods
CLASSIC ONCONEURONAL ANTIBODY			
Hu (ANNA1)	SCLC	Enzephalomyelitis, PCD, LE, brainstemencephalitis	Fixed TBA, LA/IB
Ri (ANNA2)	Mammary, SCLC	Brainstemencephalitis, OMS	Fixed TBA; LA/IB
Yo (PCA1)	Ovary, mammary	PCD	Fixed TBA; LA/IB
CV2 (CRMP5)	SCLC, thymoma	Encephalomyelitis, optic neuropathy, PCD, LE	Fixed TBA; LA/IB; fixed CBA
Amphiphysin	SCLC, mammary	SPS, rigidity, encephalomyelitis	Fixed/unfixed TBA; LA/IB
Ma-1/2	Testis, adenocarcinoma lung	LE, brainstemencephalitis	Fixed TBA; LA/IB
DNER/TR	Hodgkin	PCD	Fixed/unfixed TBA; LA/IB; fixed CBA
NON-PARANEOPLASTIC ANTIBODY			
GAD65/67	Rarley	SPS, cerebellar ataxia, LE, epilepsy	Fixed TBA; LA/IB, fixed CBA, RIA, ELISA
TUMOR MARKERS			
SOX1 (AGNA)	SCLC	Encephalomyelitis, PCD	Fixed TBA; LA/IB
ZIC4	SCLC	Cerebellar ataxia	Fixed TBA; LA/IB

ANNA, anti-neuronal nuclear antibody; SCLC, small cell lung cancer; PCD, paraneoplastic cerebellar degeneration; LE, limbic encephalitis; TBA, tissue based assay; LA/IB, line assay/immunoblot; OMS, opsoclonus-myoclonus syndrome; PCA, purkinje cell autoantibody; CRMP, collapsin response mediator protein; SPS, stiff-person syndrome; DNER, delta/notch-like epidermal growth factor-related receptor; CBA, cell-based assay; GAD65, glutamic acid decarboxylase 65; RIA, radioimmuno assay; ELISA, enzyme-linked immunosorbent assay; AGNA, anti-glial nuclear antibody; ZIC4, zinc-finger protein 4.

missed in the line assay may be different from the typical CV2 antibodies (19).

Tumor- and Non-tumor Associated Intracellular Antibodies

Importantly, the fixed TBA is also able to detect rare antibodies such as for example anti-protein-kinase Cgamma (PKCgamma) (20), anti-carbonic anhydrase-related protein VIII (CARP VIII) (21) or anti-rhoGTPase-activating protein 26 (ARGHAP26) (22) that bind intracellular proteins highly expressed in Purkinje cells and were originally identified in patients presenting with subacute autoimmune cerebellar ataxia. Currently, the detection of these antibodies is only possible with in-house assays and the results of the TBA can either be confirmed with in-house immunoblots or fixed cell-based assays. The PKCgamma, CARP VIII and ARGHAP26 are potentially paraneoplastic antibodies and a positive antibody-test should prompt tumor search. Recently, a novel astrocytic IgG autoantibody targeting glial fibrillary acidic protein (GFAP) has been identified in the CSF and serum of 16 patients with relapsing steroid-responsive meningoencephalitis with or without myelitis and was clinically characterized in a series of 102 patients (23, 24). The antibody was identified in the TBA showing an immunofluorescence staining of a subpopulation of astrocytes confined to pia, subpia, midbrain foci, periventricular region and rostral migratory stream and subsequently characterized in the fixed CBA as GFAP-specific. An underlying tumor can be found in 22% of patients, which include teratoma, carcinoid, salivary pleomorphic adenoma, prostate carcinoma, and melanoma. Some patients may have coexisting antibodies such as anti-NMDAR or aquaporin-4 (AQP4) antibodies, which may indicate an underlying teratoma. Although the antigen is intracellularly located, patients show good response to immunotherapy. Future investigations are necessary to clarify the role of antibodies in disease evolution, give insight into T-cell antigen specificities, and reveal possible genetic factors.

Anti-GAD Antibodies

The glutamic acid decarboxylase (GAD) is an enzyme that catalyzes the transformation of glutamate into gamma-amino-butyric-acid (GABA). Two isoforms have been described, the 65 and the 67 kDa isoform. Both can be found in GABAergic neurons in the brain, the 65 kDa isoform is additionally expressed in islet cells of the pancreas. Low titers of GAD65 antibodies can occur in about 1% of healthy controls and in up to 80% of patients with diabetes mellitus type I (25). Currently available commercial test methods focus on the detection of the GAD65 isoform and include ELISA, radioimmunoassay, TBA, and line assays. The ELISA and RIA are more sensitive than TBA or line assays and can detect very low titers of GAD65, however, only high titers (usually $>$2,000 U/ml) are considered to be associated with autoimmune neurological disorders including stiff-person syndrome, ataxia, epilepsy, limbic encephalitis, and other syndromes (25). It has long been believed that screening for GAD65 antibodies is sufficient for identifying patients with GAD-autoimmunity. Interestingly, a recent study with GAD65-antibody positive patients with neurological disorders reported that GAD67 antibodies were present in the CSF even if the serum was negative for GAD67 antibodies, indicating an intrathecal antibody synthesis (26). Later it has been shown that few patients harbor antibodies only against the GAD67 isoform. The clinical picture of patients with GAD67 antibodies in serum and/or CSF is currently believed to be indistinguishable from the phenotype associated with GAD65 antibodies but the patients would be missed if GAD65 specific assays are employed such as line assays or RIA (27). Currently GAD67 can only be detected by in-house assays that either use TBA, in-house immunoblots or fixed cell-based assays.

TABLE 2 | Antibodies against surface antigens.

Antigen	Tumor	Main clinical symptoms	Predominant antibody subclass	Most widely used test methods
CENTRAL NERVOUS SYSTEM				
NMDAR	Ovarian teratoma (58% in patients >18 years)	Encephalitis	IgG1	Unfixed TBA; live/fixed CBA
LGI1	Rarely (thymoma)	LE, faciobrachial dystonic seizures, hyponatremia	IgG4/IgG1	Unfixed TBA; live/fixed CBA
CASPR2	Thymoma (38%)	LE, cerebellar ataxia, Morvan syndrome, peripheral nerve hyperexcitability	IgG4/IgG1	Unfixed TBA; live/fixed CBA
AMPAR	SCLC, breast, thymoma (60%)	LE, psychosis	IgG1	Unfixed TBA; live/fixed CBA
$GABA_BR$	SCLC (50%)	LE, ataxia	IgG1	Unfixed TBA; live/fixed CBA
$GABA_AR$	Thymoma, others (25%)	Status epilepticus, seizures, encephalitis	IgG1	Unfixed TBA; live CBA
mGluR1	Hematologic diseases (30–40%)	Cerebellar ataxia	NA	Unfixed TBA; live/fixed CBA
mGluR5	55% paraneoplastic (Hodgkin, SCLC)	Limbic dysfunction, movement disorders;	IgG1/IgG3	Unfixed TBA; live/fixed CBA
DPPX (Kv4.1)	Follicular B cell lymphoma, CLL	Hallucinations, agitation, myoclonus, tremor, seizures, diarrhea	IgG4/IgG1	Unfixed TBA; live/fixed CBA
IgLON5	–	Non-REM and REM-sleep disorder, brainstem and limbic dysfunction	IgG4/IgG1	Unfixed TBA; live/fixed CBA
GlyR	Lung cancer	SPS, PERM, epilepsy	IgG1	Unfixed TBA; live CBA
Dopamine 2R	–	Basal ganglia encephalitis, Sydenham's Chorea	NA	Unfixed TBA; live CBA
Neurexin3alpha	–	Seizures, orofacial dyskinesias	IgG1	Unfixed TBA; fixed CBA
PQ-type VGCC	SCLC	LEMS, PCD	NA	RIA
ANTIBODIES IN DEMYELINATION				
AQP4	Rarely	NMOSD, LETM, ON	IgG1	Unfixed TBA; live/fixed CBA; ELISA
MOG	–	ADEM, ON, LETM (conus), TM, NMOSD, seizures	IgG1	Live/fixed CBA
Antigen	**Associated diseases**	**Main syndrome**	**Predominant antibody subclass**	**Most widely used test methods**
PERIPHERAL NERVOUS SYSTEM				
Neurofascin155		Atypcial CIDP with distal sensomotoric neuropahty, tremor, ataxia, CNS-demyelination	IgG4	Unfixed TBA; fixed CBA; teased fibers; ELISA
Neurofascin186	IgG4-related disease; nephrotic syndrome	Subacute onset, severe phenotype, sensory ataxia	IgG4/IgG3	Fixed CBA; teased fibers; ELISA
Contactin1	Rarely nephrotic syndrome	Atypical CIDP with GBS-like onset, tremor, ataxia	IgG4/IgG3*	Unfixed TBA; fixed CBA; teased fibers; ELISA
CASPR1		CIDP, GBS, neuropathic pain	IgG4, IgG3*	Unfixed TBA; fixed CBA; teased fibers

**IgG3 were found in patients with GBS or in the acute phase of CIDP and may switch to IgG4 in the chronic phase of the disease (5, 6). NMDAR, N-methyl-D-aspartate receptor; TBA, tissue based assay; CBA, cell-based assay; LGI1, leucine-rich glioma-inactivated 1; LE, limbic encephalitis; CASPR2, contactin-associated protein-like 2; AMPAR, amino-3-hydroxy-5-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; GABA A/B R, gamma-aminobutyric acid A/B receptor; mGluR1/5, metabotropic glutamate receptor type 1/5; NA, not available; DPPX, dipeptidyl-peptidase-like protein-6; CLL, chronic lymphatic leukemia; GlyR, Glycine receptor; SPS, stiff-person syndrome; PERM, progressive encephalomyelitis with rigidity and myoclonus; P/Y-type VGCC, P/Q-type voltage gated calcium channel; LEMS, Lambert-Eaton myasthenic syndrome; RIA, radioimmuno assay; AQP4, aquaporin 4; NMOSD, neuromyelitis spectrum disorder; LETM, longitudinally transverse myelitis; ON, optic neuritis; MOG, myelin oligodendrocyte glycoprotein; ADEM, acute disseminated encephalomyelitis; TM, transvers myelitis; CIDP, chronic inflammatory demyelinating polyneuropathy; GBS, Guillain-Barré syndrome.*

AUTOANTIBODIES IN ANTI-NEURONAL AND ANTI-GLIAL SURFACE AUTOIMMUNITY

Background

Autoantibodies directed against surface antigens often recognize conformation dependent epitopes and their detection depends on methods that preserve the three-dimensional structure of the antigen such as CBA or unfixed/postfixed TBA. In clinical laboratories, CBAs are used most frequently. The CBA consists of human or murine cells that are transfected with human complementary DNA (cDNA) and express the target antigen on their surface. Sera or CSF from patients are evaluated for the presence of antibodies by binding to these expressed antigens.

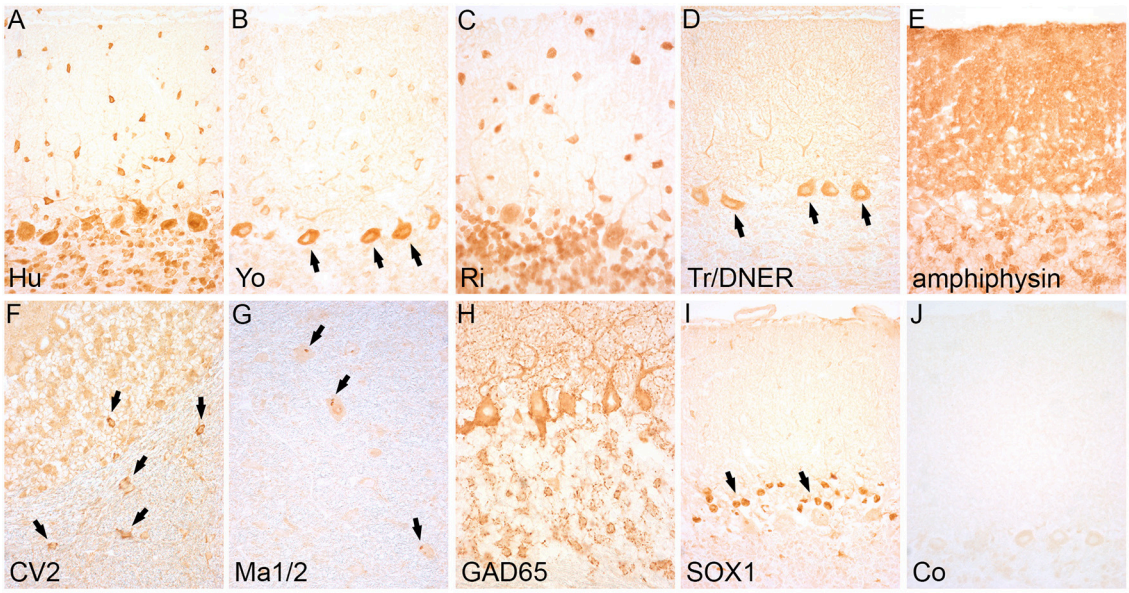


FIGURE 1 | Staining pattern of antibodies targeting intracellular antigens. Indirect immunohistochemistry (avidin-biotin peroxidase method) on rat cerebellum shows a specific staining pattern of intracellular antibodies: **(A)** Anti-Hu-antibodies label the cytoplasm and nuclei of Purkinje and granule cells. **(B)** Anti-Yo antibodies show labeling of the cytoplasm of Purkinje cells (arrows) and stellate and basket cells in the molecular layer. **(C)** Anti-Ri-antibodies show the same staining pattern like Hu-antibodies in the cerebellum (differentiation is possible by staining enteric neurons of the gut that are positive with anti-Hu but negative with anti-Ri-antibodies). **(D)** Anti-Tr/DNER antibodies strongly label the Purkinj cell somata and dendrites (arrows). **(E)** Anti-amphiphysin antibodies show an intensive synaptic staining pattern in the molecular layer of the cerebellum. **(F)** Anti-CV2-antibodies mark a subgroup of oligodendrocytes in the cerebellar cortex and white matter (arrows). **(G)** Anti-Ma1/2-antibodies show a dot-like staining pattern in large neurons of the brainstem (arrows). **(H)** Anti-GAD65-antibodies display a dot-like staining of the base of Purkinje cells and a rosette-like staining pattern in the granular layer of the cerebellar cortex **(I)** Anti-SOX1-antibodies stain the nuclei of Bergmann glia in the cerebellar cortex (arrows). **(J)** Serum of a healthy control remains negative. Magnification: **(A–J)**: x400.

CBAs are commercially available and either offered as set that allows screening of several autoantibodies within one test run [e.g., combined testing of NMDAR, AMPAR, GABA(B)R, LGI1, CASPR2, and DPPX] or as individual tests (e.g. IgLON5). The unfixed/postfixed TBAs use rodent (mouse or rat) brain tissue that contains the hippocampus and cerebellum. Sera or CSF from patients are evaluated for the presence of antibodies by binding to the rodent brain tissue and subsequently visualized either via an avidin-biotin method and light microscopy or immunofluorescence. This approach has been successful in discovering most of the autoantibodies described in the past decade. The TBAs for testing surface antibodies are commercially available or can be produced in-house and can be used as screening tool or to confirm the results of the CBA.

Challenges in Antibody Detection

Selection of the Appropriate Assay

One of the first neurological autoimmune diseases that were defined by the presence of pathogenic surface autoantibodies was myasthenia gravis associated with anti-acetylcholine receptor antibodies (AChR) (28). Later, surface antibodies to the P/Q type voltage-gated calcium channel (PQ-type VGCC) were identified in patients with Lambert-Eaton myasthenic syndrome (LEMS) (29, 30). Both antibodies were discovered by using RIA assays in which the antigens were labeled with ^{125}I-specific neurotoxins and precipitated with patient's antibodies (31). Synthetic peptide binding studies in LEMS patients demonstrated that three epitope regions of the external linker peptides S5-S6 of domain II and IV of the alpha-1A subunit of the PQ-type VGCC were essential for creating reactivity in 9/12 patients. These epitopes are considered to be linear and test methods that lack correct membrane topology are suitable for their detection (32). In contrast, other pathogenic surface antibodies mostly recognize conformational epitopes and test methods that measure antibodies against linear or refolded epitopes often produce contradictory results, including variable frequencies of seropositivity in patients with diverse clinical syndromes and healthy controls. The RIA may give false positive results due to two issues: (1) the availability of intracellular epitopes may pick up irrelevant antibodies. For example only 56% of the serum samples that were tested positive in a RIA for voltage-gated potassium channel (VGKC) complex antibodies contained antibodies against the extracellular domain of LGI1 or CASPR2, while a considerable amount of LGI1/CASPR2-negative samples were directed against cytosolic epitopes of the VGKC (33, 34). (2) False positive results may also derive from the presence of autoantibodies against the ^{125}I-neurotoxin itself (33) and false negative results may derive from an overlap of the antibody binding epitope with the binding site for the ^{125}I-neurotoxin, a known phenomenon in mysthenia gravis and AChR antibodies (35–37). These difficulties emphasize the importance of test validation with different screening methods that ensure the

exclusive recognition of the conformational epitope of the respective antigen and excludes interference with confounding components in the assay such as neurotoxins. The CBA allows the screening for conformation-dependent antibodies and enables the unequivocal identification of a specific surface antibody. The sensitivity of the CBA can be increased with different strategies such as 1. Using live CBAs instead of fixed cells as fixation methods may damage some epitopes (see **Table 1**), 2. Clustering of the antigen at high densitiy for example by co-transfecting clustering proteins such as rapsyn in the clustered AChR antibody assay (38) or 3. Increasing the number of recognizable antigens by adding further subunits of a receptor such as e.g., the gamma2 subunit of the GABA(A)R (39). The disadvantage of live CBAs is that they are technically demanding and time-consuming and their use is limited to specialized centers. The commercial CBAs are used by most clinical laboratories, however, not all antibodies can be tested with this method so far, either because the antibodies were only recently discovered and commercial CBAs may not be (readily) available, or the development of commercial assays is challenging due to methodological issues or the lack of sufficient numbers of positive controls. Another method that allows the screening for conformation-dependent antibodies is the unfixed/postfixed TBA. The TBA is a highly sensitive test method and can be used for initial screening and subsequent confirmation of positives by an antigen-specific CBA, may help to confirm the result of the CBA in case of doubtful results and is able to identify novel antibodies. A systematic comparison of the sensitivity and specificity of TBA and CBA was performed in a single-center study for anti-NMDAR antibodies and found an equal sensitivitiy for TBA and CBA (100%) in CSF, while in serum TBA was more sensitive (91.6%) than fixed CBA (86.8%) (40). Multicenter studies will be necessary to compare different assays for more target antigens and to evaluate assay reliability and reproducibility.

Search for Antigenic Targets in Autoimmune Neurological Diseases

Some patients with autoimmune neurological syndromes remain antibody negative despite some evidence that they are antibody-mediated. Unfixed/postfixed TBAs can detect most of the currently known surface antibodies involved in autoimmune encephalitis such as NMDAR, AMPAR, LGI1, CASPR2, GABA(B)R, GABA(A)R, mGluR1, mGluR5, DPPX, Tr/DNER, Neurexin3alpha, and IgLON5 (41). In addition, anti-glial antibodies such as AQP4 antibodies can be detected (42). One limitation may be that the unfixed TBA is based on rodent brain, and antibodies that recognize only human-specific epitopes may not be detected. This is the case in most patients with anti-myelin-oligodendrocyte-glycoprotein (MOG)-antibodies (43). Furthermore, some limitations in the detection by unfixed/postfixed TBA have been described for autoantibodies against the dopamine2 receptor (D2R), glycine receptor (GlyR), and P/Q-type VGCC that are only poorly visible with this technique (41) As a consequence, it is recommended to use specific CBAs (GlyR, MOG, D2R) (44–46) or a RIA (P/Q-type VGCC) (47) for the detection of these antibodies. A potential limitation may be that some antibodies require the use of live CBAs and fixation-dependent staining protocols are inappropriate to reveal a specific antigenic epitope (see **Table 2**).

Search for Antigenic Targets in Demyelinating Diseases

Anti-AQP4- and anti-MOG-antibodies are autoantibodies against glial cells that are associated with a specific spectrum of demyelinating diseases. Anti-AQP4-antibodies were the first antibodies with a clearly defined target that were identified in patients with demyelinating diseases (48) and now serve as biomarker for the diagnosis of patients with neuromyelitis optica spectrum disorders (NMOSD) (49). The incidence of AQP4-antibody positive NMO ranges from 0.05 to 0.4 per 100,000 (50). The antibodies were originally discovered by using indirect immunofluorescence on rodent brain tissue showing a characteristic staining pattern of astrocyte end feet around blood vessels, along the pial surfaces and Virchow-Robin spaces (48, 51). Meanwhile, the standard for most clinical laboratories for testing AQP4-antibodies is the use of CBAs either in form of commercially available fixed CBAs with the AQP4-M1 isoform or in-house live CBAs using the AQP4-M23 isoform. A large multicenter study systematically compared different AQP4 assays including CBAs, TBAs, flow cytometry, and ELISA and found the CBA as most sensitive and specific test method, with some benefit of using the AQP4-M23 isoform and additionally described high sensitivity and specificity for immunohistochemistry and flow cytometry in some specialized centers (52). Recently, the search for novel antibodies in demyelinating diseases by using monoclonal recombinant antibodies from patients with NMOSD revealed an anti-endothelial cell antibody against the endoplasmic reticulum chaperon GRP78 that may compromise the blood-brain barrier (53). Further studies will be necessary to clarify a potential role in initiating the inflammatory cascade and disease activity of NMOSD.

Anti-MOG antibodies were defined in patients with acute disseminated encephalomyelitis (ADEM), uni- or bilateral optic neuritis, transverse myelitis, longitudinally extensive transverse myelitis, and neuromyelitis optica. In children, one third of patients with an acute demyelinating syndrome are MOG-antibody positive (44, 54, 55). Most of the patient's antibodies recognize a human-specific epitope and TBAs based on rodent tissue are not suitable for their detection. Human MOG-antibodies were recently tested on human brain tissue and 88% of samples showed a staining of white matter (56), this approach could provide a promising screening tool in the search for novel antibodies. Currently anti-MOG-antibodies are either tested with commercial or in-house live CBAs employing HEK cells transfected with full-length human MOG (57–59). Further multicenter studies of different assays will be necessary to compare the sensitivity and specificity and identify difficulties in different test methods.

Search for Antigenic Targets in Paranodopathies—A Novel Subgroup of Autoimmune Peripheral Neuropathies

In patients with autoimmunity that primarily affects nervous tissue outside the CNS, the TBA can be expanded to the

respective target region. For example, in patients with peripheral neuropathy, screening on teased sciatic nerve fiber preparations from rodents can detect antibodies against proteins in the node of Ranvier (**Figures 2A–C**) (5, 60, 61). The node of Ranvier is a highly specialized structure that is important for the saltatory conduction of impulses in myelinated nerve fibers. A large number of adhesion molecules are involved in the formation of the axon-myelin junctions and compartmentalization of voltage-gated potassium channels and serve as potential target for autoimmunity (62). Autoimmune diseases associated with antibodies against proteins in the paranodal region of the node of Ranvier are subsumed as paranodopathies and define an exciting group of autoimmune peripheral neuropathies clinically presenting as atypical chronic inflammatory demyelinating polyneuropathy (CIDP) or Guillain-Barré-syndrome (GBS) that may benefit from treatment with rituximab (**Table 2**) (63). Based on results of teased nerve fiber screening, it is supposed that up to 40% of CIDP patients harbor antibodies against components of the myelin or the axon (64). Some antibodies such as anti-neurofascin155 or anti-contactin1 are detectable in teased nerve fibers and in the unfixed TBA (hippocampus and molecular layer of cerebellum) (6, 61, 65), while others may only be detectable in teased nerve fibers (5).

Significance of Primary Cell Cultures in Suspected Autoimmune Encephalitis

A complementary method to the screening of surface antibodies on tissue based assays are live cultures of neurons. These neurons can be used to identify a binding between an individual's antibody and a specific surface peptide on the intact neuronal membrane. A positive staining of the cells gives evidence that the detected autoantibody recognizes a surface antigen and is likely to play a pathogenic role in the disease (41). This method is used in research laboratories and may (1) help in the diagnostic procedure to differentiate between surface or intracellular reactivity in samples with doubtful results in the TBA and (2) can be used to identify the target antigen by performing immunoprecipitation of the patient's serum together with the cell culture and subsequently identify the co-precipitated target antigen by mass spectrometry. Rat hippocampal neurons are the most frequently used cell culture system for the visualization of anti-neuronal surface antibodies, however, not all neuronal surface proteins are expressed in these cells and the absence of binding should not necessarily imply the absence of surface reactivities. Moreover, anti-glial antibodies are not displayed. Alternatively, other neuronal or mixed glioneuronal cell cultures may be useful to demonstrate a neuronal or glial surface autoantibody. Anti-contactin1 and anti-CASPR1 antibodies were shown to label both rat hippocampal neurons and dorsal root ganglion cells (5, 66), in contrast, anti-AQP4-antibodies may only be detectable in glioneuronal cell cultures including rat retinal cell cultures that contain Mueller cells (**Figures 3A–O**) (67, 68). The screening of samples with suspected seronegative autoimmune encephalitis on different live cell cultures might enable to broaden the spectrum of identifyable antibodies and provide a promising approach for discovering novel autoantibodies against surface antigens.

SELECTION OF APPROPRIATE SAMPLE TYPES

A critical step to successfully detect anti-neuronal or anti-glial antibodies is the combined testing of serum and CSF in an individual patient. This has several reasons. First, the detectability of specific antibodies may differ between

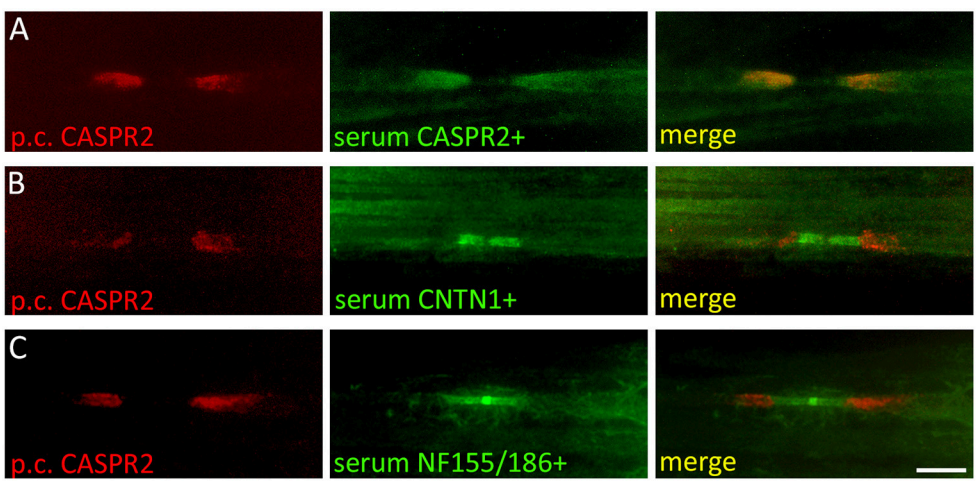


FIGURE 2 | Screening of autoantibodies on teased nerve fibers in patients with peripheral neuropathies. Rat sciatic nerve fibers were immunostained with a polyclonal rabbit anti-CASPR2 antibody (red) and serum from a patient with **(A)** anti-CASPR2 antibodies (green), **(B)** anti-contactin1 antibodies (green), and **(C)** anti-neurofascin155/186 antibodies (green). CASPR2 labels the juxtaparanodal region of the node of Ranvier, contactin1 the paranodal and neurofascin155/186 the paranodal and nodal region. CNTN1, contactin1; NF155/186, neurofascin155/186; Scale bar = 10 μm.

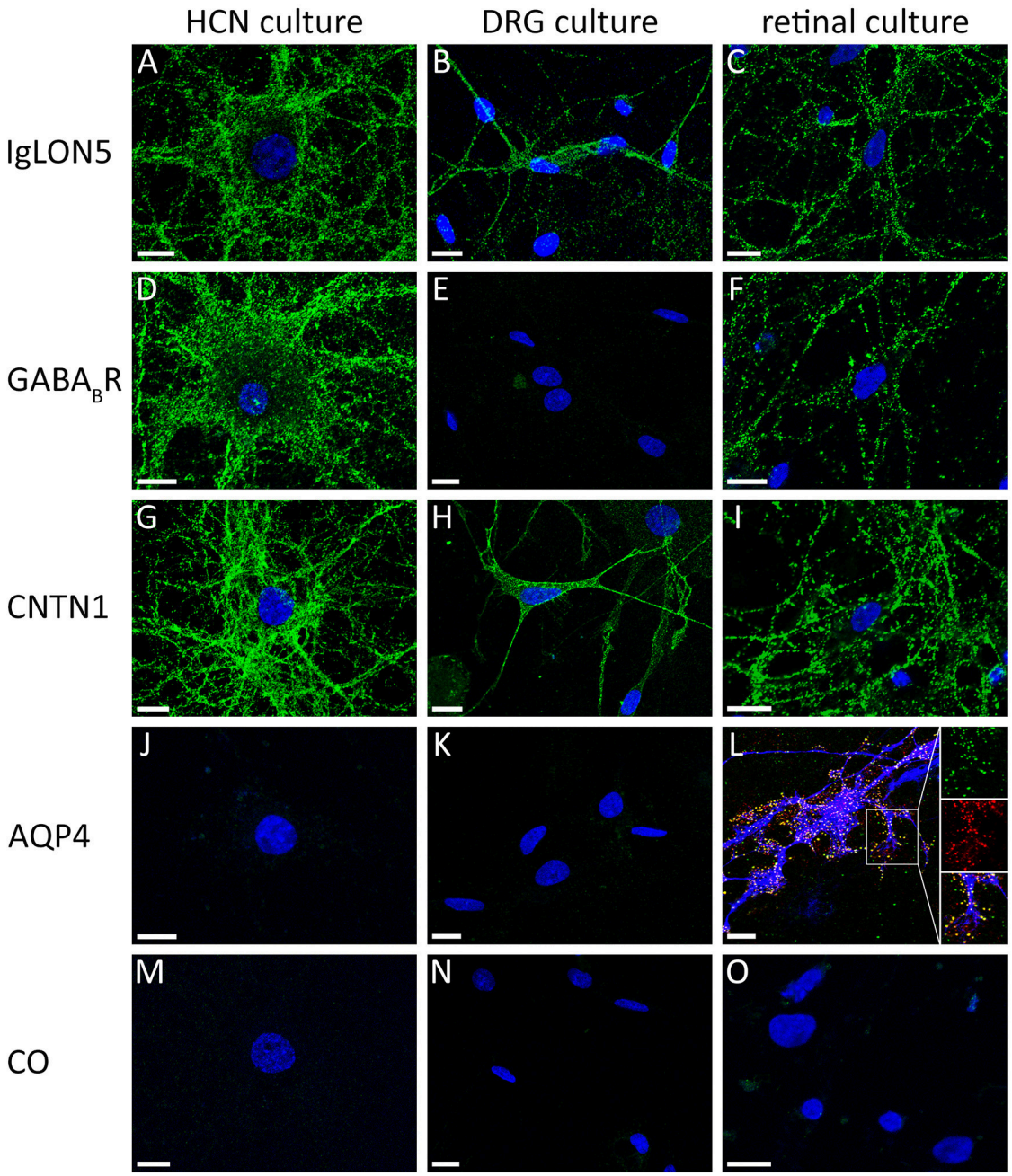


FIGURE 3 | Comparison of reactivity of different antibodies against cell surface antigens on different primary neuronal and glioneuronal cell cultures. **(A)** The serum of a patient with anti-IgLON5 antibodies shows an intensive labeling of live nonpermeabilized rat hippocampal neurons, **(B)** rat dorsal root ganglion cells (DRGs), and **(C)** dissociated rat retinal cell culture. In contrast, **(D)** the serum of a patient with anti-GABA(B)R antibodies labels hippocampal neurons but not **(E)** DRGs. **(F)** The retinal cell culture is strongly GABA(B)R positive. **(G–I)** A serum of a patient with anti-contactin1 antibodies labels all three types of cell cultures. A serum of a patient with **(J)** anti-aquaporin4 antibodies is negative on hippocampal neurons and **(K)** DRGs, but **(L)** labels the end feet membranes of GFAP-positive Müller cells (red: rabbit polyclonal anti-AQP4 antibody; green: serum of a patient with AQP4 antibodies; blue: mouse monoclonal anti-GFAP antibody). **(M–O)** A healthy control is negative. HCN, hippocampal neurons; DRG, dorsal root ganglion cells; retinal culture, dissociated rat retinal cell culture; CNTN1, contactin1; AQP4, aquaporin-4; CO, healthy control; Scale bar = 10 μm.

serum and CSF. Some antibodies may be easier identifyable in CSF than serum, for example antibodies against the NMDAR, GABA(B)R or AMPAR. In a study of 577 patients with anti-NMDAR encephalitis, in one out of 7 patients antibodies were only detectable in CSF and testing restricted to serum would have misdiagnosed the patients as seronegative

(69). Other autoimmunities may present with a substantial systemic autoantibody production such as patients with GAD65 antibodies, but they may additionally harbor antibodies against the GAD67 isoform in CSF and few cases were described with a restricted autoimmunity to GAD67. It will be important to collect more cases with exclusive GAD67 reactivity to see whether they present specific neurological features. Finally, some antibodies are more prevalent in serum than in CSF. These are for example anti-AQP4 or anti-MOG-antibodies (44). ADEM may be an important differential diagnosis for anti-neuronal autoimmune encephalitis and the testing for anti-MOG-antibodies only in CSF may lead to false negative results and delay in diagnosis.

Second, serum and CSF might harbor different sets of antibodies and in this constellation the antibodies in CSF may correlate better with the neurological symptoms than those in serum, as it has been shown in a study of patients with GABA(A)R antibodies (70).

Third, testing of serum and CSF may have methodological implications. It has been shown that testing of antibodies only in serum harbors the risk for increased background and unspecific cross-reactivity that may result in contradictory test interpretations (71, 72). To avoid misinterpretations or delay in diagnosis the testing of both serum and CSF is recommended (73).

TESTING OF THE SPECIFIC IMMUNOGLOBULIN ISOTYPES

Antibodies in human plasma belong to different isotypes according to their type of heavy chains and include IgG, IgA, IgM, IgE, and IgD. The IgG is the most abundant antibody isotype and can be classified into four subclasses IgG1, 2, 3, and 4. Pathogenic mechanisms in anti-neuronal autoimmune encephalitis were mainly associated with antibodies of the IgG isotype that can have different effects on the targeted antigen. The IgG1-3 subclasses may alter the synaptic structure by cross-linking and internalization of the receptor such as in anti-NMDAR (74) or anti-AMPAR encephalitis (75), serve as antagonist of baclofen in anti-GABA(B)R autoimmunity (41), or reduce the amount of receptor at the synapse such as in anti-GABA(A)R autoimmunity (70). In contrast, antibodies of the IgG4 subclass mainly seem to mechanically interfere between the receptor-ligand interaction resulting in the blockade of protein-protein interaction (76). Recently, antibodies of the IgA and IgM isotype against the NMDAR were found in up to 22% of patients with different neurological diseases and in healthy controls by using fixed CBAs and it was hypothesized that the symptomatic relevance of the antibodies is related to a compromised blood-brain barrier that allows access to the brain (77–81). Moreover, it was demonstrated that NMDAR antibodies regardless of the clinical presentation of the donor (healthy or ill) and immunoglobulin class could provoke receptor internalization in human-induced pluripotent stem cell-derived neurons and reduced the glutamate-evoked currents in NMDAR expressing Xenopus oocytes (79). However, the functional significance of IgA and IgM NMDAR antibodies and their ability to internalize the NMDAR could not be confirmed in a subsequent study using CBAs, unfixed TBAs, and immunostaining of live primary hippocampal neurons (82). Since robust association with anti-NMDAR encephalitis was only shown for IgG antibodies, the antibody testing in clinical practice should be focused on the IgG antibodies.

SUMMARY

The expanding field of antibody-mediated autoimmunity allows the identification of a vast range of neuronal and glial autoantibodies, which enables a more precise diagnosis of specific syndromes and disease subtypes. It is important to know that testing for onconeuronal antibodies requires other methods (line assays, fixed TBAs) than surface antibodies (CBAs and unfixed/postfixed TBAs). The highest sensitivity and specificity of a test result can be achieved by cross-validation with different test methods and the combined testing of serum and CSF samples. Test results should always be interpreted in context with the clinical presentation. In case of an unexpected positive or negative result, re-testing of the sample or performing confirmatory tests might be considered. The screening for surface antibodies on unfixed TBA can detect a large number of anti-neuronal and some anti-glial antibodies with some limitation for anti-GlyR, anti-D2R, and anti-MOG-antibodies. In patients with peripheral neuropathies, the screening can be expanded to teased nerve fibers to detect antibodies against proteins of the node of Ranvier. Moreover, the staining of primary cultures of neurons or glioneuronal cell cultures may give evidence that the detected autoantibody recognizes a surface antigen and enables the characterization of novel surface antibodies. The accurate and rapid detection of autoantibodies in CSF and serum may initiate immunotherapies to improve patients outcome.

AUTHOR CONTRIBUTIONS

GR and RH have access to all the data and take responsibility for the data, accuracy of the data analysis, design and conceptualization of the review. CS, DD, VP, JL, SG, SM, PR, PS, HK, and IK: analysis and interpretation of the data, drafting, and revising the manuscript for intellectual content.

ACKNOWLEDGMENTS

This work was partly supported by the Medical Scientific Fund of the Mayor of the City of Vienna, Project 15022, the Jubiläumsfonds der Österreichischen Nationalbank, Project 16919, and the Austrian Science Fund (FWF): I 3334-B27. IK was supported by an Erwin Schrödinger Fellowship by the Austrian Science Fund (FWF): J 3545-B13.

REFERENCES

1. Dalmau J, Graus F. Antibody-mediated encephalitis. *N Engl J Med.* (2018) 378:840–51. doi: 10.1056/NEJMra1708712
2. Bien CG, Vincent A, Barnett MH, Becker AJ, Blümcke I, Graus F, et al. Immunopathology of autoantibody-associated encephalitides: clues for pathogenesis. *Brain* (2012) 135(Pt 5):1622–38. doi: 10.1093/brain/aws082
3. Werner C, Pauli M, Doose S, Weishaupt A, Haselmann H, Grünewald B, et al. Human autoantibodies to amphiphysin induce defective presynaptic vesicle dynamics and composition. *Brain* (2016) 139(Pt 2):365–79. doi: 10.1093/brain/awv32
4. Graus F, Saiz A, Dalmau J. Antibodies and neuronal autoimmune disorders of the CNS. *J Neurol.* (2010) 257:509–17. doi: 10.1007/s00415-009-5431-9
5. Doppler K, Appeltshauser L, Villmann C, Martin C, Peles E, Krämer HH, et al. Auto-antibodies to contactin-associated protein 1 (Caspr) in two patients with painful inflammatory neuropathy. *Brain* (2016) 139(Pt 10):2617–30. doi: 10.1093/brain/aww189
6. Doppler K, Appeltshauser L, Wilhelmi K, Villmann C, Dib-Hajj SD, Waxman SG, et al. Destruction of paranodal architecture in inflammatory neuropathy with anti-contactin-1 autoantibodies. *J Neurol Neurosurg Psychiatry* (2015) 86:720–8. doi: 10.1136/jnnp-2014-309916
7. van Sonderen A, Thijs RD, Coenders EC, Jiskoot LC, Sanchez E, de Bruijn MA, et al. Anti-LGI1 encephalitis: clinical syndrome and long-term follow-up. *Neurology* (2016) 87:1449–56. doi: 10.1212/WNL.0000000000003173
8. Graus F, Cordon-Cardo C, Posner JB. Neuronal antinuclear antibody in sensory neuronopathy from lung cancer. *Neurology* (1985) 35:538–43. doi: 10.1212/WNL.35.4.538
9. Solimena M, Folli F, Aparisi R, Pozza G, De Camilli P. Autoantibodies to GABA-ergic neurons and pancreatic beta cells in stiff-man syndrome. *N Engl J Med.* (1990) 322:1555–60. doi: 10.1056/NEJM199005313222202
10. Rosenfeld MR, Eichen JG, Wade DF, Posner JB, Dalmau J. Molecular and clinical diversity in paraneoplastic immunity to Ma proteins. *Ann Neurol.* (2001) 50:339–48. doi: 10.1002/ana.1288
11. Pittock SJ, Lucchinetti CF, Lennon VA. Anti-neuronal nuclear autoantibody type 2: paraneoplastic accompaniments. *Ann Neurol.* (2003) 53:580–7. doi: 10.1002/ana.10518
12. Pittock SJ, Lucchinetti CF, Parisi JE, Benarroch EE, Mokri B, Stephan CL, et al. Amphiphysin autoimmunity: paraneoplastic accompaniments. *Ann Neurol.* (2005) 58:96–107. doi: 10.1002/ana.20529
13. Honnorat J, Antoine JC, Derrington E, Aguera M, Belin MF. Antibodies to a subpopulation of glial cells and a 66 kDa developmental protein in patients with paraneoplastic neurological syndromes. *J Neurol Neurosurg Psychiatry* (1996) 61:270–8. doi: 10.1136/jnnp.61.3.270
14. Peterson K, Rosenblum MK, Kotanides H, Posner JB. Paraneoplastic cerebellar degeneration. I. A clinical analysis of 55 anti-Yo antibody-positive patients. *Neurology* (1992) 42:1931–7. doi: 10.1212/WNL.42.10.1931
15. Graus F, Dalmau J, Valldeoriola F, Ferrer I, Rene R, Marin C, et al. Immunological characterization of a neuronal antibody (anti-Tr) associated with paraneoplastic cerebellar degeneration and Hodgkin's disease. *J Neuroimmunol.* (1997) 74:55–61. doi: 10.1016/S0165-5728(96)00205-6
16. Graus F, Delattre JY, Antoine JC, Dalmau J, Giometto B, Grisold W, et al. Recommended diagnostic criteria for paraneoplastic neurological syndromes. *J Neurol Neurosurg Psychiatry* (2004) 75:1135–40. doi: 10.1136/jnnp.2003.034447
17. Graus F, Dalmou J, Rene R, Tora M, Malats N, Verschuuren JJ, et al. Anti-Hu antibodies in patients with small-cell lung cancer: association with complete response to therapy and improved survival. *J Clin Oncol.* (1997) 15:2866–72. doi: 10.1200/JCO.1997.15.8.2866
18. Storstein A, Monstad SE, Haugen M, Mazengia K, Veltman D, Lohndal E, et al. Onconeural antibodies: improved detection and clinical correlations. *J Neuroimmunol.* (2011) 232:166–70. doi: 10.1016/j.jneuroim.2010.10.009
19. Sabater L, Saiz A, Dalmau J, Graus F. Pitfalls in the detection of CV2 (CRMP5) antibodies. *J Neuroimmunol.* (2016) 290:80–3. doi: 10.1016/j.jneuroim.2015.11.009
20. Hoftberger R, Kovacs GG, Sabater L, Nagy P, Racz G, Miquel R, et al. Protein kinase Cgamma antibodies and paraneoplastic cerebellar degeneration. *J Neuroimmunol.* (2013) 256:91–3. doi: 10.1016/j.jneuroim.2012.12.002
21. Hoftberger R, Sabater L, Velasco F, Ciordia R, Dalmau J, Graus F. Carbonic anhydrase-related protein VIII antibodies and paraneoplastic cerebellar degeneration. *Neuropathol Appl Neurobiol.* (2014) 40:650–3. doi: 10.1111/nan.12118
22. Doss S, Numann A, Ziegler A, Siebert E, Borowski K, Stöcker W, et al. Anti-Ca/anti-ARHGAP26 antibodies associated with cerebellar atrophy and cognitive decline. *J Neuroimmunol.* (2014) 267:102–4. doi: 10.1016/j.jneuroim.2013.10.010
23. Fang B, McKeon A, Hinson SR, Kryzer TJ, Pittock SJ, Aksamit AJ, et al. Autoimmune glial fibrillary acidic protein astrocytopathy: a novel meningoencephalomyelitis. *JAMA Neurol.* (2016) 73:1297–1307. doi: 10.1001/jamaneurol.2016.2549
24. Flanagan EP, Hinson SR, Lennon VA, Fang B, Aksamit AJ, Morris PP, et al. Glial fibrillary acidic protein immunoglobulin G as biomarker of autoimmune astrocytopathy: analysis of 102 patients. *Ann Neurol.* (2017) 81:298–309. doi: 10.1002/ana.24881
25. Saiz A, Blanco Y, Sabater L, Gonzalez F, Bataller L, Casamitjana R, et al. Spectrum of neurological syndromes associated with glutamic acid decarboxylase antibodies: diagnostic clues for this association. *Brain* (2008) 131(Pt 10):2553–63. doi: 10.1093/brain/awn183
26. Gresa-Arribas N, Arino H, Martinez-Hernandez E, Petit-Pedrol M, Sabater L, Saiz A, et al. Antibodies to inhibitory synaptic proteins in neurological syndromes associated with glutamic acid decarboxylase autoimmunity. *PLoS ONE* (2015) 10:e0121364. doi: 10.1371/journal.pone.0121364
27. Guasp M, Sola-Valls N, Martinez-Hernandez E, Gil MP, Gonzalez C, Brieva L, et al. Cerebellar ataxia and autoantibodies restricted to glutamic acid decarboxylase 67 (GAD67). *J Neuroimmunol.* (2016) 300:15–7. doi: 10.1016/j.jneuroim.2016.09.019
28. Lindstrom JM, Seybold ME, Lennon VA, Whittingham S, Duane DD. Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates, and diagnostic value. *Neurology* (1976) 26:1054–9. doi: 10.1212/WNL.26.11.1054
29. Vincent A, Lang B, Newsom-Davis J. Autoimmunity to the voltage-gated calcium channel underlies the Lambert-Eaton myasthenic syndrome, a paraneoplastic disorder. *Trends Neurosci.* (1989) 12:496–502. doi: 10.1016/0166-2236(89)90109-4
30. Lennon VA, Lambert EH. Autoantibodies bind solubilized calcium channel-omega-conotoxin complexes from small cell lung carcinoma: a diagnostic aid for Lambert-Eaton myasthenic syndrome. *Mayo Clin Proc.* (1989) 64:1498–504. doi: 10.1016/S0025-6196(12)65705-X
31. Vincent A, Roberts M, Willison H, Lang B, Newsom-Davis J. Autoantibodies, neurotoxins and the nervous system. *J Physiol Paris* (1995) 89:129–36. doi: 10.1016/0928-4257(96)80110-0
32. Parsons KT, Kwok WW. Linear B-cell epitopes in lambert-eaton myasthenic syndrome defined by cell-free synthetic peptide binding. *J Neuroimmunol.* (2002) 126:190–5. doi: 10.1016/S0165-5728(02)00063-2
33. Lang B, Makuch M, Moloney T, Dettmann I, Mindorf S, Probst C, et al. Intracellular and non-neuronal targets of voltage-gated potassium channel complex antibodies. *J Neurol Neurosurg Psychiatry* (2017) 88:353–61. doi: 10.1136/jnnp-2016-314758
34. van Sonderen A, Schreurs MW, de Bruijn MA, Boukhrissi S, Nagtzaam MM, Hulsenboom ES, et al. The relevance of VGKC positivity in the absence of LGI1 and Caspr2 antibodies. *Neurology* (2016) 86:1692–99. doi: 10.1212/WNL.0000000000002637
35. Besinger UA, Toyka KV, Homberg M, Heininger K, Hohlfeld R, Fateh-Moghadam A. Myasthenia gravis: long-term correlation of binding and bungarotoxin blocking antibodies against acetylcholine receptors with changes in disease severity. *Neurology* (1983) 33:1316–21. doi: 10.1212/WNL.33.10.1316
36. Saxena A, Stevens J, Cetin H, Koneczny I, Webster R, Lazaridis K, et al. Characterization of an anti-fetal AChR monoclonal antibody isolated from a myasthenia gravis patient. *Sci Rep.* (2017) 7:14426. doi: 10.1038/s41598-017-14350-8
37. Hara H, Hayashi K, Ohta K, Itoh N, Nishitani H, Ohta M. Detection and characterization of blocking-type anti-acetylcholine receptor antibodies in sera from patients with myasthenia gravis. *Clin Chem.* (1993) 39:2053–7.

38. Leite MI, Jacob S, Viegas S, Cossins J, Clover L, Morgan BP, et al. IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis. *Brain* (2008) 131(Pt 7):1940–52. doi: 10.1093/brain/awn092
39. Pettingill P, Kramer HB, Coebergh JA, Pettingill R, Maxwell S, Nibber A, et al. Antibodies to GABAA receptor alpha1 and gamma2 subunits: clinical and serologic characterization. *Neurology* (2015) 84:1233–41. doi: 10.1212/WNL.0000000000001326
40. Gresa-Arribas N, Titulaer MJ, Torrents A, Aguilar E, McCracken L, Leypoldt F, et al. Antibody titres at diagnosis and during follow-up of anti-NMDA receptor encephalitis: a retrospective study. *Lancet Neurol.* (2014) 13:167–77. doi: 10.1016/S1474-4422(13)70282-5
41. Dalmau J, Geis C, Graus F. Autoantibodies to synaptic receptors and neuronal cell surface proteins in autoimmune diseases of the central nervous system. *Physiol Rev.* (2017) 97:839–87. doi: 10.1152/physrev.00010.2016
42. Hoftberger R, Sabater L, Marignier R, Aboul-Enein F, Bernard-Valnet R, Rauschka H, et al. An optimized immunohistochemistry technique improves NMO-IgG detection: study comparison with cell-based assays. *PLoS ONE* (2013) 8:e79083. doi: 10.1371/journal.pone.0079083
43. Mayer MC, Breithaupt C, Reindl M, Schanda K, Rostasy K, Berger T, et al. Distinction and temporal stability of conformational epitopes on myelin oligodendrocyte glycoprotein recognized by patients with different inflammatory central nervous system diseases. *J Immunol.* (2013) 191:3594–604. doi: 10.4049/jimmunol.1301296
44. Reindl M, Di Pauli F, Rostasy K, Berger T. The spectrum of MOG autoantibody-associated demyelinating diseases. *Nat Rev Neurol.* (2013) 9:455–61. doi: 10.1038/nrneurol.2013.118
45. Dale RC, Merheb V, Pillai S, Wang D, Cantrill L, Murphy TK, et al. Antibodies to surface dopamine-2 receptor in autoimmune movement and psychiatric disorders. *Brain* (2012) 135(Pt 11):3453–68. doi: 10.1093/brain/aws256
46. Carvajal-Gonzalez A, Leite MI, Waters P, Woodhall M, Coutinho E, Balint B, et al. Glycine receptor antibodies in PERM and related syndromes: characteristics, clinical features and outcomes. *Brain* (2014) 137(Pt 8):2178–92. doi: 10.1093/brain/awu142
47. Motomura M, Lang B, Johnston I, Palace J, Vincent A, Newsom-Davis J. Incidence of serum anti-P/O-type and anti-N-type calcium channel autoantibodies in the Lambert-Eaton myasthenic syndrome. *J Neurol Sci.* (1997) 147:35–42. doi: 10.1016/S0022-510X(96)05303-8
48. Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* (2004) 364:2106–12. doi: 10.1016/S0140-6736(04)17551-X
49. Wingerchuk DM, Banwell B, Bennett JL, Cabre P, Carroll W, Chitnis T, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology* (2015) 85:177–89. doi: 10.1212/WNL.0000000000001729
50. Pandit L, Asgari N, Apiwattanakul M, Palace J, Paul F, Leite MI, et al. Demographic and clinical features of neuromyelitis optica: a review. *Mult Scler.* (2015) 21:845–53. doi: 10.1177/1352458515572406
51. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med.* (2005) 202:473–7. doi: 10.1084/jem.20050304
52. Waters P, Reindl M, Saiz A, Schanda K, Tuller F, Kral V, et al. Multicentre comparison of a diagnostic assay: aquaporin-4 antibodies in neuromyelitis optica. *J Neurol Neurosurg Psychiatry* (2016) 87:1005–15. doi: 10.1136/jnnp-2015-312601
53. Shimizu F, Schaller KL, Owens GP, Cotleur AC, Kellner D, Takeshita Y, et al. Glucose-regulated protein 78 autoantibody associates with blood-brain barrier disruption in neuromyelitis optica. *Sci Transl Med.* (2017) 9:eaai9111. doi: 10.1126/scitranslmed.aai9111
54. Baumann M, Sahin K, Lechner C, Hennes EM, Schanda K, Mader S, et al. Clinical and neuroradiological differences of paediatric acute disseminating encephalomyelitis with and without antibodies to the myelin oligodendrocyte glycoprotein. *J Neurol Neurosurg Psychiatry* (2015) 86:265–72. doi: 10.1136/jnnp-2014-308346
55. Jurynczyk M, Messina S, Woodhall MR, Raza N, Everett R, Roca-Fernandez A, et al. Clinical presentation and prognosis in MOG-antibody disease: a UK study. *Brain* (2017) 140:3128–38. doi: 10.1093/brain/awx276
56. Peschl P, Schanda K, Zeka B, Given K, Bohm D, Ruprecht K, et al. Human antibodies against the myelin oligodendrocyte glycoprotein can cause complement-dependent demyelination. *J Neuroinflamm.* (2017) 14:208. doi: 10.1186/s12974-017-0984-5
57. Mader S, Gredler V, Schanda K, Rostasy K, Dujimovic I, Pfaller K, et al. Complement activating antibodies to myelin oligodendrocyte glycoprotein in neuromyelitis optica and related disorders. *J Neuroinflamm.* (2011) 8:184. doi: 10.1186/1742-2094-8-184
58. Jarius S, Ruprecht K, Kleiter I, Borisow N, Asgari N, Pitarokoili K, et al. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 1: frequency, syndrome specificity, influence of disease activity, long-term course, association with AQP4-IgG, and origin. *J Neuroinflamm.* (2016) 13:279. doi: 10.1186/s12974-016-0717-1
59. Jarius S, Ruprecht K, Stellmann JP, Huss A, Ayzenberg I, Willing A, et al. MOG-IgG in primary and secondary chronic progressive multiple sclerosis: a multicenter study of 200 patients and review of the literature. *J Neuroinflamm.* (2018) 15:88. doi: 10.1186/s12974-018-1108-6
60. Querol L, Nogales-Gadea G, Rojas-Garcia R, Martinez-Hernandez E, Diaz-Manera J, Suarez-Calvet X, et al. Antibodies to contactin-1 in chronic inflammatory demyelinating polyneuropathy. *Ann Neurol.* (2013) 73:370–80. doi: 10.1002/ana.23794
61. Devaux JJ, Miura Y, Fukami Y, Inoue T, Manso C, Belghazi M, et al. Neurofascin-155 IgG4 in chronic inflammatory demyelinating polyneuropathy. *Neurology* (2016) 86:800–7. doi: 10.1212/WNL.0000000000002418
62. Stathopoulos P, Alexopoulos H, Dalakas MC. Autoimmune antigenic targets at the node of Ranvier in demyelinating disorders. *Nat Rev Neurol.* (2015) 11:143–56. doi: 10.1038/nrneurol.2014.260
63. Querol L, Rojas-Garcia R, Diaz-Manera J, Barcena J, Pardo J, Ortega-Moreno A, et al. Rituximab in treatment-resistant CIDP with antibodies against paranodal proteins. *Neurol Neuroimmunol Neuroinflamm.* (2015) 2:e149. doi: 10.1212/NXI.0000000000000149
64. Querol L, Devaux J, Rojas-Garcia R, Illa I. Autoantibodies in chronic inflammatory neuropathies: diagnostic and therapeutic implications. *Nat Rev Neurol.* (2017) 13:533–47. doi: 10.1038/nrneurol.2017.84
65. Querol L, Nogales-Gadea G, Rojas-Garcia R, Diaz-Manera J, Pardo J, Ortega-Moreno A, et al. Neurofascin IgG4 antibodies in CIDP associate with disabling tremor and poor response to IVIg. *Neurology* (2014) 82:879–86. doi: 10.1212/WNL.0000000000000205
66. Miura Y, Devaux JJ, Fukami Y, Manso C, Belghazi M, Wong AH, et al. Contactin 1 IgG4 associates to chronic inflammatory demyelinating polyneuropathy with sensory ataxia. *Brain* (2015) 138(Pt 6):1484–91. doi: 10.1093/brain/awv054
67. Nagelhus EA, Veruki ML, Torp R, Haug FM, Laake JH, Nielsen S, et al. Aquaporin-4 water channel protein in the rat retina and optic nerve: polarized expression in Muller cells and fibrous astrocytes. *J Neurosci.* (1998) 18:2506–19. doi: 10.1523/JNEUROSCI.18-07-02506.1998
68. Zeka B, Hastermann M, Kaufmann N, Schanda K, Pende M, Misu T, et al. Aquaporin 4-specific T cells and NMO-IgG cause primary retinal damage in experimental NMO/SD. *Acta Neuropathol Commun.* (2016) 4:82. doi: 10.1186/s40478-016-0355-y
69. Titulaer MJ, McCracken L, Gabilondo I, Armangue T, Glaser C, Iizuka T, et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. *Lancet Neurol.* (2013) 12:157–65. doi: 10.1016/S1474-4422(12)70310-1
70. Petit-Pedrol M, Armangue T, Peng X, Bataller L, Cellucci T, Davis R, et al. Encephalitis with refractory seizures, status epilepticus, and antibodies to the GABAA receptor: a case series, characterisation of the antigen, and analysis of the effects of antibodies. *Lancet Neurol.* (2014) 13:276–86. doi: 10.1016/S1474-4422(13)70299-0
71. Armangue T, Santamaria J, Dalmau J. When a serum test overrides the clinical assessment. *Neurology* (2015) 84:1379–81. doi: 10.1212/WNL.0000000000001425
72. Zandi MS, Paterson RW, Ellul MA, Jacobson L, Al-Diwani A, Jones JI, et al. Clinical relevance of serum antibodies to extracellular N-methyl-D-aspartate receptor epitopes. *J Neurol Neurosurg Psychiatry* (2015) 86:708–13. doi: 10.1136/jnnp-2014-308736
73. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol.* (2016) 15:391–404. doi: 10.1016/S1474-4422(15)00401-9

74. Hughes EG, Peng X, Gleichman AJ, Lai M, Zhou L, Tsou R, et al. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *J Neurosci.* (2010) 30:5866–75. doi: 10.1523/JNEUROSCI.0167-10.2010
75. Peng X, Hughes EG, Moscato EH, Parsons TD, Dalmau J, Balice-Gordon RJ. Cellular plasticity induced by anti-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor encephalitis antibodies. *Ann Neurol.* (2015) 77:381–98. doi: 10.1002/ana.24293
76. Koneczny I. A new classification system for IgG4 autoantibodies. *Front Immunol.* (2018) 9:97. doi: 10.3389/fimmu.2018.00097
77. Dahm L, Ott C, Steiner J, Stepniak B, Teegen B, Saschenbrecker S, et al. Seroprevalence of autoantibodies against brain antigens in health and disease. *Ann Neurol.* (2014) 76:82–94. doi: 10.1002/ana.24189
78. Hammer C, Stepniak B, Schneider A, Papiol S, Tantra M, Begemann M, et al. Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood-brain barrier integrity. *Mol Psychiatry* (2014) 19:1143–9. doi: 10.1038/mp.2013.110
79. Castillo-Gomez E, Oliveira B, Tapken D, Bertrand S, Klein-Schmidt C, Pan H, et al. All naturally occurring autoantibodies against the NMDA receptor subunit NR1 have pathogenic potential irrespective of epitope and immunoglobulin class. *Mol Psychiatry* (2017) 22:1776–84. doi: 10.1038/mp.2016.125
80. Zerche M, Weissenborn K, Ott C, Dere E, Asif AR, Worthmann H, et al. Preexisting serum autoantibodies against the NMDAR subunit NR1 modulate evolution of lesion size in acute ischemic stroke. *Stroke* (2015) 46:1180–6. doi: 10.1161/STROKEAHA.114.008323
81. Doss S, Wandinger KP, Hyman BT, Panzer JA, Synofzik M, Dickerson B, et al. High prevalence of NMDA receptor IgA/IgM antibodies in different dementia types. *Ann Clin Transl Neurol.* (2014) 1:822–32. doi: 10.1002/acn3.120
82. Hara M, Martinez-Hernandez E, Arino H, Armangue T, Spatola M, Petit-Pedrol M, et al. Clinical and pathogenic significance of IgG, IgA, and IgM antibodies against the NMDA receptor. *Neurology* (2018) 90:e1386–94. doi: 10.1212/WNL.0000000000005329

Conflict of Interest Statement: RH received speaker honoraria from Euroimmun. The Medical University of Vienna receives payments for antibody assays and antibody validation assays (aquaporin-4 and other anti-neuronal and anti-glial antibodies) organized by Euroimmun (Lübeck, Germany).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

MINI REVIEW
published: 05 September 2018
doi: 10.3389/fneur.2018.00706

Systematic Review: Syndromes, Early Diagnosis, and Treatment in Autoimmune Encephalitis

*Christina Hermetter, Franz Fazekas and Sonja Hochmeister**

Department of Neurology, Medical University of Graz, Graz, Austria

In recent years, new antibodies have been discovered which mediate autoimmune encephalitis. This immunological response can be triggered by an infection or a tumor. Classical onconeuronal antibodies are directed against intracellular neuronal agents but recently, a novel group of antibodies to neuronal cell-surface and synaptic antigens associated with different CNS-syndromes, has been discovered. Interestingly, the syndromes in this group can be successfully treated with immunotherapy and frequently do not have underlying tumors. The aim of this review is to describe the current state of knowledge about autoimmune encephalitis, in order to provide clinicians with a concise, up-to-date overview. Thus, a comprehensive literature search was performed in medical databases. The literature was carefully studied and new findings focusing on the symptoms, diagnosis and treatment were summarized and interpreted. Even though it might be challenging in some cases, the awareness of certain symptom constellations and demographic information, in combination with laboratory- and MRI-results, allows clinicians to make the diagnosis of probable autoimmune encephalitis at an early stage. Treatment can therefore be initiated faster, which significantly improves the outcome. Further investigations could define the underlying pathogenic mechanisms. Randomized controlled trials, paired with increasing clinical experience, will be necessary to improve the identification of affected patients, treatment strategies, and outcomes in the years to come.

Keywords: autoimmune encephalitis, antibodies, surface antigens, clinical relevance, treatment

OPEN ACCESS

Edited by:
Johann Sellner,
Universitätsklinikum Salzburg, Austria

Reviewed by:
David Andrew Fulcher,
Australian National University, Australia
Eoin Flanagan,
Mayo Clinic, United States

****Correspondence:***
Sonja Hochmeister
sonja.hochmeister@medunigraz.at

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *31 March 2018*
Accepted: *03 August 2018*
Published: *05 September 2018*

Citation:
Hermetter C, Fazekas F and Hochmeister S (2018) Systematic Review: Syndromes, Early Diagnosis, and Treatment in Autoimmune Encephalitis. Front. Neurol. 9:706. doi: 10.3389/fneur.2018.00706

INTRODUCTION

Autoimmune encephalitis (AIE) may be associated with the presence of specific autoantibodies. In cases in which an autoantibody is detected in the CSF or serum, AIE can be divided into two groups, depending on the localization of the target antigen. In addition to the "well"-defined classical paraneoplastic syndromes with antibodies which target intracellular proteins (e.g., anti-Hu, anti-Yo, anti-Ri), a new group of antibodies associated with AIE and their correlating symptoms, have been defined. They interact directly with cell-surface neuronal receptors or synaptic proteins (1, 2). In the case of classical, paraneoplastic syndromes, the disease is triggered by an anti-tumor immune reaction and is considered to result primarily from a CD8+T-cell

response with the antibodies being likely to arise secondarily to the cellular T-cell-driven damage directed at the intracellular molecules. The new group of antibodies against surface antigens, seems to be directly pathogenic and may change their target's structure or function, with resulting consequences on its behavior or tissue destruction, by receptor internalization or blockage, redistribution from the synaptic to the extra synaptic site, or interference with the ligand-receptor interaction. But the underlying causes for the pathogenic pathways leading to the antibodies accessing the CNS and the associated immune response are, as yet, poorly understood. The role of T-cells has not yet been fully established in detail (3, 4). Different mechanisms have been proposed, one including various infectious triggers which "prime" the immune system by activating T or/and B cells against similar epitopes by way of molecular mimicry, as in the case of Herpes simplex virus-encephalitis associated with NMDA-R-encephalitis (5, 6). Unlike in classical paraneoplastic syndromes, in cell-surface or synaptic antibody-syndromes the presence of a tumor is variable, they respond to multimodal immunotherapy and seem to have a better overall prognosis (7, 8). Awareness and knowledge is emerging rapidly through clinicians, due to a large number of case reports, as well as the performance of retrospective data analysis. However, in many cases, the diagnosis and the treatment remain challenging.

In this review we therefore focus on the clinical perspective of the symptoms, essential aspects of an early diagnosis and differential diagnosis, as well as the treatment options in adults.

CHARACTERISTIC CLINICAL SYNDROMES

Initially, the clinical features of different types of AIE may overlap. The symptoms include epileptic seizures, movement disorders, psychiatric, and cognitive alterations (9).

AIE Associated With Antibodies Against Neuronal Cell Surface Antigens

NMDA-R Encephalitis Prototype

To date, the best recognized subtype is N-methyl D-aspartate-receptor (NMDA-R) encephalitis. About 80% of the patients are young and female (median 20 years). Typically, symptoms emerge in stages. Patients usually develop virus-like prodromal symptoms, with headaches, lethargy and fever, followed by progressive behavioral changes, memory deficits, confusion, and psychosis within 2 weeks.

This progresses to language problems, epileptic seizures, a range of movement disorders and eventually, global encephalopathy, and dysregulation of autonomic functions may occur, with severe complications such as hyperthermia, cardiac arrhythmias, blood pressure instability, or coma (due to decreased NMDA-R influence in the brainstem), requiring intensive care unit management (2, 10). tumors can be found in one third of the patients. Women of reproductive age are mainly affected due to an ovarian teratoma while, in the elderly, it is more often a carcinoma. Other tumors that have been described are rare and include neuroblastoma, Hodgkin lymphoma, tumors of the breast, thymus and lung (10). Most of the patients require long-term hospitalization and subsequent rehabilitation. Depending on the early diagnosis, beginning of full treatment and time to tumor removal, full recovery usually takes up to 18 months. One group reported 75% with a modified ranking scale from 1 to 2, in a cohort of 360 patients, in which symptoms like memory or language deficits seemed to be the last to recover (10).

The limbic system is a predilection in autoimmune encephalitis and is the most consistently affected structure. Target proteins associated with classical limbic encephalitis are AMPA, GABAb, LGI1, and GAD. Additional clinical findings may allow further differentiation between these different types (11, 12).

Anti-AMPA-R (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor) encephalitis typically progresses rapidly but in some cases, only psychiatric symptoms are present. Patients have a high risk of underlying tumors (lung, breast or thymus) (10). Immunotherapy is often successful initially but relapses occur frequently (1).

Patients with **anti-GABAb** encephalitis have a high association with neoplasms, including small-cell lung cancer or neuroendocrine tumors (11). Additional characteristic features are early and frequent prominent seizures or status epilepticus. Some patients might also exhibit ataxia and opsoclonus-myoclonus-syndrome. The syndrome usually responds well to immunotherapy (1, 10).

In addition to the symptoms of classical, limbic encephalitis, common features of leucine-rich glioma-inactivated protein 1(**LGI-1**) encephalitis are hypernatremia and rapid-eye-movement sleep disturbance. Prior to the encephalitis syndrome, patients frequently have highly repetitive, unilateral faciobrachial dystonic seizures (FBDS). The seizures are often refractory to anticonvulsive treatment but improve with immunotherapy. In comparison to NMDA-R-encephalitis, patients with LGI1-encephalitis usually seem to respond faster at the beginning of immunotherapy, although the long-term outcome tends to be less favorable. tumors known to be associated with LGI1-encephalitis are bronchial carcinoma and thymoma (1, 7, 10).

Like GABAb, the **GABAa**-antibody type has a high risk of severe seizures or often intractable status epilepticus, requiring pharmacologically induced coma (8, 10). The MRI often shows hyperintense lesions outside the limbic system, in contrast to all other forms (11).

Antibodies directed against **Glycine receptor** (Gly-R) and **DPPX** (dipeptidyl-peptidase-like protein-6) have been described in patients with brainstem and spinal cord hyperexcitability disorders, such as PERM-syndrome (progressive encephalomyelitis with rigidity and myoclonus). Gly-R antibodies were also found in a few cases of stiff-person-syndrome. Prodromal diarrhea with substantial weight loss is commonly reported in the DPPX- group (1). DPPX is also expressed in the myenteric plexus (13).

It is important to differentiate anti-contactin-associated protein-2 (**CASPR2**) encephalitis from motor neuron diseases. In rare cases, it is also associated with limbic encephalitis. It is more commonly associated with Morvan syndrome, a rare disease combining peripheral nerve hyperexcitability, neuromyotonia, autonomic disturbance and sometimes encephalopathy. Neuromyotonia is often associated with painful peripheral neuropathy but bulbar weakness can also occur. The same associated tumor entities have been described as for the LGI1-type (7, 10, 13).

Antibodies against metabotropic glutamate receptor 5 (**mGLUR5**) have been found in patients with Hodgkin lymphoma and Ophelia syndrome (limbic encephalitis with predominate memory deficits) and antibodies against mGLUR1, in cerebellar ataxia. Immunotherapy is often successful and full recoveries are achieved (1, 13).

The **Adenylate-kinase 5** antibody syndrome usually presents with isolated, severe short-term memory loss. There is no association with cancer but the response to immunotherapy is poor (14).

AIE Associated With Antibodies Against Intracellular Antigens

This group includes the classical onconeuronal antibodies (e.g., anti-Hu, Ri, Yo, Ma2/Ta and Amphiphysin) with their well-characterized syndromes, which will not be discussed any further here (for a comprehensive overview we refer to excellent review articles e.g., *Paraneoplastic neurological syndromes and autoimmune encephalitis* Stich and Rauer, 2014) and the glutamic acid decarboxylase 65 (GAD) antibodies.

High antibody titres against **GAD65**, a non-paraneoplastic intracellular antigen, are associated with different neurological symptoms including limbic encephalitis, seizures and cerebellar ataxia. They are also common in stiff-person-syndrome. There is usually no underlying tumor. Low titres of GAD antibodies can also occur in healthy and in up to 80% of type 1 diabetes mellitus patients (14).

Some of the neuronal antibodies are associated with concurrent thyroid antibodies. Thyroid antibodies are not specific and can also be present in 13% of healthy individuals. SREAT (Steroid-responsive encephalopathy with autoimmune thyroiditis) might be a differential diagnosis at disease onset, since the symptoms can be similar, but it can ultimately be excluded by the detection of neuronal surface antibodies (14).

Acute disseminated encephalomyelitis (ADEM) could be associated with neuronal antibodies and is an important differential diagnosis, as clinical and/or MRI findings can overlap. In an epidemiological study of the prevalence of autoimmune encephalitides, it was shown that MOG antibodies were among the neuronal autoantibodies with high specificity and were one of the most commonly detected (15). MRI can help to further differentiate ADEM from other autoimmune encephalitis particularly in the follow up, as ADEM should show no new clinical or MRI findings 3 months after the onset of symptoms (see *Diagnostic criteria for ADEM* Graus et al, 2017). In addition, cases of autoimmune encephalitis combined with demyelinating disorders have been reported many times. Therefore, patients with atypical features like optic neuritis or demyelination in MRI or otherwise prominent neuropsychiatric symptoms, should be tested for concurrent disorders (AQP4- and MOG antibodies plus NMDA-R antibodies) (14, 16, 17).

Other antibodies associated with encephalitis are listed in **Table 1**, for the purpose of completeness.

DIAGNOSIS

The clinical diagnosis of AIE can be challenging. Initially, the symptoms of different types of AIE can overlap. Occasionally, combinations with headaches, mild hyperthermia, and frequent CSF pleocytosis, can also mislead to empiric antiviral or antibiotic treatment until the results for infectious encephalitis are completed, or the diagnosis is delayed by the resemblance to psychiatric illnesses (1, 8). Psychiatric disorders are generally the most common symptoms at AIE onset (19). Their symptoms are multiple and nonspecific; psychotic symptoms, hallucinations, paranoid thoughts, catatonia, behavioral and mood disorders can be present and can change during the course of the disease. Physicians in general need to be aware of this and initiate accurate diagnosis and treatment early on. Also, the benefits of psychotropic drug treatment are very limited in cases of AIE. Some medications, especially first-generation antipsychotics, might be even harmful (20).

A careful history taking may be helpful, as prodromal symptoms often occur (1, 8).

The following symptoms suggests a "probable" autoimmune encephalitis before antibody detection: A combination of characteristic clinical features in most cases but with different severity or dominance, together with additional information and specific findings such as age and gender, specific movement disorders (e.g., facial-brachial dystonic seizures), accompanied comorbidities (hyponatremia, diarrhea, and work-up or history for tumor), neuroimaging findings or EEG patterns, good empiric treatment response and no reasonable alternative diagnosis (1, 11). Antibody detection is unlikely to be an early diagnostic criterion because results take several days at least and are not available at disease onset. It is a confirmatory diagnostic test, however, the test can also be negative in up to 50% of autoimmune encephalitis series (14, 17).

Possible complications include coma, hyperkinesia (injuries, ventilation problems), autonomic dysfunction and prolonged need for artificial respiration and intensive care treatment (2).

The most important differential diagnosis to rule out is infectious encephalitis. If the clinical suspicion is high, an initially negative PCR should be retested (e.g., HSV PCR can be negative when tested within 24 h of onset) (14). Other differential diagnoses include metabolic or endocrine encephalopathies, psychiatric disorders, malignant neuroleptic syndrome, and rheumatic diseases (Sjögren-Syndrome or Lupus) (2).

The following diagnostic criteria have been reviewed and updated by a panel of experts in autoimmune encephalitis and should guide clinicians in making an early diagnosis, which is not dependent on the autoantibody status. In order to further classify the subtype with comorbidities, the malignancy association, and prognosis of autoantibodies remain crucial (14).

TABLE 1 | Antibodies related to autoimmune encephalitis [onconeuronal antibodies are excluded; summarized from (7, 14, 18)].

Antibody	Syndromes	MRI: T2/Flair Sequences	Tumor	F/M	Age (Median)
NMDA-R	Prodromal stage, global encephalopathy	Normal or transient non-region specific changes (∼33%)	10–50%, (age dependent) ovarian teratoma	4:1	1–85 (21)
LGI1	Faciobrachial dystonic seizures, limbic encephalitis, hyponatremia, sleep disorders, myoclonia	Hyperintense signal in medial temporal lobes and basal ganglia (>80%)	<10–20% Bronchial carcinoma, thymoma	1:2	30–80 (60)
AMPA-R	Limbic encephalitis (predominant psychosis), seizures	Hyperintense signal in medial temporal lobes (90%)	70% Bronchial- or Mamma carcinoma, Thymoma	9:1	38–87 (60)
GABAb-R	Limbic encephalitis, seizures	Hyperintense signal in medial temporal lobes (>60%)	60% Bronchial carcinoma, neuroendocrine tumors	1:1	24–75 (62)
CASPR2	Morvan syndrome, neuromyotonia, polyneuropathy, bulbar weakness, limbic encephalitis	Normal or Hyperintense signal in medial temporal lobes (∼40%)	<20–40% bronchial carcinoma, thymoma	1:4	46–77 (60)
Glycine-R	PERM, Myelopathy, Stiff person syndrome	Normal or nonspecific changes (∼10%)	∼10% Lymphoma, thymoma	6:5	5–69 (43)
mGLUR5	Ophelia syndrome	Normal or hyperintense signal in various brain regions (∼50%)	Hodgkin lymphoma	1:2	35
GAD*	Stiff person syndrome, limbic encephalitis, seizures, cerebellar ataxia	n/k	25% Thymoma, small cell lung carcinoma	n/k	n/k
GABAa-R	Encephalitis with refractory seizures	Hyperintense signal in multiple cortical and subcortical regions	25% Thymoma	n/k	n/k
DPPX	Encephalitis, diarrhea, hyperplexia	Normal or nonspecific changes	<10% Lymphoma	n/k	n/k
Dopamine-2-R	Basal ganglia encephalitis with abnormal movements, gait disturbance	Hyperintense signal in basal ganglia	n/k	1:1	2–15 (6)
Neurexin-3 α	Encephalitis	Normal	n/k	n/k	n/k
IgLON5	NREM and REM sleep disorder, brain stem dysfunction	Normal	n/k	n/k	n/k
mGLUR1	Cerebellar ataxia	Normal or cerebellar atrophy	A few cases described, Hodgkin disease	n/k	n/k
nACH-R	Encephalitis, postural tachycardia syndrome, Chronic intestinal pseudo-obstruction	Not applicable	30% thymoma, mamma/bladder/rectum/bronchial carcinoma, lymphoma	2:1	20–76 (58)
MOG	Acute disseminated encephalomyelitis	Diffuse, poorly demarcated, large (>1–2 cm) lesions predominantly in the white matter	0%	n/k	n/k
Adenylate-kinase 5	Isolated severe short-term memory loss, no seizures	Not applicable	No association	n/k	n/k

**Intracellular target, all other antibodies listed have cell-surface/synaptic targets. Abb.:n/K not known.*

Diagnostic criteria for possible autoimmune encephalitis

1. subacute onset (usually within a few weeks but less than 3 months) with change in personality or level of consciousness and symptoms suggesting involvement of the limbic system including working memory deficits, psychiatric symptoms and seizures.
2. At least one of the following:
   - A new focal clinical CNS event
   - EEG with epileptic or slow-wave activity
   - CSF pleocytosis
   - MRI findings suggestive of encephalitis*
3. Reasonable exclusion of alternative causes**.

**Hyperintense signal on T2-weighted/FLAIR highly restricted to one or both medial temporal lobes or in multifocal areas involving gray or white matter compatible with demyelination or inflammation (see below), **CNS infections, septic encephalopathy, metabolic encephalopathy, drug toxicity, cerebrovascular disease, neoplastic disorders, Creutzfeldt-Jakob disease, epileptic disorders, rheumatologic disorders, mitochondrial diseases [summarized from (14, 17, 21)].*

Diagnostic criteria for definite autoimmune encephalitis

1. Subacute onset (usually within a few weeks but less than 3 months) with change in personality or level of consciousness and symptoms suggesting involvement of the limbic system including working memory deficits, psychiatric symptoms and seizures.
2. At least one of the following:
   - EEG with epileptic or slow-wave activity
   - CSF pleocytosis.
3. Typical MRI findings: Bilateral hyperintensities on T2-weighted/FLAIR sequence highly restricted to the medial temporal lobes.
4. Reasonable exclusion of alternative causes.

If all of the above criteria match, the definitive diagnosis can be made (14).

Diagnostic criteria for autoantibody-negative possible autoimmune encephalitis

1. Rapid progression (less than 3 months) of working memory deficits, psychiatric symptoms, altered mental status.
2. At least two of the following:
   - MRI findings suggestive of encephalitis
   - Brain biopsy showing inflammatory infiltrates and excludes other disorders
   - CSF pleocytosis, CSF-specific oligoclonal bands and/or elevated CSF IgG Index.
3. Exclusion of well-defined syndromes of autoimmune encephalitis (e.g., ADEM, Bickerstaff's encephalitis).
4. Reasonable exclusion of alternative causes.

If all of the above criteria match, the definitive diagnosis can be made (14).

Obligatory Diagnostic Tools

At onset of the symptoms, CSF and serum analysis show a mild to moderate lymphocytic pleocytosis (<100 cells/μl) in 60–80% of patients. One third of patients show mild to moderate increased protein concentration, and 50% of patients show oligoclonal bands (1, 14). However, unremarkable CSF findings do not exclude the diagnosis (22).

The sensitivity of antibody testing has only been investigated in a few types of AIE, primarily in NMDA-R-encephalitis. Different studies have shown that patients with NMDA-R encephalitis had no detectable antibodies in serum in 14% of cases, but they all had them in CSF. The situation seems to be similar for other autoantibodies. LGI1-encephalitis is exceptional and often shows normal CSF findings (1, 6, 14).

Therefore, both serum AND CSF should always be tested for antibodies.

Antibody titres may correlate with clinical severity, but determining the clinical relevance of an antibody based on the titre is not recommended. The clinical picture and additional comorbidities are more reliable for evaluating a treatment response, course and prognosis (23).

The IgG-antibody subtype is classified as being pathogenic in most of the established syndromes. IgA and IgM-antibodies have unclear significance and have also been described in many other psychiatric disorders and in healthy controls (8).

In summary, antibody testing can never replace clinical judgement; the finding of antibodies *only* in serum or non-IgG-isotypes together with an atypical clinical picture for the identified antibody, should be interpreted cautiously (21).

Clinicians need to be aware of the pitfalls in antibody testing, like those mentioned above in respect of GAD antibodies and thyroid antibodies. Another example are the voltage-gated potassium channel ("VGKC") antibodies. VGKC complex antibodies were the first surface receptor antibodies associated with AIE to be detected. Recent studies have differentiated LGI1 and Caspr2 antibodies related to VGKC complex antibodies. They are associated with a limited subset of syndromes, whereas other VGKC antibodies have an unknown specificity and might occur in any type of cell damage. They are also described in patients with non-autoimmune, pre-existing conditions. One group investigated the clinical relevance of VGKC positivity in patients without LG1 and Caspr2 antibodies in comparison with VGKC negatives, according to clinical criteria, in order to determine evidence for autoimmune inflammation in both groups. When antibodies to LG1/Caspr2 were lacking, there were no differences between the groups, implying that VGKC positivity is not a clear marker for autoimmune-mediated pathogenesis and does not contribute to diagnosis in clinical practice (4, 24).

MRI is frequently normal or shows only slight alterations. Common findings are not specific but typically show high uni- or bilateral T2/Flair-signals, especially in the medial temporal lobe with extrahippocampal cortical or subcortical lesions, without restricted diffusion or hemorrhage (11). A definitive distinction between infectious (e.g., HSV, tuberculosis) and autoimmune encephalitis is usually not possible from MRI alone (7, 8, 17). HSV encephalitis is less restricted to the limbic system and often shows restricted diffusion abnormalities and contrast uptake (14). In the case of LG1 encephalitis, hyperintensities in the basal ganglia are common (22). **Figure 1** shows common MRI findings in different types of encephalitis.

The EEG is often abnormal. Apart from extreme delta brush (a generalized rhythmic delta activity with superimposed fast activity), which if present, is characteristic for NMDA-R-encephalitis, there are no pathognomonic patterns (8). Unspecific, frequent findings include general slowing, epileptic potentials or status and periodic lateralized epileptiform discharges (PLEDs) (17).

Tumor screening is essential. The range and frequency of associated malignancies differs according to the autoantibody detected. Depending on the autoantibody or clinical syndrome, specific tests (ultrasound, CT or MRI) should be performed based on their sensitivity (8). All patients may need chest, abdomen and pelvic-CT scans. Females should also undergo gynecological exams, breast, and ovarian ultrasound, and if negative, pelvic MRI for small teratomas. Males should undergo urological evaluation and ultrasound. Positive antibody-detection is highly associated with malignancies in older patients (>60 years). If CT/MRI and ultrasound do not yield any findings, a whole-body 18F-Fluorodeoxyglucose (FDG)-PET should be considered (17, 22, 26).

PET neuroinflammation imaging might play an important role in the future, as new radiotracers are currently being developed in clinical studies; their potential in assessing neuroinflammation still requires evaluation but might provide deeper insights into the complex immunopathology (27, 28).

The early detection of tumors is important, not only for the prognosis but also as treatments with immunotherapy could complicate tumor detection (e.g., lymphoma) (8).

Infections as Possible Triggers

Herpes-simplex-encephalitis (HSE) is usually monophasic. Nevertheless, around 25% of cases that have been successfully treated with antiviral therapy show relapse after several weeks. In some cases, this might be due to viral reactivation but in others, especially those which presented with new symptoms, the new CSF samples showed NMDA-R antibodies without viral reactivation and the symptoms resolved after immunotherapy. Therefore, HSE patients that worsen after resolved infectious encephalitis, should be tested for infectious and autoimmune encephalitis (12).

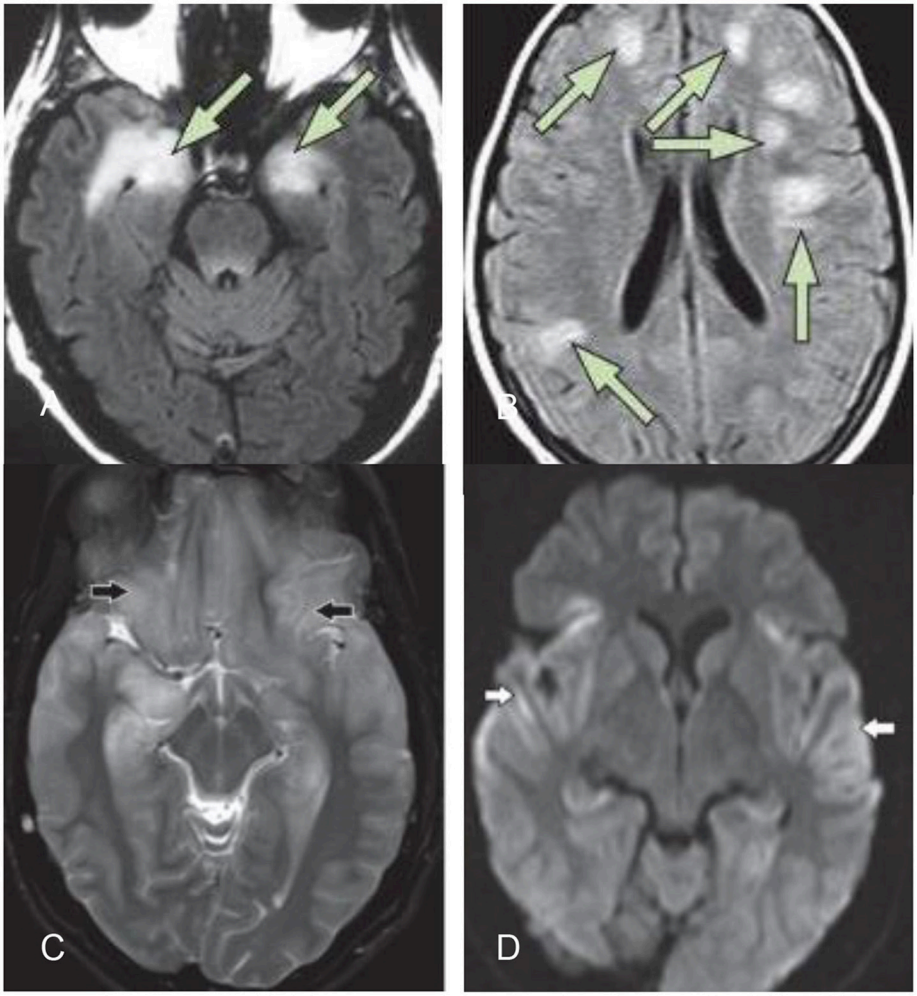


FIGURE 1 | (A) Typical MRI of limbic encephalitis with bilateral hyperintensities in the medial temporal lobe on T2-weighted fluid–attenuated inversion recovery imaging **(B)** typical MRI of ADEM (14). **(C,D)** Herpes simplex virus encephalitis: bilateral symmetric cortical swelling and hyperintensity on T2 weighted image involving the anteromedial temporal lobes, insular cortex, orbital gyri (black arrows) with restricted diffusion in the involved areas (white arrows) (25).

Untreated Campylobacter jejuni infections can induce ganglioside-autoantibody mediated diseases, including Guillain-Barré-syndrome, Miller-Fisher-syndrome and in the CNS, Bickerstaff encephalitis. Characteristic findings are subacute onset, progressive impairment of consciousness, ataxia, and ophthalmoplegia. MRI shows brainstem abnormalities in 23% of cases, VGKC-antibodies may be present but are uncharacteristic findings and also frequently detectable in non-autoimmune diseases. Anti-GQ1b antibodies are confirmatory and make a clear distinction possible (5, 14).

TREATMENT AND PROGNOSIS

There are currently no randomized, controlled trials based on standard immunotherapy protocols, however, many retrospective and some prospective studies have clearly suggested the efficacy of immunomodulatory therapy. Seventy percent of patients respond to gradual immunotherapy escalation. Co-existing tumors, age, and delay in treatment are additional factors which determine the outcome. In general, young patients have a better outcome (5, 29).

First line therapy consists of corticosteroids plus IVIG and/or plasma exchange (PLEX)/immunoadsorption. Previous studies have shown that the use of high-dose corticosteroids is initially associated with better clinical outcome. In contrast to steroids, IVIG, and plasmapheresis/immunoadsorption are unlikely to worsen infectious encephalitis. In cases where there is a reasonable suspicion of autoimmune encephalitis, a multimodal immunological treatment may be started prior to CSF-antibody results, especially when the MRI findings reinforce the diagnosis (2, 8).

Although in many studies corticosteroids appear to be effective in AIE, the largely antibody-mediated disease pathogenesis needs to be considered. The effect of corticosteroids on B cells and Igs is limited and additional treatment may be required (30).

TABLE 2 | Immunomodulatory agents, dosing regime, and adverse effects.

	Treatment	Regimen	Adverse effects
	FIRST LINE IMMUNOTHERAPY		
Depending on the individual clinical response and tolerability	Methylprednisolone *AND/OR* NO CLEAR EFFECT AFTER THE LAST DOSE	1 g/day for 3–5 days and sometimes stepwise reduction over weeks	Insomnia, psychiatric, hyperglycaemia, electrolyte imbalances, hypertension, peptic ulcera, infections, osteoporosis, cataracts, Cushing syndrome
	IVIG *AND/OR*	0,4 g/kg/day for 5 days, probably followed by weekly or every 5 weeks	Headaches, renal failure, thrombotic events, anaphylaxis (IgA-deficiency!)
	PLEX/Immunoadsorption	1 Session every other day for 5–7 cycles	Hypotension, electrolyte imbalances, coagulopathy, due to central line: pneumothorax, infection, hemorrhage, thrombosis
		NO EFFECT AFTER ONE WEEK	
	SECOND LINE IMMUNOTHERAPY		
	Rituximab *OR*	375 mg/m^2 weekly for 4 weeks	Allergic reaction, opportunistic infection, reactivation of infections(e.g. tuberculosis or hepatitis B), PML
	Cyclophosphamide	750 mg/m^2 monthly for 3–6 months	Gastrointestinal, hair loss, mucositis, haemorrhagic cystitis, myelosuppression, infertility
	STEROID-SAVING AGENTS FOR MAINTENANCE THERAPY (IF STEROIDS LED TO CLINICAL IMPROVEMENT)		
	Mycophenolate *OR*	Initially 500 mg twice daily, target 1000 mg twice daily	Gastrointestinal, hypertension, infections, myelosuppression, lymphoma, peripheral oedema, PML, cytomegalovirus infection, skin malignancies
	Azathioprine	Initially 1,5 mg/kg once daily or divided twice daily, target 2–3 mg/kg/day	Gastrointestinal, fever, rash, myalgias, hair loss, cytopenia, hepatoxicity, lymphoma, infections, hypersensitivity reactions
	ALTERNATIVE THERAPY* (IF INADEQUATE RESPONSES TO SECOND LINE THERAPIES)		
	Tocilizumab	*initially 4 mg/kg, followed by an increase to 8 mg/kg monthly based on clinical response*	
	Low-dose interleukin-2	*1,5 million IU/day, 4 subcutaneous injections with 3-week interval*	

No clear recommendation! There are observational studies that resulted in clinical improvement with this alternative therapy, but these results remain to be confirmed, more evidence is needed before making final conclusions (30).

So far, there is no strong evidence of a difference in efficacy between IVIG and plasmapheresis. It must be considered in the therapy plan that IVIG can be removed by PLEX. Therefore, PLEX immediately after IVIG therapy is not recommended (22).

Selective immunoadsorption represents another extracorporal antibody depletion method which has been proven in a few clinical studies to be effective as part of the multimodal immunotherapy of AIE, leading to clinically relevant improvement. Compared to PLEX, immunoadsorption allows a more targeted removal of proteins and avoids the disadvantages of plasma substitution (e.g., risk of infection or allergic reactions) and the impact on coagulation. All coagulation factors were significantly reduced by 50–70% after PLEX, whereas after immunoadsorption, single factors were not or only moderately, reduced. Documented side effects of immunoadsorption were nonspecific and related to intravenous lines (22, 31).

IVIG is more convenient for the patient and is cost-effective, compared to invasive options for antibody depletion. It is also more readily available for immediate therapy.

If there is little or no clinical improvement, second-line therapy should be implemented with Rituximab or Cyclophosphamide, with the former having a favorable side effect profile (8, 21). Different immunosuppressing drugs can be considered for long-term treatment (7). As, so far, there is no evidence to suggest the superiority of any specific regimen, **Table 2** provides an overview of the immunomodulatory treatments and possible adverse effects, to support clinicians in the decision-making process for the individual patient.

The optimal duration of these treatments is unknown. The clinical picture and issue of relapse rates (e.g., known high relapse rates in LGI1-R-encephalitis) should be considered and might lead to a longer or continuous treatment with Rituximab or Cyclophosphamide. Relapses should be treated with the same treatment scheme as the first clinical presentation (2).

In tumor-associated autoimmune encephalitis, surgical treatment should be initiated as soon as possible. It can relieve the symptoms effectively and favors the long-term outcome (2). A worsening of symptoms could, in contrast, suggest an incomplete resection, recurrence or secondary metastasis (5, 21).

Seizures may be very difficult to control and pharmacologically induced coma is frequently needed until the autoimmune disease regresses (6). So far, there is no evidence that one anticonvulsive medication is more efficacious than others. Due to the neuropsychiatric side effects of Levetiracetam, it might be difficult to determine whether, for example, acute agitation is due to the disease or pharmacologically reinforced. Lamotrigine, Benzodiazepines and Lacosamide can be used, as they do not seem to have a strong impact on cognitive function (22).

Follow up measurement of antibody titres during therapy, especially in serum, are not useful for treatment decisions, as

they can test low in the initial analysis, even if the patient is in a coma. They can persist for years, even when the patient has fully recovered. However, in cases of relapses, it might be helpful to determine the course of the antibody titre (1).

Overall, encephalitis cases associated with surface-antibodies have a better prognosis than those associated with intracellular antibodies. However, in all cases, early stage treatment is crucial (1, 2).

Clinicians need to be aware that neurological symptoms may appear a long time before a tumor is detectable (e.g., micro teratomas), therefore, if the initial screening was negative, repeated follow-ups must be performed. Surveillance imaging at intervals of 4 to 6 months for at least 4 years are suggested (7, 17).

CONCLUSION

The recognition of certain symptom constellations is crucial. When patients present with a clinical picture of encephalitis or sudden altered mental state, it is extremely important to consider an underlying autoimmune pathogenesis early on. If the listed criteria support the diagnosis of possible autoimmune encephalitis, treatment can be implemented early and prior to the onset of severe complications. Once the antibody results are available, the treatment can be re-evaluated and adapted.

The underlying mechanisms for activation and autoimmune response in the CNS are still unclear. Further investigations are needed to gain sufficient insights into how immune mechanisms affect nervous system functions. In addition, randomized, controlled trials could help to establish more specific therapies for the different subtypes of AIE.

AUTHOR CONTRIBUTIONS

CH and SH wrote the manuscript, FF contributed by helpful discussion. All authors have read and approved the manuscript.

REFERENCES

1. Leypoldt F, Armangue T, Dalmau J. Autoimmune encephalopathies. *Ann N Y Acad Sci*. (2015) 1338:94–114. doi: 10.1111/nyas.12553
2. Günther A, Schubert J, Brämer D, Witte OW. Autoimmune encephalitis. [Article in German] *Dtsch Med Wochenschr*. (2016) 141:1244–9. doi: 10.1055/s-0041-108858
3. Weissert R. Adaptive immunity is the key to the understanding of autoimmune and paraneoplastic inflammatory central nervous system disorders. *Front Immunol*. (2017) 8:336. doi: 10.3389/fimmu.2017.00336
4. Höftberger R, Lassmann H. Immune-mediated disorders. *Handb Clin Neurol*. (2017). 145:285–99. doi: 10.1016/B978-0-12-802395-2.00020-1
5. Platt MP, Agalliu D, Cutforth T. Hello from the other side: how autoantibodies circumvent the blood-brain barrier in autoimmune encephalitis. *Front Immunol*. (2017) 21:8:442. doi: 10.3389/fimmu.2017.00442
6. Höftberger R. Neuroimmunology: an expanding frontier in autoimmunity. *Front Immunol*. (2015) 6:206. doi: 10.3389/fimmu.2015.00206
7. Stich O, Rauer S. [Paraneoplastic neurological syndromes and autoimmune encephalitis]. [Article in German]. *Nervenarzt* (2014) 85:485–98. doi: 10.1007/s00115-014-4030-x
8. Lancaster E. The diagnosis and treatment of autoimmune encephalitis. *J Clin Neurol*. (2016) 12:1–13. doi: 10.3988/jcn.2016.12.1.1
9. Bauer J, Bien CG. Neuropathology of autoimmune encephalitides. *Handb Clin Neurol*. (2016) 133:107–20. doi: 10.1016/B978-0-444-63432-0.00007-4
10. Newman MP, Blum S, Wong RC, Scott JG, Prain K, Wilson RJ, et al. Autoimmune encephalitis. *Intern Med J*. (2016) 46:148–57. doi: 10.1111/imj.12974
11. Kelley BP, Patel SC, Marin HL, Corrigan JJ, Mitsias PD, Griffith B. Autoimmune encephalitis: pathophysiology and imaging review of an overlooked diagnosis. *AJNR Am J Neuroradiol*. (2017) 38:1070–8. doi: 10.3174/ajnr.A5086
12. Selmi C, Barin JG, Rose NR. Current trends in autoimmunity and the nervous system. *J Autoimmun*. (2016) 75:20–9. doi: 10.1016/j.jaut.2016.08.005
13. Dalmau J, Rosenfeld MR. Autoimmune encephalitis update. *Neuro Oncol*. (2014) 16:771–8. doi: 10.1093/neuonc/nou030
14. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. Clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol*. (2016) 15:391–404. doi: 10.1016/S1474-4422(15)00401-9
15. Dubey D, Pittock SJ, Kelly CR, McKeon A, Lopez-Chiriboga AS, Lennon VA, et al. Autoimmune encephalitis epidemiology and a comparison to infectious encephalitis. *Ann Neurol*. (2018) 83:166–77. doi: 10.1002/ana.25131
16. Liu CY, Zhu J, Zheng XY, Ma C, Wang X. Anti-N-Methyl-D-aspartate receptor encephalitis: a severe, potentially reversible autoimmune encephalitis. *Mediat Inflamm*. (2017) 2017:6361479. doi: 10.1155/2017/6361479
17. Lee SK, Lee ST. The laboratory diagnosis of autoimmune encephalitis. *J Epilepsy Res*. (2016) 6:45–52. doi: 10.14581/jer.16010
18. Dalmau J, Geis C, Graus F. Autoantibodies to synaptic receptors and neuronal cell surface proteins in autoimmune diseases of the central nervous system. *Physiol Rev*. (2017) 97:839–87. doi: 10.1152/physrev.00010.2016
19. Steiner J, Prüß H, Köhler S, Hasan A, Falka P. Autoimmune encephalitis with psychotic symptoms. Diagnostics, warning signs and practical approach. [Article in German] *Nervenarzt* (2018) 89:530–8. doi: 10.1007/s00115-018-0499-z
20. Honnorat J, Plazat LO. Autoimmune encephalitis and psychiatric disorders. *Revue Neurol*. (2018) 174:228–36. doi: 10.1016/j.neurol.2017.11.004
21. Lewerenz J, Jarius S, Wildemann B, Wandinger KP, Leypoldt F. Autoantibody-associated and autoimmune encephalitis and cerebellitis: clinical presentation, diagnostic work-up and treatment]. [Article in German] *Nervenarzt* (2016) 87:1293–9 doi: 10.1007/s00115-016-0235-5
22. Dubey D, Blackburn K, Greenberg B, Stuve O, Vernino S. Diagnostic and therapeutic strategies for management of autoimmune encephalopathies. *Exp Rev Neuroth*. (2016) 8:937–49. doi: 10.1080/14737175.2016.1189328
23. Abboud H, Rossman I, Mealy MA, Hill E, Thompson N, Banerjee A, et al. Neuronal autoantibodies: differentiating clinically relevant and clinically irrelevant results. *J Neurol*. (2017) 264:2284–92. doi: 10.1007/s00415-017-8627-4
24. Van Sonderen A, Schreurs M, De Bruijn M, Boukhrissi S, Nagtzaam M, Hulsenboom E, et al. The relevance of VGKC positivity in the absence of LGI1 and Caspr2 antibodies. *Neurology* (2016) 86:1692–99. doi: 10.1212/WNL.0000000000002637
25. Sureka J, Jakkani RK. Clinico-radiological spectrum of bilateral temporal lobe hyperintensity: a retrospective review. *Br J Radiol*. (2012) 85:e782–92. doi: 10.1259/bjr/30039090
26. Derlin T, Grünwald V, Steinbach J, Wester HJ, Ross TL. Molecular imaging in oncology using positron emission tomography. *Dtsch Arztebl Int*. (2018) 115:175–81. doi: 10.3238/arztebl.2018.0175
27. Janssen B, Vugts DJ, Windhorst AD, Mach RH. PET Imaging of microglial activation—beyond targeting TSPO. *Molecules* (2018) 23:607. doi: 10.3390/molecules23030607

28. Alam M, Lee J, Lee SY. Recent progress in the development of TSPO PET ligands for neuroinflammation imaging in neurological diseases. *Nucl Med Mol Imaging* (2017) 51:283–96. doi: 10.1007/s13139-017-0475-8
29. Varley J, Taylor J, Irani SR. Autoantibody-mediated diseases of the CNS: structure, dysfunction and therapy. *Neuropharmacology* (2017) 132:71–82. doi: 10.1016/j.neuropharm.2017.04.046
30. Shin YW, Lee ST, Park KI, Jung KH, Jung KY, Lee SK et al. Treatment strategies for autoimmune encephalitis. *Ther Adv Neurol Disord*. (2018) 11:1–19. doi: 10.1177/1756285617722347
31. Fassbender C, Klingel R, Köhler W. Immunoadsorption for autoimmune encephalitis. *Artherosclerosis Sup*. (2017) 30:257–63. doi: 10.1016/j.atherosclerosissup.2017.05.041

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

METHODS
published: 22 August 2018
doi: 10.3389/fneur.2018.00661

Optimization of an Anti-NMDA Receptor Autoantibody Diagnostic Bioassay

Nan-Chang Chiu[1,2,3], Yi-Jie Lin[1], Ruu-Fen Tzang[2,3,4], Ying-Syuan Li[5], Hui-Ju Lin[5], Subir Das[6], Caleb G. Chen[3,7], Chiao-Chicy Chen[3,4] and Kate Hsu[5]**

[1] Division of Pediatric Neurology, Department of Pediatrics, MacKay Children's Hospital, Taipei, Taiwan, [2] MacKay Junior College of Medicine, Nursing, and Management, New Taipei City, Taiwan, [3] MacKay Medical College, New Taipei City, Taiwan, [4] Department of Psychiatry, MacKay Memorial Hospital, Taipei, Taiwan, [5] MacKay Memorial Hospital Transfusion Medicine & Immunogenetics Laboratories, Tamsui, Taiwan, [6] Institute of Biophotonics, National Yang-Ming University, Taipei, Taiwan, [7] Department of Internal Medicine, MacKay Memorial Hospital, Taipei, Taiwan

OPEN ACCESS

Edited by:
Morten Blaabjerg, University of Southern Denmark Odense, Denmark

Reviewed by:
Reinhild Klein, Universität Tübingen, Germany
Anna Fogdell-Hahn, Karolinska Institutet (KI), Sweden

***Correspondence:**
Chiao-Chicy Chen
cchen@tmu.edu.tw
Kate Hsu
khsu1@mmh.org.tw

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *12 April 2018*
Accepted: *24 July 2018*
Published: *22 August 2018*

Citation:
Chiu N-C, Lin Y-J, Tzang R-F, Li Y-S, Lin H-J, Das S, Chen CG, Chen C-C and Hsu K (2018) Optimization of an Anti-NMDA Receptor Autoantibody Diagnostic Bioassay. Front. Neurol. 9:661. doi: 10.3389/fneur.2018.00661

Anti-N-methyl-D-aspartate receptor (anti-NMDAR) encephalitis is one of the most frequently encountered autoimmune encephalitis. The pathogenesis of both anti-NMDAR encephalitis and schizophrenia involve down-regulation of NMDA receptors. Whether autoantibody-mediated destruction of neuronal NMDA receptors is associated with schizophrenia or first-episode psychosis (FEP) remains unclear, as the current findings from different groups are inconsistent. The main culprits are likely due to heterogeneity of autoantibodies (autoAbs) in a patient's blood or cerebrospinal fluid (CSF), as well as due to limitation of the current detection methods for anti-NMDAR autoAbs. Here, we optimized the current diagnostic method based on the only commercially-available anti-NMDAR test kit. We first increased detection sensitivity by replacing reporter fluorophore fluorescein isothiocyanate (FITC) in the kit with Alexa Fluor 488, which is superior in resisting photobleaching. We also found that using an advanced imaging system could increase the detection limit, compared to using a simple fluorescence microscope. To improve test accuracy, we implemented secondary labeling with a well-characterized mouse anti-NR1 monoclonal antibody (mAb) after immunostaining with a patient's sample. The degree of colocalization between mouse and human antisera in NMDAR-expressing cells served to validate test results to be truly anti-NMDAR positive or false-positive. We also incorporated DNA-specific DAPI to simultaneously differentiate autoAbs targeting the plasma membrane from those targeting cell nuclei or perinuclear compartments. All the technical implementation could be integrated in a general hospital laboratory setting, without the need of specialized expertise or equipment. By sharing our experience, we hope this may help improve sensitivity and accuracy of the mainstream method for anti-NMDAR detection.

Keywords: Anti-N-methyl-D-aspartate receptor (anti-NMDAR) encephalitis, autoantibody (autoAb), autoimmune encephalitis, schizophrenia, diagnostic test, fluorescein isothiocyanate (FITC), Alexa Fluor 488, antigen (Ag)

INTRODUCTION

Diagnosis of anti-NMDAR autoimmune encephalitis requires identification of pathogenic anti-NMDAR autoAbs in a clinical sample (1). Because anti-NMDAR autoAbs could target neuronal receptor and impair glutamatergic transmission, psychotic and cognitive disturbing symptoms are prominent in anti-NMDAR encephalitis (2–5). Not surprisingly, early presentation of anti-NMDAR encephalitis shares symptoms of schizophrenia. For neuropsychiatrists, it has been an intriguing research topic to determine whether autoantibodies against NMDA receptor might contribute to the pathogenesis of a subset of schizophrenia through autoimmune-mediated neuroinflammation.

In an early study of 571 patients diagnosed with anti-NMDAR autoAbs, 23 of them (4%) presented no neurological symptoms but isolated psychiatric episodes (6). As many patients with anti-NMDAR encephalitis are first seen by psychiatrists for their initial prominent psychiatric symptoms, these 4% of the anti-NMDAR-positive patients with only psychiatric symptoms conceivably might be diagnosed as psychosis or even schizophrenia in psychiatric clinics. In our hospital psychiatric clinics in Taiwan, we have identified anti-NMDAR autoAbs in first-visited patients who showed abrupt and atypical psychosis with autonomic disturbance. After the correct diagnosis, their psychiatric symptoms were eventually cured by immunosuppressive treatments, emphasizing the extreme importance of correctly sorting out these patients (7, 8).

Similar results were found by research teams in U.K. and Japan (9–11). In U.K., Zandi and colleague reported the presence of serum anti-NMDAR autoantibodies in 6.5% of the patients with schizophrenia (9); Lennox et al. reported anti-NMDAR IgG in 3% of 228 patients with FEP and not in the blood samples of 105 healthy controls (11). In Japan, Tsutsui and colleague found anti-NMDAR autoAbs in the sera of four out of 51 patients with schizophrenia and schizoaffective disorder (7.8%) (10). However, there are contradicting findings from groups in Germany (0.7% anti-NMDAR IgG in 1081 schizophrenic patients and 0.4% in 1272 healthy subjects) (12), in another Taiwan hospital (0% in 78 patients with first-episode schizophrenia and 0% in 234 patients with chronic schizophrenia) (13), and in Turkey (0% in 49 schizophrenic patients and 0% in 48 healthy subjects) (14). Thus, whether anti-NMDAR autoantibodies could be associated with pure psychiatric illness (e.g., schizophrenia) remains an open question.

Though autoantibodies present in autoimmune patients are intrinsically complex and heterogeneous with a diverse range of specificities and affinities to autoantigens, the root of these controversies likely also involves the various detection approaches that different research groups took. The current detection methodology for anti-NMDAR autoAbs, whether developed commercially or in-house in individual academic labs, all utilizes NR1/NR2-expressing cultured cells for immunofluorescence labeling (9–11, 15). In this approach, negative controls are untransfected or untransduced cultured cells; tester cells are NR1/NR2-expressing cultured cells. A test result is considered positive if the blood or CSF sample from a patient shows reactivity to heterologously-expressed NMDA receptor, and not to negative-control cells.

Research groups that incorporate their in-house immunostaining protocols generally reported higher occurrence rates of anti-NMDAR autoAbs in patients with schizophrenia or psychosis, and absence or lower frequencies of the antibodies in healthy controls (9, 11, 15–17). Because the in-house protocols generally use live NR1/NR2b-expressing cultured cells, they provide a broader and more realistic range of antigenic sites than chemically-fixed cells from a commercial kit. However, heterologous expression of NMDA receptor in cultured cells requires ketamine, which is inaccessible to most laboratories including ours. So we also used the conventional kit for anti-NMDAR tests.

As suggested in recent Commentaries and Replies to journal articles, the different results from different groups might also be related to the imperfect performance of the commercial kit that many of them relied on (4, 9, 17–22). Based on our experience with the commercial reagents, there were definitely rooms for improvement. In our early trials with these reagents, we noticed that the fluorescent signals that reported antibody-antigen (Ab-Ag) interaction quenched quickly under a conventional fluorescence microscope. We initially often had to repeat a test several times, especially for clinical samples that were eventually determined to have low titers of anti-NMDAR autoAbs. This report described our approaches to increase sensitivity and accuracy of anti-NMDAR detection based on the conventional approach.

MATERIALS AND METHODS

Ethics Statement

The study was carried out in accordance with the principles of the Declaration of Helsinki, and was approved by the Institutional Review Board (IRB) of Taiwan Mackay Memorial Hospital (MMH)(MMH-IRB registration numbers: 14MMHIS068 & 14MMHIS282). Written informed consent was obtained from all participating subjects.

Optimization for Detection of Anti-NMDAR AutoAbs

Optimization was based on the recommended protocol of the anti-Glutamate Receptor (type NMDA) IIFT kit (EUROIMMUN, Lubeck, Germany). Similar to the Manufacturer's Instruction, 30 μl of a clinical sample (either undiluted or 1:2 diluted CSF, or 10-fold diluted blood serum or plasma) was incubated with a pair of Tester and Negative-Control BIOCHIPs for 1 hour at room temperature, followed by two washes with PBS-Tween 20 (all provided by the kit) for 5 min. Tester and Negative-Control BIOCHIPs are mini chips coated with fixed, NMDAR-expressing cultured cells and unexpressed cells, respectively. These paired chips are embedded on a microscope slide. In the protocol provided by the kit, ab-ag interaction was probed by secondary labeling with 25 μl of FITC-conjugated anti-human antisera (included in the kit) for 30 min at room temperature, followed by washes.

To increase detection sensitivity, we substituted secondary FITC-conjugated anti-human antisera (provided in the kit) with Alexa fluor 488-conjugated anti-human immunoglobin (1:100 dilution; Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Detailed optimization for the concentration of detection probe Alexa Fluor 488, incubation time, and dilution of clinical samples was provided in the online Supplemental Figures. **Figure S1** showed the optimal dilution of Alexa Fluor 488-conjugated anti-human immunoglobin to be 1:100. **Figure S2** showed the optimal length of time for incubating a clinical sample with BIOCHIPs to be 1 hour. **Figure S3** showed how an autoAb titer is determined using a blood plasma sample. For titer determination, a plasma/serum sample is generally diluted at 1:10, 1:32, 1:100, 1:320, and up to 1:640. An autoAb titer is the highest possible dilution that still allows for visualization of the fluorescence signals from the antibody-antigen interaction. **Figure S4** showed the ideal dilution of a CSF test to be 1:2 or no dilution (the latter identical to the manufacturer's suggestion) using our immunostaining protocol.

Double Immunolabeling With Mouse Anti-NR1 mAb

For clinical samples with ambiguous test results using the single-labeling method (described above), the samples could be re-tested or tried with the double-labeling method to verify the accuracy of single-staining results. For double labeling, a specific mouse anti-NR1 mAb is incorporated to mark subcellular locations that express NMDA receptor. The rationale is that if a patient's sample does not react to the same subcellular regions as the mouse mAb, then this patient does not have anti-NR1 autoAbs. The first part of the double-labeling protocol was identical to single labeling described above. Briefly, 30 μl of a clinical sample was incubated with a pair of Negative-Control and Tester BIOCHIPs for 1 hour, followed by washes. An optional fixative step with 0.01% glutaraldehyde for 30 seconds could be employed prior to second labeling with mouse anti-NR1 mAb clone 54.1 (1:2000 dilution; Merck-Millipore, Temecula, CA, USA). The second staining with this specific mouse mAb lasted for 1 hour, followed by washes. Though human and mouse antisera were incubated with BIOCHIPs sequentially, their individual labeling with the detection fluorophores were administered simultaneously. Specifically, after sequential probing with human and mouse antisera, the BIOCHIPs were then incubated in 25 μl of a mixture of Alexa fluor 488-conjugated anti-human and Alexa fluor 568-conjugated anti-mouse immunoglobin for 30 min (both at 1:1000 dilution; both from Jackson ImmunoResearch Laboratories), followed by washes.

For labeling of cell nuclei, instead of using glycerol from the commercial kit, immunostained BIOCHIPs were sealed with a glycerol-based mountant containing DAPI (Thermo Fisher Scientific, Waltham, MA, USA).

Fluorescence Imaging and Calculation

The images were taken from (1) an inverted fluorescence phase contrast microscope (OLYMPUS IX71) coupled with the SPOT RT3 microscope digital camera and imaging processing system (Diagnostic Instruments Inc., Sterling Heights, MI, USA); (2) the TissueFAXS Cell Analysis System (TissueGnostics GmbH, Vienna, Austria). In double-labeling experiments, the degree of colocalization (R_{coloc}) between green and red fluorescence was calculated by the Colocalization Threshold plugin (ImageJ). R_{coloc} is the Pearson's correlation coefficient for images above thresholds: $R_{coloc} \sim 1$ refers to a perfectly positive correlation; $R_{coloc} \sim 0$ refers to the complete absence of a correlation (23). For verification of image-based test results, the batch number of each BIOCHIP-embedded slide was recorded. Immunostained

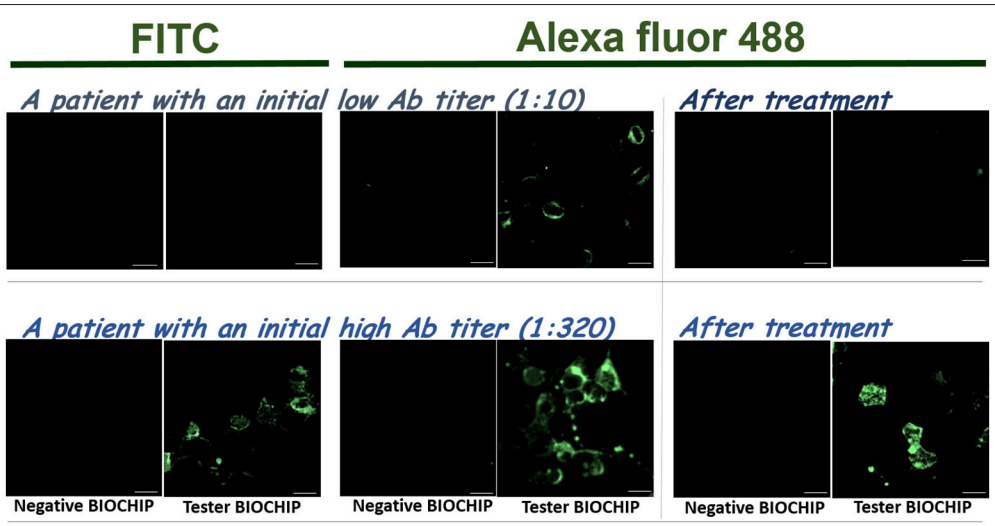


FIGURE 1 | Replacement of secondary anti-human antisera-conjugated fluorophore FITC (left) with Alexa Fluor 488 (right) improved the sensitivity of anti-NMDAR autoAb detection based on EUROIMMUN's anti-Glutamate Receptor IIFT. The experimental procedure mostly followed the recommendation from the manufacturer. **Top:** Comparison using a clinical sample with a low content of anti-NMDAR autoAbs (titer 1:10). **Bottom:** Comparison using a sample with a high titer of anti-NMDAR antisera (titer 1:320). The right pairs are from the same patients after effective immunosuppressive treatments. Scale bars, 20 μm.

BIOCHIPs were examined independently by at least two lab specialists under a fluorescence microscope. The criteria and workflow for the optimized anti-NMDAR autoAb diagnostic test are outlined at the end of this paper.

RESULTS

Improvement of Anti-NMDAR Detection Sensitivity

To improve detection sensitivity, we compared the reporter fluorescence probe provided in the kit, FITC, with functionally-equivalent Alexa fluor 488. Though both fluorophores are nearly identical in spectral properties (excitation max 490 nm / emission max 525 nm) and quantum yields (∼0.9), Alexa fluor 488 is significantly more photostable and less sensitive to environmental changes (e.g., pH), and has higher initial brightness (24, 25). In **Figure 1**, labeling with either FITC or Alexa fluor 488 gave strong signals for a sample with a high titer of anti-NMDAR autoAbs (**Figure 1**, bottom). However, for a sample with a low anti-NMDAR titer, labeling with Alexa fluor 488 showed distinctive differences between negative-control and NMDAR-positive BIOCHIPs, while that differences were much less distinguishable with FITC (**Figure 1**, top). After effective immunosuppressive treatments, both cases showed visibly reduced titers of anti-NMDAR autoAbs (**Figure 1**, right panels). For the patient with an initial low autoAb titer (1:10), his autoAbs after the treatments became almost undetectable even with more sensitive Alexa Fluor 488 (**Figure 1**, right panels).

Table 1 showed that Alexa Fluor 488 replacing FITC as the detection probe improved detection sensitivity, and generally allowed higher sample dilution or titers. Labeling with more sensitive Alexa fluor 488 also allows a broader range of autoAb detection, as reflected by the broader range of autoAb titers in the same patient samples that were also tested with FITC (**Table 1**). We also tested 26 stable psychiatric patients (23 schizophrenia and 3 bipolar disorder) from the hospital psychiatric day-care center, and found anti-NMDAR autoAb present in one out of the 26 patients (with a weak blood titer at 1:32). This patient suffered from chronic schizophrenia, and did not meet the criteria for possible autoimmune encephalitis (1). Thus, we were able to identify anti-NMDAR autoAb in ∼3.8% psychiatric patients using the improved, kit-based method. Our positive rate for anti-NMDAR autoAb in psychiatric patients was similar to the rates reported by various groups using their more sensitive, in-house-developed methods described in the Introduction section (9, 10, 15–17). For comparison, our improved tests on 101 healthy control samples did not yield a positive result (**Table 1**).

From our early trials using five different types of fluorescent microscopes/imaging systems in our department, we found that the choice of an imaging system affected detection sensitivity. Most of the imaging systems were able to resolve samples with high antibody titers (e.g., 1:100 or higher) (**Figure 2**, middle). A high-end optic/imaging system could further resolve relatively weak signals from samples with low Ab titers (**Figure 2**, top).

TABLE 1 | Comparison of the sensitivity of anti-NMDAR test between the two detection probes–FITC and Alexa Fluor 488.

Subject*	Status of anti-NMDAR encephalopathy	Anti-NMDAR titer#	
		FITC	Alexa Fluor 488
pt 1	Cured	1:10	1:32
pt 2	Cured	1:10	1:10
pt 3	Cured	1:10	1:32
pt 4	Recurrent	1:32	1:320
pt 5	Recurrent	1:32	1:100
pt 6	Cured	Indeterminate	1:10
Psychiatric day-care patients** *(n = 26)*	–	–	25 negative; 1 positive (blood titer 1:32)##
Healthy controls *(n = 101)*	–	–	All negative *(101/101)*

**Patient (pt) subjects diagnosed of anti-NMDAR encephalitis fulfilled the diagnostic criteria listed in Graus et al. (1).*

***These were stable psychiatric patients attending programs at MMH Psychiatric Day-Care Centre: 23/26 schizophrenia; 3/26 bipolar disorder.*

 # The anti-NMDAR titer was determined by the highest possible dilution of a patient's plasma or serum sample which could still reveal fluorescence signals from anti-NMDAR autoAb labeling.

The only blood anti-NMDAR-positive patient is a stable patient with schizophrenia, whose symptoms do not meet the criteria for possible autoimmune encephalitis (1).

Improvement of Anti-NMDAR Detection Accuracy by Co-labeling With a Mouse Anti-NR1 mAb

There was a need to improve the accuracy of anti-NMDAR detection. We occasionally encountered uncertain readouts that showed no staining in the negative-control cells but positive signals with unusual patterns in NMDAR-expressing cells. These unusual staining patterns could appear punctate, absent from the plasma membrane, or present in unexpected subcellular locations (e.g., cell nuclei). The percentage of fluorescence-labeled cells in the BIOCHIPs could also be used to roughly assess the accuracy of a test result. We estimated experimentally that not all but about 30–50% of the cells on the EUROIMMUN's Tester BIOCHIP express NMDA receptor. So experimenters should be alert when only sporadic cells or over 50% of the cells on a Tester BIOCHIP are fluorescently labeled.

To improve the accuracy of anti-NMDAR detection, it is critical to verify whether ambiguously positive signals indeed result from binding to NMDA receptor, and not from interaction with cellular components other than NMDA receptor on a Tester BIOCHIP. We implemented second labeling with a well-characterized mouse anti-NR1 mAb clone 54.1 to specifically locate heterologously-expressed NMDA receptor (15), after labeling the BIOCHIP with a clinical sample. This mouse anti-NR1 was stained with red fluorescent Alexa Fluor 568, while bound human autoAbs were stained with green fluorescent Alexa fluor 488 (**Figure 3**). Noticeably, for some clinical samples,

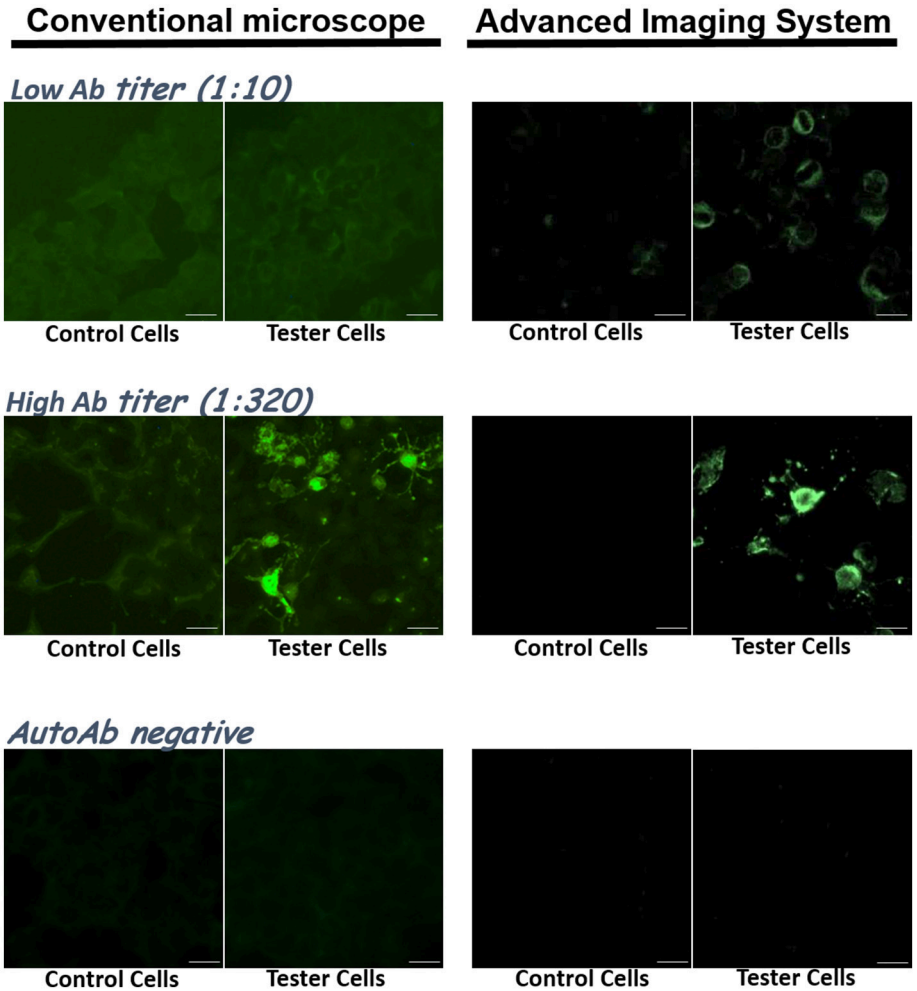


FIGURE 2 | The choice of optic/imaging systems could affect resolution and sensitivity of anti-NMDAR detection. Clinical samples were tested with the IIFT kit, and their images taken by a conventional fluorescence microscope (left) and by an advanced imaging system from TissueGnostics GmbH (right) were compared. **Top:** images from a sample with a low anti-NMDAR titer (1:10). **Middle:** images from a sample with a high anti-NMDAR titer (1:320). **Bottom:** images from an anti-NMDAR-negative sample. Scale bars, 20 μm.

this mouse anti-NR1 might compete with a patient's autoAbs for binding to NMDA receptor, resulting in a low degree of colocalization between the mouse and the human antisera. To circumvent the issue, we added a brief fixative step following clinical sample labeling and before labeling with mouse anti-NR1. This fixative step could prevent mouse anti-NR1 from outcompeting a patient's autoAbs for binding to NMDA receptor (**Figure 3**), since mouse mAb clone 54.1 generally exhibits higher affinities to NMDA receptor than most human anti-NMDAR antisera. In some tests, indeed this additional fixative step increased % colocalization between mouse and human anti-NMDAR antisera [**Figure 4**—patient A: R_{coloc} ~0 (without fixation) vs. R_{coloc} ~0.17 (with fixation)].

By marking heterologously-expressed NMDA receptor with red fluorescence on a Tester BIOCHIP, this double-labeling approach allowed us to identify "false-positive" results. As demonstrated in the second example in **Figure 4**, this initial test result by single staining showed green fluorescent cells in the Tester BIOCHIP and no signals in the Negative-Control BIOCHIP. Our experimenter however noticed that almost all the cultured cells on the Tester BIOCHIP were green fluorescent, and decided to re-test this sample by double staining. Experimentation with either double-labeling approaches, with or without fixation following clinical sample labeling, failed to identify any colocalization between the human sample and the mouse mAb in NMDAR+ cells on the Tester BIOCHIP. This clinical sample was thus considered "false-positive," since the green fluorescent signals shown on the Tester BIOCHIP did not result from binding to NMDA receptor.

This double-labeling approach could also be used to verify CSF test results. As demonstrated in the second example in **Figure 5**, incorporation of a fixative step after staining with the patient's sample and before staining with the mouse anti-NR1 also increased % colocalization (**Figure 5** bottom—patient B:

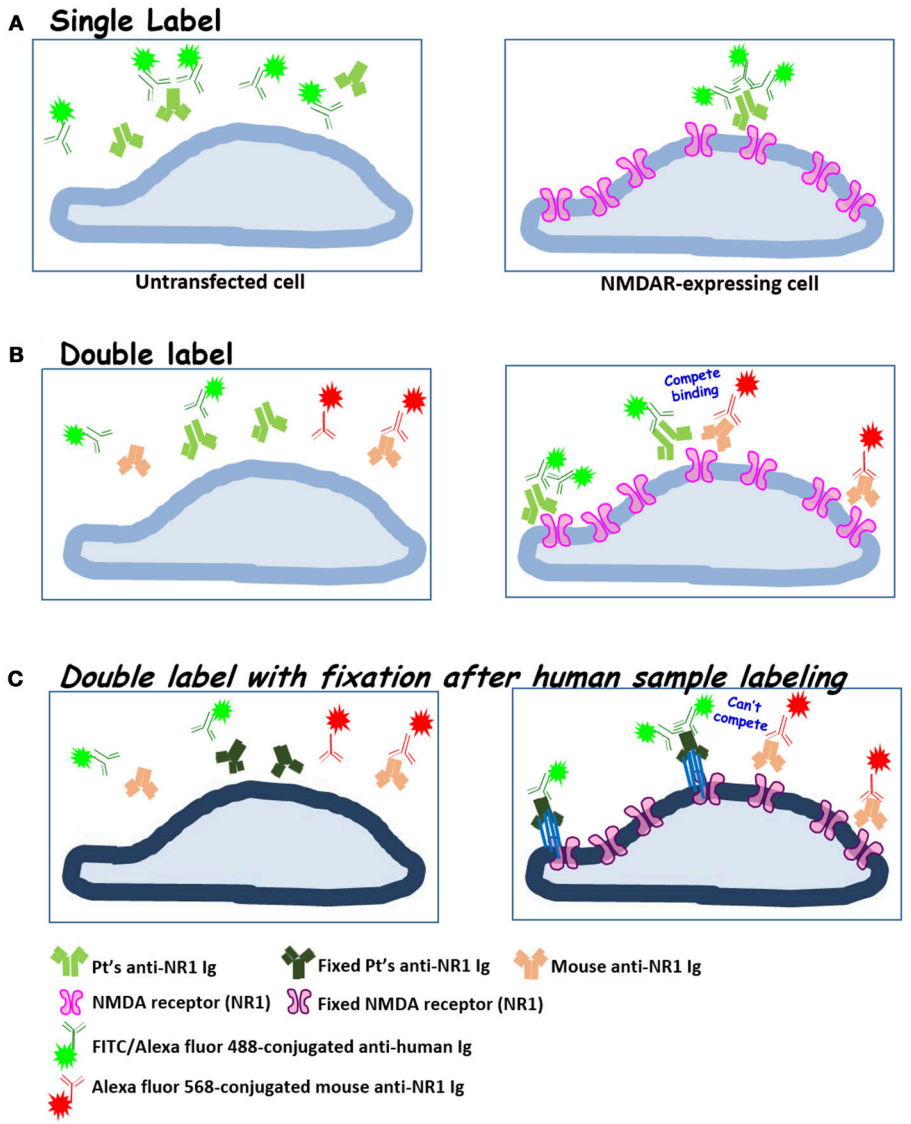


FIGURE 3 | The diagrams illustrate our experimental approaches to validate anti-NMDAR test results by double labeling with a well-characterized mouse anti-NR1 mAb. **(A)** Single labeling with human blood or CSF samples (standard protocol); **(B)** sequential double labeling that starts with a human sample (green) and then a mouse anti-NMDAR mAb (orange-red); **(C)** incorporation of brief fixation (blue bars indicating chemical crosslinkers) after clinical sample labeling. Mouse anti-NR1 mAb thus can no longer compete with human antisera for binding to NMDA receptor, as in **(B)**. Fixed cell membrane and proteins were represented in darker hues.

R_{coloc} ~0.09 [without fixation]→ R_{coloc} ~0.28 (with fixation)). Intriguingly, the CSF autoAbs of patient A exhibited similar degrees of colocalization with the mouse anti-NR1, regardless of whether there was a fixative step after initial sample labeling [**Figure 5** top—patient A: R_{coloc} ~0.51 (without fixation) vs. 0.46 (with fixation)]. But for the blood sample of patient A, the degree of antibody colocalization was enhanced with fixation (**Figure 4**, top). These differences suggest that the avidities or the composition of anti-NMDAR autoAbs from the CSF and from the blood samples of patient A were different, because they were both compared experimentally to the same mouse anti-NR1 clone.

Differentiation of Anti-NMDAR From Anti-nuclear AutoAbs

We also improved the accuracy of anti-NMDAR diagnostics by adding DAPI. This allowed experimenters to evaluate immunostaining patterns of clinical antisera, and to simultaneously identify all cell nuclei and estimate the percentage of positively-stained cells in a tester BIOCHIP. As NMDA receptors are expressed on the plasma membrane and the membranes of endoplasmic reticulum and the Golgi apparatus, the degree of colocalization between anti-NMDAR antisera and cell nuclei should be zero or extremely low (**Figure 6** top: an NMDAR-positive sample with $R_{coloc\ to\ DAPI}$ ~0).

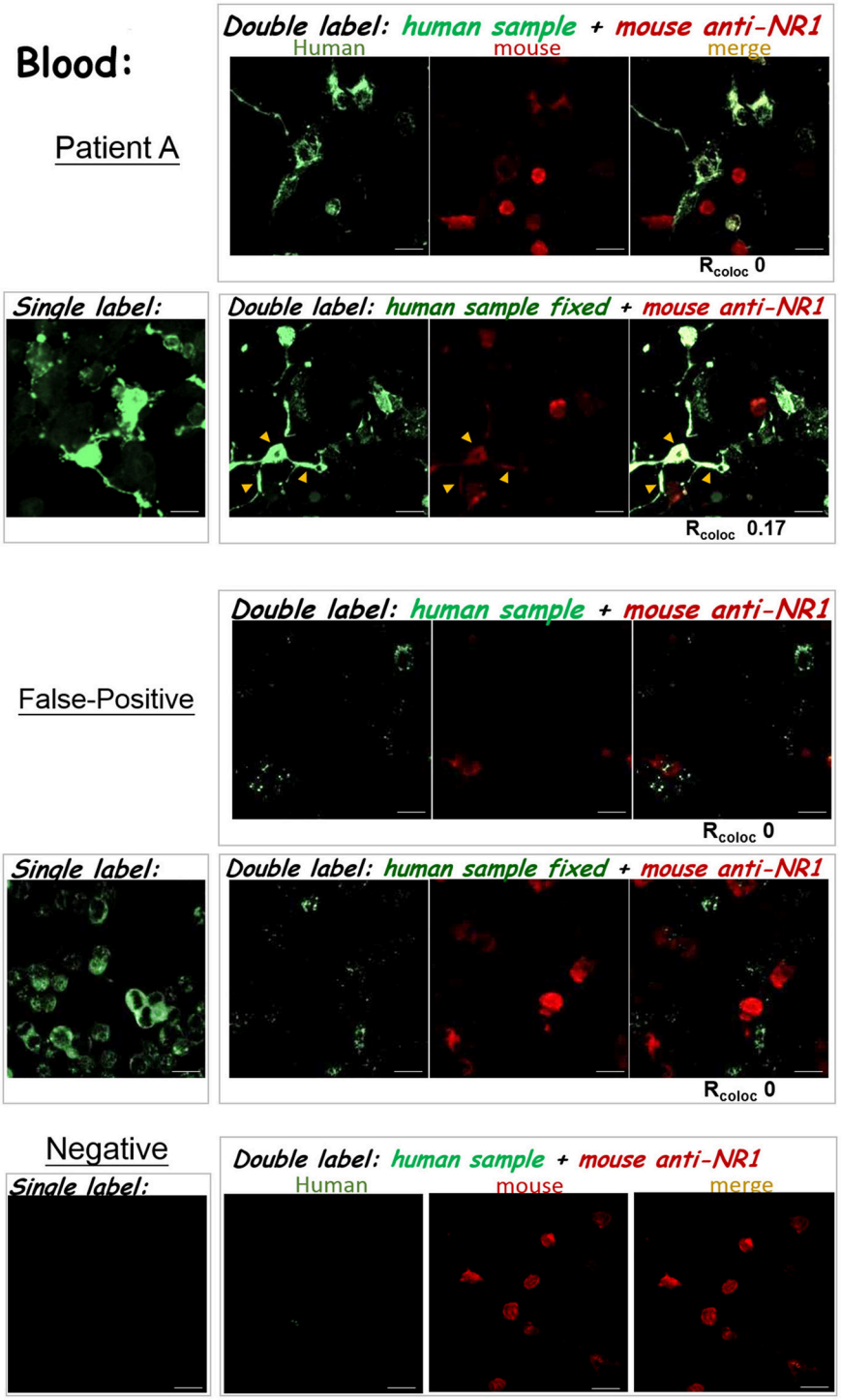


FIGURE 4 | The results of anti-NMDAR autoAb tests were confirmed by double labeling. The three experimental protocols utilizing the IIFT kit (as illustrated in **Figures 3A–C**) were compared. The degree of green/red colocalization, represented by R_{coloc}, was indicated beneath each dual-color merged image. **Top**—Patient A: The degrees of colocalization between a diluted plasma sample from patient A and the mouse anti-NR1 mAb improved after incorporating glutaraldehyde fixation following sample labeling (R_{coloc} ~0 → ~0.17). **Middle**—False-positive: A diluted blood sample showed positive signals by the standard single-labeling protocol (left images), and were later deemed "false-positive" by both double-labeling tests (right panels). This sample with "false-positive" results failed to colocalize with heterologously-expressed NMDAR by either tests illustrated in **Figures 3B,C**. **Bottom**—anti-NMDAR-negative: No green fluorescence was shown in the Tester BIOCHIPs by single or double labeling with an anti-NMDAR-negative plasma sample. Yellow arrowheads pointed to sites of colocalization of green and red fluorescence (overlay in yellow-orange color). Scale bars, 20 μm.

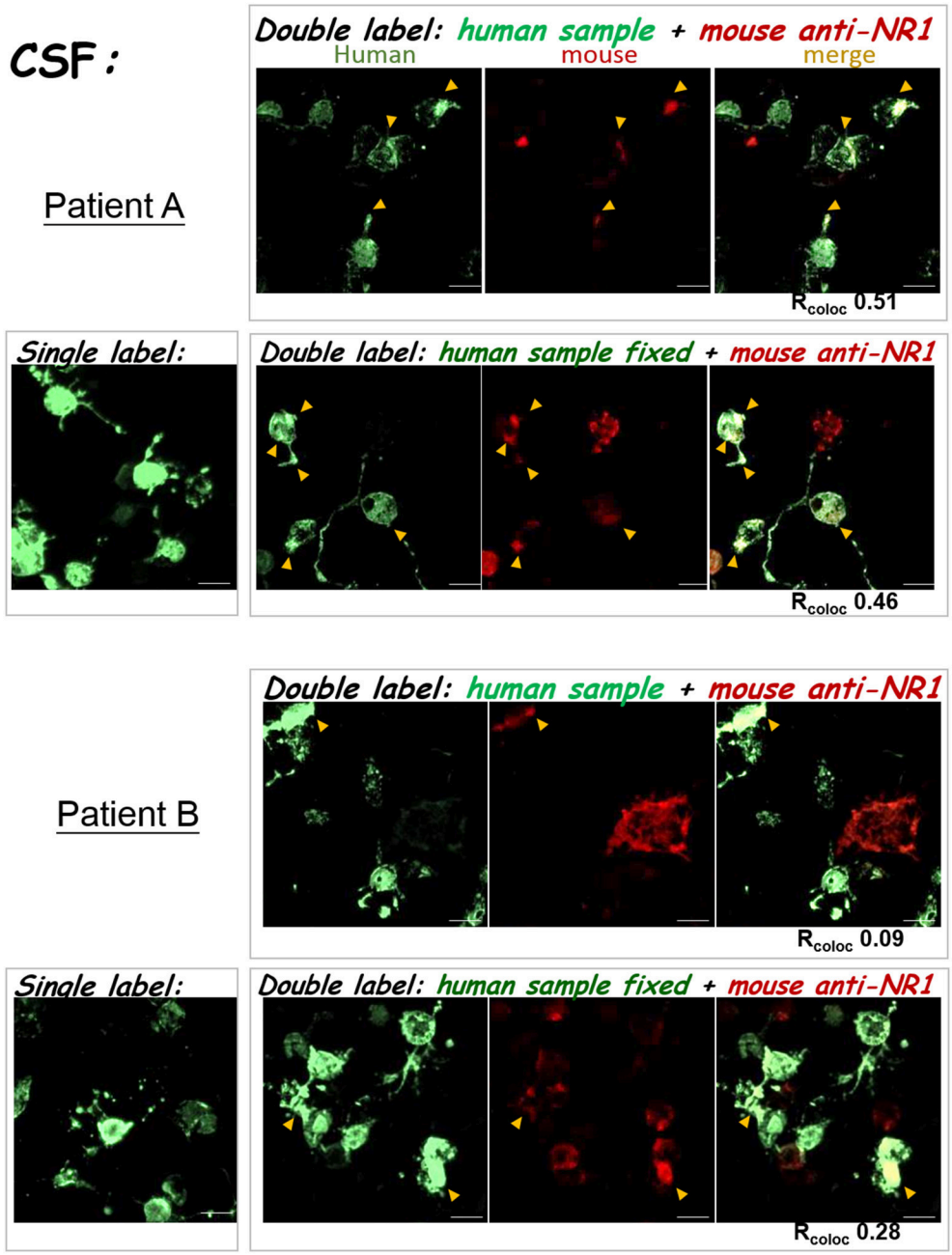


FIGURE 5 | Double labeling with the mouse anti-NR1 mAb confirmed test results for clinical CSF samples. The left images were single-labeling results for the two CSF samples. The right images were from the two double-stain protocols (as in **Figures 3B,C**). **TOP:** CSF test results from Patient A. **BOTTOM:** CSF test results from patient B. Yellow arrowheads pointed to sites of colocalization of green and red fluorescence (overlay in yellow-orange color). Scale bars, 20 μm.

By DAPI labeling, we had found an anti-NMDAR-positive sample that also showed substantial colocalization to cell nuclei in the Tester BIOCHIP (**Figure 6** bottom: $R_{coloc\ to\ DAPI}$ ~0.46), but not to cell nuclei in the Negative-Control BIOCHIP. Notably, heterologous expression of NMDA receptors requires NR2, which is also an autoimmune target in neuropsychiatric systemic lupus erythematosus (NPSLE) (26, 27). So we suggested further testing and evaluation for this patient. Thus, incorporation of DAPI stain in an anti-NMDAR test could potentially help differentiate anti-NMDAR encephalitis from other types of autoimmune insults in the CNS.

A Workflow for Lab Testing of Anti-NMDAR AutoAbs

Table 2 summarizes anti-NMDAR tests performed with our optimized single-labeling protocol and some further validated

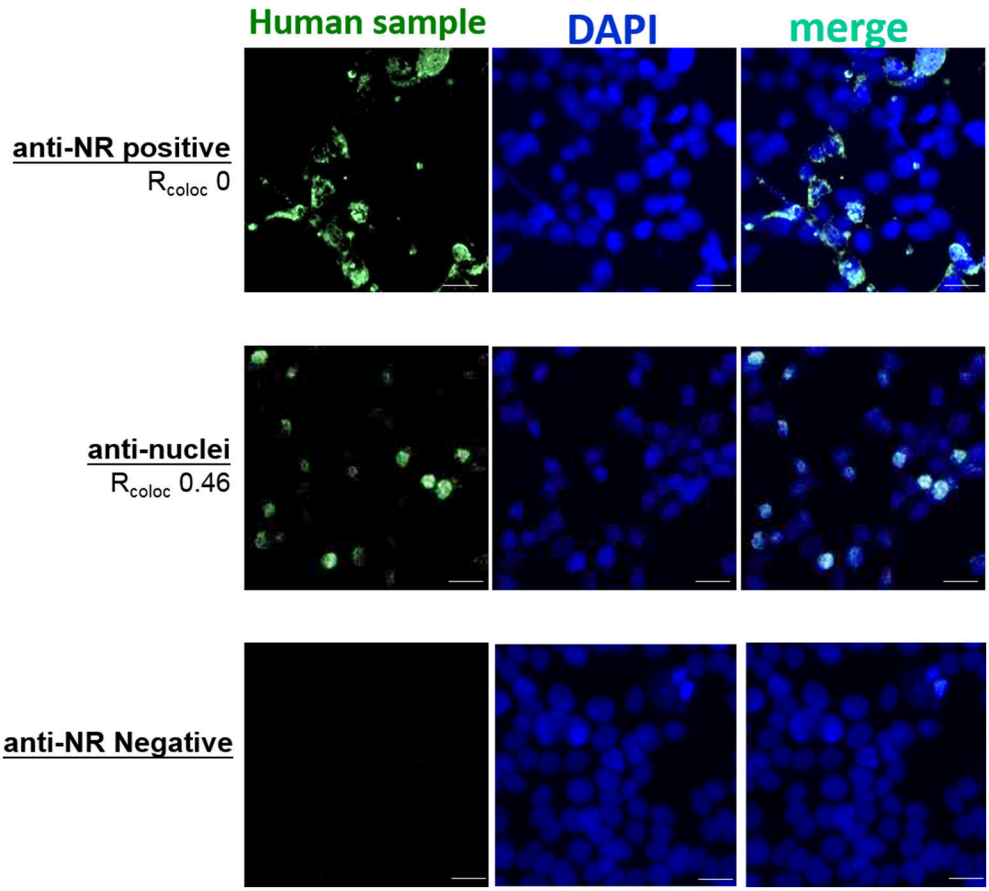


FIGURE 6 | Inclusion of DAPI stain in anti-NMDAR tests helped differentiate immunostaining patterns by anti-NMDAR antibodies from that by nucleic acid-reactive substances. The degrees of colocalization between labeling of a clinical sample (green fluorescence) and labeling by DAPI (blue fluorescence) were expressed in R_{coloc}. The images from an anti-NMDAR (NR)-positive clinical sample (top example: $R_{coloc\ to\ DAPI}$ ~0) and a clinical sample primarily reacting to cell nuclei (middle example: $R_{coloc\ to\ DAPI}$ ~0.46) were put together for comparison. The images from an anti-NMDAR-negative sample showed no green fluorescence (bottom example). Scale bars, 20 μm.

with the double-labeling protocols. For convenience and consistency of lab testing, our workflow (**Figure 7**) utilized all the reagents from the anti-Glutamate Receptor IIFT kit but the secondary FITC probe and glycerol. We replaced FITC with superior fluorophore Alexa Fluor 488 as the detection probe (**Figure 1**). We also replaced glycerol with a DAPI-containing mounting medium, which allowed assessment of % fluorescence-labeled cells and correct interpretation of immunostaining patterns (**Figure 6**).

Indeterminate results (**Figure 7**) may cast doubts on whether some component in the clinical sample could indeed bind specifically to NMDA receptor. So if a significant fraction of the control cells that lack NMDAR expression (e.g., >5%) is labeled with green fluorescence, this is NMDAR-independent binding and could confound interpretation of the results from NMDAR+ cells (Tester BIOCHIPs). If the fraction of fluorescent cells exceeds 50% on the Tester BIOCHIP, this result should also be dealt with caution since the fraction of NMDAR+ cells on a commercial Tester BIOCHIP rarely exceeds 50% (**Figure 7**). Additionally, if the fluorescent pattern is atypical that of the normal expression of a surface receptor (e.g., lack of plasma membrane expression), the fluorescent signal is likely NMDAR-independent, too.

Our workflow is supplemented with a "double-labeling" option in case when single-labeling experiments show "indeterminate" results and require verification. Double labeling with the well-characterized mouse anti-NMDAR mAb marks the subcellular location of heterologously-expressed NMDA receptor, allowing an experimenter to determine whether the fluorescent signal is NMDAR-specific or not by direct visual assessment of the merged image of green and red fluorescence (**Figure 7**). As compiled in **Table 2**, the double-labeling tests were performed either for validation of ambiguous results from standard single stain, or for assessment of possible epitope shifts, particularly in serious, recurrent patients.

DISCUSSION

The recent emergence of anti-NMDAR encephalitis (28), which is frequently encountered in psychiatric services, reminds

TABLE 2 | A summary of the anti-NMDAR autoAb tests done with our optimized approach for patients suspected of anti-NMDAR-related autoimmune encephalopathy.

Blood anti-NMDAR test results	Number of blood samples tested*	Number of CSF samples tested*	Number of CSF results matched to blood results#	%CSF-blood result matches	Number of double-stain tests performed
Positive**	**37**	**20**	**17**	**85% (17/20)**	**5**
initial titer 1:10	*17*	*9*	*8*	89% (8/9)	*1*
initial titer 1:32	*13*	*4*	*3*	75% (3/4)	*1*
initial titer 1:100	*4*	*4*	*3*	75% (3/4)	*1*
initial titer 1:320	*3*	*3*	*3*	100% (3/3)	*2*
Negative	**39**	**8**	**8**	**100% (8/8)**	**2**

All patients suspected of anti-NMDAR encephalitis or referred by other hospitals were first tested with blood samples using our modified protocol based on EUROIMMUN IIFT (as outlined in **Figure 7). Suspected patients presenting milder symptoms (e.g., predominantly psychiatric presentation) did not usually provide CSF samples, unless their blood test results later suggested a likelihood for the disease. So the number of CSF testing was lower than that of blood testing for the negative and lower titer groups.*

***The initial titer was generally determined with the blood sample retrieved when a patient was first suspected of anti-NMDAR encephalitis or referred by other hospitals. We only provide positive or negative findings for CSF samples.*

#The three cases that show discordance between blood and CSF test results were all due to negative CSF findings but positive blood findings.

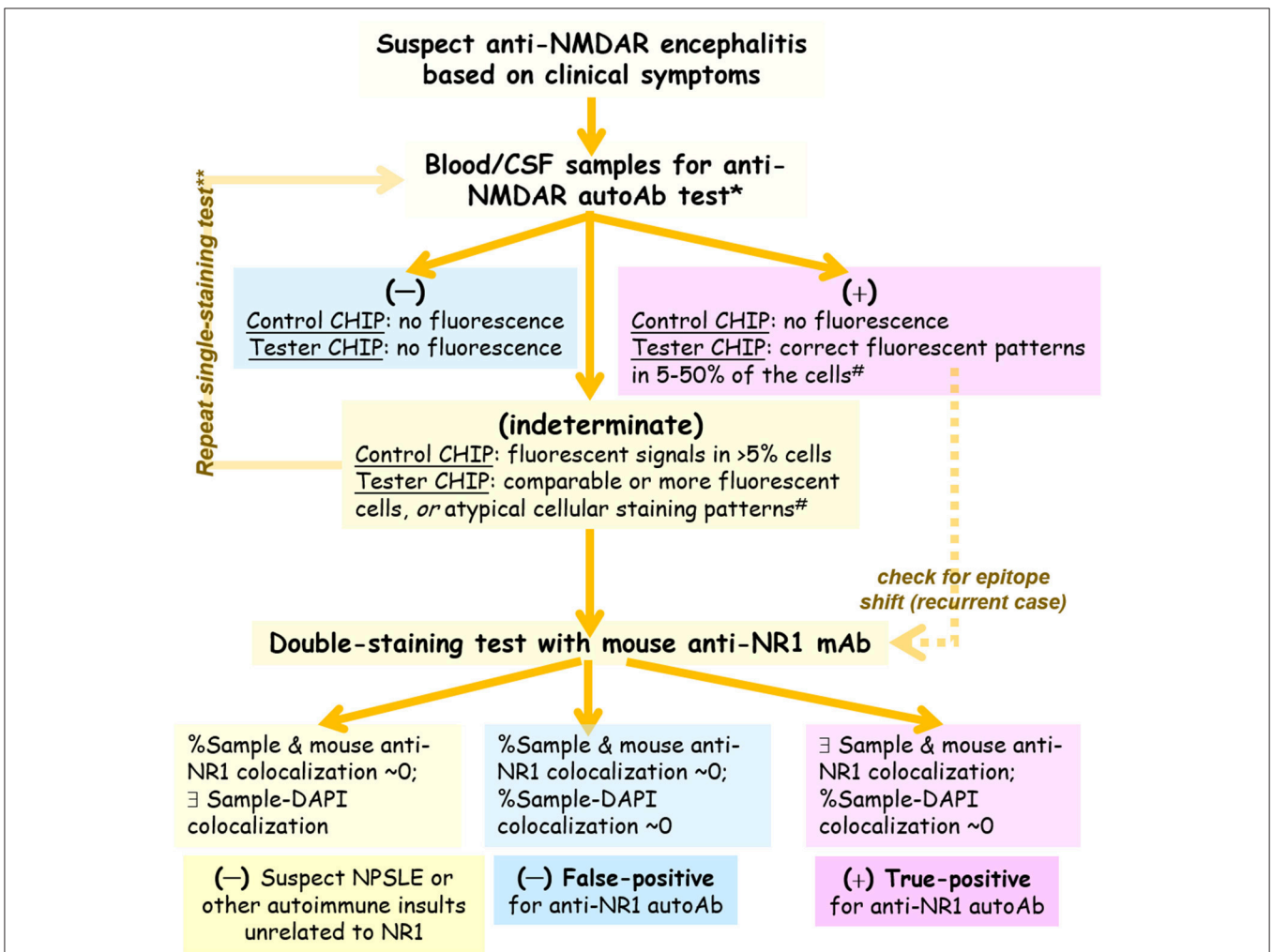


FIGURE 7 | An optimized workflow for anti-NMDAR diagnostic bioassay. *The anti-NMDAR autoAb test utilizes reagents from EUROIMMUN's IIFT kit, with two changes: (1) the detection probe FITC provided by the kit is replaced by Alexa fluor 488 for higher sensitivity; (2) glycerol provided by the kit is replaced by a DAPI-containing mountant for marking cell nuclei. #An atypical cellular staining pattern does not reflect the normal subcellular localization of membrane receptor such as NMDAR (e.g., fluorescent signals absent from the plasma membrane or present inside cell nuclei). **After reporting an indeterminate result to the physician in charge, he or she may request re-testing using the same sample or using a newly-withdrawn sample.

us the challenges in differential diagnosis of schizophrenia spectrum disorders. The similar clinical presentations between schizophrenia and the early phase of anti-NMDAR encephalitis also raise questions on whether their pathobiological mechanisms could overlap to a certain degree. But whether anti-NMDAR autoAbs are significantly present in patients with acute psychosis or schizophrenia remains highly controversial, in part due to complexities of patient samples and mediocre sensitivity/specificity of the current mainstream method for anti-NMDAR diagnostic bioassay. Here, we tackled the latter technical issue, and developed approaches to increase its sensitivity and accuracy.

We experimentally showed that replacement of secondary probe FITC with superior Alexa fluor 488 enhanced detection sensitivity (**Figure 1**). From our experience, we also recommend the use of an advanced fluorescence imaging system, which generally provides higher image resolution and sensitivity than a basic fluorescence microscope (**Figure 2**).

To improve the accuracy of anti-NMDAR diagnostics, we developed two validation protocols for samples with initial ambiguous results. Both protocols employed second labeling with a mouse anti-NR1 mAb, after clinical sample labeling (**Figure 3**). The ensuing colocalization test helped validate or disprove uncertain results from the conventional single-stain method. These two verification protocols not only helped identify cases with "false-positive" results, but also allowed us to track whether the binding affinities of autoAbs from different stages of the disease had changed relative to the same mouse mAb (**Figure 4 5**). Simultaneous labeling with DAPI specified locations of cell nuclei and helped validate or differentiate autoimmune targets at the subcellular level (**Figure 6**). Last, these improvements on the diagnostic sensitivity and accuracy should reduce the extra efforts and cost required for repeated testing, especially for samples with indeterminate results from single stain.

Our workflow (**Figure 7**) provides a general guideline for cell-based detection of neuronal autoAbs. Because NMDA receptor is a large membrane protein, ideally the protein retains its native, membrane-bound conformation best when heterologously expressed in an appropriate mammalian cell line. Non-cell-based, conventional ELISA that requires purified NMDA receptor or its protein fragment or peptide as the source of antigen conceivably is not ideal for this purpose. Another emerging technology—Meso Scale Discovery Electrochemiluminescence (MSD-ECL), has the potential to detect multiple antibodies with ultra-sensitivity. For detection of neuronal autoAbs, MSD-ECL also needs to adopt cell-based expression systems for correctly-folded membrane proteins (autoantigens), similar to the widely-used BIOCHIP methodology. Despite all the technical difficulties ahead, multiplex MSD-ECL is perhaps the only approach that will allow simultaneous detection and differentiation of various types of autoAb-mediated autoimmune encephalitis that is much in need clinically.

LIMITATIONS

In this method paper, we implemented modification to the mainstream anti-NMDAR autoAb diagnostic method to improve detection sensitivity and accuracy. This was a study aiming to optimize the current lab diagnostic protocols using clinical samples primarily from patients suspected of anti-NMDAR encephalitis referred by neurologists and psychiatrists. We thus did not screen the prevalence of anti-NMDAR autoAbs in a large cohort of psychiatric illness (such as schizophrenia).

Test for the presence of anti-NMDAR autoAbs is critical for the diagnosis of anti-NMDAR autoimmune encephalitis. However, the presence of anti-NMDAR autoAbs in one's body fluid does not equate to anti-NMDAR-mediated disease, if relevant clinical symptoms are lacking. This is because some autoAbs may not be pathogenic if they never encounter the antigen, or if their interaction with endogenous NMDA receptor does not affect normal functions of the receptor or have any pathophysiological impacts. AutoAbs can also be transient and exhibit epitope shifts. So when a test outcome is unexpected, because of the complexity of the disease or its lab diagnostics, re-test or double-labeling verification should be considered (**Figure 7**).

AUTHOR CONTRIBUTIONS

KH designed the experiments and wrote the paper. N-CC, Y-JL, R-FT, and C-CC conducted the human trial. H-JL and Y-SL performed experiments and analyzed the data. KH and CC supported experimentation. SD and KH performed imaging analyses.

FUNDING

This work was supported by grants from Taiwan Ministry of Science & Technology (MOST 105-2314-B-195-004), and from Mackay Memorial Hospital (MMH-103-117, MMH-105-22, MMH-106-15, MMH-107-33).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2018.00661/full#supplementary-material

REFERENCES

1. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol.* (2016) 15:391–404. doi: 10.1016/S1474-4422(15)00401-9
2. Kayser MS, Dalmau J. Anti-NMDA receptor encephalitis in psychiatry. *Curr Psychiatry Rev.* (2011) 7:189–93. doi: 10.2174/157340011797183184
3. Dalmau J. NMDA receptor encephalitis and other antibody-mediated disorders of the synapse: the 2016 Cotzias lecture. *Neurology* (2016) 87:2471–82. doi: 10.1212/WNL.0000000000003414

4. Zandi MS, Lennox B, Vincent A. N-Methyl-D-aspartate receptor autoantibodies in psychiatric illness. *Biol Psychiatry* (2016) 79:e61. doi: 10.1016/j.biopsych.2015.03.034
5. Al-Diwani A, Pollak TA, Langford AE, Lennox BR. Synaptic and neuronal autoantibody-associated psychiatric syndromes: controversies and hypotheses. *Front Psychiatry* (2017) 8:13. doi: 10.3389/fpsyt.2017.00013
6. Kayser MS, Titulaer MJ, Gresa-Arribas N, Dalmau J. Frequency and characteristics of isolated psychiatric episodes in anti-N-methyl-d-aspartate receptor encephalitis. *JAMA Neurol.* (2013) 70:1133–9. doi: 10.1001/jamaneurol.2013.3216
7. Tzang R, Hsu K, Chang Y, Liu T. Immunotherapy for anti-NMDAR encephalitis: A review of paraneoplastic, autoimmune encephalopathy. *J Cancer Res Pract.* (2017) 4:1–4. doi: 10.1016/j.jcrpr.2016.08.003
8. Tzang RF, Chang CH, Chang YC, Hsu K, Chen CC. Immune modulating therapy: An effective add-on intervention for psychosis of anti-N-methyl-d-aspartate receptor encephalitis in Taiwan. *Psychiatry Clin Neurosci.* (2018) 72:624–25. doi: 10.1111/pcn.12679
9. Zandi MS, Irani SR, Lang B, Waters P, Jones PB, Mckenna P, et al. Disease-relevant autoantibodies in first episode schizophrenia. *J Neurol.* (2011) 258:686–8. doi: 10.1007/s00415-010-5788-9
10. Tsutsui K, Kanbayashi T, Tanaka K, Boku S, Ito W, Tokunaga J, et al. Anti-NMDA-receptor antibody detected in encephalitis, schizophrenia, and narcolepsy with psychotic features. *BMC Psychiatry* (2012) 12:37. doi: 10.1186/1471-244X-12-37
11. Lennox BR, Palmer-Cooper EC, Pollak T, Hainsworth J, Marks J, Jacobson L, et al. Prevalence and clinical characteristics of serum neuronal cell surface antibodies in first-episode psychosis: a case-control study. *Lancet Psychiatry* (2017) 4:42–8. doi: 10.1016/S2215-0366(16)30375-3
12. Hammer C, Stepniak B, Schneider A, Papiol S, Tantra M, Begemann M, et al. Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood-brain barrier integrity. *Mol Psychiatry* (2014) 19:1143–9. doi: 10.1038/mp.2013.110
13. Chen CH, Cheng MC, Liu CM, Liu CC, Lin KH, Hwu HG. Seroprevalence survey of selective anti-neuronal autoantibodies in patients with first-episode schizophrenia and chronic schizophrenia. *Schizophr Res.* (2017) 190:28–31. doi: 10.1016/j.schres.2017.03.012
14. Timucin D, Ozdemir O, Parlak M. The role of NMDAR antibody in the etiopathogenesis of schizophrenia. *Neuropsychiatr Dis Treat.* (2016) 12:2327–32. doi: 10.2147/NDT.S113872
15. Irani SR, Bera K, Waters P, Zuliani L, Maxwell S, Zandi MS, et al. N-methyl-D-aspartate antibody encephalitis: temporal progression of clinical and paraclinical observations in a predominantly non-paraneoplastic disorder of both sexes. *Brain* (2010) 133:1655–67. doi: 10.1093/brain/awq113
16. Beck K, Lally J, Shergill SS, Bloomfield MA, Maccabe JH, Gaughran F, et al. Prevalence of serum N-methyl-D-aspartate receptor autoantibodies in refractory psychosis. *Br J Psychiatry* (2015) 206:164–5. doi: 10.1192/bjp.bp.113.142216
17. Pathmanandavel K, Starling J, Merheb V, Ramanathan S, Sinmaz N, Dale RC, et al. Antibodies to surface dopamine-2 receptor and N-methyl-D-aspartate receptor in the first episode of acute psychosis in children. *Biol Psychiatry* (2015) 77:537–47. doi: 10.1016/j.biopsych.2014.07.014
18. Steiner J, Teegen B, Schiltz K, Bernstein HG, Stoecker W, Bogerts B. Prevalence of N-methyl-D-aspartate receptor autoantibodies in the peripheral blood: healthy control samples revisited. *JAMA Psychiatry* (2014) 71:838–9. doi: 10.1001/jamapsychiatry.2014.469
19. Titulaer MJ, Dalmau J. Antibodies to NMDA receptor, blood-brain barrier disruption and schizophrenia: a theory with unproven links. *Mol Psychiatry* (2014) 19:1054. doi: 10.1038/mp.2014.25
20. Ehrenreich H, Steiner J. Reply: to PMID 24853231. *Ann Neurol.* (2015) 77:184. doi: 10.1002/ana.24232
21. Kayser MS. Fact or fiction? Examining a role for N-methyl-D-aspartate receptor autoantibodies in psychiatric illness. *Biol Psychiatry* (2015) 77:506–7. doi: 10.1016/j.biopsych.2014.12.005
22. Ehrenreich H. Autoantibodies against the N-Methyl-d-aspartate receptor subunit NR1: untangling apparent inconsistencies for clinical practice. *Front Immunol* (2017) 8:181. doi: 10.3389/fimmu.2017.00181
23. Dunn KW, Kamocka MM, Mcdonald JH. A practical guide to evaluating colocalization in biological microscopy. *Am J Physiol Cell Physiol.* (2011) 300:C723–742. doi: 10.1152/ajpcell.00462.2010
24. Panchuk-Voloshina N, Haugland RP, Bishop-Stewart J, Bhalgat MK, Millard PJ, Mao F, et al. Alexa dyes, a series of new fluorescent dyes that yield exceptionally bright, photostable conjugates. *J Histochem Cytochem.* (1999) 47:1179–88. doi: 10.1177/002215549904700910
25. Mahmoudian J, Hadavi R, Jeddi-Tehrani M, Mahmoudi AR, Bayat AA, Shaban E, et al. Comparison of the photobleaching and photostability traits of alexa fluor 568- and fluorescein isothiocyanate- conjugated antibody. *Cell J* (2011) 13:169–72.
26. Jeltsch-David H, Muller S. Neuropsychiatric systemic lupus erythematosus: pathogenesis and biomarkers. *Nat Rev Neurol.* (2014) 10:579–96. doi: 10.1038/nrneurol.2014.148
27. Ogawa E, Nagai T, Sakuma Y, Arinuma Y, Hirohata S. Association of antibodies to the NR1 subunit of N-methyl-D-aspartate receptors with neuropsychiatric systemic lupus erythematosus. *Mod Rheumatol.* (2016) 26:377–83. doi: 10.3109/14397595.2015.1083163
28. Dalmau J, Tuzun E, Wu HY, Masjuan J, Rossi JE, Voloschin A, et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann Neurol.* (2007) 61:25–36. doi: 10.1002/ana.21050

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

BRIEF RESEARCH REPORT
published: 10 August 2018
doi: 10.3389/fneur.2018.00656

Anti-ARHGAP26 Autoantibodies Are Associated With Isolated Cognitive Impairment

Frederik Bartels[1,2], *Harald Prüss*[1,3] *and Carsten Finke*[1,2]*

[1] Department of Neurology, Charité – Universitätsmedizin Berlin, Berlin, Germany, [2] Berlin School of Mind and Brain, Humboldt-Universität zu Berlin, Berlin, Germany, [3] German Center for Neurodegenerative Diseases (DZNE) Berlin, Berlin, Germany

Autoantibodies against the RhoGTPase-activating protein 26 (ARHGAP26) were originally identified in the context of subacute autoimmune cerebellar ataxia. Further studies identified a wider clinical spectrum including psychotic, affective, and cognitive symptoms. Only a few patients reported so far had evidence of a tumor association. A prospective analysis between January 2015 and December 2017 at the Dept. of Neurology at Charité—Universitätsmedizin Berlin identified 14 patients with ARHGAP26 autoantibodies on a cell-based assay, of which three patients had additional brain immunohistochemistry staining of cerebellar molecular layer and Purkinje cells, who were therefore considered antibody-positive. In all three patients, ARHGAP26 autoantibodies were associated with tumors. In two patients, an isolated cognitive impairment without additional neurological deficits was observed. These cases thus further extend the clinical spectrum associated with ARHGAP26 autoantibodies and strengthen a potential paraneoplastic context.

Keywords: ARHGAP26, GRAF1, anti-Ca, medusa-head antibodies, neuronal autoantibodies, cognitive impairment

OPEN ACCESS

Edited by:
Thomas Seifert-Held, Graz University Hospital, Austria

Reviewed by:
Anne-Katrin Pröbstel, University of California, San Francisco, United States
Romana Höftberger, Medizinische Universität Wien, Austria

***Correspondence:**
Carsten Finke
carsten.finke@charite.de

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *30 April 2018*
Accepted: *23 July 2018*
Published: *10 August 2018*

Citation:
Bartels F, Prüss H and Finke C (2018) Anti-ARHGAP26 Autoantibodies Are Associated With Isolated Cognitive Impairment. Front. Neurol. 9:656. doi: 10.3389/fneur.2018.00656

INTRODUCTION

Autoantibodies against the RhoGTPase-activating protein 26 (ARHGAP26) were originally identified in patients with subacute autoimmune cerebellar ataxia (ACA). The first patient was described in 2010 and presented with limb and gait ataxia, dysarthria and diplopia developing over a period of 2 weeks, followed by hyperekplexia, depression, restlessness, and anxiety. Further tests revealed normal CSF cell count, but intrathecal IgG synthesis and subsequent cerebellar atrophy on MRI. No tumor was detected (1). Two more patients with ARHGAP26 autoantibodies and cerebellar ataxia were reported 3 years later (2). In one of these patients, antibody detection lead to the discovery of ovarian cancer, suggesting ARHGAP26 autoantibodies as a potential marker of a paraneoplastic neurological syndrome (PNS). The other reported patient had weight loss, but no further tumor workup was performed. In an additional case, ARHGAP26 antibodies were not only associated with cerebellar ataxia, but also with cognitive and affective symptoms, indicating a broader clinical spectrum (3). This notion was further supported by the report of a fifth case with recurrent psychotic episodes, but no signs of cerebellar ataxia (4). Recently, two more cases have been described including one with cerebellar ataxia and a history of both breast cancer and melanoma and a second case with cerebellar ataxia, tremor, myoclonus, depression, and mild cognitive deficits (5).

In summary, ARHGAP26 autoantibodies were primarily reported in patients with cerebellar ataxia, but have also been associated with additional clinical features such as psychotic symptoms, depression, and cognitive decline. A tumor was detected in some of these patients, suggesting a potential paraneoplastic etiology.

Here, we report three new cases with predominant cognitive impairment and associated malignancy, further extending the clinical spectrum associated with ARHGAP26 autoantibodies and strengthening their potential paraneoplastic context.

METHODS

Patients

Patients with a suspected autoimmune-mediated brain disorder seen at the Department of Neurology at Charité—Universitätsmedizin Berlin and 1,055 additional tumor patients were prospectively screened for neuronal autoantibodies between January 2015 and December 2017. Tumor patients had a confirmed diagnosis of melanoma, prostate, lung, breast, gastric/esophageal, or colon cancers, leukemia, or lymphoma and were recruited at the corresponding departments. Patient charts were retrospectively reviewed. The study was approved by the ethics committee of Charité—Universitätsmedizin Berlin and all patients gave written informed consent for publication.

Antibody Detection

ARHGAP26 autoantibodies were detected by cell-based assay (CBA) and immunohistochemistry. Patients were only considered antibody-positive if both assays were positive. The CBA used fixed human recombinant HEK293-cells expressing ARHGAP26 (Euroimmun AG, Lübeck, Germany). For immunohistochemistry, cryosections of brain tissue (rat hippocampus, rat cerebellum, monkey cerebellum, Euroimmun AG) were incubated with patient serum/CSF using indirect immunofluorescence. The previously described IgG cerebellar staining pattern of the molecular layer and Purkinje cells (PC) was considered positive on immunohistochemistry (1).

CBA revealed 14 ARHGAP26-positive patients with serum titers between 1:10 and 1:10,000. Of those, samples from three patients showed typical cerebellar staining on immunohistochemistry and were therefore considered antibody-positive in this study.

Neuropsychological Assessment and Cerebral MRI

Detailed cognitive assessment was performed using neuropsychological tests evaluating working memory, verbal, and visuospatial long-term memory, attention, language, executive functions, and premorbid intelligence level. In case 2, the Montreal Cognitive Assessment (MOCA) was performed, including tests for short-term memory, visuospatial abilities, executive function, attention, and language (6). In case 1, MRI data was acquired on a 1.5T Symphony Vision scanner (Siemens, Erlangen, Germany) using a coronal T2w TIRM sequence and a contrast-enhanced MPGRAGE sequence.

RESULTS

Case 1

This 84 year-old patient presented with progressive anterograde amnesia developing within a few weeks, followed by Broca's aphasia, loss of appetite, weight loss, intermittent hyponatremia, gait ataxia, and emotional instability. Neurological examination showed vertical and horizontal saccadic eye movements with occasional ocular flutter, generalized muscular atrophy, brisk tendon reflexes, gait ataxia, marked dysdiadochokinesia, and impaired fine-motor skills. Detailed neuropsychological testing revealed mild cognitive impairment with deficits of attention, word fluency, working, and anterograde verbal memory. Cerebral MRI showed marked generalized atrophy and signs of microangiopathy **(Figures 1A,B)**. Basic CSF studies were unremarkable, protein 14-3-3 was negative. The patient had a medical history of monoclonal gammopathy (MGUS) (IgM-lambda) and multiple cardiovascular risk factors including arterial hypertension (AHT), coronary artery disease (CAD) and minor posterior circulation strokes without persistent neurological deficits. Serological testing revealed ARHGAP26 autoantibodies with a 1:1,000 titer and a typical staining pattern on brain tissue, i.e., IgG staining of cerebellar molecular layer and PCs.

The patient was started on oral methylprednisone 500 mg/d for 3 days, followed by 40 mg/d for 4 weeks and subsequent weekly reduction by 10 mg. In light of a possible PNS, further diagnostic workup was recommended. Follow-up studies revealed a decrease in serum titer to 1:32 with no antibodies detected in CSF. Again, CSF basic studies were unremarkable, but CSF-specific oligoclonal bands (OCBs) were positive. Phospho-TAU/TAU, beta-amyloid and beta-amyloid ratio were unremarkable. Cerebral MRI remained unchanged and a whole-body PET-CT revealed no tumor signs. The patient received four cycles of plasmapheresis with mild improvements of short-term memory and was discharged for rehabilitation.

Three months later, the patient presented with agitation and depression. At this point, serum testing showed an ARHGAP26 antibody titer of 1:100 and the previously documented MGUS (IgM-lambda). Further hematologic work-up revealed a B-cell lymphoma, for which the patient was started on obinutuzumab and chlorambucil.

Case 2

This 73-year-old patient with prostate cancer presented with slowly progressive memory decline over the last years, mainly having trouble remembering new names and appointments. He had been diagnosed with prostate cancer 10 years before and hepatic metastases were detected a few months prior to presentation. He had a history of asthma and migraine, but had been without symptoms for over 20 years. At the time of presentation, his prostate cancer was treated with docetaxel.

His neurological examination was unremarkable, except for mild tandem gait imbalance. The Montreal Cognitive Assessment (MOCA) revealed mild cognitive impairment with 22/30 points (normal $\geq$26) with deficits in language, abstraction,

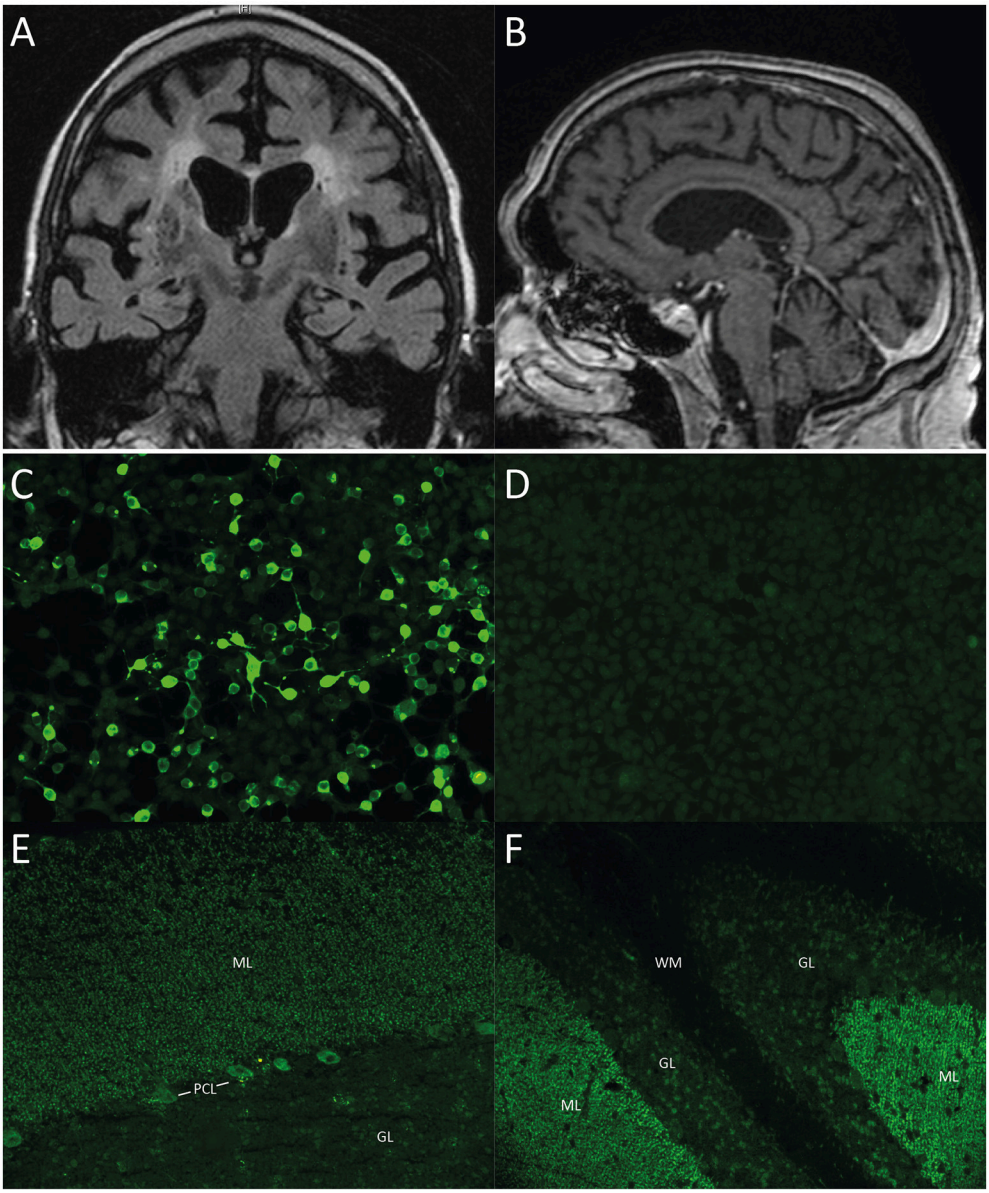


FIGURE 1 | (A, B) Cerebral MRI of case 1. **(A)** Coronal T2 TIRM image and **(B)** Contrast-enhanced MPGRAGE image showing whole brain atrophy apparent in the frontal and parietal cortex as well as in the insular region with proportional atrophy of the hippocampus and the cerebellum as well as signs of microangiopathy. **(C–F)** Immunohistochemistry, representative images of case 2. All images taken at 200x magnification. **(C)** Cell-based assay with recombinant ARHGAP26-expressing HEK293-cells, 1:100 dilution, showing binding of patient serum IgG to ARHGAP26-expressing cells. **(D)** Empty-vector control cells after incubation with patient serum (1:100 dilution) demonstrates no binding of patient IgG. **(E)** Cerebellum monkey, 1:100 dilution with patient serum IgG, showing staining pattern of molecular layer (ML) and Purkinje cell layer (PCL). **(F)** Cerebellum rat, 1:100 dilution, Note patient serum IgG binding to molecular layer, Purkinje cell layer (PCL), but not white matter (WM). ML, molecular layer; PCL, Purkinje cell layer; GL, granular layer; WM, white matter.

verbal memory, and orientation. ARHGAP26 antibodies were detected in serum with a CBA (titer 1:3,200) **(Figures 1C,D)**. Immunohistochemistry identified the typical cerebellar staining of the molecular layer and PCs (dilution 1:1,000) **(Figures 1E,F)**. Interestingly, rat hippocampal staining showed a fine granular-to-smooth pattern (1:320). 6 month later, immunohistochemistry remained highly indicative of ARHGAP26 (1:3,200), while the CBA titer increased to 1:10,000. The patient received no immunosuppressive therapy and died a few months later of metastasized prostate cancer.

Case 3

This 77-year-old man with gastric adenocarcinoma und lung metastases showed cognitive impairment in a detailed neuropsychological work-up. He was diagnosed with gastric carcinoma following abdominal pain 2 years prior

TABLE 1 | Clinical and diagnostic characteristics of all reported ARHGAP26-positive patients.

	Age	Gender	Symptoms					Cerebral MRI	CSF studies	ARHGAP26 antibody		Tumor
			Cerebellar	Psychotic	Affective	Cognitive	Other			Titer serum	Titer CSF	
Patient 1[a]	33	Female	Limb and gait ataxia, nystagmus, dysarthria	–	Depression		Hyperekplexia	Cerrebellar atrophy	Pleocytosis(44/μl). BBB disruption, OCBs pos	1:6,000	1:2,000	–
Patient 2[b]	68	Female	Gait ataxia, dysarthria, nystagmus, dizziness, nausea/vomiting, cerebellar ataxia	–	–	–	–	Empty sella, Cerrebellar atrophy	–	1:32,000	–	Ovarian cancer
Patient 3[b]	38	Male	Dysarthria, gait ataxia, dysarthria, nystagmus, dizziness				Weight loss	Cerrebellar atrophy	5 cells/ul: OCBs pos	1:3,200	–	–
Patient 4[c]	24	Male	Ataxia, dysarthria, nystagmus, oscillopsia		Flattended affect	Cognitive impairment (deficits in attention, executive function, working memory, verbal learning and recall, and spatial recognition)	Weight loss headache	Cerrebellar atrophy	BBB disruption, OCBs pos	1:20,000	1:240	–
Patient 5[d]	34	Female	–	Reccurent psychotic symptoms (impressive and aggressive behaviors, altered personality, socially inappropriate actions, mutism, apathy)	Suicidal thoughts	–	Headache	Normal	Normal	1:1,000	pos.	–
Patient 6[e]	57	Female	Limb and gait ataxia, saccadic eye movement, dizziness	–	–	–	Cutaneous hematoma	Normal	Normal	1:32	–	History of breast cancer, melanoma
Patient 7[e]	37	Female	Limb and gait ataxia, nystagmus, dysarthria	–	Depression	–	–	Normal	BBB disruption, OCBs pos	1:100	–	–
Case 1 (Patient 8)	84	Male	Limb and gait ataxia, saccadic eye movement, ocular flutter	–	Emotional instability	Cognitive impairment (deficits in attention, working memory, semantic word fluency, and anterograde verbal memory) Cognitive impairment (memory deficit)	Hyperekplexia myoclonic jerks. Loss of appetite, weight loss, intermittent hyponatremia	Genralized atrophyl	OCBs pos	1:1,000	–	B-cell lymhoma

(Continued)

TABLE 1 | Continued

	Age	Gender	Symptoms					Cerebral MRI	CSF studies	ARHGAP26 antibody		Tumor
			Cerebellar	Psychotic	Affective	Cognitive	Other			Titer serum	Titer CSF	
Case 2 (Patient 9)	73	Male	–	–	–	–	–	–	–	1:10,000	–	Prostrate cancer
Case 3 (Patient 10)	77	Male	–	–	–	Cognitive impairment (deficits in short-term memory, attention and executive function)	–	–	–	1:100	–	Gastric adenocarcinoma

[a](1); [b](2); [c](3); [d](4); [e](5).

to presentation. The patient, a smoker with 30 pack-years, had a history of CAD, AHT, peripheral artery disease, and chronic obstructive pulmonary disease. Staging revealed a pulmonary nodule that was consistent with a distant metastasis of the gastric adenocarcinoma on biopsy. The patient was started on chemotherapy with four cycles of FLOT regimen (fluorouracil, leucovorin, oxaliplatin, and docetaxel), followed by gastric resection and radiotherapy of the lung metastasis with additional four cycles of adjuvant FLOT chemotherapy.

At the time of presentation, there was no evidence of local carcinoma recurrence. The pulmonary nodule remained stable. Neurological examination was unremarkable. Cognitive testing showed deficits in short-term memory, attention, and executive function. Serum testing revealed autoantibodies against ARHGAP26 on CBA (1:100) and immunohistochemistry (1:100).

Table 1 summarizes clinical and diagnostic features of all previously reported ARHGAP26-positive patients including the cases above.

DISCUSSION

We here describe three new ARHGAP26-positive cases with isolated cognitive impairment in two patients and a tumor association in all three cases, suggesting a broader clinical spectrum and highlighting the importance to screen antibody-positive patients for malignancies.

The previously described clinical spectrum associated with ARHGAP26 autoantibodies includes cerebellar ataxia, but also psychotic, affective and cognitive symptoms (1–5). In line with the initial context of cerebellar ataxia, immunohistochemistry revealed binding to cerebellar molecular layer and PC cytoplasm and membrane (1). While a pathogenic role of ARHGAP26 autoantibodies remains unknown, tissue staining would be consistent with a clinical effect of pure cerebellar syndrome. However, other associated clinical symptoms such as depression, psychotic behavior and cognitive deficits would be more difficult to explain. Here, we observed predominant cognitive impairment in three ARHGAP26-positive patients - with in fact isolated cognitive deficits without other neurological symptoms in two of the patients. Affected cognitive domains included attention, short-term and working memory, verbal memory, semantic word fluency, and executive function.

One possible explanation for cognitive deficits in ARHGAP26-positive patients is that autoantibodies not only bind to cerebellum, but also other brain structures such as limbic regions including the hippocampus. Indeed, ARHGAP26 was found to be expressed in a subset of hippocampal neurons (4, 7). In a previous ARHGAP26-positive case with isolated psychiatric symptoms the authors concluded that the lack of cerebellar symptoms in that patient suggests other brain regions to be involved (4). Furthermore, they pointed out that in other antibody-associated neurological disorders, a wide clinical spectrum of one single antibody (e.g., anti-Hu, anti-AQ4) is common, presumably due to widespread expression of the antigen. Interestingly, in our case 2, immunohistochemistry revealed hippocampal staining,

suggesting a potential autoimmune response to the limbic system, although the presence of another, yet undefined antibody cannot be excluded.

Alternatively, cognitive symptoms could directly be mediated by cerebellar dysfunction, as conceptualized by the cerebellar cognitive–affective syndrome (Schmahmann's syndrome) (8–10). Schmahmann's syndrome occurs in patients with isolated cerebellar disease and includes deficits of attention, working and verbal memory, visuo-spatial cognition, executive function, language as well as behavior and affect (11). It is therefore well-suited to explain the cognitive and affective symptoms in patients with ARHGAP26 antibodies. However, isolated cognitive deficits in Schmahmann's syndrome have not been reported so far. Associated anatomical regions include large bilateral or pancerebellar damage (8). Due to the lack of post-mortem studies in ARHGAP26-positive patients, the targeted cerebellar regions are unknown, but MRI studies revealed generalized cerebellar atrophy. Therefore, it seems plausible that isolated cognitive dysfunction could be part of a cerebellar cognitive-affective syndrome in ARHGAP26-positive patients (3).

An underlying tumor was only found in two of the seven previously reported cases (2, 5), whereas all three of the here described patients had a cancer diagnosis before or revealed on further work-up. Autoantibodies targeting intracellular antigens are frequently associated with underlying malignancy and can precede cancer diagnosis by up to 15 months (12, 13). Therefore, delayed tumor detection in previous cases cannot be excluded. Indeed, repeated tumor screening in case 1 led to the diagnosis of B-cell lymphoma 12 months after initial presentation. With these three new cases, now 50% of all reported ARHGAP26-positive patients had a tumor-association, emphasizing the importance to screen for underlying malignancy. Interestingly, ARHGAP26 was found to be expressed in most samples of prostate cancer, suggesting a possible trigger in case 2 (7).

Immunosuppressive therapy was only administered in one patient. Here, mild improvement of short-term memory was observed after steroids and plasmapheresis, even before tumor detection and treatment. This suggests a potential benefit of immunosuppressive therapy. Tumor treatment was initiated or continued in all patients. While one patient died of his prostate cancer, long-term outcome of the other patients remains to be seen. Previous cases reported little effect of immunosuppressive therapy at best, ideally stabilizing patients with the existing deficits (5). Long-term clinical follow-up and evaluation of patient outcome after immunosuppressive and tumor therapy should be addressed in future studies.

We considered patients antibody-positive only when being positive in both assays, CBA and immunohistochemistry on brain sections. Of the 11 patients that were positive on CBA only (i.e., without the corresponding immunohistochemistry pattern), three underwent neuropsychological assessment. Interestingly, two of these patients also had isolated cognitive impairment. It is unclear whether in these cases the CBA is more sensitive than immunohistochemistry, similar to assays with other antibodies such as against the NMDA receptor. Alternatively, antibodies may bind to an epitope that is present only on recombinantly expressed antigens in the CBA or which is lost on brain sections, e.g., due to tissue processing including fixation. Further clinical correlations with more patients are required to disentangle the significance of isolated positive CBAs.

In summary, we here describe three new cases with ARHGAP26 autoantibodies with tumor association that presented with predominant cognitive impairment. This allows for the following conclusions for clinical practice: (1) The spectrum of ARHGAP26-associated symptoms is broader than initially expected and also includes isolated cognitive impairment; (2) A positive ARHGAP26 antibody-test should prompt the search for an underlying malignancy; and (3) ARHGAP26-mediated autoimmune encephalopathy is a potential, yet rare differential diagnosis in patients with cognitive impairment.

AUTHOR CONTRIBUTIONS

Data was collected by FB, HP, and CF. Figures and tables were created by FB with support of HP and CF. Manuscript was written and edited by FB, HP, and CF.

ACKNOWLEDGMENTS

This research was supported by the Berlin School of Mind and Brain. We acknowledge support from the German Research Foundation (DFG) and the Open Access Publication Fund of Charité—Universitätsmedizin Berlin.

REFERENCES

1. Jarius S, Wandinger KP, Horn S, Heuer H, Wildemann B. A new purkinje cell antibody (anti-Ca) associated with subacute cerebellar ataxia: immunological characterization. *J Neuroinflammation* (2010) 7:21. doi: 10.1186/1742-2094-7-21
2. Jarius S, Martínez-García P, Hernandez AL, Brase JC, Borowski K, Regula JU, et al. Two new cases of anti-Ca (anti-ARHGAP26/GRAF) autoantibody-associated cerebellar ataxia. *J Neuroinflammation* (2013) 10:7. doi: 10.1186/1742-2094-10-7
3. Doss S, Nümann A, Ziegler A, Siebert E, Borowski K, Stöcker W, et al. Anti-Ca/anti-ARHGAP26 antibodies associated with cerebellar atrophy and cognitive decline. *J Neuroimmunol.* (2014) 267:102–4. doi: 10.1016/j.jneuroim.2013.10.010
4. Jarius S, Wildemann B, Stöcker W, Moser A, Wandinger KP. Psychotic syndrome associated with anti-Ca/ARHGAP26 and voltage-gated potassium channel antibodies. *J Neuroimmunol.* (2015) 286:79–82. doi: 10.1016/j.jneuroim.2015.07.009
5. Wallwitz U, Brock S, Schunck A, Wildemann B, Jarius S, Hoffmann F. From dizziness to severe ataxia and dysarthria: new cases of anti-Ca/ARHGAP26 autoantibody-associated cerebellar ataxia suggest a broad clinical spectrum. *J Neuroimmunol.* (2017) 309:77–81. doi: 10.1016/j.jneuroim.2017.05.011
6. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. (2005). The Montreal Cognitive Assessment, MoCA: a brief

screening tool for mild cognitive impairment. *J Am Geriatr Soc.* 53:695–9. doi: 10.1111/j.1532-5415.2005.53221.x
7. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Tissue-based map of the human proteome. *Science* (2015) 347:1260419. doi: 10.1126/science.1260419
8. Manto M, Mariën P. Schmahmann's syndrome - identification of the third cornerstone of clinical ataxiology. *Cerebel Ataxias* (2015) 2:2. doi: 10.1186/s40673-015-0023-1
9. Schmahmann JD, Weilburg JB, Sherman JC. The neuropsychiatry of the cerebellum–insights from the clinic. *Cerebellum* (2007) 6:254–67. doi: 10.1080/14734220701490995
10. Schmahmann JD, Sherman JC. The cerebellar cognitive affective syndrome. *Brain* (1998) 121(Pt. 4):561–79.
11. Hoche F, Guell X, Vangel MG, Sherman JC, Schmahmann JD. The cerebellar cognitive affective/Schmahmann syndrome scale. *Brain* (2018) 141:248–70. doi: 10.1093/brain/awx317
12. Lancaster E, Dalmau J. Neuronal autoantigens–pathogenesis, associated disorders and antibody testing. *Nat Rev Neurol.* (2012) 8:380–90. doi: 10.1038/nrneurol.2012.99
13. Peterson K, Rosenblum MK, Kotanides H, Posner JB. Paraneoplastic cerebellar degeneration. I. A clinical analysis of 55 anti-Yo antibody-positive patients. *Neurology* (1992) 42:1931–7.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

CASE REPORT
published: 13 July 2018
doi: 10.3389/fneur.2018.00579

Clinical Management of Epilepsy With Glutamic Acid Decarboxylase Antibody Positivity: The Interplay Between Immunotherapy and Anti-epileptic Drugs

Kari-Matti Mäkelä[1]*, *Aki Hietaharju*[1], *Antti Brander*[2] and *Jukka Peltola*[1]

[1] Department of Neurology, University of Tampere, Tampere University Hospital, Tampere, Finland, [2] Department of Radiology, Medical Imaging Centre, Tampere University Hospital, Tampere, Finland

Background: There is scanty guidance in the literature on the management of patients with glutamic acid decarboxylase (GAD65) antibody associated autoimmune epilepsy (GAD-epilepsy). GAD-epilepsy is a rare distinct neurological syndrome with a wide clinical spectrum. We describe six GAD-epilepsy patients with special emphasis on the treatment timing and the relationship between immunologic and anti-epileptic therapy.

Methods: Six patients diagnosed with GAD-epilepsy in Tampere University Hospital who had received immunotherapy from 2013 to 2017 were retrospectively analyzed from patient records. Data about symptom onset, including antibody levels, magnetic resonance imaging (MRI), electroencephalograms, immunotherapy and anti-epileptic treatment timing and treatment responses were collected and analyzed. Kruskall-Wallis test was used in the statistical evaluation.

Results: All patients were female aged 9–54 at symptom onset. Three had hypothyroidism, none had diabetes, two had migraine. Five patients had very high (>2,000 IU/ml) and one had high (52–251 IU/ml) GAD65 antibody titers. All patients presented with seizure disorders. Patients who received early initiation of immunotherapy (3–10 months) responded well to treatment; patients in whom the immunotherapy was started later (15–87 months) did not respond ($p = 0.0495$). The first patient was seizure-free after 1 year of regular intravenous immunoglobulin and one antiepileptic drug (AED). The second patient developed unilateral temporal lobe T2 signal changes in MRI; she responded well to immunotherapy, experiencing a significant reduction in seizure frequency and resolution of MRI abnormalities. However, seizures continued despite trials with several AEDs. The third patient responded well to immunoadsorption and rituximab with one AED, with lowering of GAD65 titers (from >2,000 to 300). There was a long delay in the diagnosis of GAD-epilepsy in the three patients who had developed refractory epilepsy, one with hippocampal sclerosis. They all received immunotherapy but none responded. However, AED modification or vagus nerve stimulation reduced the seizure frequency in two patients. Epilepsy surgery was ineffective.

OPEN ACCESS

Edited by:
Thomas Seifert-Held,
Graz University Hospital, Austria

Reviewed by:
Anna Fogdell-Hahn,
Karolinska Institutet (KI), Sweden
Nico Melzer,
Universität Münster, Germany

***Correspondence:**
Kari-Matti Mäkelä
karimatti.makela@gmail.com

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *03 May 2018*
Accepted: *26 June 2018*
Published: *13 July 2018*

Citation:
Mäkelä K-M, Hietaharju A, Brander A and Peltola J (2018) Clinical Management of Epilepsy With Glutamic Acid Decarboxylase Antibody Positivity: The Interplay Between Immunotherapy and Anti-epileptic Drugs. Front. Neurol. 9:579. doi: 10.3389/fneur.2018.00579

Conclusions: These results highlight the importance of early detection of GAD65 antibodies in refractory epilepsy as immunotherapy can be effective if administered in the early stages of the disease when it can prevent permanent brain tissue damage.

Keywords: clinical management, glutamic acid decarboxylase antibody, limbic encephalitis, autoimmune epilepsy, case series

INTRODUCTION

Autoimmunity is increasingly being recognized as a cause of epilepsy (1). Glutamic acid decarboxylase 65-kilodalton isoform (GAD65) antibodies have been associated with multiple non-neurological and neurological syndromes including autoimmune epilepsy (2).

GAD65 is an intracellular antigen, highly expressed in the presynaptic terminals of inhibitory neurons in the central nervous system (CNS) and in pancreatic β-cells (3). GAD65 antibodies possibly serve as a surrogate marker for organ specific autoimmune disorders mediated by cytotoxic T cells (4). However, there might also be some currently unknown pathogenic surface-antigens targeted against hippocampi co-existing with the GAD65 antibody and contributing to temporal-lobe epilepsy (TLE) (5). Furthermore, the related pathological processes can lead to hippocampal sclerosis and refractory epilepsy (6). Moreover, widespread white matter changes have been observed in GAD65 antibody related limbic encephalitis (LE) (7).

Recently, anti-neuronal antibodies were detected in 20.5% of epilepsies of unknown etiology and of these, 64% were high titer GAD65 antibodies (8). Previously, it has been estimated that between 1.7% (9) and 8.7% (10) of epilepsy patients are harboring GAD65 antibodies.

GAD65 antibody associated autoimmune epilepsy (GAD-epilepsy) is a rare but distinct neurological syndrome with a wide clinical spectrum ranging from mild non-pharmacoresistant epilepsy (10) to refractory TLE (11), LE (12), and also extra-limbic encephalitis (ELE) (13). It seems that indolent GAD65 autoimmunity can develop into more severe forms over time (14).

The literature contains only a few case reports dealing with the management of refractory GAD-epilepsy (15). In addition to anti-epileptic drugs (AEDs), a plethora of immunotherapies has been tried with variable or unsatisfactory results (11, 15, 16). Overall, the response to immunotherapy is poor and only a few patients achieve seizure-freedom (17).

Since there is no clear guidance in the literature with respect to the timing or on the combination of immunotherapy with AEDs in the management of GAD-epilepsy, here we describe six GAD-epilepsy cases treated with immunotherapy during different disease stages and compare the results of immunotherapy with those achieved by AEDs.

MATERIALS AND METHODS

Study Cohort

Patients treated in Tampere University Hospital Department of Neurology for GAD-epilepsy between the years 2012 and 2017 were studied. The clinical data was analyzed retrospectively from patient records. The initial diagnosis was suspected due to the clinical symptoms and then supported by highly elevated titers of serum GAD65 antibodies. Written informed consent was obtained from the participants for the publication of this case series.

Statistics

All statistical calculations were done in R version 3.4.3 (www.r-project.org). Kruskall-Wallis test was used to compare treatment results in immunotherapy responders vs. non-responders.

Laboratory and Imaging Studies

GAD65 antibody levels were analyzed in Fimlab laboratories (Tampere, Finland) with standard clinical methods. In most patients, Euroimmun (Luebeck, Germany) anti-GAD ELISA (IgG) was used according to the manufacturer's protocol. Prior to 2014, the Medizym (Berlin, Germany) anti-GAD ELISA (IgG) was used according to the manufacturer's protocol. Most serum and cerebrospinal fluid (CSF) neuronal autoantibody panels were determined in Wieslab (Malmö, Sweden) with standard methods. In patient 1, CSF neuronal antibodies were analyzed in the Institut D'Investigacions Biomédiques August Pi I Sunyer, (Hospital Clinic, University of Barcelona, Spain). Other laboratory studies were undertaken with standard laboratory methods at Fimlab laboratories. Brain magnetic resonance images (MRI) were obtained according to a dedicated epilepsy protocol on a 3 Tesla scanner. Electroencephalograms (EEG) were obtained with standard protocols.

Therapeutic Interventions

Immunotherapy, including immunoadsorption, was administered in all patients by following generally accepted clinical principles. Accordingly, AED treatment was provided to all patients in order to achieve maximum seizure control and tolerability. Selective amygdalo-hippocampectomy (SAH) and vagus nerve stimulator (VNS) were offered to some drug-resistant patients after they had undergone comprehensive pre-surgical diagnostics according to the current standards.

Treatment Outcomes

Outcome variables were the seizure or other main symptom frequencies estimated from patient records such that an over 50% symptom reduction was considered as a good treatment response; changes in the GAD65 antibody titer levels were also determined.

TABLE 1 | Individual patient characteristics, serological and cerebrospinal fluid studies.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age at onset, years	54	19	20	9	14	16
Sex	Female	Female	Female	Female	Female	Female
Symptom onset	2014/2	2012/7	2014/6	2007/12	2011/3	2014/1
Immunotherapy initiated	2014/5	2013/5	2014/10	2015/3	2012/6	2016/3
Comorbidities	Hypothyroidism	Migraine	Hypothyroidism, migraine	Hypothyroidism	-	-
GAD65 ab, serum, IU/ml	52–251	over 2,000	over 2,000	over 2,000	over 2,000	over 2,000
GAD65 ab, CSF	Negative	Positive	Negative	Negative	Positive	Not done
Serum studies, positive	TPO, VGKC (low), B2GP (low)$^{*\#£\$\%\prime\prime}$	All negative$^{¤\$\#+x}$	All negative$^{\mu\#-x\&z}$	ICA (5120 IU/ml)$^{*£\#\wedge i}$	ANA$^{*\#£\wedge x\&}$	ANA$^{*\#\prime\prime c}$
CSF studies, positive	VGKC (low)*	All negative$^{¤!}$	All negative$^{\mu}$	All negative*	All negative*	All negative*
CSF (WBC, protein, IgG-index, oligoclonal bands)	1, 1443-923, elevated, no	11-3, normal, elevated, yes	All normal	Normal, normal, normal, yes	Normal, elevated, elevated, yes	6, normal, normal, yes

The individual laboratory studies are indicated with superscripts; only positive results are shown.

*ab, antibody; aCL, anticardiolipin ab; ANA, anti-nuclear antibody; ANCA, anti-neutrophil cytoplasmic antibody; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; B2GP, Beta-2 Glycoprotein 1 Antibodies; caspr2, contactin-associated protein-like 2; CCP, cyclic citrullinated peptide ab; CSF, cerebrospinal fluid; C, complement; DNA, Deoxyribonucleic acid; ENA, Extractable nuclear antigen; GABA, gamma-aminobutyric acid; GAD65, Glutamic acid decarboxylase 65-kilodalton isoform; HbA1c, Hemoglobin A1c; HHV, Human herpesvirus; HIV, Human immunodeficiency virus; HSV-PCR, herpes simplex virus polymerase chain reaction; ICA, islet cell antibodies; LGI1, leucine-rich glioma inactivated 1; mGluR, metabotropic glutamate receptor; NMDA, N-Methyl-D-aspartate receptor; MPO, myeloperoxidase ab; PR3, anti-proteinase 3; RF, rheumatoid factor; RNP, ribonucleoprotein; SSA, anti-Sjögren's-syndrome-related antigen A; SSB, anti-Sjögren's-syndrome-related antigen B; TPO, thyroid peroxidase; TSH, thyreotropin; TTGA, Tissue transglutaminase ab; VGKC, voltage gated potassium channel; WBC, white blood cells; *AMPA-1, caspr2, GABA-B, LGI1, mGluR1, mGluR5, NMDA; $^{\#}$ampiphysin, ANA, ANCA, DNA, ENA $^{\$}$borrelia, aCL, B2GP; $^{£}$CV2, Hu, Ma1, Ma2, Ri, Sox1, Yo; ¤ NMDA,VGKC, AMPA-1, GABA-B, HHV-6; $^{\mu}$ NMDA, VGCK; $^{\%}$HIV, 14-3-3; +TTGA; $^{-}$MPO; $^{\prime\prime}$TPO; $^{\wedge}$RNP, SSA, SSB; ^{i}ICA $^{!}$HSV-PCR; ^{x}C3, C4; $^{\&}$RF; ^{z}CCP, HbA1c, TSH, thyroxine; c, cryoglobulin.*

RESULTS

All six patients were female aged 9–54 at symptom onset (**Table 1**) and presented with seizure disorders (**Table 2**). Patients 1–3 displayed a positive response whereas patients 4–6 exhibited a negative response to immunotherapy; in the former group, the mean delay from symptom onset to immunotherapy initiation was only 5.7 months (range = 3–10 months) whereas in the latter group, it was significantly longer, 66 months (range = 15–87 months) $p = 0.0495$.

A 54-year-old woman (patient 1; **Figure 1A**) presented in the emergency department with a few weeks' history of cognitive decline and fluctuating vertigo, aphasia and tremor. The neurological examination detected a fine tremor in all limbs and total aphasia. The EEG revealed non-convulsive status epilepticus (NCSE) without definitive lateralizing or localizing features and this was treated with intravenous immunoglobulin (IVIg) and IV AEDs. The NCSE resolved within 24 h. However, she experienced several relapses which mostly started with speech difficulties leading to total aphasia, confusion, anxiety, mild gait abnormality and tremor. NCSE relapsed three times and of these two were treated successfully with IVIg. One NCSE was successfully treated with propofol. Ultimately, the patient was suffering only a mild speech impairment and gait disturbance at the end of her immunotherapy cycle. Because of no relapses for 3 years with IVIg, the gradual reduction of dosage and increase of treatment interval is ongoing. The patient is still on AED monotherapy.

A 19-year-old woman (patient 2; **Figure 1B**) was brought to the emergency department with daily focal impaired awareness seizures (FIAS) (18, 19) and complaints of memory impairment. TLE was diagnosed and the patient was almost symptom-free for 6 months with one AED, experiencing only mild aura symptoms once a month. Her seizure frequency increased and a second AED was initiated but with no clear response. GAD-epilepsy was diagnosed during further examinations and her response to immunotherapy was dramatic, resulting in almost complete resolution of seizures. A follow-up MRI revealed a novel left temporomesial signal change and edema correlating with the EEG findings (**Figure 2**). In later follow-up MRIs after repeated immunotherapy, the signal changes had started to resolve and in due course, disappeared completely. A mild memory impairment was confirmed in the neuropsychological examination; this did not respond to immunotherapy. The patient continued to have only a few FIAS daily. Immunotherapy was eventually terminated since it did not provide any further reduction in her seizure activity and the MRI abnormalities had resolved. This caused neither increase in seizure frequency nor worsening of her condition. She is still experiencing regular FIAS and is being treated with four AEDs.

A 20-year-old woman (patient 3; **Figure 1C**) presented in the emergency department after focal to bilateral tonic-clonic seizures (FBTCS). On arrival, she had mild left sided weakness and aphasia which soon resolved and she was discharged. For a few weeks before the seizure, she had experienced mild cognitive symptoms, mainly confusion. Headache, left-sided weakness and the feelings of confusion relapsed without there being any seizures. GAD-epilepsy was diagnosed early and immunotherapy initiated to prevent worsening of the symptoms. AED was provided mainly for migraine prevention. There

TABLE 2 | Seizure types, imaging studies, treatments, and treatment responses.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Seizure types (18)	NCSE	FIAS	FBTCS, FIAS, FAS	FIAS	FIAS	FBTCS, FAS
Other symptoms	Fast cognitive decline, vertigo, tremor, dystonia, aphasia, hallucinations.	Memory defect, depression, vertigo.	Headache, cognitive impairment, tremor, anxiety, left sided weakness.	Cognitive slowing, nausea, depression.	Memory problems, compulsive thoughts, fear, anxiety.	Eczema, joint symptoms.
Epilepsy type	Focal (onset unknown)	Focal (bitemporal)	Focal (temporal)	Focal (bitemporal)	Focal (bitemporal)	Focal (multifocal)
EEG	During the SE episode slow wave discharges bilaterally with frontal maximum.	Ictally left or right temporal lobe discharges.	Interictal normal, no ictal recordings.	Ictally left or right temporal lobe discharges.	Ictally left or right temporal lobe discharges.	Ictally left or right widespread discharges without definitive localizing features.
MRI	Normal	Left temporomesial T2 signal change which resolved after treatment.	Normal	Normal	Left hippocampal sclerosis.	Marginal right hippocampal atrophy.
Immunotherapies	IVIg, MP, PR, RTX, MMF	IVIg, MP, PR, MMF, RTX, IA	IVIg, MP, IA, RTX	IVIg, MP, IA, RTX	IVIg, AZP, IA, RTX	IVIg, HCQ
Current AEDs	LCM	CBZ, LCM, LEV, ZNS	TPM	OCZ, ZNS	AZM, LCM, ZNS	ECZ, LEV, OCZ
Prior AEDs	CBZ, LEV, LZP, PEH, TPM, VLP, CLB	OCZ	–	LEV	CBZ	CBZ, CLB, LEV, OCZ, PRG, VLP
Epilepsy surgery	No	No	No	Yes, left temporal lobe, no HS. VNS.	Yes, left temporal lobe, HS.	No
Treatment response	Symptom-free after 1 year with regular IVIG.	Good response to IA, nevertheless refractory epilepsy.	Good response to IA and RTX, however multiple relapses.	No response to late immunotherapy, response to VNS.	No response to late immunotherapy, surgery or AED.	No response to late immunotherapy, response to AED.

AED, anti-epileptic drug; AZM, acetazolamide; AZP, azathioprine; CBZ, carbamazepine; CLB, clobazam; CP, Cyclophosphamide; ECZ, eslicarbazepine; EEG, Electroencephalography; FAS, Focal aware seizure; FBTCS, Focal to bilateral tonic–clonic seizure; FIAS, Focal impaired awareness seizure; HS, hippocampal sclerosis; HCQ, hydroxychloroquine; IA, immunoadsorption; IVIg, intravenous immunoglobulin; LCM, lacosamide; LEV, levetiracetam; LZP; lorazepam; MMF, mycophenolate mofetil; MP, methylprednisolone; MRI, Magnetic resonance imaging; NCSE, non-convulsive status epilepticus; OCZ, oxcarbazepine; PEH, phenytoin; PR, prednisolone; PRG, pregabalin; RTX, rituximab; TPM, topiramate; VNS, vagus nerve stimulation; VLP, sodium valproate; ZNS, zonisamide.

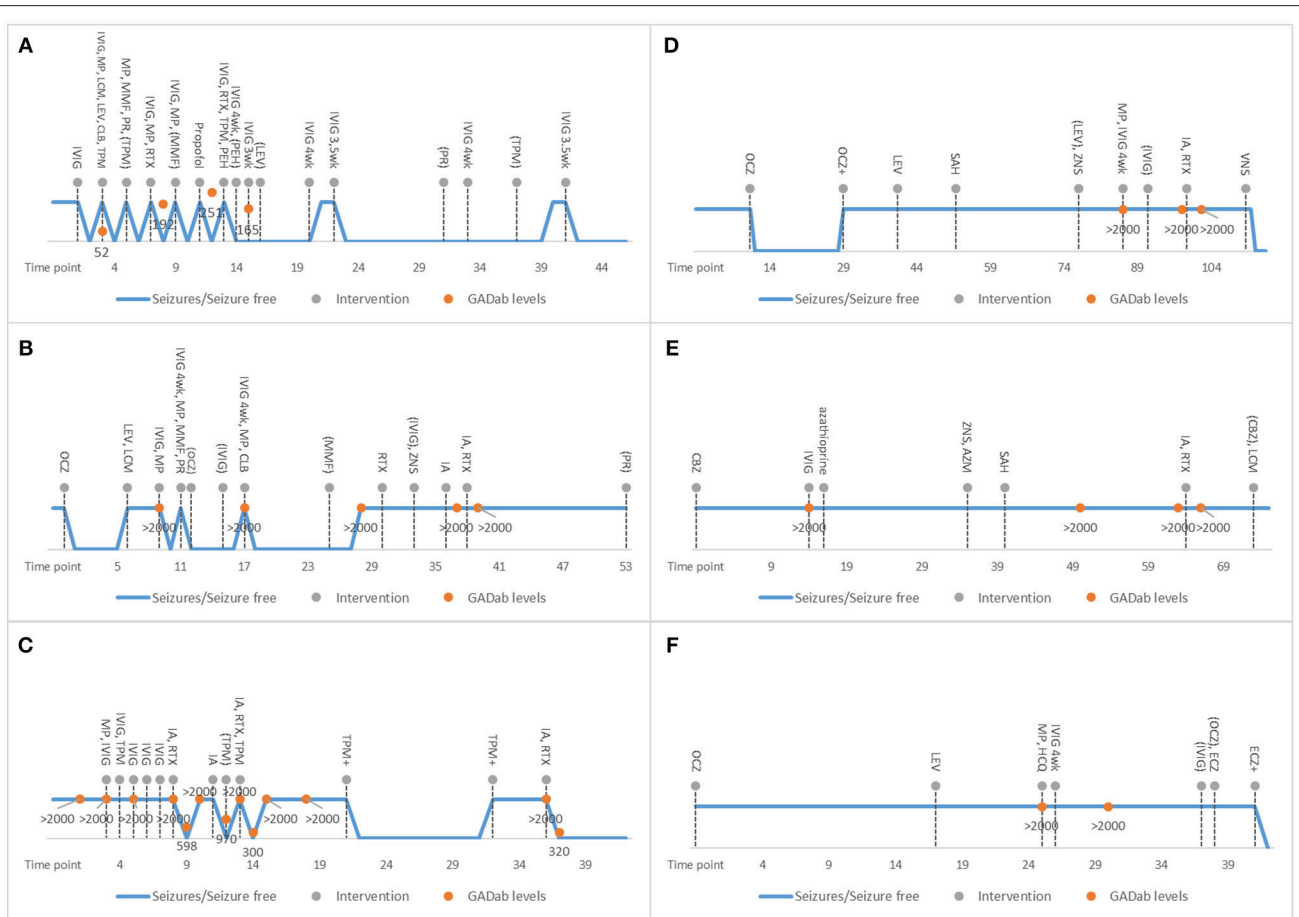


FIGURE 1 | Individual characteristics of treatment responses and therapies provided in the studied GAD-epilepsy patients are shown. X-axis shows the time-points in months starting from symptom onset. The blue line displays seizures / no seizures. Dotted lines refer to the interventions. Orange dots are GAD65 antibody levels. Discontinuation of therapies is shown in parenthesis. 4 wk means 4-week intervals. Patient 1 **(A)** responded well to early initiation of immunotherapy. With patient 2 **(B)**, there was longer delay before immunotherapy and she continued to experience seizures even after several AED and immunotherapy trials. However, her MRI pathology resolved. Patient 3 **(C)** responded well to immunoadsorption with decreasing of GAD65 antibody levels after every trial. Patients 4–6 **(D–F)** did not respond to late immunotherapy. In patient 4, a vagus nerve stimulator ultimately reduced seizure levels. In patient 6, AED modification reduced her seizure levels. AED, antiepileptic drugs; AZM, acetazolamide; AZP, azathioprine; CBZ, carbamazepine; CLB, clobazam; CP, Cyclophosphamide; ECZ, eslicarbazepine; GAD65, Glutamic acid decarboxylase 65-kilodalton isoform; HCQ, hydroxychloroquine; IA, immunoadsorption; IVIG, intravenous immunoglobulin; LCM, lacosamide; LEV, levetiracetam; LZP; lorazepam; MMF, mycophenolate mofetil; MP, methylprednisolone; OCZ, oxcarbazepine; PEH, phenytoin; PR, prednisolone; RTX, rituximab; SAH, selective amygdalohippocampectomy; TPM, topiramate; VNS, vagus nerve stimulation: wk, week; VLP, sodium valproate; ZNS, zonisamide;.

was no clear response to the initial immunotherapies and they had to be stopped due to adverse effects. The patient started to suffer anxiety and fear-like emotions after a second FBTCS. She was provided with secondary immunotherapy with immunoadsorption (IA) and there was clear resolution of symptoms and also a lowering of GAD65 antibody levels. However, she continued to experience focal unaware seizures (FAS) with mild right sided arm twitching and there was a return of the high GAD65 antibody titer levels; therefore, IA was repeated with a good response.

A 9-year-old girl (patient 4; **Figure 1D**) presented with nausea, abdominal pain and excessive swallowing and TLE was diagnosed. She was symptom-free with one AED for 1 year until she started to have 40 FIAS on a monthly basis. Multiple AEDs and epilepsy surgery did not reduce her seizure frequency. High GAD65 antibody levels were detected when performing an extensive serology panel before VNS implantation 7 years after symptom onset. Since primary immunotherapy achieved no effects, secondary immunotherapy with IA and rituximab was tried but with no symptom relief and no effect on GAD65 antibody levels. Immunotherapy was discontinued and a VNS implanted, which when combined with two AEDs, achieved an initial response, i.e., the patient became seizure-free.

A 14-year-old girl (patient 5; **Figure 1E**) presented with FIAS and TLE was diagnosed. Brain MRI revealed left hippocampal sclerosis. Multiple AEDs and epilepsy surgery did not reduce her seizure frequencies. GAD-epilepsy was diagnosed 15 months after symptom onset. She received primary immunotherapy but it offered no benefits. Some years later, IA and rituximab were tried but these neither eased her symptoms nor reduced her antibody

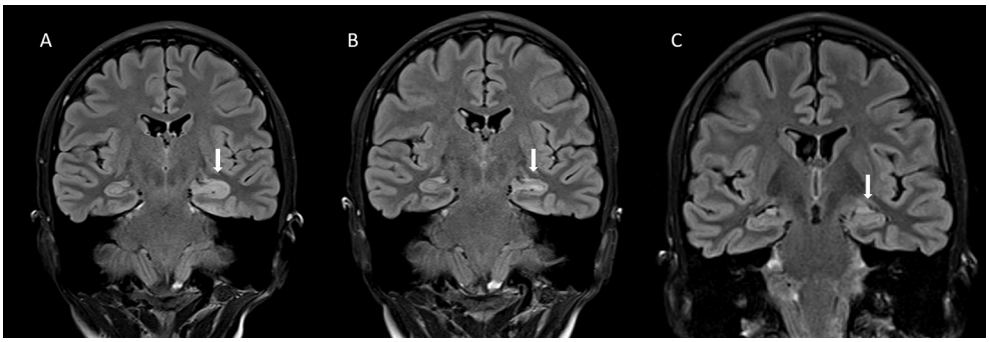


FIGURE 2 | (A) The coronal fluid attenuation inversion recovery (FLAIR) magnetic resonance (MRI) -image taken during the acute stage of the illness shows an abnormally hyperintense and swollen head of the left hippocampus (arrow). **(B)** Five months later, the finding has mostly resolved, although slight hyperintensity of the left hippocampal head can still be seen (arrow). **(C)** In a control image, 3 years and 8 months after the acute stage, the abnormal finding has totally resolved (arrow). There are no signs of atrophy in the primarily affected area.

levels. She is still experiencing regular FIAS despite therapy with three AEDs.

A 16-year-old girl (patient 6; **Figure 1F**) presented with FBTCS, eczema and joint pain. Despite treatment with two AEDs, she continued to experience FIAS and high serum GAD65 antibody levels were detected 26 months after symptom onset. Primary immunotherapy had no effect on seizures and it was discontinued due to adverse effects. Hydroxychloroquine eased her joint symptoms and this therapy was continued but she still experienced FIAS. With AED modification, her seizure levels declined and thus secondary immunotherapy was not tried.

DISCUSSION

We have described the clinical management of six patients with GAD-epilepsy. Three patients responded well to early immunotherapy initiated within 10 months after symptom onset and one patient's brain MRI abnormalities resolved after regular immunotherapy. Immunotherapy achieved no objective benefit in three patients who already had developed refractory epilepsy. Instead, AED modification or VNS implantation achieved better clinical results than immunotherapy in patients in whom the diagnosis of GAD-epilepsy had been delayed. Epilepsy surgery was ineffective in these patients.

Even though the biological process is most likely a continuum, our results suggest that the clinical course of GAD-epilepsy forms three major stages. In the first stage, reversible acute immunoactivation causes the first seizure (20). In this stage, the main focus of management should be placed on immunotherapy since this can prevent permanent brain tissue damage and stop the epilepsy from becoming refractory, as was seen with patients 1 and 3. In the second stage of GAD-epilepsy, there is already subtle irreversible brain tissue damage (4), which causes refractory epilepsy (Patient 2). During the second stage, immunotherapy can still be highly effective as was seen with the resolution of brain MRI abnormalities in patient 2. However, it seems that after the resolution of the immunoactivation, the focus in management should shift to managing the refractory epilepsy. In the third stage, there has been progressive damage leading to hippocampal sclerosis and to a more diffuse brain damage and cognitive symptoms (7). In this stage, immunotherapy seems to be ineffective and the emphasis should be on the management of the refractory epilepsy.

All of the evidence surrounding the management of GAD-epilepsy has been based on small case reports and the treatment results have been variable (15). The patients in our study largely resemble previous study populations with a female sex predominance and young age. In patients with diabetes, GAD65 antibody titer levels of over 200 IU/ml are considered high (21). In GAD-epilepsy, both high and very high (over 1,000 IU/ml) GAD65 antibody titer levels have been detected (2) which is in accordance with the findings in our patients. CSF was abnormal in all but one of our patients. Especially patients 2 and 5 showed significant immunoactivation in the CSF. Malignancy is rarely associated with GAD-epilepsy (15) as was also shown in our data. Many GAD65 antibody positive patients harbor other autoantibodies indicative of polyautoimmunity (22). Accordingly, two of our patients had ANA and one harbored TPO-antibodies. GAD-epilepsy patients can also develop diabetes or other neurological GAD65 antibody associated syndromes (3) although this was not observed in our patients. Even in non-diabetic patients, the GAD65 antibody positivity is strongly associated with thyroid disease (23) which was also present in 50% of our patients. Patient 1 had low titer antibodies against the VGKC complex but tested negative for Caspr2 and LGI1. This finding is of uncertain clinical value (24). In our previous study, we did not detect the presence of VGKC antibodies in GAD-epilepsy patients (25).

In most case reports, IVIg and MP are the standard first line immunotherapies administered (11, 15, 26) in GAD-epilepsy as was the case with our patients. Some patients have benefited also from plasma exchange (PLEX) (26, 27). The effects of IVIg and immunoadsorption have been usually unsatisfactory. However, in many of these studies, there has been a long delay from symptom onset to treatment (11, 26). We used immunoadsorption successfully in patient 3. CSF-filtration has also been tried, however with a long delay from symptom onset (11). Second line therapy usually includes cyclophosphamide and rituximab (11, 26). We administered rituximab as second line therapy but not cyclophosphamide in view of its adverse effects in

young female patients. Other immunosuppressive agents such as azathioprine and mycophenolate mofetil (MMF) have often been tried (11) with varying results, as also in our patients. Moreover, natalizumab has been tried to block T-cell entry into the CNS (11). In one case report, GAD-epilepsy was successfully managed with basiliximab (28); this was attributed to a reduction in the numbers of activated T-cells via interleukin-2 receptor blockade. Rituximab has an indirect inhibiting effect on pathogenic T-cells (29) which could in part explain its effect as the pathology of GAD-epilepsy seems to be mediated by cytotoxic T cells (4).

It is generally accepted that immunotherapy in GAD-epilepsy should be initiated as soon as possible (15), however there is no clear evidence defining when immunotherapy will no longer be effective. In many previous studies, there has been a long delay to diagnosis and immunotherapy initiation. For example, when there was a 4.5 ($\pm$0.4) year delay in immunotherapy, only every fifth patient showed any improvement (26). Furthermore, when the median disease duration was 18 months, it was reported that treatment results were poor (11).

Our results suggest that one obtains optimal results when immunotherapy is initiated during the early stages of acute immunoactivation when no brain MRI changes are yet visible as was seen with patients 1 and 3. In some case reports it has been shown similarly that early initiation of immunotherapy provides complete seizure freedom (30). Thus, there is convincing evidence that early immunotherapy can be effective in the first stage of GAD-epilepsy.

In the second stage of GAD-epilepsy, there is already irreversible brain tissue damage causing refractory epilepsy as was observed in patient 2 and in many previous case series which have demonstrated a poor treatment response to immunotherapy (11). However, we could show that the already developed brain MRI abnormalities could be resolved after regular immunotherapy. In some case reports, immunotherapy has also achieved a similar resolution of the MRI abnormalities (16, 31). There is one case report describing the empirical initiation of MP, IVIg, plasmapheresis, rituximab and cyclophosphamide in refractory status epilepticus which later proved to be GAD-epilepsy (16). After 1 month, that patient was almost symptom-free with only occasional breakthrough seizures with regular rituximab infusions and 5 AEDs with resolution of the MRI abnormalities (16). This evidence is suggesting that even during the second stage of GAD-epilepsy, immunotherapy can reverse brain tissue damage and possibly prevent a more severe clinical course of GAD-epilepsy. However, in this stage, the management of GAD-epilepsy shifts from immunotherapy to managing the refractory epilepsy.

In third stage of GAD-epilepsy, there already has occurred permanent progressive damage, possibly hippocampal sclerosis and permanent cognitive symptoms. One of our patients with late GAD-epilepsy diagnosis had developed hippocampal sclerosis, as has often been shown before (6) as the cytotoxic process seems to initially involve limbic areas (4). Moreover, widespread white matter changes have been detected in GAD-LE (7) suggesting that there is also a more widespread pathology. Late immunotherapy in refractory GAD-epilepsy had little effect, which is in line with previous evidence (26). However, there is one case report which claimed that PLEX exerted a clear effect 7 years after symptom onset even though MP and IVIg had no effect (27) and in one study, basiliximab showed temporal resolution of seizures also 7 years after diagnosis (28). For these reasons, immunotherapy should be tried at least shortly, even in late GAD-epilepsy diagnosis.

AED selection in GAD-epilepsy is undertaken according to the normal clinically accepted principles in attempts to achieve maximum seizure control and tolerability (15). Only a few GAD-epilepsy patients become seizure-free exclusively with AEDs (32). AEDs also have immunomodulatory effects which could in part explain their effect on the autoimmune epilepsies (32). All but one of our patients required multiple AEDs. However, after the symptoms were controlled with immunotherapy, some AEDs could be discontinued. Moreover, we recommend that when immunotherapy is no longer effective, it is advisable to concentrate on the management of epilepsy. One of our patients responded well to VNS which has not been shown previously in GAD-epilepsy patients. Epilepsy surgery was performed on two of our patients but it exerted no clear effect on seizure levels and this resembles the situation in other GAD-epilepsy patients (6). The better response to VNS than to epilepsy surgery might be because of the diffuse pathology in GAD-epilepsy (7). In all three of our refractory patients, however, the epileptic focus was eventually bilateral, pointing to an insidious continuing cytotoxic process. It seems that early immunotherapy can halt the destructive process and epilepsy surgery could be avoided.

A clear limitation of our study is the low number of patients and the retrospective nature of the study design. However, GAD-epilepsy is a rare entity and large patient materials are difficult to obtain. Moreover, our patients showed varying symptoms. Previously only GAD-TLE or GAD-LE patients have been studied. In this study, we combined GAD-epilepsy patients with different presentations and also the diagnoses had been made with varying delays. However, this also shows that GAD-epilepsy should be suspected in many different clinical scenarios and we have provided new evidence on the timing of the treatments.

In conclusion, these results highlight the importance of early detection of GAD65 antibodies in refractory epilepsy as immunotherapy can be effective during the early stages of the disease and it can possibly prevent the development of permanent brain tissue damage.

ETHICS STATEMENT

A case report is a medical/educational activity that does not meet the DHHS definition of research, which is: a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge. Therefore, the activity does not have to be reviewed by a IRB.

AUTHOR CONTRIBUTIONS

K-MM, AH, AB, JP conceived and designed the study. K-MM, AH, AB, JP analyzed the data. AB analyzed MRI images. K-MM, AH, AB, JP wrote the paper.

REFERENCES

1. Bauer J, Becker AJ, Elyaman W, Peltola J, Ruegg S, Titulaer MJ, et al. Innate and adaptive immunity in human epilepsies. *Epilepsia* (2017) 58(Suppl. 3):57–68. doi: 10.1111/epi.13784
2. Peltola J, Kulmala P, Isojarvi J, Saiz A, Latvala K, Palmio J, et al. Autoantibodies to glutamic acid decarboxylase in patients with therapy-resistant epilepsy. *Neurology* (2000) 55:46–50. doi: 10.1212/WNL.55.1.46
3. McKeon A, Tracy JA. GAD65 neurological autoimmunity. *Muscle Nerve* (2017) 56:15–27. doi: 10.1002/mus.25565
4. Bien CG, Vincent A, Barnett MH, Becker AJ, Blumcke I, Graus F, et al. Immunopathology of autoantibody-associated encephalitides: clues for pathogenesis. *Brain* (2012) 135:1622–38. doi: 10.1093/brain/aws082
5. Fouka P, Alexopoulos H, Akrivou S, Trohatou O, Politis PK, Dalakas MC. GAD65 epitope mapping and search for novel autoantibodies in GAD-associated neurological disorders. *J Neuroimmunol.* (2015) 281:73–7. doi: 10.1016/j.jneuroim.2015.03.009
6. Glover RL, DeNiro LV, Lasala PA, Weidenheim KM, Graber JJ, Boro A. ILAE type 3 hippocampal sclerosis in patients with anti-GAD-related epilepsy. *Neurol Neuroimmunol Neuroinflamm.* (2015) 2:e122. doi: 10.1212/NXI.0000000000000122
7. Wagner J, Schoene-Bake JC, Witt JA, Helmstaedter C, Malter MP, Stoecker W, et al. Distinct white matter integrity in glutamic acid decarboxylase and voltage-gated potassium channel-complex antibody-associated limbic encephalitis. *Epilepsia* (2016) 57:475–83. doi: 10.1111/epi.13297
8. Dubey D, Alqallaf A, Hays R, Freeman M, Chen K, Ding K, et al. Neurological autoantibody prevalence in epilepsy of unknown etiology. *JAMA Neurol.* (2017) 74:397–402. doi: 10.1001/jamaneurol.2016.5429
9. Brenner T, Sills GJ, Hart Y, Howell S, Waters P, Brodie MJ, et al. Prevalence of neurologic autoantibodies in cohorts of patients with new and established epilepsy. *Epilepsia* (2013) 54:1028–35. doi: 10.1111/epi.12127
10. Falip M, Carreno M, Miro J, Saiz A, Villanueva V, Quilez A, et al. Prevalence and immunological spectrum of temporal lobe epilepsy with glutamic acid decarboxylase antibodies. *Eur J Neurol.* (2012) 19:827–33. doi: 10.1111/j.1468-1331.2011.03609.x
11. Malter MP, Frisch C, Zeitler H, Surges R, Urbach H, Helmstaedter C, et al. Treatment of immune-mediated temporal lobe epilepsy with GAD antibodies. *Seizure* (2015) 30:57–63. doi: 10.1016/j.seizure.2015.05.017
12. Malter MP, Helmstaedter C, Urbach H, Vincent A, Bien CG. Antibodies to glutamic acid decarboxylase define a form of limbic encephalitis. *Ann Neurol.* (2010) 67:470–8. doi: 10.1002/ana.21917
13. Najjar S, Pearlman D, Najjar A, Ghiasian V, Zagzag D, Devinsky O. Extralimbic autoimmune encephalitis associated with glutamic acid decarboxylase antibodies: an underdiagnosed entity? *Epilepsy Behav.* (2011) 21:306–13. doi: 10.1016/j.yebeh.2011.03.038
14. Fauser S, Uttner I, Arino H, Scherbaum WA, Saiz A, Lewerenz J. Long latency between GAD-antibody detection and development of limbic encephalitis–a case report. *BMC Neurol.* (2015) 15:177. doi: 10.1186/s12883-015-0435-9
15. Daif A, Lukas RV, Issa NP, Javed A, VanHaerents S, Reder AT, et al. Antiglutamic acid decarboxylase 65 (GAD65) antibody-associated epilepsy. *Epilepsy Behav* (2018) 80:331–6. doi: 10.1016/j.yebeh.2018.01.021
16. Khawaja AM, Vines BL, Miller DW, Szaflarski JP, Amara AW. Refractory status epilepticus and glutamic acid decarboxylase antibodies in adults: presentation, treatment and outcomes. *Epileptic Disord.* (2016) 18:34–43. doi: 10.1684/epd.2016.0797
17. Spatola M, Dalmau J. Seizures and risk of epilepsy in autoimmune and other inflammatory encephalitis. *Curr Opin Neurol.* (2017) 30:345–53. doi: 10.1097/WCO.0000000000000449
18. Fisher RS, Cross JH, D'Souza C, French JA, Haut SR, Higurashi N, et al. Instruction manual for the ILAE 2017 operational classification of seizure types. *Epilepsia* (2017) 58:531–42. doi: 10.1111/epi.13671
19. Fisher RS, Cross JH, French JA, Higurashi N, Hirsch E, Jansen FE, et al. Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. *Epilepsia* (2017) 58:522–30. doi: 10.1111/epi.13670
20. Liimatainen S, Lehtimaki K, Palmio J, Alapirtti T, Peltola J. Immunological perspectives of temporal lobe seizures. *J Neuroimmunol.* (2013) 263:1–7. doi: 10.1016/j.jneuroim.2013.08.001
21. Schloot NC, Pham MN, Hawa MI, Pozzilli P, Scherbaum WA, Schott M, et al. Inverse Relationship between organ-specific autoantibodies and systemic immune mediators in type 1 diabetes and type 2 diabetes: action LADA 11. *Diabetes Care* (2016) 39:1932–9. doi: 10.2337/dc16-0293
22. Liimatainen S, Peltola M, Sabater L, Fallah M, Kharazmi E, Haapala AM, et al. Clinical significance of glutamic acid decarboxylase antibodies in patients with epilepsy. *Epilepsia* (2010) 51:760–7. doi: 10.1111/j.1528-1167.2009.02325.x
23. Sorgjerd EP, Thorsby PM, Torjesen PA, Skorpen F, Kvaloy K, Grill V. Presence of anti-GAD in a non-diabetic population of adults; time dynamics and clinical influence: results from the HUNT study. *BMJ Open Diabetes Res Care* (2015) 3:e000076. doi: 10.1136/bmjdrc-2014-000076
24. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol.* (2016) 15:391–404. doi: 10.1016/S1474-4422(15)00401-9
25. Liimatainen S, Peltola J, Hietaharju A, Sabater L, Lang B. Lack of antibodies to NMDAR or VGKC-complex in GAD and cardiolipin antibody-positive refractory epilepsy. *Epilepsy Res.* (2014) 108:592–6. doi: 10.1016/j.eplepsyres.2013.12.015
26. Hansen N, Widman G, Witt JA, Wagner J, Becker AJ, Elger CE, et al. Seizure control and cognitive improvement via immunotherapy in late onset epilepsy patients with paraneoplastic versus GAD65 autoantibody-associated limbic encephalitis. *Epilepsy Behav* (2016) 65:18–24. doi: 10.1016/j.yebeh.2016.10.016
27. Mazzi G, Roia DD, Cruciatti B, Mata S, Catapano R. Plasma exchange for anti GAD associated non paraneoplastic limbic encephalitis. *Transfus Apher Sci.* (2008) 39:229–33. doi: 10.1016/j.transci.2008.09.005
28. Widman G, Golombeck K, Hautzel H, Gross CC, Quesada CM, Witt JA, et al. Treating a GAD65 antibody-associated limbic encephalitis with basiliximab: a case study. *Front Neurol.* (2015) 6:167. doi: 10.3389/fneur.2015.00167
29. Dalakas MC. B cells as therapeutic targets in autoimmune neurological disorders. *Nat Clin Pract Neurol.* (2008) 4:557–67. doi: 10.1038/ncpneuro0901
30. Incecik F, Herguner OM, Besen S, Yilmaz M. Autoimmune encephalitis associated with glutamic acid decarboxylase antibodies: a case series. *Acta Neurol Belg.* (2018). doi: 10.1007/s13760-018-0880-5. [Epub ahead of print].
31. Kobayakawa Y, Tateishi T, Kawamura N, Doi H, Ohyagi Y, Kira J. A case of immune-mediated encephalopathy showing refractory epilepsy and extensive brain MRI lesions associated with anti-glutamic acid decarboxylase antibody. *Rinsho Shinkeigaku* (2010) 50:92–7. doi: 10.5692/clinicalneurol.50.92
32. Feyissa AM, Lopez Chiriboga AS, Britton JW. Antiepileptic drug therapy in patients with autoimmune epilepsy. *Neurol Neuroimmunol Neuroinflamm.* (2017) 4:e353. doi: 10.1212/NXI.0000000000000353

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 08 June 2018
doi: 10.3389/fneur.2018.00434

Evaluation of Clinical and Paraclinical Findings for the Differential Diagnosis of Autoimmune and Infectious Encephalitis

Judith N. Wagner[1], Ognian Kalev[2], Michael Sonnberger[3], Ingomar Krehan[4] and Tim J. von Oertzen[1]*

[1] Department of Neurology 1, Kepler University Hospital, Linz, Austria, [2] Department of Neuropathology, Kepler University Hospital, Linz, Austria, [3] Department of Neuroradiology, Kepler University Hospital, Linz, Austria, [4] Department of Neurology 2, Kepler University Hospital, Linz, Austria

OPEN ACCESS

Edited by:
Johann Sellner, Christian Doppler Klinik, Universitätsklinikum Salzburg, Austria

Reviewed by:
Matthias Klein, Klinikum der Universität München, Germany
Christian G. Bien, Epilepsie-Zentrum, Krankenhaus Mara, Germany

****Correspondence:***
Judith N. Wagner
judith.wagner@kepleruniklinikum.at

Specialty section:
This article was submitted to Neurocritical and Neurohospitalist Care, a section of the journal Frontiers in Neurology

Received: *29 March 2018*
Accepted: *23 May 2018*
Published: *08 June 2018*

Citation:
Wagner JN, Kalev O, Sonnberger M, Krehan I and von Oertzen TJ (2018) Evaluation of Clinical and Paraclinical Findings for the Differential Diagnosis of Autoimmune and Infectious Encephalitis. Front. Neurol. 9:434. doi: 10.3389/fneur.2018.00434

Background: The differential diagnosis of autoimmune and infectious encephalitis is notoriously difficult. For this study, we compare the presenting clinical symptoms and paraclinical test results of autoimmune and infectious encephalitis patients. A clinical algorithm for the diagnosis of autoimmune encephalitis has recently been published. We test these Graus criteria on our cohort for diagnostic sensitivity and specificity within the first week of presentation.

Methods: We included all patients seen at our department within a 10-year-period who were diagnosed with encephalitis. The discharge diagnoses served as the reference standard for testing the clinical algorithm for two conditions: use of all the clinical information available on a patient during the first week of hospital admission assuming undefined autoantibody status and microbiological test results (C1) vs. consideration of all the information available on a patient, including the results of serological and microbiological testing (C2).

Results: Eighty-four patients (33 autoimmune, 51 infectious encephalitis) were included in the study. Fifty-one (17 autoimmune, 34 infectious) had a definite clinical diagnosis. The two groups differed significantly for the presence of headache, fever, epileptic seizures, and CSF cell-count at presentation. Application of the clinical algorithm resulted in a low sensitivity (58%) and very low specificity (8%) for the diagnosis of possible autoimmune encephalitis. The latter increased considerably in the subgroups of probable and definite autoimmune encephalitis. Whereas the sensitivity of the individual diagnostic categories was clearly time-dependent, the specificity rested foremost on the knowledge of the results of microbiological testing. Anti-CASPR2- and -LGI1-associated autoimmune encephalitis and tick-borne virus encephalitis presented particular diagnostic pitfalls.

Conclusions: We define clinical symptoms and paraclinical test results which prove valuable for the differentiation between infectious and autoimmune encephalitis. Sensitivity and specificity of the clinical algorithm clearly depended on the amount of time

passed after hospital admission and knowledge of microbiological test results. Accepting this limitation for the acute setting, the algorithm remains a valuable diagnostic aid for antibody-negative autoimmune encephalitis or in resource-poor settings. The initiation of immune therapy however should not be delayed if an autoimmune etiology is considered likely, even if the diagnostic criteria of the algorithm are not (yet) fulfilled.

Keywords: encephalitis, autoimmune disease, limbic encephalitis, neuroinfectiology, neuroimmunology

INTRODUCTION

Encephalitis is an inflammatory process affecting the cerebral parenchyma. It is associated with considerable morbidity and mortality, causing focal neurological deficits, cognitive and neuropsychiatric defects, and epilepsy (1–6). The etiology can be infectious (most often viral) or autoimmune. This field has been a very dynamic one during the last decade due to the rapidly expanding spectrum of antibodies causing autoimmune encephalitis [AE; (7)] as well as to the discovery of new infectious agents or redistribution of the geographic range of known pathogens (8). The diagnosis of AE is frequently difficult as the paraclinical testing is often unremarkable: the rate of false negative magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) analysis is particularly high in the elderly with antibodies against CASPR2 and LGI1 (9). An abnormal MRI has been described in only 30% of patients with anti-NMDA-receptor-encephalitis (NMDARE) and with CASPR2-associated AE, an abnormal CSF in 20–40% of AE patients with CASPR2- and LGI1-antibodies (10). Furthermore, about 50% of all AE patients are antibody-negative (11). But unremarkable cerebral imaging and CSF analysis may also occur in infectious encephalitis (IE), particularly in the immunocompromised (12, 13). Despite of advanced molecular and serological diagnostic techniques, the causative pathogen cannot be detected in up to 60% (14). Hence, the etiology of encephalitis remains unresolved in ∼50% of all cases (15, 16).

This poses a significant dilemma as AE and IE require opposite therapeutic strategies and the early institution of therapy is associated with a more favorable outcome (17–21). In a position paper published in 2016, Graus et al. acknowledge the importance of enabling the clinician to define an early diagnosis and ground it on clinical symptoms at the time of presentation and standard paraclinical tests that are readily available (22). The authors developed an algorithm allowing for a diagnosis of probable or even definite AE solely on the grounds of clinical presentation, MRI, CSF analysis, and EEG. The aim of this paper is to test the sensitivity and specificity of this algorithm on our cohort of encephalitis patients. In particular, we aim to elucidate whether it is helpful in distinguishing AE and IE during the early stage (i.e., first week) of hospital admission. We also compare the prevalence of individual presenting symptoms and results of paraclinical tests between the two etiological groups so as to define additional markers distinguishing between them early in the course of the disease.

METHODS

Patients

We included all patients seen at our department from 2007 to 2017 who were diagnosed with a recognized sero-clinical encephalitic syndrome (such as brachiofacial dystonic seizures with detection of LGI1-antibodies) or fulfilled the criteria of the Consensus Statement of the International Encephalitis Consortium (23) for possible, probable or confirmed encephalitis:

- Major Criterion (required):

Patients presenting to medical attention with altered mental status (defined as decreased or altered level of consciousness, lethargy or personality change) lasting ≥24 h with no alternative cause identified.

- Minor Criteria (2 required for possible encephalitis; ≥3 required for probable or confirmed encephalitis):
  - Documented fever ≥38°C (100.4°F) within the 72 h before or after presentation
  - Generalized or partial seizures not fully attributable to a preexisting seizure disorder
  - New onset of focal neurologic findings
  - CSF WBC count ≥5/cubic mm
  - Abnormality of brain parenchyma on neuroimaging suggestive of encephalitis that is either new from prior studies or appears acute in onset
  - Abnormality on electroencephalography that is consistent with encephalitis and not attributable to another cause

All patient records at our department were reviewed by an experienced neurologist (JW). The relevant information was extracted from our electronic clinical information system. Patients were included in the study if they had

a) The diagnosis of definite[c] IE or AE: defined by detection of the causative pathogen/ antibody in a patient with an appropriate clinical picture OR
b) The diagnosis of a probable[c] infectious or autoimmune encephalitis: defined by a typical clinical course—i.e., monophasic <4 weeks ± prodromal symptoms in infectious and polyphasic/undulating/monophasic >4 weeks in autoimmune encephalitis and/or a clear response to either antimicrobial or immunosuppressive therapeutic agents

Qualifiers such as "probable," "possible," "definite" are specified with a superscript "c" if they refer to the clinical criteria

delineated above and with "a" if they refer to the algorithm by Graus et al.

Patients were excluded if they had a purulent encephalomeningitis, if the diagnosis did not meet the level of certainty specified above or if the diagnosis made after reviewing the entirety of a patient's records differed from the initial diagnosis at discharge.

All analyses were performed separately on the group of patients with a definite[c] diagnosis as well as on the entire cohort (probable[c] + definite[c]). Unless otherwise specified, results pertain to the former group.

Study Definitions

The confirmed discharge diagnosis was used as the reference against which the clinical algorithm was tested. We defined two conditions:

- Condition C1: the clinical information available on a patient during week 1 of his or her hospital admission was considered; their autoantibody status and the results of specific microbiological tests were assumed to be unknown
- Condition C2: all the information available on a patient was considered, including the results of serological and microbiological testing

T0 was defined as the time of onset of symptoms as reported by the patient or his family. T1 was the time of admission to our hospital. In case the patient was transferred from another hospital and all the information was available to us, we would consider T1 to refer to the external admission.

Diagnostics

Autoantibody testing was performed using immunofluorescence and line blotting for intracellular antibodies and immunofluorescence on commercially available cell-based assays for extracellular antibodies. Immunofluorescence was carried out on EUROIMMUN tissue biochips for paraneoplastic neuronal antibodies and EUROIMMUN biochips with transfected cells for antibodies against neuronal receptors. Antibodies against intracellular antigens were also tested with EUROIMMUN line blot. For Ganglioside IgG- and IgM-antibodies detection the "Buhlmann GanglioCombi" enzyme-linked immunosorbent assay was used. Standard laboratory procedures were followed according to the manufacturer's instructions. The assays were evaluated by experienced neuropathologists. The diagnostic panels represented the standard selection of antigens described at the respective points in time (for details see Supplementary Table 1). Currently, immunological testing comprises antibodies against Hu, Yo, Ri, PNMA2 (Ma2/ta), CV2, amphiphysin, PCA2, TR, SOX1, Zic4, Recoverin, GAD, Myelin, Titin, MAG, GM1, GM2, GD1a, GD1b, GQ1b, and anti-glial nuclear antibodies as well as NMDAR-/CASPR2-/GABA B-/LGI1-/AMPA-GluR1/2- and DPPX-antibodies. Antibody screening was performed in all patients discharged with the diagnosis of AE and in 11 patients with the final diagnosis of IE. Standard microbiological screening comprised PCR and/or serology for herpes simplex virus type 1 and 2 (HSV1, HSV2), cytomegalovirus (CMV), varicella zoster virus (VZV), Epstein-Barr virus (EBV), tick-borne encephalitis virus (TBEV), borrelia and cultures for bacteria and mycobacteria (CSF and serum). It was performed in all IE cases and in all but two patients with a diagnosis of AE. Further microbiological testing was guided by clinical judgement. MRI, EEG and CSF analyses were performed according to standard protocols.

Statistical Analysis

Excel and MedCalc statistical software were used for evaluation of patient data. We calculated absolute frequencies and percentages for categorical variables and the median and range for continuous variables. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for different categories defined by Graus et al. using our confirmed discharge diagnoses as a reference standard. For further sensitivity and specificity analyses, a receiver operating characteristic (ROC) curve analysis was performed. Groups were compared using the Chi-Square- and Mann–Whitney-*U*-Test. Statistical significance was assumed for $p < 0.05$.

The study was approved by the ethics committee of Upper Austria.

RESULTS

Eighty-four (44 male) patients seen in our department between January 2007 and December 2017 fulfilled the inclusion criteria. In 71 patients the discharge diagnosis was made before the publication of the diagnostic algorithm by Graus et al. making a bias unlikely. Thirty-three were diagnosed with autoimmune encephalitis (17 definite[c] AE), 51 with infectious encephalitis (34 definite[c] IE). Diagnoses in antibody negative AE included parainfectious AE/ADEM (3), Bickerstaff encephalitis (2), and seronegative limbic/autoimmune encephalitis (11).

Epidemiological and Clinical Data for the Entire (Probable[c] + Definite[c]) Cohort

Median age was 58 years (AE; range 13–87) and 57 years (IE; range 14–83), respectively. Median time lapse between T0 and T1 was 5 days (range 1–270) for AE and 3 days (range 1–100) for IE, median time to last follow-up defined as lapse between T1 and the last time the patient was seen at our department for any reason was 427 (range 5–2,364) for AE and 44 days (range 3–2,510) for IE. Eight (AE) and 2 (IE) patients were diagnosed with neoplastic disease: 1 patient each with Non-Hodgkin Lymphoma and chronic lymphatic leukemia in IE and cancer of unknown primary (2 patients), ovarial teratoma (2 patients), pulmonary adenocarcinoma (1 patient), pulmonary neuroendocrine tumor (1 patient), mesothelioma (1 patient), and prostate carcinoma (1 patient) in AE. A subset of our AE patients have been described before (24, 25).

Epidemiological and Clinical Data for the Definite[c] Cohort

This cohort included 51 patients (34 IE; 26 male). Median age was 57 years (AE; range 13–73) and 61 years (IE; range 16–77), respectively. Median time lapse between T0 and T1 was 10 days (range 1–270) for AE and 4 days (range 1–100) for IE,

median time to last follow-up defined as lapse between T1 and the last time the patient was seen at our department for any reason was 920 (range 46–2,364) for AE and 58 days (range 3–2,510) for IE. Baseline characteristics of the two diagnostic groups are summarized in **Table 1**, the microorganisms and autoantibodies detected in those patients with definite IE/AE are listed in **Tables 2A,B**.

Signs and Symptoms (Condition C1; Definite[C])

The majority of patients with IE presented with fever (94%), headache (56%), quantitative alterations of consciousness (56%), and psychiatric symptoms (including personality changes and psychomotor retardation; 82%). Speech disorders (24%), focal neurological deficits (29%), and epileptic seizures (21%) were frequently encountered as well. Focal neurological signs in IE comprised central paresis (4 patients), cerebellar symptoms (3 patients), cranial nerve palsies (4 patients) and oculomotor system disturbances (3 patients; some patients displayed two or more focal neurological symptoms).

In AE, headache, fever, alterations of consciousness and psychiatric symptoms were significantly less prevalent at presentation (0, 12, 12, and 47%, respectively). Epileptic seizures were frequently encountered (88%), rendering them the most common presenting symptom in AE. Signs and symptoms in IE and AE are summarized in **Table 3**.

Diagnostic Tests (Condition C1; Definite[C])

All patients were investigated by cranial MRI and CSF examination, and 17/17 (AE) and 27/34 (IE) by EEG at least once during the hospital stay. Comparison of the results of paraclinical testing performed during the first week after hospital admittance revealed that increased CSF cell count was significantly more common in IE patients. On further analysis of those patients who had CSF pleocytosis (i.e., 5 or more cells/μl), the IE group displayed a significantly higher median of CSF total cells. At a criterion value of ≤36 cells/μl—chosen by ROC curve analysis to maximize the Youden's index—the sensitivity of diagnosis of AE was 75%, the specificity 87.5%. The rate of positive oligoclonal bands and intrathecal immunoglobulin synthesis did not differ significantly between AE and IE patients, neither did the number of patients with pathological results on cranial MRI and EEG (regional and general slowing as well as epileptic discharges were considered pathological). The most common location of supratentorial (sub)cortical MRI changes in AE patients were the mesial temporal lobes (5/17 patients), whereas extratemporal T2-alterations in IE patients were more frequent (7/34 patients) than mesio-temporal lesions (6/34 patients). Lesions in IE were predominantly localized in the thalamus and brain stem and most often found in tick-borne encephalitis. The results of the diagnostic tests are summarized in **Tables 4**, **5**.

Signs, Symptoms, and Diagnostic Tests in the (Probable[C] + Definite[C]) Cohort

All evaluations delineated above were performed in the (probable[C] + definite[C]) cohort as well. The main difference to the analysis of the definite[C] cohort alone pertains to the frequency of cognitive symptoms and alterations of consciousness as presenting symptoms in AE vs. IE. The preponderance of patients presenting with cognitive symptoms in the entire AE cohort became significant at $p = 0.03$, whereas the difference concerning alteration of consciousness lost significance. Otherwise, all trends remained the same.

Diagnostic Algorithm (Condition C1) – (Probable[C] + Definite[C]) Cohort

Among 33 AE patients (17 definite[C] AE/16 probable[C] AE), 19 fulfilled the criteria for possible[A] AE according to Graus et al., 12 patients in whom well characterized autoantibodies

TABLE 2A | Antibodies detected in patients with definite[C] AE.

Antibody	*n*
NMDAR	5
LGI1	4
CASPR2	3
Ma2	2
Ri	1
GabaB	1
SOX1*	1
Amphiphysin*	1

The antibodies marked with an asterisk were found in the same patient.

TABLE 2B | Microorganisms detected in patients with definite[C] IE.

Microorganism	*n*
Tick-borne encephalitis virus	25
Herpes simplex virus type 1	5
Ebstein-Barr virus	2
Tropheryma whippelii	1
Varicella zoster virus	1

TABLE 1 | Characteristics of patients (definite[C]) contained in the two diagnostic groups of autoimmune (AE) and infectious (IE) encephalitis.

Diagnostic group	Total (male)	Age (median, range)	Days T0 to T1 (median, range)	Days—follow-up (median, range)	Tumor *n* (%)
Autoimmune encephalitis	17 (9)	57 (13;73)	10 (1;270)	920 (46;2364)	6 (35)
Infectious encephalitis	34 (17)	61 (17;77)	4 (1;100)	58 (3;2510)	0 (0)

T0 specifies the time of symptom onset, T1 the time of hospital admission.

TABLE 3 | Clinical symptoms of definite[C] AE and IE patients during week 1 of hospital admission.

Diagnostic group	Headache *n* (%)	Fever n (%)	Psychiatric symptoms *n* (%)	Cognitive symptomes *n* (%)	Alteration of consciousness *n* (%)	Movement disorders *n* (%)	Speech disorders *n* (%)	Autonomic dysfunction *n* (%)	Focal signs *n* (%)	Epileptic seizures *n* (%)
Autoimmune encephalitis	0 (0)	2 (12)	8 (47)	6 (35)	2 (12)	2 (12)	1 (6)	4 (24)	5 (29)	15 (88)
Infectious encephalitis	19 (56)	32 (94)	28 (82)	5 (15)	19 (56)	2 (6)	8 (24)	7 (21)	10 (29)	7 (21)
p-value	0.0001	<0.0001	0.01		0.003					<0.0001

Symptoms differing significantly between the two diagnostic groups are shaded in gray. P-values of these comparisons are given at the bottom of the table.

TABLE 4 | Results of paraclinical tests of definite[C] AE and IE patients during week 1 of hospital admission.

Diagnostic group	Pleocytosis *n* (%)	Total CSF cell count per μl (median; range)	OCB positive and/or i.th. IgG-synthesis (%)	Pathology in EEG *n* (%)	Pathology on MRI *n* (%)
Autoimmune encephalitis	8 (47)	33 (5; 200)	35	14 (82)	8 (47)
Infectious encephalitis	32 (94)	86 (7; 705)	35	23 (68)	17 (50)
p-value	0.0001	0.005			

Results differing significantly between the two diagnostic groups are shaded in gray. P-values of these comparisons are given at the bottom of the table. For total CSF cell count, only those patients showing a pleocytosis (i.e., ≥ 5 cells/μl) were considered.

TABLE 5 | Localization of hyperintense lesions on T2-/FLAIR-weighted MRI performed within 1 week of hospital admission for definite[C] AE and IE patients.

MRI changes (FLAIR)	Infectious encephalitis	Autoimmune encephalitis
Supratentorial extra-temporal	7	3
Latero-temporal	2	0
Mesio-temporal	6	5
Basal ganglia except thalamus	2	1
Thalamus	8	0
Cerebellum	0	0
Brain stem	7	0

Multiple entries per patient possible.

were detected did not enter the algorithm at this point, either because they did not meet the time criterium (i.e., progression of symptoms of <3 months) or the main clinical criterium (i.e., presentation with working memory deficits, altered mental status, or psychiatric symptoms). Three patients in this group had CASPR2-antibodies, four patients had LGI1-antibodies, and one patient each had Ma2-/Ri-/GabaB-/NMDAR- and a combination of SOX1- and amphiphysin-antibodies. They either presented with subtle cognitive deficits or personality changes after several months of symptom progression or reported seizures—rather than mental deficits—as the presenting feature. The clinical features of these 12 patients are summarized in **Table 6**.

Three of the remaining 19 AE patients qualified for the category definite[a] autoimmune limbic encephalitis due to quasi-pathognomonic bilateral, strictly mesial changes of the temporal lobe on T2-/FLAIR (fluid-attenuated inversion recovery)-weighted MRI or bilateral, mesio-temporal hypermetabolism on FDG-PET as an alternative imaging criterium approveded by Graus et al. for the diagnosis of definite[a] limbic encephalitis. All three cases (3 females, age 29–61 years) were autoantibody-negative.

One patient with the clinical diagnosis of ADEM fell into the probable[a] autoimmune category as the definite diagnosis of ADEM according to the algorithm would have required the absence of new clinical or MRI findings 3 months after symptom onset. Hence, this diagnosis cannot be made during the first week by definition.

3 patients fulfilled the criteria of clinical NMDARE, therefore being considered probable[a] autoimmune. In one of these NMDAR-antibodies could be detected, the other two patients were finally considered as autoantibody-negative AE (both tested negative for NMDAR-antibodies on 1 and 5 occasions, respectively). Four oligosymptomatic NMDAR-antibody-positive patients did not exhibit the minimum number of major symptom groups during the first week of hospital admission and therefore remained in the possible[a] AE category.

Two patients diagnosed with probable Bickerstaff encephalitis did not enter the algorithm as they failed the clinical criteria of possible[a] AE due to lack of cognitive, mental or psychiatric symptoms. They exclusively presented with ataxia and central oculomotor symptoms. GM1, GD1b and GD1a antibodies—but no GQ1b antibodies—were detected in both patients' serum.

The categories "cell-surface/onconeuronal antibodies" and "thyroid antibodies" were not considered at this point as per study design. Finally, two patients with the clinical diagnosis of antibody-negative AE actually fulfilled the corresponding criteria of the algorithm, leaving a total of 10 AE patients in the "reconsider diagnosis" category.

TABLE 6 | Characteristics of the 12 definite[C] AE patients missed by the clinical algorithm.

Sex	Age (years)	Antibody	Site of antibody detection	Main clinical complaints	T0–T1 (days)	Follow-up (days)	MRI—increased FLAIR signal	EEG	CSF—Pleocyt. (cells/μl)	Tumor
Male	57	CASPR2	Serum, CSF	Cognitive symptoms, seizures	180	1,077	Left amygdala	Left temporal epileptic discharges	Yes (7)	No
Male	30	Ma2	Serum, CSF	Cognitive symptoms, psychiatric alterations, seizures	150	987	Left temporomesial	Epileptic discharges	Yes (5)	No
Female	57	Ri	Serum	Opsoclonus	56	638	Microangiopathy	Not done	Yes (64)	Cancer of unknown origin
Male	73	LGI1	Serum	Seizures, autonomic symptoms	49	1,619	Right (para-) hippocampus	Epileptic discharges	No	Prostate carcinoma
Male	65	GABA B	Serum	Seizures, facial palsy	14	350	Normal	General slowing	No	No
Female	67	LGI1	Serum, CSF	Seizures	8	920	Right amygdala and hippocampus	General slowing	Yes (11)	No
Male	40	NMDAR	Serum, CSF	Seizures, dysarthria, dysphagia	7	385	Bilateral (sub) cortical	Epileptic discharges	No	No
Male	72	CASPR2	Serum, CSF	Seizures	150	427	Right hippocampus	Regional slowing	No	No
Male	65	LGI1	Serum	Seizures	21	267	Microangiopathy	Normal	No	No
Female	70	SOX1, Amphiphysin	Serum, CSF	Seizures, hemiparesis	10	881	Normal	Epileptic discharges	No	Neuroendocrine bronchial carcinoma
Male	64	CASPR2	Serum, CSF	Seizures	1	2,321	Normal	Normal	No	No
Female	39	LGI1	Serum	Cognitive symptoms, psychiatric alterations, seizures	270	1,431	Normal	Not done	No	No

Almost all IE patients (92%) qualified for the diagnosis of possible[a] AE under the premise that the results of microbiological testing were unknown. Four fulfilled the criteria for "clinical NMDARE—probable[a] AE" and 13 for "antibody-negative AE."

Our analysis resulted in a sensitivity of 58% and a specificity of 8% for the "possible[a] AE" category during the first week of admission under the assumption of ignorance of the autoantibody status/ microbiological test results, corresponding to a PPV of 29% and a NPV of 22%. The category "clinical NMDARE—probable[a] AE" resulted in a sensitivity of 20% and a specificity of 92% (PPV = 14%, NPV = 95%), the category "definite[a] limbic AE" in a sensitivity of 13% and a specificity of 100% (the 23 AE patients not diagnosed with parainfectious encephalitis/ADEM, NMDARE or Bickerstaff encephalitis were considered as limbic encephalitis). In total, 9 (1/8) of all AE patients were diagnosed as probable[a] or definite[a] AE under condition C1, corresponding to a sensitivity of 27%.

Diagnostic Algorithm (Condition C1) – Definite[c] Cohort

Among 17 definite[c] AE, 5 fulfilled the criteria for possible[a] AE according to Graus et al., 1 patient fulfilled the criteria of clinical NMDARE, therefore being considered probable[a] autoimmune. None of the remaining patients fulfilled the criteria of antibody-negative AE.

Almost all IE patients (94%) qualified for the diagnosis of possible[a] AE under the premise that the results of microbiological testing were unknown. Three fulfilled the criteria for "clinical NMDARE—probable[a] AE." They were diagnosed with HSV1 encephalitis (2 patients) and TBE (1 patient). Ten patients fulfilled the criteria for "antibody-negative AE" (HSV1 encephalitis in 2 patients, TBE in 8 patients).

The subanalysis of the definite[c] group of patients only rendered a sensitivity of 29% and a specificity of 6% for the "possible[a] AE" category during the first week of admission under the assumption of ignorance of the autoantibody status/ microbiological test results, corresponding to a PPV of 14% and a NPV of 14%. The category "clinical NMDARE - probable[a] AE" resulted in a sensitivity of 20% and a specificity of 91% (PPV = 25%, NPV = 91%). In total, 1 of all definite[c] AE patients was diagnosed as probable[a] AE under condition C1, corresponding to a sensitivity of 6%.

Diagnostic Algorithm (Condition C2) – (Probable[c] + Definite[c]) Cohort

Permitting all clinical information obtained for each patient—including test results from autoantibody and microbiological testing—to be taken into consideration, 36 IE patients were excluded from the algorithm by the "reasonable exclusion of alternative causes" criterium. Hence, the specificity of "possible[a] AE" increased to 71%. The sensitivity of this criterion changed only marginally (58–61%), whereas the sensitivity for the diagnosis of all probable[a] or definite[a] AE increased from 27 to 45% and for "clinical NMDARE - probable[a] AE" from 20 to 80%. The latter increase was due to three NMDARE patients developing one or more major symptoms after 3, 4, and 6 weeks. One NMDARE patient did not meet the "possible[a] AE" criteria due to lack of mental/psychiatric symptoms and would therefore not have been considered in the NMDARE category when strictly following the algorithm in a successive fashion. The NMDARE diagnostic panel applied in isolation would have resulted in a sensitivity of 100% under condition C2. For a graphic illustration of the application of the clinical algorithm see **Figures 1**, **2**.

Diagnostic Algorithm (Condition C2) – Definite[c] Cohort

With all clinical information taken into consideration, all 34 definite[c] IE patients were excluded from the algorithm by the "reasonable exclusion of alternative causes" criterium. Hence, the specificity of "possible[a] AE" increased to 76%. The sensitivity of this criterion was 35%. All 6 patients diagnosed as possible[a] AE went on to be diagnosed as probable[a] or definite[a] AE due to their positive antibody status. The sensitivity for "clinical NMDARE - probable[a] AE" remained at 80%.

DISCUSSION

The differential diagnosis of IE and AE is notoriously difficult, particularly at an early stage after symptom onset. Hence, the first aim of our analysis was to define a subset of presenting symptoms and paraclinical test results in order to facilitate distinguishing between these two entities. We found that they differed significantly in respect to epileptic seizures, fever, headache, psychiatric symptoms, alteration of consciousness, and CSF pleocytosis during the first week of hospital admission.

In concordance with our results, previous studies found seizures to be less frequent in IE than in AE individuals (13, 26, 27). Fever and headache have been reported to occur more often in IE patients (13). Another study showed mixed results though, with fever being less common in AE individuals than in patients with HSV1 encephalitis, but slightly more frequent than in patients with VZV encephalitis (27). The same group reported headache to be slightly more common in AE than in HSV1 encephalitis, but less frequent than in VZV encephalitis. These inconsistencies between different studies and our results are most likely due to the heterogeneity of the pathogenic agents included in the analyses. They may also result from our focus on the symptoms at the time of a patient's initial presentation rather than on all symptoms during the entire course of the disease.

Whereas alterations of consciousness are common to both IE and AE patients (13, 26), these former studies revealed a higher incidence of psychiatric symptoms in AE, seemingly contradicting our results. This is most likely due to our wide definition of psychiatric symptoms, including psychomotor slowing and lethargy. Previous reports have shown the latter to be frequent symptoms in AE as well as in IE (27).

As to the paraclinical tests, previous publications support our claim that CSF pleocytosis is less frequent and milder in AE than in IE. Comparing a cohort of NMDARE with IE patients, Gable et al. reported a higher median cell count in those with IE for most infectious pathogens with the exception of rabies (13, 26). Their findings as to the prevalence of MRI changes in

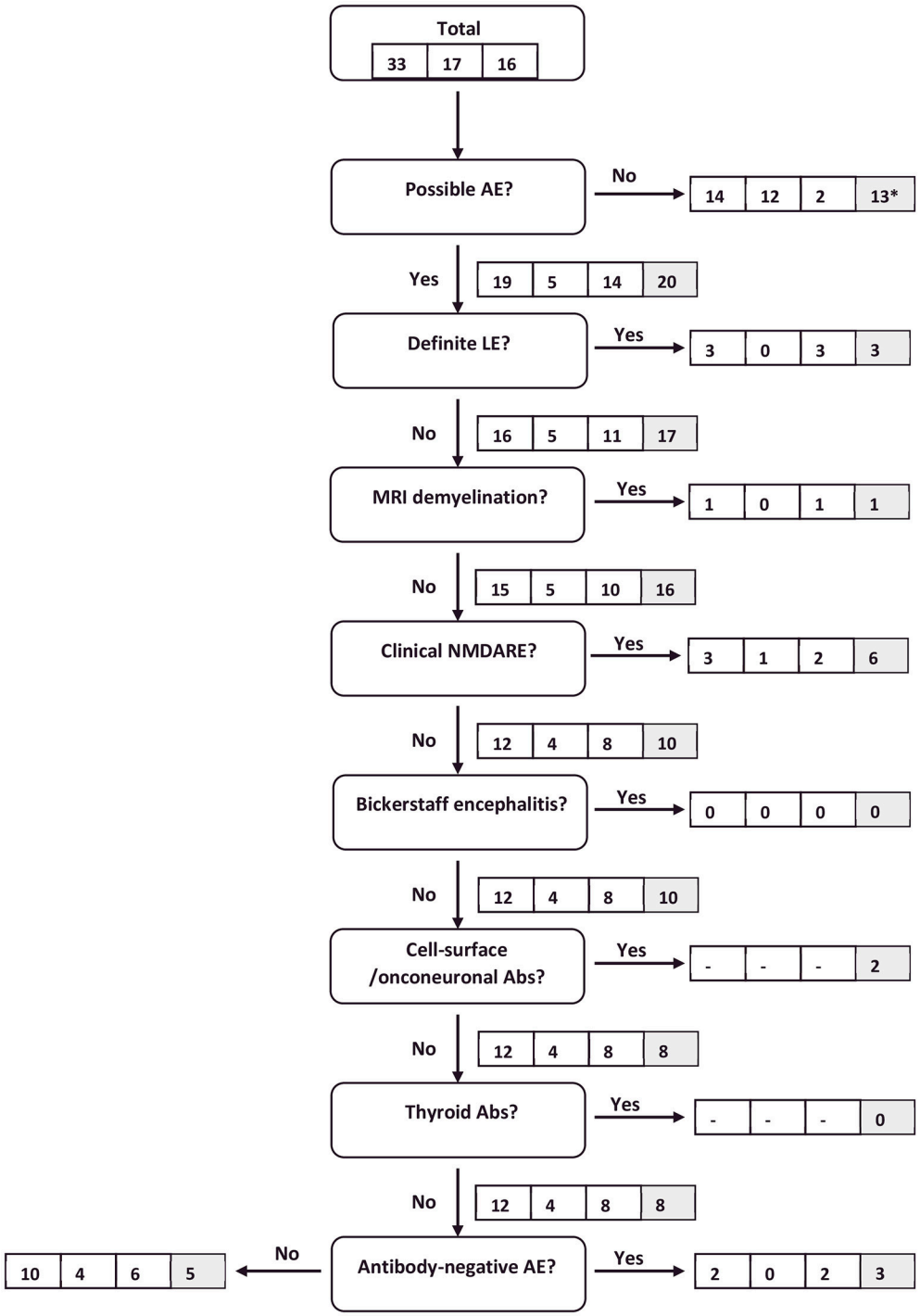


FIGURE 1 | Flow chart of AE patients following the clinical algorithm suggested by Graus et al. (22). Framed figures adhere to the following sequence: all AE patients/definite[C] AE patients/probable[C] AE patients (condition C1). Where applicable, the last box (shaded in gray) provides the respective figure for all AE patients under condition C2. *11 antibody-positive patients are included in this number. They would eventually have been diagnosed with AE based on antibody-status. However, as antibody-status does not feature in the "possible AE" criteria, they were excluded at this point.

AE patients (46%) closely resemble our results as well (13). The rate of MRI pathologies in IE reported by this group ranges from 40 to 100%, dependent on the specific pathogen. The majority of their patients displayed some form of EEG pathology (AE and IE), again confirming our findings. The relatively low percentage of oligoclonal bands/intrathecal immunoglobulin synthesis in

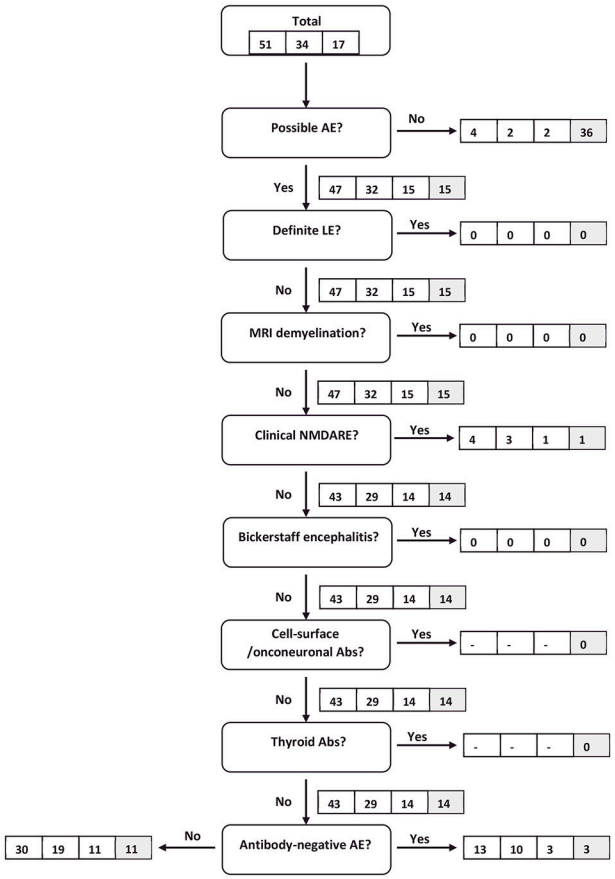


FIGURE 2 | Flow chart of IE patients following the clinical algorithm suggested by Graus et al. (22). Framed figures adhere to the following sequence: all IE patients/definite[c] IE patients/probable[c] IE patients (condition C1). Where applicable, the last box (shaded in gray) provides the respective figure for all IE patients under condition C2.

our cohort is most likely due to us reporting the CSF analysis performed during the first week of admission, when intrathecal immunoglobulin production may not yet have started.

The next step of our approach was to apply the Graus algorithm to encephalitis patients under the condition C1. We tested the definite[c] patients alone as well as the (probable[c] + definite[c]) patients to obtain both the best scientific rigor possible as well as the conclusions drawn from a larger and more heterogeneous cohort, including clinical pictures considered in the algorithm that frequently (ADEM in the adult) or always (antibody-negative encephalitis) lack definite confirmation by immunological testing. Both sensitivity and specificity were low or very low for the diagnosis of "possible[a] AE," "clinical NMDARE - probable[a] AE," and AE of all levels of diagnostic confidence for both the definite[c] and the (probable[c] + definite[c]) cohort. The respective sensitivity increased under the condition C2, particularly so for "clinical NMDARE - probable[a] AE." The same is true for the specificity, mainly due to the exclusion of IE cases with positive microbiological testing in the first step of the algorithm.

In an approach similar to ours, the algorithm was evaluated by a Chinese group on 95 patients, 64 of whom had AE (28). The remaining 31 cases included viral encephalitis (14 cases), purulent encephalitis (2), tuberculous meningoencephalitis (2), CNS tumor (3), and epileptic disorders (10). Their selection with a ratio of only 45% viral IE is most likely partially responsible for their much higher specificity of the "possible[a] AE" diagnosis (83% at days 0–14 of admission, increasing to 92% afterwards), as viral encephalitis seems to be the most difficult to be distinguished from AE compared to encephalopathies of other origin. The overall sensitivity reported by Li et al. for possible[a] AE was higher (84%) than the one we calculated in our entire collective either under condition C1 or C2. This is probably due to a higher ratio of NMDARE cases (61% of all AE) in their collective, for which the algorithm seems to have a particularly high sensitivity (29). Notably, the sensitivity reported by Li et al. for possible[a] AE, definite[a] limbic AE, and probable[a] NMDARE for the time period of up to 14 days after admission very closely resembles our data for condition C1 in the entire patient group: 60 vs. 58% (possible[a] AE), 10 vs. 13% (definite[a] limbic AE), and 16 vs. 20% (probable[a] NMDARE).

In an Australian cohort of 29 children with NMDARE, the authors found a time-dependent sensitivity of the Graus diagnostic criteria for "clinical NMDARE - probable[a] AE" of 24% after 1 week of symptoms, rising to 90% when the entire time of inpatient hospital admission was taken into account (29). The median time to fulfilling the diagnostic criteria was 2 weeks. Three children with IE (enterovirus, mycoplasma) fulfilled the criteria as well, again demonstrating the difficulties in delimiting AE from non-granulocytic IE.

Both studies and our own data confirm that the sensitivity of the clinical algorithm for the diagnosis of AE is clearly time-dependent, restricting its usefulness in the acute setting. However, it remains a valuable diagnostic aid for antibody-negative AE or in resource-poor settings, where access to advanced serological diagnostics is limited. Furthermore, the specificity of "clinical NMDARE - probable[a] AE" and "definite[a] limbic AE" is high, encouraging the initiation of immunosuppressive therapy if the respective criteria apply – even in the absence of serological proof. Considering the low sensitivity at initial presentation and the importance of early therapy, however, therapy should not be withheld until all criteria are fulfilled but rather started if an AE is deemed likely (29).

CASPR2- and LGI1-antibody associated encephalitis poses a particular diagnostic challenge. Not only are CSF and MRI unremarkable in many cases, these were also the AE to most frequently escape detection by the clinical algorithm due to their often subtle evolution. Furthermore, commercially available cell-based assays used for antibody detection seem to have the lowest sensitivity for CSF CASPR2- and LGI1-antibodies when compared to anti-$GABA_B$, -GAD65, and -NMDAR (30). The sensitivity was higher when testing the serum, although this may introduce more unspecific results. These findings should motivate to persist with the diagnostics—i.e., involve a research laboratory for further serological testing—when the

clinical suspicion of anti-CASPR2-/LGI1-encephalitis remains high despite negative diagnostics.

An interesting secondary finding pertains to the 3 patients diagnosed as "definite limbic encephalitis" on the grounds of the Graus algorithm. All of them were antibody-negative, young to middle-aged (29–61 years) women who presented with epileptic seizures (refractory epileptic status in one patient). Epidemiologically, this cohort resembles previous patient groups diagnosed with NORSE [new-onset refractory status epilepticus; see for example (31)]. However, they significantly differ from an antibody-negative AE cohort recently described, which mainly consisted of elderly males presenting with short-memory loss (32). These divergent findings insinuate that the manifestation of antibody-negative AE comprises distinct pathologies.

On the part of IE, TBE was particularly difficult to distinguish from AE. Albeit very sensitive, there may be pitfalls associated with the specific serology if TBE-virus antibodies are determined very early during the course of the disease, due to cross-reactivity with other flavivirus or to previous TBE-vaccination (33, 34). The encephalitic form of TBE virus infection often goes along with psychiatric symptoms such as psychomotor slowing and decreased vigilance. In some cases, speech disorders, epileptic seizures and/or movement disorders occur, rendering the clinical picture similar to NMDARE. Furthermore, TBE patients frequently show bilateral basal ganglia/thalamic involvement, fulfilling the Graus criteria of "MRI features suggestive of encephalitis," which they define as "brain MRI hyperintense signal on T2-weighted fluid-attenuated inversion recovery sequences [. . .] in multifocal areas involving gray matter, white matter, or both compatible with demyelination or inflammation" (22). If the serology is equivocal, TBE may therefore easily be mistaken for autoantibody-negative AE as reflected by the high proportion of TBE-patients in this group under condition C1. It would be interesting to investigate whether this is true for other flaviviruses as well. The high prevalence of TBE in our sample certainly contributed further to the low specificity of the algorithm.

Limitations of our study include that not all IE patients were investigated with the immunological panel. This is particularly relevant in the light of recent discoveries of AE being triggered by viral infection, such as post-HSV-encephalitis NMDARE (35). Similar restrictions apply to AE patients: although all of them were tested for antineuronal antibodies, the extent of the panels varied according to the respective knowledge at the time of testing. We attempted to overcome these limitations by conducting all analyses not only on the entire cohort, but also on the subgroup of those patients, in whom definite diagnoses had been possible. The conclusions pertaining to presenting clinical signs, symptoms and paraclinical test results were very similar in both groups. The problem of a low sensitivity and specificity of the Graus algorithm was more pronounced in the subgroup containing only patients with positive immunological/microbiological test results.

As to the survey of presenting symptoms, cognitive symptoms may have been underestimated in both diagnostic groups for difficulty of assessment in patients suffering from psychiatric symptoms or altered consciousness. Furthermore, mild psychiatric or cognitive changes may have been underdiagnosed if underreported by the patient and his family and unrecognized by the physician at admission. Further shortcomings result from the heterogeneity of AE and IE aetiologies in our rather small cohort as well as the retrospective nature of the study. Prospective larger investigations are warranted to further explore the intricate challenge of early diagnosis in encephalitis.

AUTHOR CONTRIBUTIONS

JW conceived the study design, is responsible for statistical analysis and data interpretation, and wrote the manuscript. OK, MS, IK, and TvO helped with data acquisition and interpretation and critically revised the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2018.00434/full#supplementary-material

REFERENCES

1. Zhao L, Zhou M, Wang B, Guo J, Chen N, He L. Clinical characteristics and outcome of clinically diagnosed viral encephalitis in southwest China. *Neurol Sci.* (2015) **36**:2191–7. doi: 10.1007/s10072-015-2333-8
2. Pewter SM, Williams WH, Haslam C, Kay JM. Neuropsychological and psychiatric profiles in acute encephalitis in adults. *Neuropsychol Rehabil.* (2007) **17**:478–505. doi: 10.1080/09602010701202238
3. Bauer J, Bien CG. Encephalitis and epilepsy. *Semin Immunopathol.* (2009) **31**:537–44. doi: 10.1007/s00281-009-0176-1
4. Michael BD, Solomon T. Seizures and encephalitis: clinical features, management, and potential pathophysiologic mechanisms. *Epilepsia* (2012) **53**(Suppl. 4):63–71. doi: 10.1111/j.1528-1167.2012.03615.x
5. Sellner J, Trinka E. Clinical characteristics, risk factors and pre-surgical evaluation of post-infectious epilepsy. *Eur J Neurol.* (2013) **20**:429–39. doi: 10.1111/j.1468-1331.2012.03842.x
6. Misra UK, Tan CT, Kalita J. Viral encephalitis and epilepsy. *Epilepsia* (2008) **49**:13–8. doi: 10.1111/j.1528-1167.2008.01751.x
7. Newman MP, Blum S, Wong RCW, Scott JG, Prain K, Wilson RJ, et al. Autoimmune encephalitis. *Intern Med J.* (2016) **46**:148–57. doi: 10.1111/imj.12974
8. Boucher A, Herrmann JL, Morand P, Buzelé R, Crabol Y, Stahl JP, et al. Epidemiology of infectious encephalitis causes in 2016. *Med Mal Infect.* (2017) **47**:221–35. doi: 10.1016/j.medmal.2017.02.003
9. Escudero D, Guasp M, Ariño H, Gaig C, Martínez-Hernández E, Dalmau J, et al. Antibody-associated CNS syndromes without signs of inflammation in the elderly. *Neurology* (2017) **89**:1471–5. doi: 10.1212/WNL.0000000000004541
10. Varley J, Taylor J, Irani SR. Autoantibody-mediated diseases of the CNS: structure, dysfunction and therapy. *Neuropharmacology* (2018) **132**:71–82. doi: 10.1016/j.neuropharm.2017.04.046
11. Lee SK, Lee S-T. The laboratory diagnosis of autoimmune encephalitis. *J Epilepsy Res.* (2016) **6**:45–50. doi: 10.14581/jer.16010
12. Rawal G, Yadav S, Wani UR, Ambastha AK. HSV Encephalitis with NORMAL CSF - a case report with review of literature. *J Clin Diagn Res*. (2015) **9**:OD06-07. doi: 10.7860/JCDR/2015/16999.6966

13. Gable MS, Sheriff H, Dalmau J, Tilley DH, Glaser CA. The frequency of autoimmune N-methyl-D-aspartate receptor encephalitis surpasses that of individual viral etiologies in young individuals enrolled in the California Encephalitis Project. *Clin Infect Dis Off Publ Infect Dis Soc Am.* (2012) **54**:899–904. doi: 10.1093/cid/cir1038
14. Kennedy PGE, Quan P-L, Lipkin WI. Viral encephalitis of unknown cause: current perspective and recent advances. *Viruses* (2017) **9**:138. doi: 10.3390/v9060138
15. Thakur KT, Motta M, Asemota AO, Kirsch HL, Benavides DR, Schneider EB, et al. Predictors of outcome in acute encephalitis. *Neurology* (2013) **81**:793–800. doi: 10.1212/WNL.0b013e3182a2cc6d
16. Bloch KC, Glaser CA. Encephalitis Surveillance through the Emerging Infections Program, 1997-2010. *Emerg Infect Dis.* (2015) **21**:1562–7. doi: 10.3201/eid2109.150295
17. Dubey D, Samudra N, Gupta P, Agostini M, Ding K, Van Ness PC, et al. Retrospective case series of the clinical features, management and outcomes of patients with autoimmune epilepsy. *Seizure* (2015) **29**:143–7. doi: 10.1016/j.seizure.2015.04.007
18. Byun J-I, Lee S-T, Jung K-H, Sunwoo J-S, Moon J, Lim J-A, et al. Effect of immunotherapy on seizure outcome in patients with autoimmune encephalitis: a prospective observational registry study. *PLoS ONE.* (2016) **11**:e0146455. doi: 10.1371/journal.pone.0146455
19. Titulaer MJ, McCracken L, Gabilondo I, Armangué T, Glaser C, Iizuka T, et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. *Lancet Neurol.* (2013) **12**:157–65. doi: 10.1016/S1474-4422(12)70310-1
20. Thompson J, Bi M, Murchison AG, Makuch M, Bien CG, Chu K, et al. The importance of early immunotherapy in patients with faciobrachial dystonic seizures. *Brain J Neurol.* (2017) **141**:348–356. doi: 10.1093/brain/awx323
21. Poissy J, Wolff M, Dewilde A, Rozenberg F, Raschilas F, Blas M, et al. Factors associated with delay to acyclovir administration in 184 patients with herpes simplex virus encephalitis. *Clin Microbiol Infect.* (2009) **15:**560–4. doi: 10.1111/j.1469-0691.2009.02735.x
22. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol.* (2016) **15**:391–404. doi: 10.1016/S1474-4422(15)00401-9
23. Venkatesan A, Tunkel AR, Bloch KC, Lauring AS, Sejvar J, Bitnun A, et al. Case definitions, diagnostic algorithms, and priorities in encephalitis: consensus statement of the international encephalitis consortium. *Clin Infect Dis.* (2013) **57**:1114–28. doi: 10.1093/cid/cit458
24. Bien CG, Mirzadjanova Z, Baumgartner C, Onugoren MD, Grunwald T, Holtkamp M, et al. Anti-contactin-associated protein-2 encephalitis: relevance of antibody titres, presentation and outcome. *Eur J Neurol.* (2017) **24**:175–86. doi: 10.1111/ene.13180
25. Dogan Onugoren M, Deuretzbacher D, Haensch CA, Hagedorn HJ, Halve S, Isenmann S, et al. Limbic encephalitis due to GABAB and AMPA receptor antibodies: a case series. *J Neurol Neurosurg Psychiatry* (2015) **86**:965–72. doi: 10.1136/jnnp-2014-308814
26. Gable MS, Gavali S, Radner A, Tilley DH, Lee B, Dyner L, et al. Anti-NMDA receptor encephalitis: report of ten cases and comparison with viral encephalitis. *Eur J Clin Microbiol Infect Dis.* (2009) **28**:1421–9. doi: 10.1007/s10096-009-0799-0
27. Granerod J, Ambrose HE, Davies NW, Clewley JP, Walsh AL, Morgan D, et al. Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study. *Lancet Infect Dis.* (2010) **10**:835–44. doi: 10.1016/S1473-3099(10)70222-X
28. Li L, Sun L, Du R, Zheng Y, Dai F, Ma Q, et al. Application of the 2016 diagnostic approach for autoimmune encephalitis from Lancet Neurology to Chinese patients. *BMC Neurol.* (2017) **17**:195. doi: 10.1186/s12883-017-0974-3
29. Ho ACC, Mohammad SS, Pillai SC, Tantsis E, Jones H, Ho R, et al. High sensitivity and specificity in proposed clinical diagnostic criteria for anti-N-methyl-D-aspartate receptor encephalitis. *Dev Med Child Neurol.* (2017) **59**:1256–60. doi: 10.1111/dmcn.13579
30. McCracken L, Zhang J, Greene M, Crivaro A, Gonzalez J, Kamoun M, et al. Improving the antibody-based evaluation of autoimmune encephalitis. *Neurol Neuroimmunol Neuroinflammation* (2017) **4**:e404. doi: 10.1212/NXI.0000000000000404
31. Iizuka T, Kanazawa N, Kaneko J, Tominaga N, Nonoda Y, Hara A, et al. Cryptogenic NORSE. *Neurol Neuroimmunol Neuroinflammation.* (2017) **4**:e396. doi: 10.1212/NXI.0000000000000396
32. Graus F, Escudero D, Oleaga L, Bruna J, Villarejo-Galende A, Ballabriga J, et al. Syndrome and outcome of antibody-negative limbic encephalitis. *Eur J Neurol.* (2018). doi: 10.1111/ene.13661. [Epub ahead of print].
33. Litzba N, Zelená H, Kreil TR, Niklasson B, Kühlmann-Rabens I, Remoli ME, et al. Evaluation of different serological diagnostic methods for tick-borne encephalitis virus: enzyme-linked immunosorbent, immunofluorescence, and neutralization assay. *Vector Borne Zoonotic Dis.* (2014) **14**:149–59. doi: 10.1089/vbz.2012.1287
34. Günther G, Haglund M, Lindquist L, Sköldenberg B, Forsgren M. Intrathecal IgM, IgA and IgG antibody response in tick-borne encephalitis. Long-term follow-up related to clinical course and outcome. *Clin Diagn Virol.* (1997) **8**:17–29.
35. Höftberger R, Armangue T, Leypoldt F, Graus F, Dalmau J. Clinical neuropathology practice guide 4-2013: post-herpes simplex encephalitis: N-methyl-Daspartate receptor antibodies are part of the problem. *Clin Neuropathol.* (2013) **32**:251–4. doi: 10.5414/NP300666

Conflict of Interest Statement: JW reports personal fees from UCB Pharma GmbH and travel funds from Boehringer Ingelheim GmbH and Daiichi Sankyo GmbH. TvO reports personal fees and non-financial support from Eisai Pharma GmbH Vienna, grants, personal fees and non-financial support from UCB Pharma GmbH Vienne, non-financial support from Medtronic Österreich GmbH, grants, personal fees and non-financial support from Novartis Pharma, personal fees from Roche Pharma, personal fees from Biogen Idec Austria, personal fees from Liva Nova, personal fees from Sanofi Aventis GmbH, and grants from Grossegger & Drbal GmbH.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer CB declared a past co-authorship with one of the authors TvO to the handling Editor.

frontiers in Neurology

CASE REPORT
published: 17 May 2018
doi: 10.3389/fneur.2018.00329

IgLON5-Associated Encephalitis With Atypical Brain Magnetic Resonance Imaging and Cerebrospinal Fluid Changes

Massimiliano Montagna[1,2], Rizvana Amir[3], Ilse De Volder[1,4], Martin Lammens[5], Jef Huyskens[6] and Barbara Willekens[1,7]**

[1] Department of Neurology, Antwerp University Hospital, Antwerp, Belgium, [2] Master After Master in Medicine, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium, [3] Department of Neurology, Sint Maarten General Hospital, Mechelen, Belgium, [4] Multidisciplinary Sleep Disorders Centre, Antwerp University Hospital, Antwerp, Belgium, [5] Department of Pathology, Antwerp University Hospital, University of Antwerp, Antwerp, Belgium, [6] Department of Radiology, Antwerp University Hospital, Antwerp, Belgium, [7] Laboratory of Experimental Hematology, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium

OPEN ACCESS

Edited by:
Johann Sellner, Christian Doppler Klinik, Universitätsklinikum Salzburg, Austria

Reviewed by:
Lucas Schirmer, University of California, San Francisco, United States
Anne-Katrin Pröbstel, University of California, San Francisco, United States

***Correspondence:**
Massimiliano Montagna
massimiliano.montagna@uza.be;
Barbara Willekens
barbara.willekens@uza.be

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *25 February 2018*
Accepted: *25 April 2018*
Published: *17 May 2018*

Citation:
Montagna M, Amir R, De Volder I, Lammens M, Huyskens J and Willekens B (2018) IgLON5-Associated Encephalitis With Atypical Brain Magnetic Resonance Imaging and Cerebrospinal Fluid Changes. Front. Neurol. 9:329. doi: 10.3389/fneur.2018.00329

IgLON5-associated encephalitis is a syndrome with different clinical presentations consisting of sleep dysfunction, bulbar dysfunction, chorea, and progressive supranuclear palsy-like symptoms whereas dysautonomy and cognitive decline usually appear in later stages of the disease. We report a case of a patient with IgLON5-associated encephalitis presenting with rapidly progressive cognitive decline and atypical inflammatory lesions on brain magnetic resonance imaging, oligoclonal bands on cerebrospinal fluid, anti-IgLON5 antibodies exclusively of the IgG1 class, and a fierce inflammatory reaction on brain biopsy, who responded favorably to immunotherapy.

Keywords: brain inflammation, IgLON5, autoimmune encephalitis, rapidly evolving dementia, akathisia, dyskinesia

INTRODUCTION

A 75-year-old female patient was admitted to the geriatric ward in March 2016 with acute confusion, somnolence, verbal aggression, and fever (see **Figure 1** for a timeline of this case report). Brain magnetic resonance imaging (MRI) showed spotty enhancement in the right temporal and frontal lobes with focal leptomeningeal enhancement and edema (**Figure 2**). An extensive workup under suspicion of leptomeningeal metastasis was negative for a primary neoplasm. A brain biopsy performed to exclude a lymphoma showed signs of severe white matter destruction with many macrophages and lymphocytosis, but no malignancy, nor signs of vasculitis (**Figure 3**). Neurological consultation nor cerebrospinal fluid (CSF) analysis was performed at this stage. Oral corticosteroid treatment led to significant regression of the symptoms, and a follow-up MRI in August 2016 showed a significant decrease in the volume of the lesions with disappearance of gadolinium enhancement.

During a second episode of acute confusion, apraxia, visual hallucinations, and somnolence in December 2016, brain MRI remained unchanged in comparison to the brain MRI performed in August. Electro-encephalography (EEG) showed large amounts of delta waves but no epileptic activity. CSF analysis showed a normal cell count, a mildly elevated protein level (48.4 mg/dl) and 15 oligoclonal bands of which 3 were matched between serum and CSF. Polymerase chain reaction analysis on CSF showed no evidence for the presence of DNA of herpes simplex 1 and 2 virus and varicella zoster virus. Metabolic and infectious etiologies were ruled out. Under suspicion of an autoimmune encephalitis, anti-IgLON5 antibodies were detected in the serum (titer 1:10,000) while other autoantibodies remained negative [the antibodies against the following antigens were tested: Hu, Yo, Ri, CV2, amphiphysin, Ma2/Ta, Zic4, GAD65, Tr(DNER), Recoverin, Sox1—method:

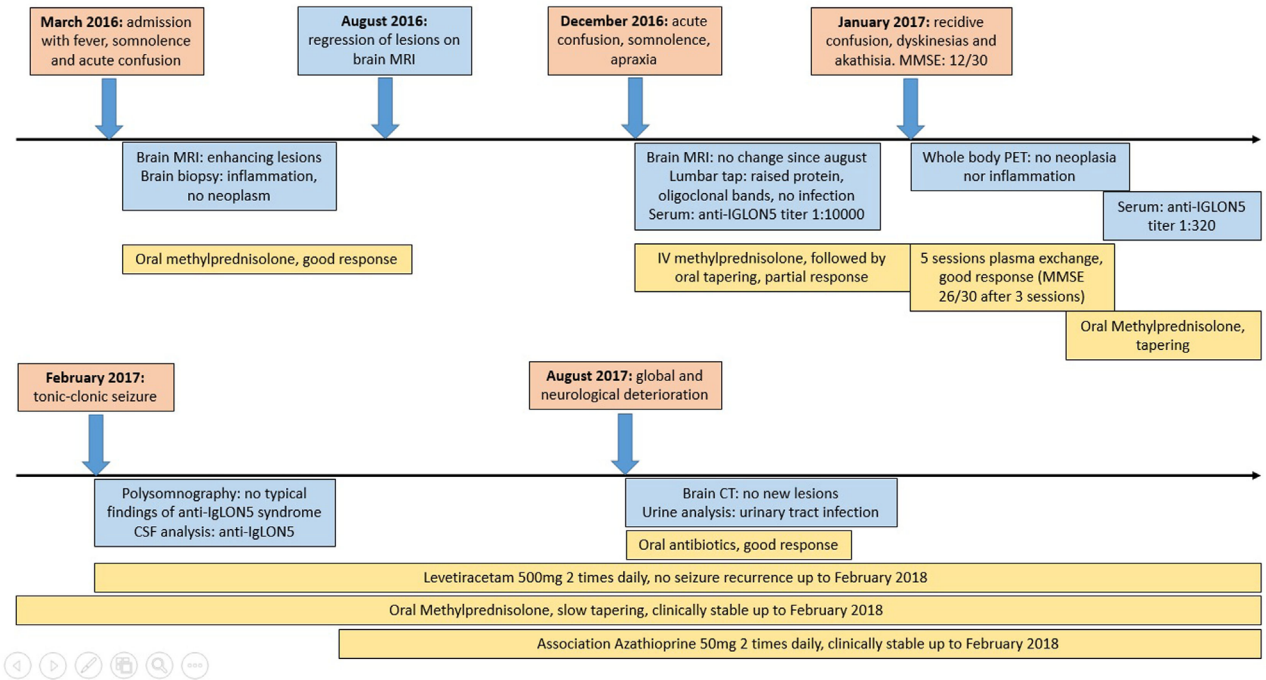


FIGURE 1 | A timeline displaying synthetically the evolution of our case: in the red boxes, the clinical events have been reported; in the blue boxes, there is a list of the significant investigations that have been performed; and in the yellow boxes, we displayed the therapies and the relative effect.

EUROLINE; MOG, NMDA-r, AMPA-r, GABA-b, LGI-1, CASPR2, DPPX, myelin, glycine receptors, mGluR1, mGluR5, GABA-a, Rho GTAase activating protein 26, CARPVIII, GluRD2, flotillin—method: immunofluorescence test] (Euroimmun AG, Lübeck, Germany).

High-dose intravenous methylprednisolone (1 g daily for 3 days) leads to a moderate improvement of the consciousness level and apraxia but had only minor effects on the hallucinations. Oral steroids were tapered slowly over several weeks, but in January 2017 she relapsed. On examination, she was logorrheic, incoherent with lower limb dyskinesias and akathisia. The Mini Mental State Examination (MMSE) score was 12/30. A whole body positron emission tomography/computed tomography scan showed no evidence of inflammation or neoplasia.

Five sessions of plasma exchange were performed: after three sessions the MMSE improved to a score of 26/30, hallucinations and dyskinesias completely disappeared. The titer of anti-IgLON5 antibodies decreased to 1:320 after this treatment. One month after discharge the patient was readmitted due to a tonic–clonic seizure for which levetiracetam was started. A polysomnographic examination (PSG) was performed but showed no evidence for stridor, finalistic movements, or repetitive rapid periodic leg movements.

Cerebrospinal fluid analysis, performed at this point, showed the presence of anti-IgLON5 antibodies. The presence of the anti-IgLON5 antibodies on serum and CSF was confirmed in the laboratory of Prof. Dr. Dalmau and Prof. Dr. Graus [Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona]. No other autoantibodies were detected. Using previously reported techniques (1), IgLON5 antibodies of the patient recognized an epitope in the Ig-like domain 2 of IgLON5. The IgG antibody subclass was exclusively IgG1. A re-examination of the brain biopsy showed no presence of Tau-protein deposition. HLA typing showed that our patient had haplotype DQB1*0501 and DRB1*1001.

After a new corticosteroid tapering regimen and maintenance treatment with azathioprine 50 mg two times daily our patient has remained neurologically stable with no more hallucinations nor movement disorders and a slight cognitive impairment. A brain CT was performed in June 2017 due to transient neurological regress (later proved to be caused by a urinary tract infection and completely resolved after appropriate antibiotic treatment): this investigation showed no new pathological findings. After this date, no more brain imaging study was performed.

She was still ambulatory up to November 2017 but due to social reasons and general frailty with increasing help demand she was finally admitted to a nursing home. A follow-up contact in February 2018 showed a still remarkable cognitive function (MMSE score 27/30) and no evidence for relapse of epilepsy. Our patient had still episodes of visual hallucinations in the period before being admitted to the nursing home: there were then issues of probable suboptimal therapeutic compliance. After being admitted, compliance improved and visual hallucinations disappeared. At the moment of the last contact, our patient was on methylprednisolone 4 mg daily and azathioprine 50 mg two times per day orally.

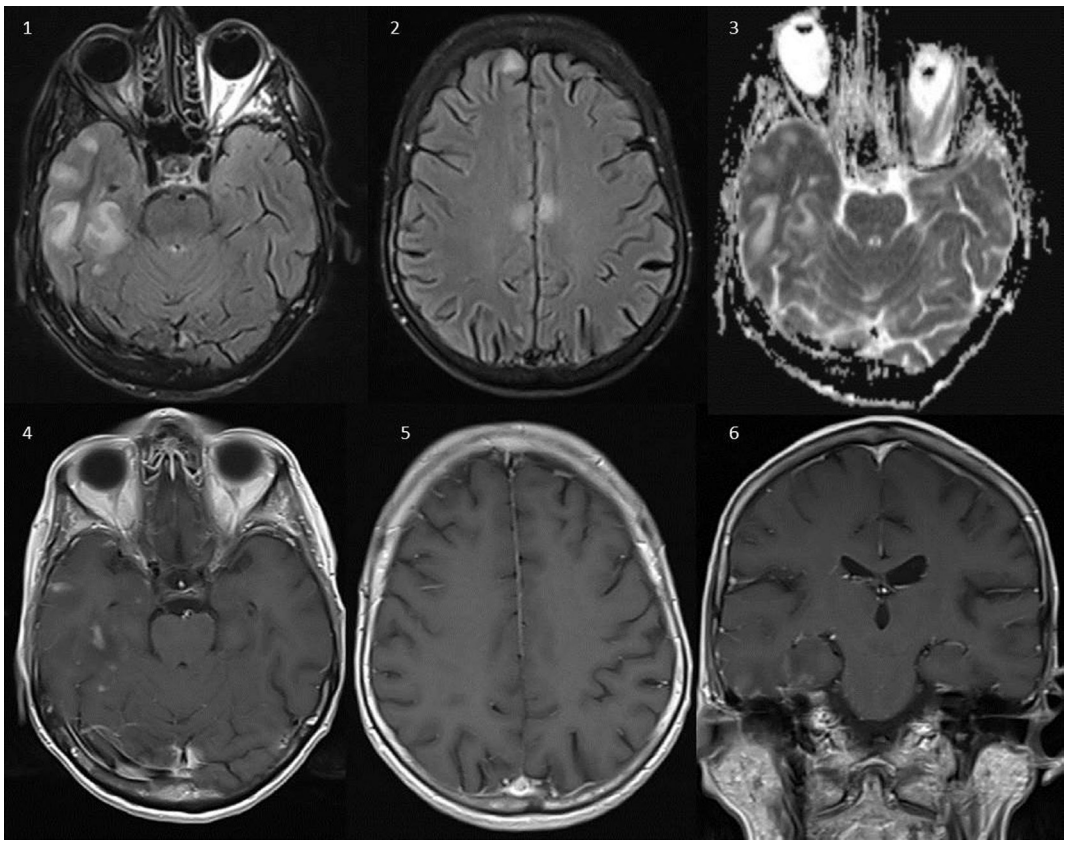


FIGURE 2 | Brain magnetic resonance imaging (Siemens Aera 1.5 T). Axial FLAIR (1, 2), axial ADC-maps (3), axial T1 after intravenous gadolinium (4, 5), and coronal T1 after intravenous gadolinium (6). Several lesions with high T2-signal on FLAIR in the right temporal lobe, bilateral in the frontal lobe, and the callosal body without signs of restricted diffusion on the ADC-maps, compatible with vasogenic edema. Several lesions in the right temporal lobe show patchy contrast enhancement after intravenous administration of gadolinium-based contrast (4–6). None of the lesions were hemorrhagic.

DISCUSSION

Anti-IgLON5 syndrome was first described in 2014 (2) as a disorder characterized by sleep dysfunction, a progressive supranuclear palsy-like syndrome (3), movement disorders (e.g., chorea) and brainstem and hypothalamic involvement leading to dysphagia and dysarthria, with a varying degree of dysautonomic features. Cognitive decline has been described mostly in a later stage of the disease. Neuropathologic findings show tau deposits in the hypothalamus and tegmentum: the relation with anti-IgLON5 has been suspected but not clarified. HLA-DRB1*1001 and HLA-DQB1*0501 association suggests an autoimmune pathogenesis (4).

In all IgLON5-positive patients PSG shows various anomalies such as abnormal sleep architecture, undifferentiated non rapid eye movement (non-REM) sleep or poorly structured stage N2, REM sleep behavior disorder, central hypoventilation, stridor, and obstructive sleep apnea. No significant abnormalities have been found on EEG, electromyography (2) and brain MRI, with the exception of slight brainstem and bilateral hippocampal atrophy (described, respectively, in three patients and one patient) (3). No clear association with an underlying neoplastic pathology has been found thus far. CSF analysis varies from normal (2), to pleiocytosis and increased protein levels (5). Only in one previously reported patient intrathecal synthesis of immunoglobulins has been described (3). Treatment with immunosuppressants showed highly variable results, with a tendency for improved response after earlier start of the treatment (2, 6, 7).

Our patient presented with atypical clinical, polysomnographic, MRI, and CSF findings. The clinical course was dominated since the onset by fluctuating cognitive symptoms, improving after immunotherapy. The atypical clinical presentation might be caused by the fact that our patient has anti-IgLON5 antibodies exclusively of the IgG1 subclass, while to date all described cases have, to the best of our knowledge, presented with either isolated IgG4 or mixed IgG1 and IgG4 subtypes, with IgG4 predominance (1, 3). As IgG1 is able to bind complement, in contrast to IgG4 subtype (8), triggering of this activation route might be an explanation for the fierce inflammatory response seen in this patient. While we cannot completely exclude a viral encephalitis as a precipitating event, as there was no CSF analysis during the first presentation, this seems unlikely as the patient improved on treatment with steroids, which would aggravate an infectious cause. Also, the brain biopsy was not suggestive for an infectious pathology. Occurrence of NMDA-receptor encephalitis after herpes simplex encephalitis has been reported, leading

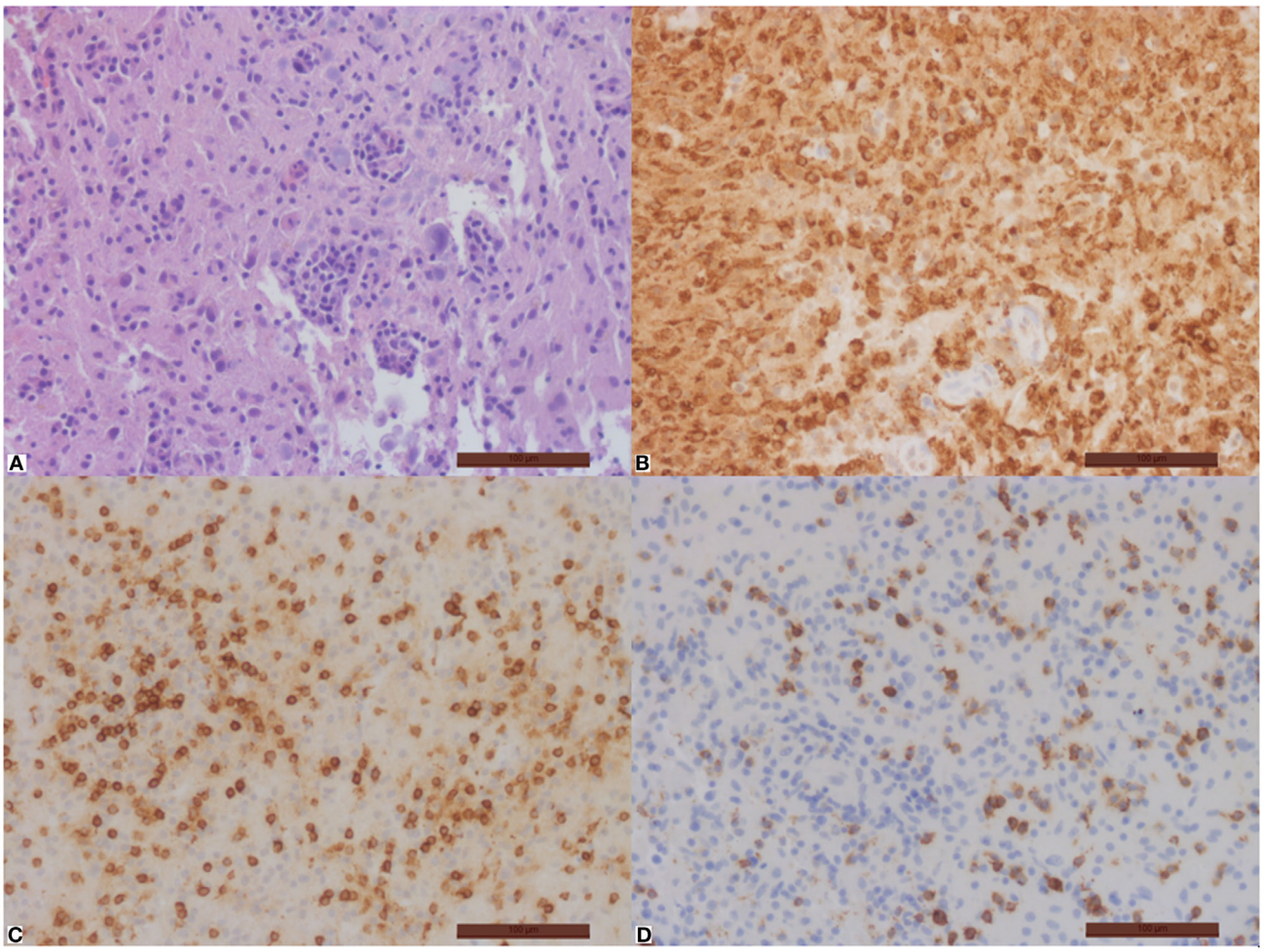


FIGURE 3 | Important inflammation of the brain tissue with disseminated macrophages **(B)** and T-cells **(C)**, no B-cells. Part of the T-cells is CD8-positive **(D)**. Hematoxylin–eosin **(A)**, immunohistochemical stain with antibodies against CD68 **(B)**, CD3 **(C)**, and CD8 **(D)**. Magnification bar = 100 µm.

to the hypothesis that the viral encephalitis was the triggering event for the development of the autoimmune encephalitis (9). Conversely, as the different subclasses of IgG can be produced in response to antigens dependently on the mechanism of sensitization, a possible preceding viral infection, not yet described in relation to IgLON5-associated encephalitis, could account for the production of IgG exclusively of class 1 in our patient (10). Typical sleep phenomena were not present in our patient though the sleep EEG was of the "undifferentiated non-REM-sleep" type. Although the presentation, clinical course, and MRI findings of this patient seem more compatible with GABA-A receptor encephalitis (11), these antibodies were undetectable, as were other known autoantibodies related to autoimmune encephalitis. While one might argue that this patient may have another unknown autoantibody implicated in the disease course, the fact that our patient has the same haplotype as in previously described cases as well as the presence of IgLON5 antibodies in serum and CSF suggests that the clinical spectrum of IgLON5-associated encephalitis is broader than what is known to date.

Our patient was treated with corticosteroids and with plasma exchange: after the latter treatment, her cognitive deficits improved dramatically and her anti-IgLON5 serum titer decreased from 1:10,000 to 1:320: this suggests that in our patient cognitive decline could be related to the titer of anti-IgLON5 antibodies and that the antibodies play a role in the pathogenesis. Moreover, the favorable treatment response to immunotherapy in this patient might also be related to the presence of IgG1 subclass antibodies, with effective removal of complement factors contributing to the treatment effect (12).

The early recognition of this autoimmune encephalitis and rapid treatment with corticosteroids and plasmapheresis may have resulted in the good outcome of this patient, compared to the non-response to immunotherapy in previously described cases. This supports the hypothesis that the antibodies are pathogenic and that neurodegeneration might be prevented by early treatment. Our patient presented with clear inflammatory changes on CSF and MRI, which might be another reason for her dramatical improvement after immunotherapy.

CONCLUDING REMARKS

IgLON5-associated encephalitis is a relatively new autoimmune encephalopathy that can present with various neurological symptoms. This case report expands the clinical spectrum of this disease and supports the use of early immunotherapy.

INFORMED CONSENT

The patient subject of this case report gave her written informed consent for the writing and publication of this case report.

ETHICS STATEMENT

The ethics committee was not consulted. Written informed consent was obtained from the patient.

AUTHOR CONTRIBUTIONS

MM was involved in the patient case, collected necessary data, drafted and finalized the manuscript. RA was involved in the patient case, delivered necessary data, and critically revised the manuscript for intellectual content. IV was involved in the patient case and critically revised the manuscript for intellectual content. ML revised the brain biopsy, provided images of the specimen, and critically revised the manuscript for intellectual content. JH revised all brain MRIs, provided a selection of images, and critically revised the manuscript for intellectual content. BW was involved in the patient case, drafting of the manuscript and critically revision of different versions for intellectual content. All the authors approved the final version of this manuscript.

ACKNOWLEDGMENTS

We would like to thank L. De Valensart Schoonmackers and A. Cant for their help in this case. A special thank goes to Prof. Dr. F. Graus, Prof. Dr. J. Dalmau, and Dr. E. Gelpi for their interesting remarks and suggestions and for their help with the re-analysis of the sera and CSF of our patient.

REFERENCES

1. Sabater L, Planagumá J, Dalmau J, Graus F. Cellular investigations with human antibodies associated with the anti-IgLON5 syndrome. *J Neuroinflammation* (2016) 13(1):226. doi:10.1186/s12974-016-0689-1
2. Sabater L, Gaig C, Gelpi E, Bataller L, Lewerenz J, Torres-Vega E, et al. A novel non-rapid-eye movement and rapid-eye-movement parasomnia with sleep breathing disorder associated with antibodies to IgLON5: a case series, characterisation of the antigen, and post-mortem study. *Lancet Neurol* (2014) 13(6):575–86. doi:10.1016/S1474-4422(14)70051-1
3. Gaig C, Graus F, Compta Y, Högl B, Bataller L, Brüggemann N, et al. Clinical manifestations of the anti-IgLON5 disease. *Neurology* (2017) 88(18):1736–43. doi:10.1212/WNL.0000000000003887
4. Gelpi E, Höftberger R, Graus F, Ling H, Holton JL, Dawson T, et al. Neuropathological criteria of anti-IgLON5-related tauopathy. *Acta Neuropathol* (2016) 132(4):531–43. doi:10.1007/s00401-016-1591-8
5. Simabukuro MM, Sabater L, Adoni T, Cury RG, Haddad MS, Moreira CH, et al. Sleep disorder, chorea, and dementia associated with IgLON5 antibodies. *Neurol Neuroimmunol Neuroinflamm* (2015) 2(4):e136. doi:10.1212/NXI.0000000000000136
6. Schroder JB, Melzer N, Ruck T, Heidbreder A, Kleffner I, Dittrich R, et al. Isolated dysphagia as initial sign of anti-IgLON5 syndrome. *Neurol Neuroimmunol Neuroinflamm* (2017) 4(1):e302. doi:10.1212/NXI.0000000000000302
7. Haitao R, Yingmai Y, Yan H, Fei H, Xia L, Honglin H, et al. Chorea and parkinsonism associated with autoantibodies to IgLON5 and responsive to immunotherapy. *J Neuroimmunol* (2016) 300:9–10. doi:10.1016/j.jneuroim.2016.09.012
8. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. *Front Immunol* (2014) 5:520. doi:10.3389/fimmu.2014.00520
9. Armangue T, Leypoldt F, Málaga I, Raspall-Chaure M, Marti I, Nichter C, et al. Herpes simplex virus encephalitis is a trigger of brain autoimmunity. *Ann Neurol* (2014) 75(2):317–23. doi:10.1002/ana.24083
10. Lowe D, Higgins R, Zehnder D, Briggs DC. Significant IgG subclass heterogeneity in HLA-specific antibodies: implications for pathogenicity, prognosis, and the rejection response. *Hum Immunol* (2013) 74(5):666–72. doi:10.1016/j.humimm.2013.01.008
11. Spatola M, Petit-Pedrol M, Simabukuro MM, Armangue T, Castro FJ, Barcelo Artiques MI, et al. Investigations in GABAA receptor antibody-associated encephalitis. *Neurology* (2017) 88(11):1012–20. doi:10.1212/WNL.0000000000003713
12. Volkin RL, Starz TW, Winkelstein A, Shadduck RK, Lewis JH, Hasiba U, et al. Changes in coagulation factors, complement, immunoglobulins, and immune complex concentrations with plasma exchange. *Transfusion* (1982) 22(1):54–8. doi:10.1046/j.1537-2995.1982.22182154218.x

Conflict of Interest Statement: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. The authors received no financial support for the research, authorship, and/or publication of this article.

frontiers in Immunology

ORIGINAL RESEARCH
published: 24 April 2018
doi: 10.3389/fimmu.2018.00873

Interleukin-27 Gene Therapy Prevents the Development of Autoimmune Encephalomyelitis but Fails to Attenuate Established Inflammation due to the Expansion of CD11b$^+$Gr-1$^+$ Myeloid Cells

Jianmin Zhu[1†], Jin-Qing Liu[2†], Zhihao Liu[2], Lisha Wu[2], Min Shi[1], Jianchao Zhang[3], Jonathan P. Davis[3] and Xue-Feng Bai[1,2]*

[1] Pediatric Translational Medicine Institute, Shanghai Children's Medical Center, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, [2] Department of Pathology and Comprehensive Cancer Center, Ohio State University, Columbus, OH, United States, [3] Department of Physiology, Ohio State University, Columbus, OH, United States

OPEN ACCESS

Edited by:
Johann Sellner,
Christian Doppler Klinik,
Universitätsklinikum Salzburg,
Austria

Reviewed by:
Markus Kleinewietfeld,
VIB-UGent Center for Inflammation Research (IRC), Belgium
Abdelhadi Saoudi,
Institut National de la Santé et de la Recherche Médicale (INSERM), France

***Correspondence:**
Xue-Feng Bai
xue-feng.bai@osumc.edu

[†]These authors have contributed equally to this work.

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology

Received: 08 November 2017
Accepted: 09 April 2018
Published: 24 April 2018

Citation:
Zhu J, Liu J-Q, Liu Z, Wu L, Shi M, Zhang J, Davis JP and Bai X-F (2018) Interleukin-27 Gene Therapy Prevents the Development of Autoimmune Encephalomyelitis but Fails to Attenuate Established Inflammation due to the Expansion of CD11b$^+$Gr-1$^+$ Myeloid Cells. Front. Immunol. 9:873. doi: 10.3389/fimmu.2018.00873

Interleukin-27 (IL-27) and its subunit P28 (also known as IL-30) have been shown to inhibit autoimmunity and have been suggested as potential immunotherapeutic for autoimmune diseases such as multiple sclerosis (MS). However, the potential of IL-27 and IL-30 as immunotherapeutic, and their mechanisms of action have not been fully understood. In this study, we evaluated the efficacy of adeno-associated viral vector (AAV)-delivered IL-27 (AAV-IL-27) and IL-30 (AAV-IL-30) in a murine model of MS. We found that one single administration of AAV-IL-27, but not AAV-IL-30 completely blocked the development of experimental autoimmune encephalomyelitis (EAE). AAV-IL-27 administration reduced the frequencies of Th17, Treg, and GM-CSF-producing CD4$^+$ T cells and induced T cell expression of IFN-γ, IL-10, and PD-L1. However, experiments involving IL-10-deficient mice and PD-1 blockade revealed that AAV-IL-27-induced IL-10 and PD-L1 expression were not required for the prevention of EAE development. Surprisingly, neither AAV-IL-27 nor AAV-IL-30 treatment inhibited EAE development and Th17 responses when given at disease onset. We found that mice with established EAE had significant expansion of CD11b$^+$Gr-1$^+$ cells, and AAV-IL-27 treatment further expanded these cells and induced their expression of Th17-promoting cytokines such as IL-6. Adoptive transfer of AAV-IL-27-expanded CD11b$^+$Gr-1$^+$ cells enhanced EAE development. Thus, expansion of CD11b$^+$Gr-1$^+$ cells provides an explanation for the resistance to IL-27 therapy in mice with established disease.

Keywords: interleukin-27, IL-30, experimental autoimmune encephalomyelitis, Th1, Th17, PD-L1, Treg cells, central nervous system

INTRODUCTION

Interleukin-27 (IL-27) is an IL-12 family of cytokines that is composed of Epstein–Barr virus-induced gene 3 (EBI3) and IL-27p28 (also known as IL-30) subunits. Produced by activated antigen-presenting cells (APCs) such as dendritic cells and macrophages (1–3), IL-27 signals through a heterodimeric receptor (IL-27R) consisting of the WSX-1 and the gp130 subunits, which is expressed in a variety

of cell types including T lymphocytes and myeloid cells (4). IL-27 has been shown to inhibit Th17 responses (5, 6), and induce IL-10 (7–9) and PD-L1 (10) expression in T cells, and has been shown to inhibit inflammation in animal models of autoimmune diseases (11, 12), including animal models of multiple sclerosis (MS) (9, 13, 14) and collagen-induced arthritis (15). These results suggest that IL-27 may be a potential immunotherapeutic for human autoimmune diseases.

Indeed, previous studies (9, 13) have revealed that IL-27 delivered systemically can inhibit the development of experimental autoimmune encephalomyelitis (EAE) in mice, an experimental model of MS. However, systemic injection of IL-27 is costly, and it is also difficult to maintain an effective concentration in the circulation. In this context, gene therapy could serve as an effective alternative approach. For instance, IL-30 gene therapy has been shown to efficiently inhibit autoimmune inflammation in the central nervous system (CNS) and eye (16), and lentiviral IL-27 gene delivery to the CNS inhibits neuroinflammation (17). Adeno-associated viral vectors (AAVs) are highly efficient delivery agents for gene therapy (18). AAV vectors can efficiently transfer genes of interest to a broad range of mammalian cell types leading to high levels of stable and long-term expression after a single application (19). AAV vectors are also known to have low immunogenicity and have been used in human clinical trials (20–22). In this study, we have evaluated the therapeutic efficacy of AAV-delivered IL-27 (AAV-IL-27) and IL-30 (AAV-IL-30) in T cell-mediated autoimmune encephalomyelitis, where the inflammation in the CNS is considered to be mediated mainly by Th17/Th1 responses and T cells producing GM-CSF (23, 24). We found that one single administration of AAV-IL-27, but not AAV-IL-30 completely prevented EAE development. Experiments involving IL-10-deficient mice and PD-1 blockade revealed that AAV-IL-27-induced IL-10 and PD-L1 expression were not required for the inhibition of EAE development. However, neither AAV-IL-27 nor AAV-IL-30 treatment inhibited EAE development and Th17 responses when given at disease onset. We found that mice with established EAE had significant expansion of $CD11b^+Gr\text{-}1^+$ myeloid cells, and AAV-IL-27 treatment further expanded these cells and induced their expression of multiple cytokines including Th17-promoting cytokines such as IL-6 and IL-23. Adoptive transfer of AAV-IL-27-expanded $CD11b^+Gr\text{-}1^+$ cells enhanced EAE development. Thus, systemic delivery of IL-27 can efficiently prevent EAE development and the priming of Th17 responses. However, the therapeutic potential of IL-27 is limited by its failure in inhibiting ongoing EAE, and shutting down established Th17 responses, presumably due to the expansion of $CD11b^+Gr\text{-}1^+$ myeloid cells.

MATERIALS AND METHODS

Mice

C57BL/6, C57BL/6 mice with targeted mutation of the IL-27Rα ($IL\text{-}27R\alpha^{-/-}$) and IL-10 ($IL\text{-}10^{-/-}$) genes were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). 2D2 TCR transgenic mice (25) were described before (26, 27). All mice were maintained in the animal facilities of The Ohio State University, and the studies were approved by the Institutional Animal Care and Use Committee.

Induction and Assessment of EAE

C57BL6, $IL\text{-}10^{-/-}$, and 2D2 mice of 8–12 weeks of age were immunized subcutaneously with 200 μg MOG 35-55 emulsified in PBS:CFA (1:1) in a total volume of 100 μL. MOG35-55 (MEVGWYRSPFSRVVHLYRNGK) was purchased from Genemed Synthesis, Inc. (South San Francisco, CA, USA). The purity of the peptide was greater than 90%. Mice also received 150 ng of pertussis toxin (List Biological, Campbell, CA, USA) in 200 μL PBS *via* the tail vein immediately after the immunization and again 48 h later. The mice were observed every day for the development of EAE symptoms using parameters as we described before (26, 27).

Production of AAV Viruses and Mice Treatment

Adeno-associated viral vector-IL-27, AAV-IL-30, and AAV-ctrl viruses were produced as we previously described (28). Briefly, IL-27 or IL-30 cDNA were inserted into an AAV carrier vector under the control of the CMV-chicken beta-actin hybrid promoter (29, 30). The IL-27 or IL-30 carrier AAV vector was compacted with a helper vector in 293K cells into the AAV serotype 8 (AAV8), which could achieve high expression in muscles (31, 32). AAV viruses were injected into mice intramuscularly (i.m.) using a dose of 2×10^{11} DRP/mouse diluted in 50 μL PBS.

ELISA

Serum samples were collected from mice treated with AAV-IL-27, AAV-IL-30, and AAV-ctrl viruses at various time points after viral injection. The presence of IL-27 or IL-30 in serum was detected using ELISA kits purchased from eBiosciences (IL-27) or R&D systems, Inc. (IL-30).

Isolation of Mononuclear Cells From Spinal Cords

Spinal cord tissues from AAV-IL-27, AAV-ctrl virus-treated or -untreated mice with EAE were removed and cut into about 2-mm pieces and incubated in 10 mM Hepes/NaOH buffer containing 1 mg/mL of collagenase IV (Sigma, St. Louis, MO, USA) for 1 h at 37°C. Then, the tissues were dispersed with syringe, filtered through a 100-mm wire mesh, and centrifuged at 2,000 rpm for 5 min at 4°C. After centrifugation, tissue pallets were re-suspended in 15 mL 30% Percoll (Pharmacia, Uppsala, Sweden), then centrifuged against 70% Percoll in a 50-mL tube for 15 min. The cell monolayer at the 30–70% Percoll interface was collected and washed once for further staining and flow cytometry analyses.

Antibodies and Flow Cytometry

FITC-, PE-, APC-, or Percp-labeled antibodies to CD4 (GK1.4), CD11b (M1/70), CD45 (30-F11), Gr-1 (RB6-8C5), Ly6C (AL-21), IL-6 (MP5-32C11), IL-10 (JES5-2A5), IL-17 (TC11-18H10), IFN-γ (XMG1.2), GM-CSF (MP1-22E9), FoxP3 (NRRF-30), PD-L1 (MIH5), IL-27Rα (2918), and isotype control antibodies were purchased from BD Biosciences (San Diego, CA, USA).

Procedures for cell surface marker staining and intracellular cytokine staining were the same as we described (26, 27). Briefly, for staining of cell surface markers, mononuclear cells from spleens, lymph nodes, and CNS were stained with various antibodies in staining buffer (PBS with 1% FCS) and incubated on ice for 30 min. After washing with staining buffer, cells were fixed in 1% paraformaldehyde in PBS. For intracellular cytokine staining, cells were stimulated in culture medium for 4 h with 100 ng/mL of phorbol 12-myristate 13-acetate and 500 ng/mL of ionomycin in the presence of Golgi^{stop} (1:1,500; BD Biosciences). Viable cells were then fixed in IC fixation buffer (eBioscience), permeabilized with 1× permeabilization buffer (eBiosciences), and stained with respective antibodies. Foxp3 staining was performed according to the manufacturer's protocol (BD Biosciences). Cells were collected on a FACSCalibur flow cytometer, and data were analyzed using the FlowJo software (Tree Star, Inc., OR, USA).

Sorting of CD11b$^+$Gr-1$^+$ Cells and Adoptive Transfer Into Mice With Established EAE

Spleen mononuclear cells from AAV-IL-27 or AAV-ctrl virus-treated mice (with or without EAE) were stained for CD11b and Gr-1, the CD11b$^+$Gr-1$^+$ cells were then sorted using the Moflo XDP sorter (Beckman Coulter, Indianapolis, IN, USA). To treat mice with EAE using CD11b$^+$Gr-1$^+$ myeloid cells, we first established EAE in C57BL6 mice, on day 10 post-immunization, mice were treated with AAV-IL-27 or AAV-ctrl virus as described above. Fourteen days after AAV treatment, mice were sacrificed and CD11b$^+$Gr-1$^+$ myeloid cells were sorted from spleens and were injected i.v. into mice with established EAE (1 million cells/per mouse; day 10 post EAE induction). The mice were observed for EAE development.

Real-Time PCR

Quantitative real-time PCR was performed using an ABI 7900-HT sequence system (PE Applied Biosystems) using previously determined conditions (33). The following primers were used for amplifying specific genes: actin: 5′-GAG ACC TTC AAC ACC CCA GC-3′ (forward) and 5′-ATG TCA CGC ACG ATT TCC C-3′ (reverse); IL-1b: 5′-CCA CCT CAA TGG ACA GAA TAT CA-3′ (forward) and 5′-CCC AAG GCC ACA GGT ATT T-3′ (reverse); IL-6: 5′-CCA GAG TCC TTC AGA GAG ATA CA-3′ (forward) and 5′-AAT TGG ATG GTC TTG GTC CTT AG-3′ (reverse); IL-12a: 5′-GAC CAA ACC AGC ACA TTG AAG-3′ (forward) and 5′-CTC CCT CTT GTT GTG GAA GAA-3′ (reverse); IL-17a: 5′-CGC AAT GAA GAC CCT GAT AGA T-3′ (forward) and 5′-CTC TTG CTG GAT GAG AAC AGA A-3′ (reverse); IL-23p19: 5′-CCA GCG GGA CAT ATG AAT CTA C-3′ (forward) and 5′-TGT GGG TCA CAA CCA TCT TC-3′ (reverse); IL-10: 5′-ACA GCC GGG AAG ACA ATA AC-3′ (forward) and 5′-CAG CTG GTC CTT TGT TTG AA-3′ (reverse); IFNg: 5′-AGC TCT TCC TCA TGG CTG TT-3′ (forward) and 5′-TTT GCC AGT TCC TCC AGA TA-3′ (reverse); TNFa: 5′-ATG AGA AGT TCC CAA ATG GC-3′ (forward) and 5′-CTC CAC TTG GTG GTT TGC TA-3′ (reverse); GM-CSF: 5′-CTG CGT AAT GAG CCA GGA AC-3′ (forward) and 5′-GTT TGT CTT CCG CTG TCC AA-3′ (reverse); S100A8: 5′-GTC CTC AGT TTG TGC AGA ATA TAA A-3′ (forward) and 5′-TAT CAC CAT CGC AAG GAA CTC-3′ (reverse); S100A9: 5′-GCA CAG TTG GCA ACC TTT ATG-3′ (forward) and 5′-CCA TCA GCA TCA TAC ACT CCT C-3′ (reverse). Each sample (RNA purified from sorted CD11b$^+$Gr1$^+$ myeloid cells or spinal cords) was assayed in triplicate, and the experiments were repeated two to three times. The relative gene expression was determined using the comparative method ($2^{-\Delta\Delta Ct}$).

Statistics

Data were expressed as means of individual determinations ±SE. Two-tailed Student's *t*-test or one way ANOVA was used for statistical analyses.

RESULTS

Systemic Delivery of IL-27 by AAV Virus Inhibits Th17 Responses and Prevents EAE Development

To determine if IL-27 or IL-30 can be used as a potential therapeutic for autoimmune diseases, we generated recombinant adeno-associated virus that express IL-27 (AAV-IL-27) or IL-30 (AAV-IL-30) and the control AAV virus (AAV-ctrl). Intramuscular injection (i.m.) of 2×10^{11} DRP/mouse of AAV-IL-27 or AAV-IL-30 achieved high and stable IL-27 (**Figure 1A**) or IL-30 (**Figure 1B**) production in the peripheral blood of mice. AAV-IL-27 treatment significantly enhanced Th1 response and slightly induced T cell production of IL-10, while reduced the frequencies of Th17 and Treg cells in spleens (**Figure 1C**). By contrast, AAV-IL-30 treatment slightly inhibited Th1 response but failed to affect the frequencies of Th17/Treg cells and T cell production of IL-10 (**Figure 1D**).

To determine if AAV-delivered IL-27 or IL-30 could block EAE development, we injected AAV-IL-27, AAV-IL-30, or AAV-ctrl virus into C57BL6 mice, 1 week later mice were immunized with MOG35-55/CFA and pertussis toxin. In AAV-ctrl virus-treated mice, EAE symptoms developed, with first symptoms showed up on day 10, while disease progressed to peak around days 14–17, then the EAE symptoms went down but maintained at a lower level for a long time (**Figures 2A,B**). While a single injection of AAV-IL-27 completely prevented EAE development in C57BL6 mice (**Figure 2A**), a single dose of AAV-IL-30 only slightly inhibited EAE development (**Figure 2B**). AAV-IL-27 treatment failed to prevent EAE in IL-27Rα$^{-/-}$ mice, suggesting that AAV-IL-27 acts through IL-27 receptor (**Figure 2C**). 2D2 TCR transgenic mice develop progressive EAE symptoms upon immunization, presumably due to the activation of overwhelming numbers of myelin-specific T cells. We therefore tested if EAE in 2D2 mice could be prevented by AAV-IL-27 treatment. As shown in **Figure 2D**, AAV-IL-27 administration slightly delayed the onset of EAE symptoms, but significantly inhibited the EAE symptoms in 2D2 mice.

To determine if AAV-IL-27 prevented EAE development by altering T cell responses, we analyzed T cell subsets in the draining lymph nodes (DLNs) and spleens from AAV-IL-27-treated mice and controls. As shown in **Figure 3**, we found that the CD4$^+$ T cells from the immune lymph nodes (**Figure 3A**) and

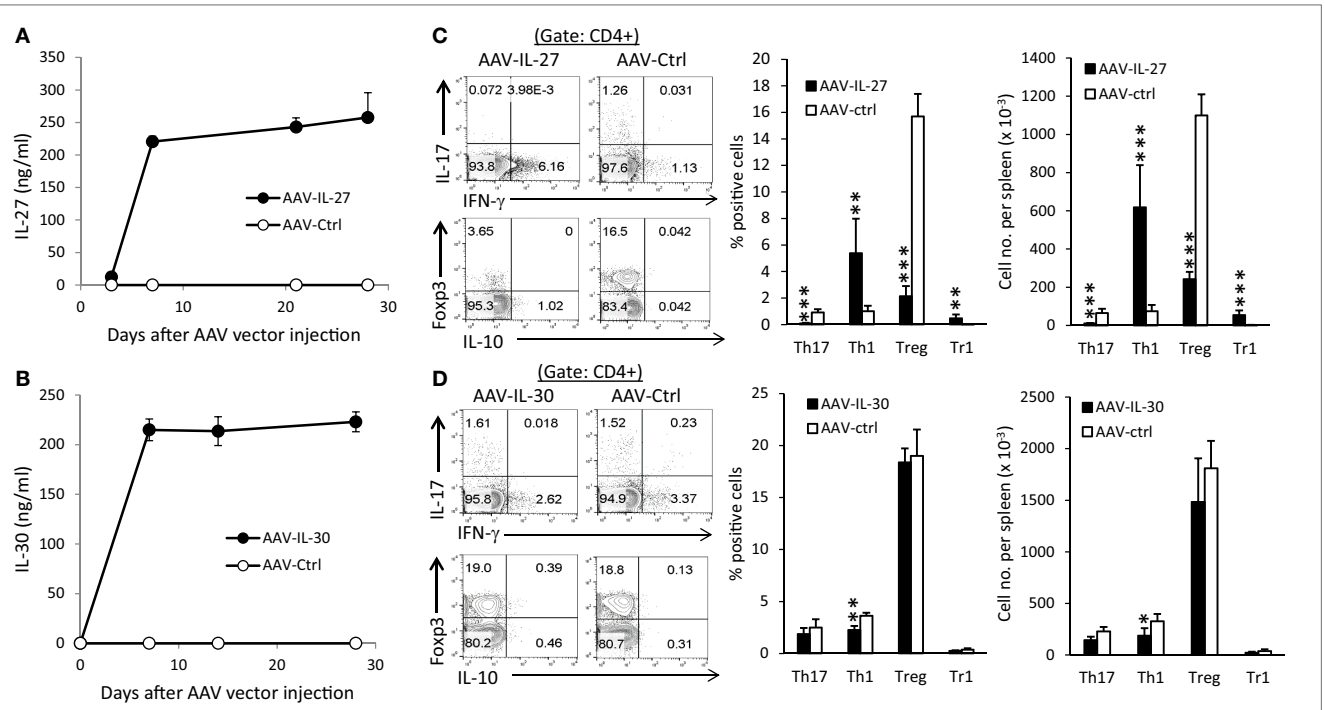


FIGURE 1 | Adeno-associated viral vector (AAV) mediated delivery of interleukin-27 (IL-27)/IL-30 and their impacts on T cell subsets. C57BL6 mice (n = 5/group) were treated with AAV-IL-27, AAV-IL-30, or AAV-ctrl virus i.m. at a dose of 2×10^{11} DRP/mouse. IL-27 **(A)** or IL-30 **(B)** levels in blood were detected by ELISA. Two weeks after AAV injection, mice (n = 5/group) were sacrificed and CD4+ T cells in spleens from AAV-IL-27 **(C)** or AAV-IL-30 **(D)** treated mice were analyzed and quantified by flow cytometry. Data shown represent two **(A,B)** and three **(C,D)** experiments with similar results. Statistical analysis was performed using the unpaired Student's t-test. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

spleens (**Figure 3B**) of AAV-IL-27-treated mice had significantly decreased GM-CSF, IL-17, and Foxp3 expressing subsets, while IL-10 and IFN-γ producing subsets increased compared with CD4+ T cells from AAV-IL-30-treated or other control groups of mice.

IL-10 and PD-L1 Independent EAE Inhibition in AAV-IL-27-Treated Mice

Since we detected increased numbers of IL-10 producing Th cells in AAV-IL-27-treated mice, we tested if AAV-IL-27 mediated inhibition of EAE *via* induction of IL-10. C57BL6 or IL-$10^{-/-}$C57BL6 mice were treated with AAV-IL-27 or AAV-ctrl virus, followed by induction of EAE *via* active immunization. While both WT and IL-$10^{-/-}$ mice treated with AAV-ctrl virus developed severe EAE symptoms (**Figure 4A**), both mice treated with AAV-IL-27 failed to develop EAE. Thus, AAV-IL-27-induced IL-10 production by T cells is insufficient to inhibit T cell-mediated EAE.

Since IL-27 was shown to induce T cell expression of PD-L1, which contributed to T cell tolerance in the EAE model (10), we tested if AAV-IL-27 induced T cell tolerance through induction of PD-L1. As shown in **Figure 4B**, we found that treatment with AAV-IL-27, but not AAV-Ctrl virus indeed induced significant expression of PD-L1 in T cells. To determine if PD-L1-PD-1 interaction among T cells mediated their tolerance, C57BL6 mice were first treated with AAV-IL-27 or AAV-ctrl virus followed by EAE induction 1 week later. On days 5, 9, 13, and 17 after EAE induction, mice receiving AAV-IL-27 treatment were also treated with 300 μg/mouse of anti-PD-1 or an isotype-matched control antibody i.p. As shown in **Figure 4C**, while mice treated with AAV-ctrl virus and control antibody exhibited EAE symptoms by day 10 and reached peak disease by day 17, mice treated with AAV-ctrl virus and anti-PD-1 developed more severe EAE, consistent with the known functions of PD-1 blockade in EAE development (34). However, mice treated with AAV-IL-27 + ctrl antibody or AAV-IL-27 + anti-PD-1 showed no EAE symptoms (**Figure 4C**). Thus, blockade of PD-L1-PD-1 interaction failed to reverse T cell tolerance induced by AAV-IL-27 treatment.

AAV-IL-27 Treatment Does Not Inhibit Established Th17 Responses and EAE

To determine if AAV-IL-27 treatment could reverse ongoing inflammation in the CNS, C57BL6 mice were immunized with MOG peptide/CFA and pertussis toxin. Ten days after immunization, when the first symptoms of EAE appeared, mice were treated with AAV-IL-27 or AAV-Ctrl virus i.m. As shown in **Figure 5A**, AAV-IL-27 treatment at day 10 after EAE induction failed to inhibit EAE development. Similarly, we found that treatment of mice on day 10 after EAE induction with AAV-IL-30 also had no effect on EAE development (**Figure 5B**). One potential explanation for failure of inhibiting EAE development could be due to lack of IL-27 receptor expression in the CNS-infiltrating CD4+ T cells. However, we found high levels of IL-27Rα expression

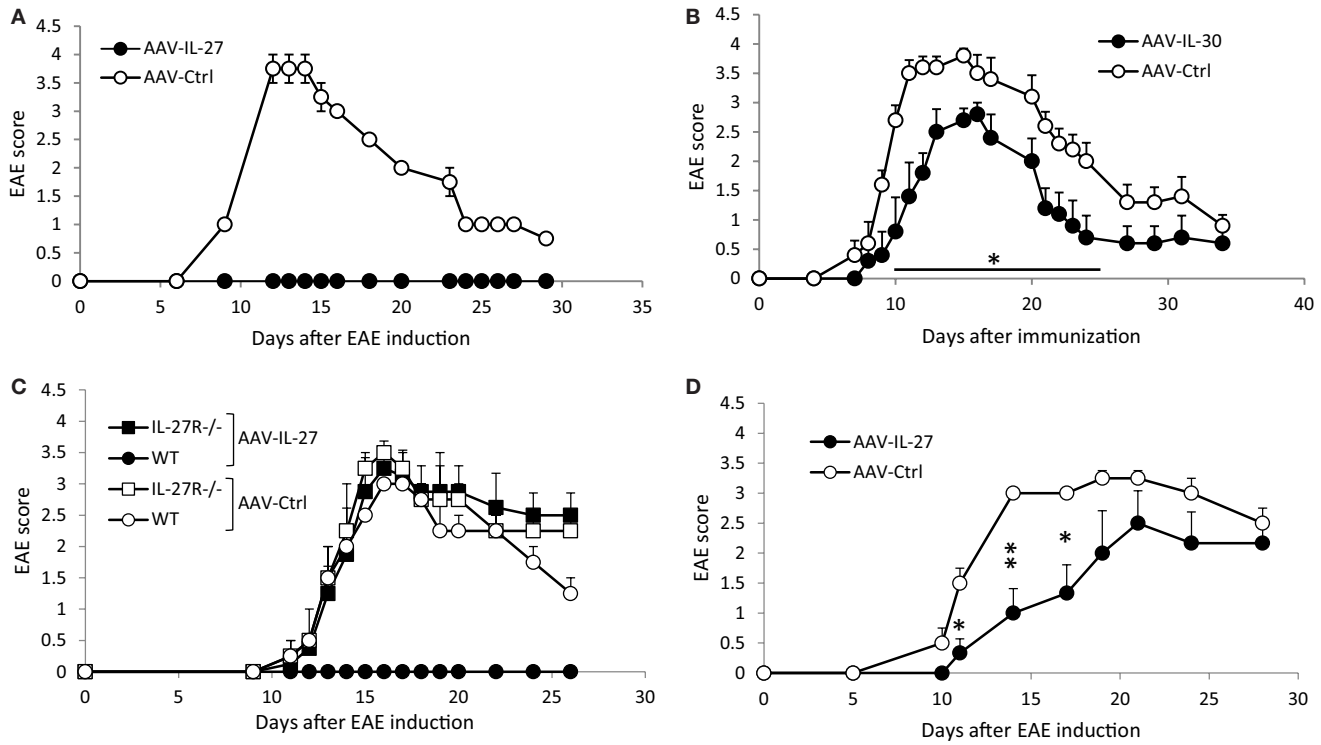


FIGURE 2 | The effects of adeno-associated viral vector (AAV)-delivered interleukin-27 (IL-27) or IL-30 in the prevention of experimental autoimmune encephalomyelitis (EAE) development. C57BL6 mice **(A,B)**, IL-27Rα$^{-/-}$ **(C)**, or 2D2 TCR transgenic mice **(D)** were treated with AAV-IL-27, AAV-IL-30, or AAV-ctrl virus i.m. at a dose of 2×10^{11} DRP/mouse. One week later, EAE was induced in the AAV-treated mice. Five mice per group were used in these experiments. Data shown represent two **(C,D)** to three **(A,B)** experiments with similar results. EAE scores are expressed as means ± SD. Statistical analysis was performed using the unpaired Student's *t*-test. $^{*}P < 0.05$; $^{**}P < 0.01$.

in the CNS-infiltrating CD4$^+$ T cells (**Figure 5C**). Moreover, we found that AAV-IL-27 treatment induced PD-L1 expression in CNS-infiltrating CD4$^+$ T cells (**Figure 5D**) and enhanced Th1 responses without significantly affecting Th17 responses in the CNS (**Figure 5E**). AAV-IL-27-treatment also significantly inhibited Treg subset without significantly affecting Tr1 subset in the CNS (**Figure 5F**). GM-CSF-producing CD4$^+$ T cells were found to be increased in the CNS of AAV-IL-27 treated mice (**Figure 5F**). Strikingly, we found that AAV-IL-27 treatment of mice with ongoing EAE upregulated many cytokine genes including GM-CSF, IL-17, IL-10, IFN-γ, IL-6, IL-1β, and TNF-α in the CNS (**Figure 5G**).

AAV-Mediated Delivery of IL-27 Induces the Expansion of CD11b$^+$Gr1$^+$ Myeloid Cells

Significant induction of cytokines such as IL-6, IL-1β, and TNF-α in the CNS suggests that AAV-IL-27 treatment may have significant impacts on myeloid cells. Indeed, through the analysis of the myeloid compartment in the peripheral lymphoid organs and CNS, we found that CD11b$^+$Gr1$^+$ myeloid cells were significantly increased in the spleens and CNS of mice with EAE, and AAV-IL-27 treatment further expanded those cells (**Figure 6A**). The impact of AAV-IL-27 on this population of cells was dramatic, as in the spleen, CD11b$^+$Gr-1$^+$ myeloid cells expanded about threefold compared to mice with untreated EAE (**Figure 6A**, right panel). Notably, we did not find expansion of CD11b$^+$Gr-1$^+$ myeloid cells in DLNs (**Figure 6A**). The expanded CD11b$^+$Gr-1$^+$ myeloid cells were mainly of the Ly6C^{low} subtype, and subtypes were not significantly different between AAV-IL-27 and AAV-ctrl-treated mice (**Figure 6B**). While we observed a major expansion of CD11b$^+$Gr-1$^+$ myeloid cells in mice with EAE that received AAV-IL-27 therapy at disease onset, in the EAE prevention model, AAV-IL-27-treated mice had much lower numbers of CD11b$^+$Gr-1$^+$ myeloid cells compared with AAV-ctrl-treated mice that developed EAE (**Figure 6C**). These results suggest that CD11b$^+$Gr-1$^+$ myeloid cells are mainly associated with disease activity. To determine if AAV-IL-27 therapy directly induce expansion of CD11b$^+$Gr-1$^+$ myeloid cells, we injected AAV-IL-27 or AAV-ctrl virus into naïve C57BL/6 mice and found that AAV-IL-27 treatment could significantly induce expansion of CD11b$^+$Gr-1$^+$ myeloid cells in naïve mice in the absence of EAE (**Figure 6D**). These data together suggest that IL-27 alone could induce expansion of CD11b$^+$Gr-1$^+$ myeloid cells, and in the presence of active EAE the expansion of these myeloid cells become more robust.

CD11b$^+$Gr-1$^+$ myeloid cells have previously been shown to inhibit or enhance EAE development (35, 36). We purified CD11b$^+$Gr-1$^+$ myeloid cells from the spleens of AAV-IL-27 and

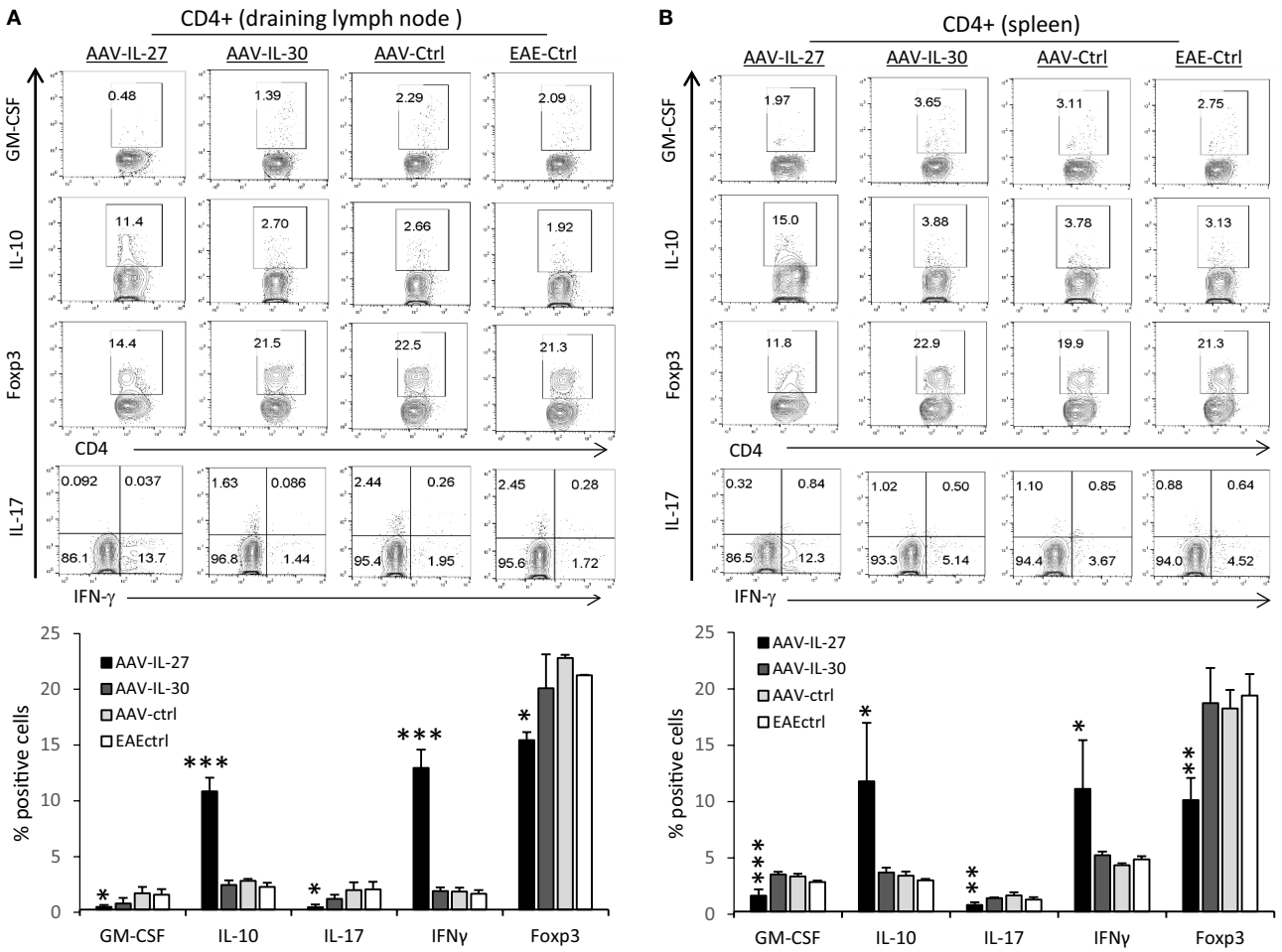


FIGURE 3 | T cell subsets in mice whose experimental autoimmune encephalomyelitis (EAE) development was prevented by adeno-associated viral vector (AAV)-delivered interleukin-27 (IL-27). C57BL6 mice (n = 5/group) were treated with AAV-IL-27, AAV-IL-30, and AAV-ctrl virus i.m. at a dose of 2×10^{11} DRP/mouse or left untreated. One week later, the mice were induced for EAE and sacrificed 2 weeks after EAE induction. T cell subsets in draining lymph nodes **(A)** and spleens **(B)** were analyzed by flow cytometry. Data were summarized and presented in the lower panels. *P < 0.05; **P < 0.01; ***P < 0.001 by one way ANOVA. Data shown represent two experiments with similar results.

AAV-ctrl virus-treated mice by FACS-based sorting, and analyzed their expression of cytokine genes. As shown in **Figure 7A**, we found that CD11b$^+$Gr-1$^+$ myeloid cells from AAV-IL-27-treated mice had increased expression of IL-6, IL-17, IL-23, S100A8, A100A9, IL-10, and TNF-α genes. IL-1β expression was decreased compared with myeloid cells from AAV-ctrl treated mice, but remained readily detectable (at 22 cycles by qPCR).

Using flow cytometry analysis, we found that IL-6 protein was readily detectable in CD11b$^+$Gr-1$^+$ myeloid cells from both AAV-IL-27 and AAV-ctrl treated EAE mice (**Figure 7B**). To test if IL-27-expanded myeloid cells affect EAE development, CD11b$^+$Gr-1$^+$ myeloid cells were FACS-purified from AAV-IL-27 or AAV-ctrl virus-treated mice with EAE and were injected i.v. into mice on day 10 post EAE induction. We found that CD11b$^+$Gr-1$^+$ myeloid cells from AAV-IL-27-treated mice more significantly enhanced EAE development (**Figure 7C**). Consistent with disease severity, we found that more CD4$^+$ T cells infiltrated into the CNS of mice receiving AAV-IL-27-expanded myeloid cells (**Figure 7D**).

DISCUSSION

In this study, we have evaluated the efficacy of AAV-delivered IL-27 (AAV-IL-27) and IL-30 (AAV-IL-30) in a murine model of MS. We found that one single administration of AAV-IL-27 completely prevented autoimmune encephalomyelitis, while significant, but incomplete protection was observed in AAV-IL-30-treated mice. AAV-IL-27 treatment inhibited Th17 responses and induced multiple inhibitory pathways in T cells. Strikingly, we found that mice with established EAE was completely resistant to AAV-IL-27 or AAV-IL-30 treatment, and AAV-IL-27 treatment induced the expansion of CD11b$^+$Gr-1$^+$ myeloid cells that could produce multiple cytokines including Th17-promoting cytokines.

The complete prevention of EAE development in C57BL6 mice by AAV-IL-27 suggests potent protective mechanisms are activated. Indeed, we observed that AAV-delivered IL-27 inhibited the priming of Th17 cells, and induced T cell expression of

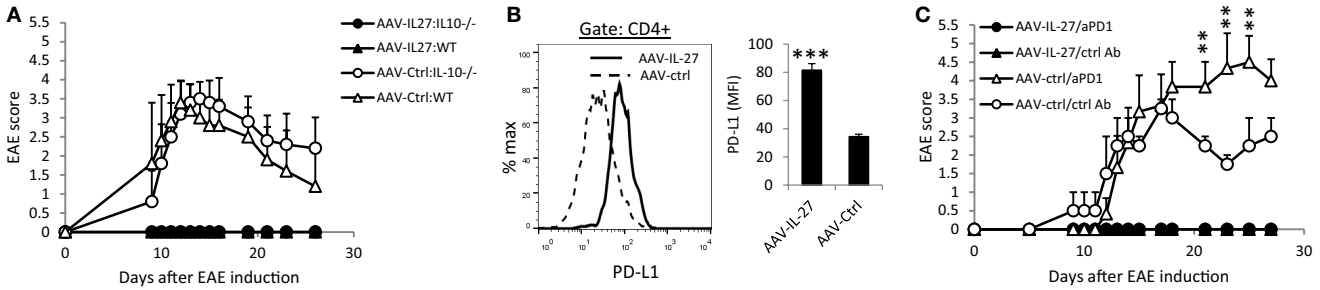


FIGURE 4 | The role of IL-10 and PD-1 blockade in adeno-associated viral vector (AAV)-interleukin-27 (IL-27) mediated prevention of experimental autoimmune encephalomyelitis (EAE). **(A)** WT or IL-10$^{-/-}$ mice were injected with AAV-IL-27 or AAV-ctrl virus i.m. at a dose of 2×10^{11} DRP/mouse. One week later, EAE was induced in the AAV virus-treated mice. Five mice per group were used in this experiment, and data shown represent two experiments with similar results. EAE scores are expressed as means ± SD. **(B)** AAV-IL-27 treatment induced T cell expression of PD-L1. Representative FACS plot (left panel) and summary (right panel) of PD-L1 expression on spleen CD4$^+$ T cells is shown. Three to four mice per group were included in this experiment, and data represent five experiments with similar results. Statistical analysis was performed using the unpaired Student's *t*-test. ****$P < 0.0001$. **(C)** PD-1 blockade failed to break T cell tolerance in AAV-IL-27-treated mice. C57BL6 mice ($n = 4$/group) were first treated with AAV-IL-27 or AAV-ctrl virus followed by EAE induction 1 week later. On days 5, 9, 13, and 17 after EAE induction, mice were also treated with 300 μg/mouse of anti-PD-1 (RMP1-14) or an isotype-matched control antibody (2A3) i.p. EAE scores are expressed as means ± SD. Data shown represent three experiments with similar results. **$P < 0.01$ by Student's *t*-test.

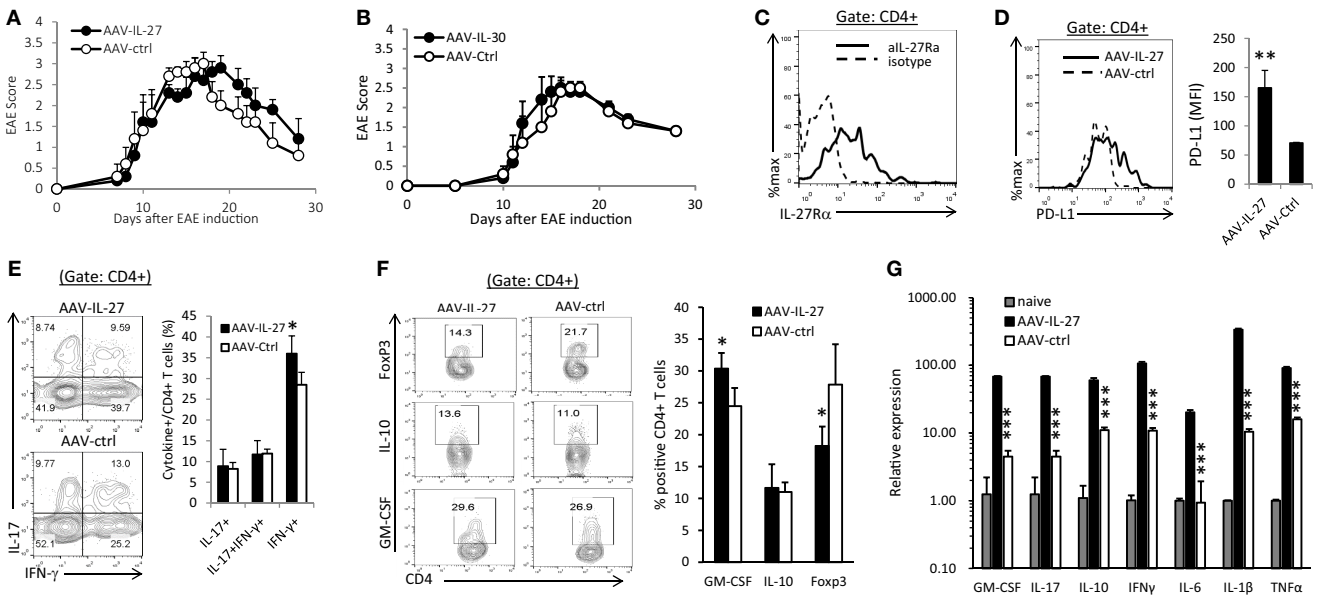


FIGURE 5 | Therapeutic effect of adeno-associated viral vector (AAV)-interleukin-27 (IL-27) or AAV-IL-30 on established experimental autoimmune encephalomyelitis (EAE). C57BL6 mice were immunized with MOG peptide/CFA and pertussis toxin. Ten days after immunization, when the first symptoms of EAE appeared, mice ($n = 5$/group) were treated with AAV-IL-27 **(A)** or AAV-IL-30 **(B)** virus i.m. The mice were then evaluated for the development of EAE signs. EAE scores are expressed as means ± SD. Data shown represent three experiments with similar results. On day 29 after EAE induction, the mice were sacrificed and the expression of IL-27Rα **(C)**, PD-L1 **(D)**, IFN-γ/IL-17A **(E)**, and FoxP3/IL-10/GM-CSF **(F)** in the central nervous system-infiltrating CD4$^+$ T cells were analyzed by flow cytometry. Data shown represent two to three experiments with similar results. Statistical analysis was performed using the unpaired Student's *t*-test. *$P < 0.05$; **$P < 0.01$. **(G)** The expression of cytokine genes in the spinal cords of AAV-IL-27 or AAV-ctrl virus-treated mice with established EAE ($n = 4$/group) were assessed by qPCR. Data shown represent two experiments with similar results. ***$P < 0.001$ by Student's *t*-test.

IL-10 and PD-L1. Inhibition of Th17 response is consistent with previous studies (37, 38) using IL-27 as therapeutic, suggesting that AAV-IL-27-mediated inhibition of Th17 response contributes to the prevention of EAE. We also observed that AAV-IL-27 treatment inhibited the frequencies of GM-CSF-producing T cells in peripheral lymphoid organs in EAE protected mice, which is consistent with the known function of IL-27 in inhibiting GM-CSF production by T cells (17, 24). Although high frequencies of IL-10-producing T cells were induced, our results suggest that AAV-IL-27-induced IL-10 production by T cells is not responsible for induction of T cell tolerance, since AAV-IL-27-treatment induced complete protection of EAE development in

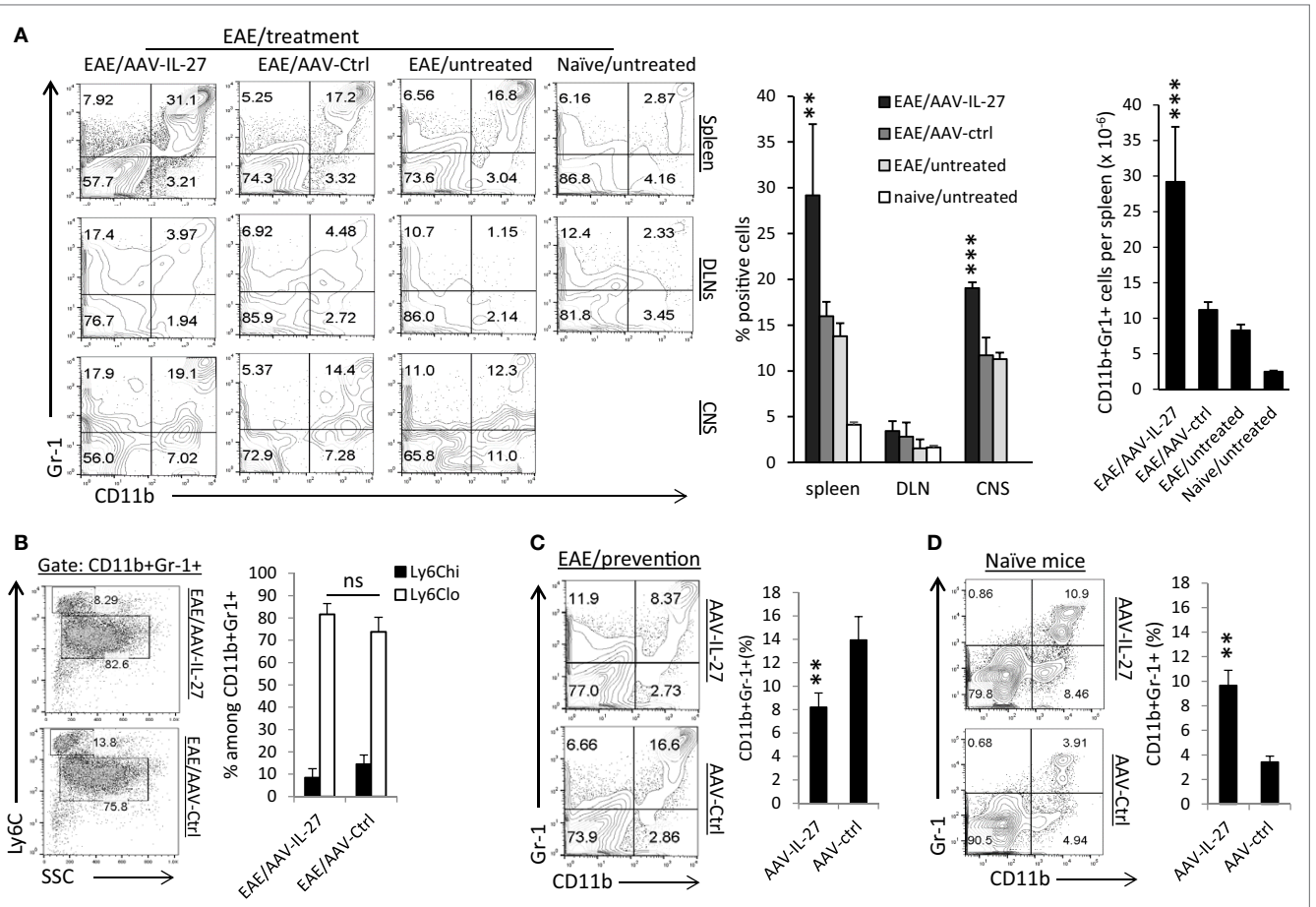


FIGURE 6 | The effects of adeno-associated viral vector (AAV)-delivered interleukin-27 (IL-27) on the expansion of CD11b$^+$Gr-1$^+$ myeloid cells. **(A)** C57BL6 mice were immunized with MOG peptide/CFA and pertussis toxin. Ten days after immunization, when the first symptoms of experimental autoimmune encephalomyelitis (EAE) appeared, mice (n = 5/group) were treated with AAV-IL-27 or AAV-ctrl virus i.m. or left untreated. By day 30 after EAE induction, mice were sacrificed, and myeloid cells in spleens (upper panel), draining lymph nodes (DLNs; middle panel) and spinal cords [central nervous system (CNS)] were analyzed by flow cytometry. Percentages and absolute numbers (spleen) of CD11b$^+$Gr-1$^+$ myeloid cells were also quantified (right panel). Data shown represent two experiments with similar results. $^{**}P < 0.01$; $^{***}P < 0.001$ by one way ANOVA test. **(B)** Subsets of myeloid cells in AAV-IL-27 or AAV-ctrl virus-treated mice with EAE. Mice (n = 6–7/group) were induced for EAE and treated as described above. By day 30 after EAE induction, mice were sacrificed, and spleen cells were stained for CD11b, Gr-1, and Ly6C, and were analyzed by flow cytometry. Statistical analysis was performed using the unpaired Student's t-test. $^*P < 0.05$. Data shown represent three experiments with similar results. **(C)** C57BL6 mice (n = 5/group) were injected with AAV-IL-27 or AAV-ctrl vectors i.m. at a dose of 2×10^{11} DRP/mouse. One week later, mice were induced for EAE. Two weeks after EAE induction, mice were sacrificed and spleen cells were analyzed for the expansion of CD11b$^+$Gr-1$^+$ myeloid cells by flow cytometry. $^{**}P < 0.01$ by Student's t-test. Data shown represent two experiments with similar results. **(D)** C57BL6 mice (n = 5/group) were injected with AAV-IL-27 or AAV-ctrl virus i.m. at a dose of 2×10^{11} DRP/mouse. Two weeks later, mice were sacrificed and spleen cells were analyzed for the expansion of CD11b$^+$Gr-1$^+$ myeloid cells by flow cytometry. $^{**}P < 0.01$ by Student's t-test. Data shown represent five experiments with similar results.

IL-10-deficient mice (**Figure 4**). IL-27-mediated PD-L1 expression in T cells has been shown to be sufficient for inducing T cell tolerance in a mouse model of human MS (10). In this study, we found that PD-L1 was induced in CD4$^+$ T cells in the peripheral lymphoid organs (**Figure 4**) and in the CNS (**Figure 5D**). However, despite the ability to enhance EAE development in AAV-ctrl treated mice, anti-PD-1 antibody treatment failed to break T cell tolerance induced by AAV-IL-27 (**Figure 4C**). Thus, PD-L1 expression in T cells is not solely responsible for AAV-IL-27-mediated blockade of EAE development.

Interleukin-27 has multi-faceted roles in T cell responses. While IL-27 has been shown to inhibit Th1 responses (39), majorities of studies have shown that IL-27 enhances Th1 responses by activating Stat1–T-bet axis (40–42). IL-27 has been shown controversial roles in Tregs (43–47), but in IL-27 transgenic mice, Treg cells are deleted (45). In this study, we found that AAV-IL-27 treatment enhanced Th1 responses and downregulated Treg frequencies. However, increased Th1 responses and reduced Treg cells did not reverse AAV-IL-27-mediated EAE protection, suggesting that IL-27-induced inhibition of Th17 priming, and may be together with the activation of other inhibitory pathways are sufficient to prevent EAE development.

A striking finding in this study is that established EAE was resistant to AAV-IL-27 treatment, and in the CNS of AAV-IL-27-treated mice, CD4$^+$ T cell production of key inflammatory cytokines such as IL-17 and GM-CSF were not affected or even

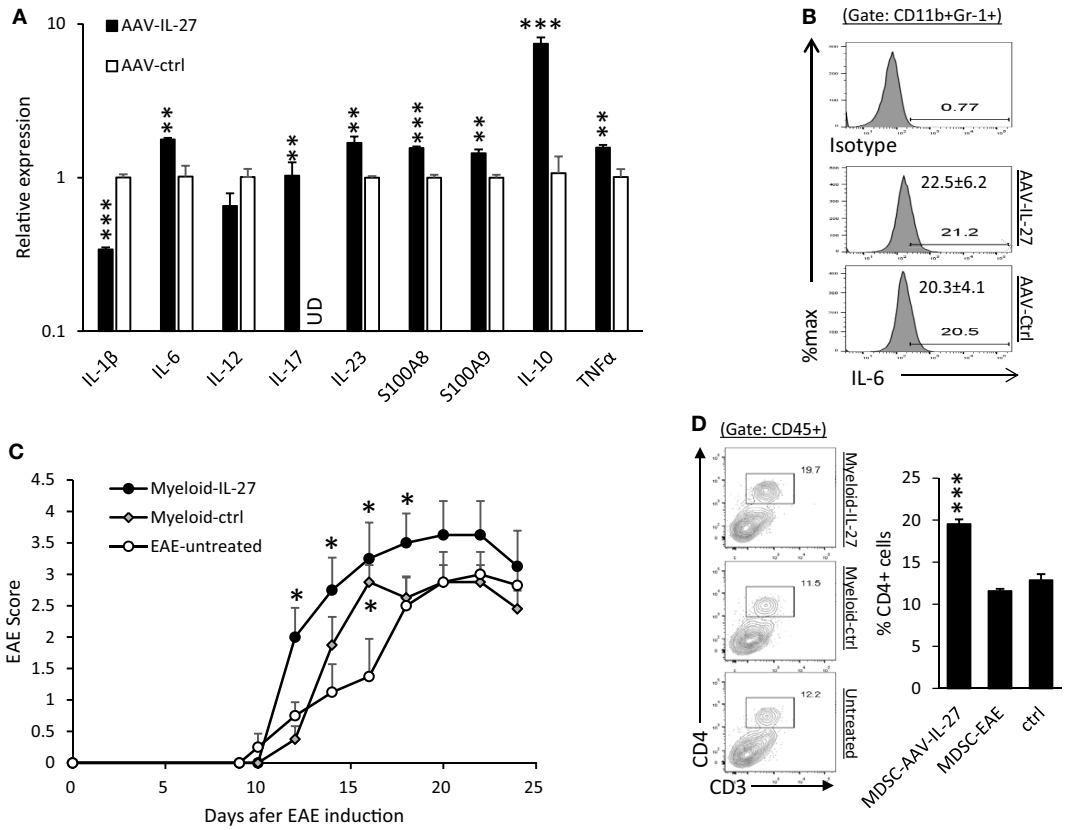


FIGURE 7 | CD11b$^+$Gr-1$^+$ myeloid cells from adeno-associated viral vector (AAV)-interleukin-27 (IL-27)-treated mice enhance experimental autoimmune encephalomyelitis (EAE) development. **(A)** Myeloid cells from AAV-IL-27-treated mice express more inflammatory cytokine genes. CD11b$^+$Gr-1$^+$ myeloid cells were FACS sorted from spleens of AAV-IL-27 and AAV-ctrl treated mice. The expression of cytokine genes was assessed by qPCR. The sorted cells from each treatment group ($n = 3$) were mixed, and results were expressed as mean ± SD from triplicates. ud, un detectable. Data shown represent two experiments with similar results. **(B)** Intracellular staining and flow cytometry were used for detecting IL-6 in myeloid cells from AAV-IL-27-treated ($n = 7$) or AAV-ctrl-treated ($n = 6$) mice. Numbers represent mean ± SD of IL-6$^+$ cells among the CD11b$^+$Gr1$^+$ population. Data shown represent two experiments with similar results. **(C)** Adoptive transfer of AAV-IL-27-treated CD11b$^+$Gr-1$^+$ myeloid cells enhances EAE development. CD11b$^+$Gr-1$^+$ myeloid cells were FACS-purified from AAV-IL-27 or AAV-ctrl virus-treated mice with EAE and were injected i.v. into mice (1 million cells/per mouse) on day 10 post EAE induction. The mice ($n = 4$/group) were observed for EAE development. *$P < 0.05$ by one way ANOVA. Data shown represent two experiments with similar results. **(D)** CD4$^+$ T cell expansion was observed in the central nervous system (CNS) of mice receiving AAV-IL-27-treated CD11b$^+$Gr-1$^+$ myeloid cells. Mice indicated in **(D)** were sacrificed on day 24 and flow cytometry was used to analyze the CNS-infiltrating leukocytes. ***$P < 0.001$ by one way ANOVA.

elevated (**Figures 5E,F**). Lack of suppression of ongoing EAE by AAV-IL-27 could be due to the blood–brain barrier (BBB) prevented IL-27 access to the CNS or due to unresponsiveness of CNS-infiltrating T cells. However, these possibilities are highly unlikely. It is known that BBB is wide open during the CNS inflammation (48, 49), and T cells in the CNS of mice with EAE expressed high levels of IL-27 receptor (**Figure 5C**). It is also unlikely that lack of suppression of ongoing inflammation is due to delayed production of IL-27 by AAV virus, since we observed that AAV-mediated IL-27 production was efficient (by day 3 > 10 ng/mL of IL-27 can be detected in blood). More importantly, we found clear evidence that the CNS T cells from AAV-IL-27-treated mice were stimulated by IL-27, which is reflected by induction of PD-L1 expression, increased Th1 and decreased Treg responses (**Figure 5**). Our results presented in **Figures 6** and **7** suggest that the resistance of ongoing Th17-mediated CNS inflammation to IL-27 therapy could be due to the expansion of CD11b$^+$Gr-1$^+$ cells. It is well established that CD11b$^+$Gr-1$^+$ cells expand during EAE development (35, 36). However, the role of this population of cells in EAE development is not clearly understood. Adoptive transfer experiment showed that these cells inhibited EAE development (35). However, other study clearly showed that these cells promoted Th17 responses *via* production of IL-1β, and depletion of this population of cells ameliorated EAE development (36). In this study, we found that mice with established EAE had significant expansion of CD11b$^+$Gr-1$^+$ cells, and AAV-IL-27 treatment further expanded these cells. Moreover, we found that AAV-IL-27 treatment could directly induce the expansion of CD11b$^+$Gr-1$^+$ cells (**Figure 6D**), and adoptive transfer of CD11b$^+$Gr-1$^+$ cells from AAV-IL-27-treated mice enhanced EAE development (**Figure 7C**). Thus, AAV-IL-27 therapy-induced expansion of CD11b$^+$Gr-1$^+$ cells enhances EAE development.

Expansion of CD11b$^+$Gr-1$^+$ myeloid cells mainly occurred in the spleens and CNS but not in DLNs, suggesting that these

cells do not regulate T cell priming but mainly act at the effector phase of EAE development. This observation partially explains why EAE development was completely prevented despite some $CD11b^+Gr\text{-}1^+$ myeloid cell expansion was observed (**Figure 6C**). The cytokine profiling of IL-27-expanded $CD11b^+Gr\text{-}1^+$ myeloid cells provides an explanation for why these cells enhance EAE development or confer resistance to IL-27 therapy. AAV-IL-27-induced myeloid cells express multiple Th17-promoting cytokines including IL-1β, IL-6, IL-17 and IL-23. IL-1β, IL-6, and IL-23 have been well established as key cytokines for Th17 cell induction/amplification and EAE development (23, 36, 50, 51). Although we observed reduced IL-1β expression in FACS-sorted, IL-27-expanded $CD11b^+Gr\text{-}1^+$ myeloid cells (**Figure 7A**; reduced but still readily detectable by qPCR at 22 cycles), the overall expression of IL-1β increased in the CNS of AAV-IL-27-treated mice (**Figure 5G**), suggesting that more $CD11b^+Gr\text{-}1^+$ myeloid cells accumulated in the CNS and served as a major source of IL-1β. AAV-IL-27-induced $CD11b^+Gr\text{-}1^+$ myeloid cells also express S100A8/S100A9, which have been implicated in the inflamed CNS of mice with EAE (52) and shown to promote IL-1β and IL-6 production by immune cells (53). Thus, AAV-IL-27 therapy induces key cytokines for Th17 response in these cells, which could amplify the pre-existing Th17 cells during the effector phase of EAE. In addition to pro-inflammatory cytokines, we also observed that IL-27-expanded $CD11b^+Gr\text{-}1^+$ myeloid cells expressed high levels of IL-10, which could explain why high levels of pro-inflammatory cytokines detected in the CNS (**Figure 5G**) did not cause much worse disease (**Figure 5A**).

Lack of suppression of ongoing autoimmunity by AAV-IL-27 is consistent with the report (54) showing that IL-27 could not inhibit established Th17 responses, but appears to be inconsistent with other reports (9, 13) demonstrating that systemic IL-27 inhibits T cell adoptive transfer EAE. At this stage, we do not know the reason for this inconsistency. AAV-mediated delivery of IL-27 is highly efficient and results in stable and high concentrations in the blood of the treated mice, and this is not easily achievable by systemic injection of IL-27 protein. It is thus necessary to determine if low and high concentrations of IL-27 induce T cell tolerance *via* different mechanisms. On the other hand, since adoptive transfer EAE involves a latent phase before EAE signs appear (55, 56), suggesting that Th17 priming/differentiation *in vivo* is still needed for causing disease after T cell transfer. Thus, the initial stage of T cell adoptive transfer should not be considered as mice having an ongoing disease, and thus it is not surprising to see that IL-27 could suppress adoptive transfer EAE.

Taken together, our study suggests that systemic delivery of IL-27 can efficiently prevent EAE development and the priming of Th17 cells. However, the therapeutic potential of IL-27 may be limited by its failure to shut down established Th17 responses and reverse ongoing inflammation, presumably due to the expansion of $CD11b^+Gr\text{-}1^+$ myeloid cells. Moreover, the depletion of Treg cells adds additional risk for IL-27-based therapy of autoimmune diseases like MS.

ETHICS STATEMENT

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of The Ohio State University (Protocol#: 2008A0093R2 and R3).

AUTHOR CONTRIBUTIONS

JZ, J-QL, ZL, and LW performed most of the experiments; MS performed IL-27 ELISA; JZ and JD helped for the production of AAV viruses. X-FB designed all experiments, performed data analyses, and wrote the manuscript.

FUNDING

This study was supported by grants from the National Institutes of Health (R21CA198037, R03CA226806) and a grant from China National Natural Science Foundation (81572805).

REFERENCES

1. Murugaiyan G, Mittal A, Weiner HL. Identification of an IL-27/osteopontin axis in dendritic cells and its modulation by IFN-gamma limits IL-17-mediated autoimmune inflammation. *Proc Natl Acad Sci U S A* (2010) 107(25):11495–500. doi:10.1073/pnas.1002099107
2. Pirhonen J, Siren J, Julkunen I, Matikainen S. IFN-alpha regulates toll-like receptor-mediated IL-27 gene expression in human macrophages. *J Leukoc Biol* (2007) 82(5):1185–92. doi:10.1189/jlb.0307157
3. Remoli ME, Gafa V, Giacomini E, Severa M, Lande R, Coccia EM. IFN-beta modulates the response to TLR stimulation in human DC: involvement of IFN regulatory factor-1 (IRF-1) in IL-27 gene expression. *Eur J Immunol* (2007) 37(12):3499–508. doi:10.1002/eji.200737566
4. Pflanz S, Hibbert L, Mattson J, Rosales R, Vaisberg E, Bazan JF, et al. WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27. *J Immunol* (2004) 172(4):2225–31. doi:10.4049/jimmunol.172.4.2225
5. Lucas S, Ghilardi N, Li J, de Sauvage FJ. IL-27 regulates IL-12 responsiveness of naive CD4+ T cells through Stat1-dependent and -independent mechanisms. *Proc Natl Acad Sci U S A* (2003) 100(25):15047–52. doi:10.1073/pnas.2536517100
6. Diveu C, McGeachy MJ, Boniface K, Stumhofer JS, Sathe M, Joyce-Shaikh B, et al. IL-27 blocks RORc expression to inhibit lineage commitment of Th17 cells. *J Immunol* (2009) 182(9):5748–56. doi:10.4049/jimmunol.0801162
7. Stumhofer JS, Silver JS, Laurence A, Porrett PM, Harris TH, Turka LA, et al. Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. *Nat Immunol* (2007) 8(12):1363–71. doi:10.1038/ni1537
8. Awasthi A, Carrier Y, Peron JP, Bettelli E, Kamanaka M, Flavell RA, et al. A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. *Nat Immunol* (2007) 8(12):1380–9. doi:10.1038/ni1541
9. Fitzgerald DC, Ciric B, Touil T, Harle H, Grammatikopolou J, Das Sarma J, et al. Suppressive effect of IL-27 on encephalitogenic Th17 cells and the effector phase of experimental autoimmune encephalomyelitis. *J Immunol* (2007) 179(5):3268–75. doi:10.4049/jimmunol.179.5.3268
10. Hirahara K, Ghoreschi K, Yang XP, Takahashi H, Laurence A, Vahedi G, et al. Interleukin-27 priming of T cells controls IL-17 production in trans via induction of the ligand PD-L1. *Immunity* (2012) 36(6):1017–30. doi:10.1016/j.immuni.2012.03.024
11. Amadi-Obi A, Yu CR, Liu X, Mahdi RM, Clarke GL, Nussenblatt RB, et al. TH17 cells contribute to uveitis and scleritis and are expanded by IL-2 and inhibited by IL-27/STAT1. *Nat Med* (2007) 13(6):711–8. doi:10.1038/nm1585
12. Kastelein RA, Hunter CA, Cua DJ. Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. *Annu Rev Immunol* (2007) 25:221–42. doi:10.1146/annurev.immunol.22.012703.104758
13. Fitzgerald DC, Fonseca-Kelly Z, Cullimore ML, Safabakhsh P, Saris CJ, Zhang GX, et al. Independent and interdependent immunoregulatory effects

of IL-27, IFN-beta, and IL-10 in the suppression of human Th17 cells and murine experimental autoimmune encephalomyelitis. *J Immunol* (2013) 190(7):3225–34. doi:10.4049/jimmunol.1200141
14. Batten M, Li J, Yi S, Kljavin NM, Danilenko DM, Lucas S, et al. Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells. *Nat Immunol* (2006) 7(9):929–36. doi:10.1038/ni1375
15. Niedbala W, Cai B, Wei X, Patakas A, Leung BP, McInnes IB, et al. Interleukin 27 attenuates collagen-induced arthritis. *Ann Rheum Dis* (2008) 67(10): 1474–9. doi:10.1136/ard.2007.083360
16. Chong WP, Horai R, Mattapallil MJ, Silver PB, Chen J, Zhou R, et al. IL-27p28 inhibits central nervous system autoimmunity by concurrently antagonizing Th1 and Th17 responses. *J Autoimmun* (2014) 50:12–22. doi:10.1016/j.jaut.2013.08.003
17. Casella G, Finardi A, Descamps H, Colombo F, Maiorino C, Ruffini F, et al. IL-27, but not IL-35, inhibits neuroinflammation through modulating GM-CSF expression. *Sci Rep* (2017) 7(1):16547. doi:10.1038/s41598-017-16702-w
18. Monahan PE, Samulski RJ. Adeno-associated virus vectors for gene therapy: more pros than cons? *Mol Med Today* (2000) 6(11):433–40. doi:10.1016/S1357-4310(00)01810-4
19. McCarty DM, Young SM Jr, Samulski RJ. Integration of adeno-associated virus (AAV) and recombinant AAV vectors. *Annu Rev Genet* (2004) 38:819–45. doi:10.1146/annurev.genet.37.110801.143717
20. Kaplitt MG, Feigin A, Tang C, Fitzsimons HL, Mattis P, Lawlor PA, et al. Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. *Lancet* (2007) 369(9579):2097–105. doi:10.1016/S0140-6736(07)60982-9
21. Daya S, Berns KI. Gene therapy using adeno-associated virus vectors. *Clin Microbiol Rev* (2008) 21(4):583–93. doi:10.1128/CMR.00008-08
22. Aalbers CJ, Tak PP, Vervoordeldonk MJ. Advancements in adeno-associated viral gene therapy approaches: exploring a new horizon. *F1000 Med Rep* (2011) 3:17. doi:10.3410/M3-17
23. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* (2003) 421(6924):744–8. doi:10.1038/nature01355
24. Codarri L, Gyulveszi G, Tosevski V, Hesske L, Fontana A, Magnenat L, et al. RORgammat drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. *Nat Immunol* (2011) 12(6):560–7. doi:10.1038/ni.2027
25. Bettelli E, Pagany M, Weiner HL, Linington C, Sobel RA, Kuchroo VK. Myelin oligodendrocyte glycoprotein-specific T cell receptor transgenic mice develop spontaneous autoimmune optic neuritis. *J Exp Med* (2003) 197(9):1073–81. doi:10.1084/jem.20021603
26. Carl JW Jr, Liu JQ, Joshi PS, El-Omrani HY, Yin L, Zheng X, et al. Autoreactive T cells escape clonal deletion in the thymus by a CD24-dependent pathway. *J Immunol* (2008) 181(1):320–8. doi:10.4049/jimmunol.181.1.320
27. Zhang X, Liu JQ, Shi Y, Reid HH, Boyd RL, Khattabi M, et al. CD24 on thymic APCs regulates negative selection of myelin antigen-specific T lymphocytes. *Eur J Immunol* (2012) 42(4):924–35. doi:10.1002/eji.201142024
28. Zhu X, Liu Z, Liu JQ, Zhu J, Zhang J, Davis JP, et al. Systemic delivery of IL-27 by an adeno-associated viral vector inhibits T cell-mediated colitis and induces multiple inhibitory pathways in T cells. *J Leukoc Biol* (2016) 100(2):403–11. doi:10.1189/jlb.3A1215-540R
29. Liu Y, Okada T, Nomoto T, Ke X, Kume A, Ozawa K, et al. Promoter effects of adeno-associated viral vector for transgene expression in the cochlea in vivo. *Exp Mol Med* (2007) 39(2):170–5. doi:10.1038/emm.2007.19
30. Song S, Laipis PJ, Berns KI, Flotte TR. Effect of DNA-dependent protein kinase on the molecular fate of the rAAV2 genome in skeletal muscle. *Proc Natl Acad Sci U S A* (2001) 98(7):4084–8. doi:10.1073/pnas.061014598
31. Wang Z, Zhu T, Qiao C, Zhou L, Wang B, Zhang J, et al. Adeno-associated virus serotype 8 efficiently delivers genes to muscle and heart. *Nat Biotechnol* (2005) 23(3):321–8. doi:10.1038/nbt1073
32. Qiao C, Li J, Jiang J, Zhu X, Wang B, Li J, et al. Myostatin propeptide gene delivery by adeno-associated virus serotype 8 vectors enhances muscle growth and ameliorates dystrophic phenotypes in mdx mice. *Hum Gene Ther* (2008) 19(3):241–54. doi:10.1089/hum.2007.159
33. Bai XF, Liu J, Li O, Zheng P, Liu Y. Antigenic drift as a mechanism for tumor evasion of destruction by cytolytic T lymphocytes. *J Clin Invest* (2003) 111(10): 1487–96. doi:10.1172/JCI17656
34. Salama AD, Chitnis T, Imitola J, Ansari MJ, Akiba H, Tushima F, et al. Critical role of the programmed death-1 (PD-1) pathway in regulation of experimental autoimmune encephalomyelitis. *J Exp Med* (2003) 198(1):71–8. doi:10.1084/jem.20022119
35. Ioannou M, Alissafi T, Lazaridis I, Deraos G, Matsoukas J, Gravanis A, et al. Crucial role of granulocytic myeloid-derived suppressor cells in the regulation of central nervous system autoimmune disease. *J Immunol* (2012) 188(3):1136–46. doi:10.4049/jimmunol.1101816
36. Yi H, Guo C, Yu X, Zuo D, Wang XY. Mouse CD11b+Gr-1+ myeloid cells can promote Th17 cell differentiation and experimental autoimmune encephalomyelitis. *J Immunol* (2012) 189(9):4295–304. doi:10.4049/jimmunol.1200086
37. Sasaoka T, Ito M, Yamashita J, Nakajima K, Tanaka I, Narita M, et al. Treatment with IL-27 attenuates experimental colitis through the suppression of the development of IL-17-producing T helper cells. *Am J Physiol Gastrointest Liver Physiol* (2011) 300(4):G568–76. doi:10.1152/ajpgi.00329.2010
38. Hanson ML, Hixon JA, Li W, Felber BK, Anver MR, Stewart CA, et al. Oral delivery of IL-27 recombinant bacteria attenuates immune colitis in mice. *Gastroenterology* (2014) 146(1):210–21.e13. doi:10.1053/j.gastro.2013.09.060
39. Villarino AV, Hunter CA. Biology of recently discovered cytokines: discerning the pro- and anti-inflammatory properties of interleukin-27. *Arthritis Res Ther* (2004) 6(5):225–33. doi:10.1186/ar1227
40. Chen Q, Ghilardi N, Wang H, Baker T, Xie MH, Gurney A, et al. Development of Th1-type immune responses requires the type I cytokine receptor TCCR. *Nature* (2000) 407(6806):916–20. doi:10.1038/35024131
41. Morishima N, Owaki T, Asakawa M, Kamiya S, Mizuguchi J, Yoshimoto T. Augmentation of effector CD8+ T cell generation with enhanced granzyme B expression by IL-27. *J Immunol* (2005) 175(3):1686–93. doi:10.4049/jimmunol.175.3.1686
42. Schneider R, Yaneva T, Beauseigle D, El-Khoury L, Arbour N. IL-27 increases the proliferation and effector functions of human naive CD8+ T lymphocytes and promotes their development into Tc1 cells. *Eur J Immunol* (2011) 41(1):47–59. doi:10.1002/eji.201040804
43. Neufert C, Becker C, Wirtz S, Fantini MC, Weigmann B, Galle PR, et al. IL-27 controls the development of inducible regulatory T cells and Th17 cells via differential effects on STAT1. *Eur J Immunol* (2007) 37(7):1809–16. doi:10.1002/eji.200636896
44. Huber M, Steinwald V, Guralnik A, Brustle A, Kleemann P, Rosenplanter C, et al. IL-27 inhibits the development of regulatory T cells via STAT3. *Int Immunol* (2008) 20(2):223–34. doi:10.1093/intimm/dxm139
45. Wojno ED, Hosken N, Stumhofer JS, O'Hara AC, Mauldin E, Fang Q, et al. A role for IL-27 in limiting T regulatory cell populations. *J Immunol* (2011) 187(1):266–73. doi:10.4049/jimmunol.1004182
46. Kim G, Shinnakasu R, Saris CJ, Cheroutre H, Kronenberg M. A novel role for IL-27 in mediating the survival of activated mouse CD4 T lymphocytes. *J Immunol* (2013) 190(4):1510–8. doi:10.4049/jimmunol.1201017
47. Hall AO, Beiting DP, Tato C, John B, Oldenhove G, Lombana CG, et al. The cytokines interleukin 27 and interferon-gamma promote distinct Treg cell populations required to limit infection-induced pathology. *Immunity* (2012) 37(3):511–23. doi:10.1016/j.immuni.2012.06.014
48. Fabis MJ, Scott GS, Kean RB, Koprowski H, Hooper DC. Loss of blood-brain barrier integrity in the spinal cord is common to experimental allergic encephalomyelitis in knockout mouse models. *Proc Natl Acad Sci U S A* (2007) 104(13):5656–61. doi:10.1073/pnas.0701252104
49. Bennett J, Basivireddy J, Kollar A, Biron KE, Reickmann P, Jefferies WA, et al. Blood-brain barrier disruption and enhanced vascular permeability in the multiple sclerosis model EAE. *J Neuroimmunol* (2010) 229(1–2):180–91. doi:10.1016/j.jneuroim.2010.08.011
50. Samoilova EB, Horton JL, Hilliard B, Liu TS, Chen Y. IL-6-deficient mice are resistant to experimental autoimmune encephalomyelitis: roles of IL-6 in the activation and differentiation of autoreactive T cells. *J Immunol* (1998) 161(12):6480–6.
51. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* (2006) 441(7090):235–8. doi:10.1038/nature04753
52. Turvey ME, Koudelka T, Comerford I, Greer JM, Carroll W, Bernard CC, et al. Quantitative proteome profiling of CNS-infiltrating autoreactive CD4+ cells reveals selective changes during experimental autoimmune encephalomyelitis. *J Proteome Res* (2014) 13(8):3655–70. doi:10.1021/pr500158r

53. Simard JC, Cesaro A, Chapeton-Montes J, Tardif M, Antoine F, Girard D, et al. S100A8 and S100A9 induce cytokine expression and regulate the NLRP3 inflammasome via ROS-dependent activation of NF-kappaB(1.). *PLoS One* (2013) 8(8):e72138. doi:10.1371/journal.pone.0072138
54. El-behi M, Ciric B, Yu S, Zhang GX, Fitzgerald DC, Rostami A. Differential effect of IL-27 on developing versus committed Th17 cells. *J Immunol* (2009) 183(8):4957–67. doi:10.4049/jimmunol.0900735
55. Bai XF, Liu JQ, Liu X, Guo Y, Cox K, Wen J, et al. The heat-stable antigen determines pathogenicity of self-reactive T cells in experimental autoimmune encephalomyelitis. *J Clin Invest* (2000) 105(9):1227–32. doi:10.1172/JCI9012
56. Bai XF, Li O, Zhou Q, Zhang H, Joshi PS, Zheng X, et al. CD24 controls expansion and persistence of autoreactive T cells in the central nervous system during experimental autoimmune encephalomyelitis. *J Exp Med* (2004) 200(4):447–58. doi:10.1084/jem.20040131

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

CASE REPORT
published: 11 April 2018
doi: 10.3389/fimmu.2018.00703

Overinterpretation and Overtreatment of Low-Titer Antibodies Against Contactin-Associated Protein-2

*Christian G. Bien**

Epilepsy Center Bethel, Krankenhaus Mara, Bielefeld, Germany

Antibodies (abs) against neural or glial antigens have become important diagnostic markers of autoimmune encephalitides. A key requirement for interpretation of any test in clinical medicine is specificity. In this work, a 35-year-old female patient with low-titer contactin-associated protein-2 abs not satisfying clinical criteria of autoimmune encephalitis is reported. The patient had a recurrent depressive disorder and, at the time of the ab study, a moderate depressive episode. Overinterpretation and misinterpretation of patient's complaints and paraclinical study results fueled the idea of an autoimmune encephalitis. It is suggested to check patients with supposedly positive ab test results critically for clinical criteria, titer cutoffs, and ab-typical epidemiological features like age and sex.

Keywords: contactin-associated protein-2 antibodies, depression, cell-based assays, neural antibodies, immunotherapy, diagnostic specificity

OPEN ACCESS

Edited by:
Johann Sellner,
Christian Doppler Klinik,
Universitätsklinikum Salzburg,
Austria

Reviewed by:
Maria Isabel Da Silva Leite,
University of Oxford,
United Kingdom
Markus Reindl,
Innsbruck Medical University,
Austria

***Correspondence:**
Christian G. Bien
christian.bien@gmx.de

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology

Received: *04 March 2018*
Accepted: *22 March 2018*
Published: *11 April 2018*

Citation:
Bien CG (2018) Overinterpretation and Overtreatment of Low-Titer Antibodies Against Contactin-Associated Protein-2. Front. Immunol. 9:703. doi: 10.3389/fimmu.2018.00703

BACKGROUND

Immunoglobulin G (IgG) antibodies (abs) against neural or glial antigens have become important diagnostic markers of autoimmune encephalitides or acquired demyelinating central nervous system (CNS) syndromes. A key question in clinical applications is their disease specificity: the clinician needs to be sure that a positive ab result is not an irrelevant finding; e.g., a non-specific product of some other physiological or pathological process, or even a laboratory artifact (that may be unmasked by re-testing the sample). In 2016, two approaches to detect false-positive ab results were described. First, a recent Position Paper authored by international experts delineated a clinical approach for the diagnosis of autoimmune encephalitis that is independent of ab findings and can be used as a plausibility check of a positive ab test result (1). A patient who does not meet the criteria for "possible autoimmune encephalitis," but is positive for a neural ab, should be carefully studied for alternative explanations for his or her condition. Second, some abs are considered non-specific if they occur below a certain serum titer. This has been suggested or studied for abs against glycine receptors (2), glutamic acid decarboxylase (3) and contactin-associated protein-2 (CASPR2) in the context of the clinical suspicion of autoimmune encephalitis (4). In this work, a patient with low-titer CASPR2 abs not satisfying both specificity criteria described above is reported who repeatedly underwent expensive and potentially harmful treatments.

CASE PRESENTATION

For a graphical presentation of the case, see **Figure 1**. At the end of 2014, at the age of 35 years, the patient of interest (female of German-Indonesian descent) developed symptoms of depression. No first-degree relatives of the patient suffered from any neuropsychiatric or autoimmune disorders.

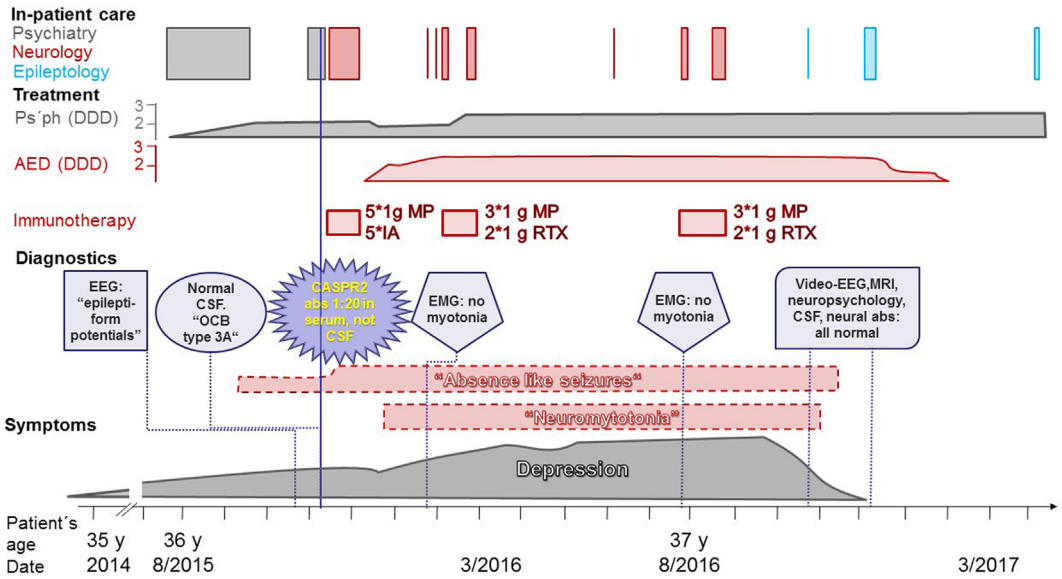


FIGURE 1 | Disease course of the patient. Gray: real symptoms, adequate treatment; red: non-real neurological symptoms (dashed border), treatments in vain; purple: diagnostic findings. Abbreviations: abs, antibodies; AED, antiepileptic drugs; CASPR2, contactin-associated protein-2; CSF, cerebrospinal fluid; DDD, defined daily doses; EEG, electroencephalogram; EMG, electromyogram; IA, immunoadsorptions; MP, intravenous methylprednisolone; MRI, magnetic resonance imaging; Ps'ph, psychopharmacological agents; RTX, rituximab; y, years.

Some years before, a psychiatrist had diagnosed this patient with a depressive episode and treated her accordingly. She had been trained as a commercial clerk. Due to her high performance and extraordinary commitment, she had been appointed managing director of five companies in Asia with 1,000 employees. After a few years in this position and along with a difficult marriage situation and a toddler, the depressive symptoms evolved. At the age of almost 36 years, self-medication with sedatives and hypnotics was no longer sufficient. Suicidal ideations tormented her. She asked for in-patient admission to a German psychosomatic hospital. The medical report lists the following symptoms: depressive mood, anhedonia, lack of drive, fatigue, concentration and distraction problems, low self-confidence, feelings of guilt, and suicidal thoughts. The diagnosis of recurrent depressive disorder (currently termed moderate depressive episode) was made (ICD-10: F33.1). Despite increasing doses of antidepressant and neuroleptic medication, her mood deteriorated, she complained of memory loss (never formally assessed), and she suffered from a sudden nervous breakdown. She said she would kill herself and her four-year old son. She was transferred to a closed ward of a university psychiatric department.

At the psychiatric department, she reported episodes during which she felt detached from the surrounding world. These "bubbles" (as she called them) occurred several times per day. At that time, a doctor from the university's department of neurology investigated her on the psychiatric ward. The doctor suspected these "bubbles" were epileptic seizures with impaired consciousness, and he ordered a routine electroencephalogram (EEG). The EEG was interpreted as displaying right-hemispheric epileptiform potentials with a tendency to generalize. However, subsequent analysis of the suspicious EEG epochs by the author of this report revealed physiological activity without any potentials suggestive of epileptiform activity (**Figure 2**). The original EEG reading led to the interpretation of the "bubbles" as focal epileptic seizures. In retrospect, one would probably interpret them as derealization epochs as part of the depressive episode (5). Magnetic resonance imaging (MRI) did not show a potential epileptogenic lesion. Lumbar puncture revealed <1 cell/µl. Neither the IgG index (0.46, normal values <0.7) nor the Reiber diagram suggested an intrathecal IgG synthesis. Isoelectric focusing produced a weak positive result with <4 autochthonous cerebrospinal fluid (CSF) bands, reported by the laboratory as "oligoclonal bands type 3a" (6). Serum and CSF were tested for neural abs using a biochip by Euroimmun (Lübeck, Germany) with human embryonic kidney (HEK) cells expressing the following antigens: *N*-methyl-D-aspartate receptors (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR), leucine-rich glioma inactivated protein 1 (LGI1), CASPR2, and γ-aminobutyric acid-B receptors ($GABA_BR$) (7). Serum bound to the CASPR2-expressing cells up to a dilution of 1:20 (endpoint titer). CSF was ab negative. This finding was perceived as a serendipitous turning point in the management of the patient. The previous psychiatric diagnosis was discarded and the diagnosis of anti-CASPR2 autoimmune encephalitis as a causally treatable explanation for the depression and the seizures was made.

The patient was transferred to the university's neurology department and received five immunoadsorptions, five intravenous (i.v.) boluses of 1 g methylprednisolone (MP) and was started on levetiracetam. She newly reported experiencing pseudohallucinations, but the depression appeared improved; thus, the antidepressant pharmacotherapy was reduced, and the patient was discharged home. Some weeks later, she visited a neurologist in private practice because of recent-onset twitches in her legs.

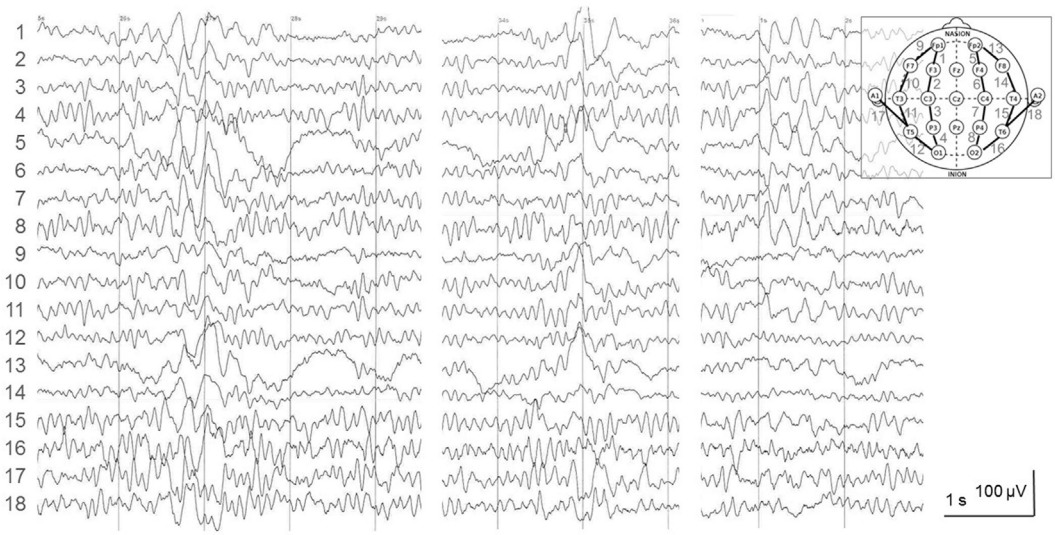


FIGURE 2 | Electroencephalogram (EEG) recorded 12 days prior to the lumbar puncture and the autoantibody diagnostics. The depicted epochs were recorded 30–80 s after onset of hyperventilation (HV), a standard provocation maneuver of epileptiform activity in the EEG laboratory. The EEG was interpreted as follows: "irregular alpha-EEG with right fronto-centro-parieto focus and singular as well as grouped spike-wave complexes with abnormal rhythmizing; strong activation under HV with conduction to the contralateral side and short generalizations." In fact, this is a normal 9/s-alpha-EEG with physiological high-amplitude slowing under HV. It has been described before that is one typical reason for EEG misinterpretation leading to the erroneous diagnosis of epilepsy (8).

She asked if this may be neuromyotonia. Neither the neurologist's report nor later medical reports stated that a physician had seen muscle twitches upon physical examination. Myotonic discharges could not be shown by an electromyogram (EMG). Because of ongoing episodes thought to be epileptic seizures, lamotrigine was added. With an increasingly depressed mood, the patient returned to the same neurology department, where neuromyotonia was added to her diagnoses. Three boluses of 1 g i.v. MP plus two infusions of 1 g rituximab (RTX) were administered. After a brief improvement in mood but with ongoing seizures and neuromyotonia, her depression returned. Approximately half a year after the first i.v. MP/RTX course, she received (despite a second negative EMG study) another such treatment.

One year after the diagnosis of anti-CASPR2 encephalitis, with ongoing leg twitching and "bubbles" but improving mood, she visited the author's clinic (hospital case no. 16810070). During a routine EEG, she had her habitual "bubble" with reduced and slowed responsiveness. An ongoing normal alpha rhythm was recorded (Video S1 in Supplementary Material). A subsequent epileptological in-patient evaluation revealed normal findings in long-term video-EEG, 3Tesla MRI of the brain, and neuropsychological, psychiatric, CSF, and ab investigations. The latter included investigations of CSF and serum by indirect immunofluorescence on mouse brain, on transfected, fixed HEK cells (Euroimmun, Lübeck, Germany), or on immunoblot (Euroimmun, Lübeck, Germany) for abs against the following antigens: NMDAR, LGI1, CASPR2, glycine receptor, AMPAR subunit 2, IgLON family member 5, $GABA_BR$, metabotropic glutamate receptor 5, dipeptidyl-peptidase-like protein-6, neuropil abs without further specification, Hu, Ri, Yo, CV2, amphiphysin, Ma2, GAD, recoverin, Sox1, Zic4, Delta/Notch-like EGF-related receptor; for the protocols, see Ref. (4). The depression had remitted. There were no more "bubbles" or leg twitches, and the antiepileptic medication was tapered. An in-patient follow-up with repetition of the aforementioned studies again did not reveal any abnormalities. The patient was discharged with "status post depressive episode" as the only diagnosis. Altogether, the patient spent 48 days in neurological in-patient care in vain, received immunotherapies at a price of approximately 27,000 €, and took antiepileptic drugs for 14 months (2.5 defined daily doses for most of this period). Fortunately, no enduring complications occurred. Written informed consent was obtained from the patient for the publication of this case report.

DISCUSSION

This patient was erroneously diagnosed in November 2015 with anti-CASPR2 encephalitis, 3 months before the recommendations for a clinical approach to autoimmune encephalitides were published online (1) and 11 months prior to the online publication stating that CASPR2 ab titers (as measured by the Euroimmun assay) in patients diagnosed with autoimmune encephalitis should be much higher than a 1:20 endpoint titer; more specifically, patients with a non-encephalitic MRI (as in this patient) need to have a CASPR2 ab serum titer >1:1,000 to have a >70% likelihood of an autoimmune encephalitis (4).

In retrospect, this patient did not pass the clinical threshold for "possible autoimmune encephalitis" because the psychiatric symptoms did not progress subacutely (1). Also, the previous diagnosis of a recurrent depressive disorder as an alternative diagnosis would have put the diagnosis of autoimmune encephalitis in doubt [see Panel 1 in Ref. (1)]. Additionally, the CASPR2 serum ab titer was too low to permit the diagnosis of an autoimmune encephalitis. Even at the time when the autoimmune encephalitis

diagnosis was made, the age and sex of the patient should have raised doubts, since anti-CASPR2 encephalitis is mainly a disorder of men at least 50 years of age (9); this has been confirmed in more recent publications (4, 10, 11).

As an additional (not infrequent) problem, overinterpretation due to overreading of the EEG led to the erroneous diagnosis of epileptiform activity (12), which supported the diagnosis of epilepsy, which was thought to emanate from autoimmune encephalitis. The indeterminate result of "oligoclonal bands type 3a" may have further fueled the idea of a CNS autoimmune process. Meanwhile, it has become clear that less than half of patients with anti-CASPR2 encephalitis who underwent CSF studies studied have intrathecal IgG synthesis (4, 10).

Detection of CASPR2 abs in this patient by a neurologist in the psychiatry department was obviously a striking event. It is tempting to speculate that this contributed to the diagnostic error that this patient had neuromyotonia. Neuromyotonia is a specific feature of Morvan syndrome, which is usually associated with CASPR2 abs (13). The patient reported "leg twitching" only after the detection of the abs. Even though no doctor ever documented the twitches and two EMGs were unable to detect them, the diagnosis of neuromyotonia was noted in the subsequent medical reports (and further corroborated the idea of an anti-CASPR2 encephalitis).

Like a previous report (14), this case study underlines the importance of specificity when making the diagnosis of an autoimmune encephalitis. Clinical criteria (1), titer cutoffs (4), and typical epidemiological features like age and sex may contribute to specific diagnoses of autoimmune encephalitides. Nevertheless, it is possible to detect novel ab-related syndromes. A successful example was the delineation of faciobrachial dystonic seizures in patients with LGI1 abs (15). This example shows that more than one individual is needed to establish such a new association.

ETHICS STATEMENT

This is a retrospective single case study of a patient who was personally treated by the author. Such a publication is covered by the Gesundheitsdatenschutzgesetz (GDSG NRW, German law on healthcare data protection). The patient signed a consent form.

AUTHOR CONTRIBUTIONS

There is only one author who did all the work. CGB designed the work; he acquired all data, analyzed, and interpreted them. He wrote the manuscript and provides approval for the publication of the content. He is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fimmu.2018.00703/full#supplementary-material.

VIDEO S1 | Routine electroencephalogram (EEG) with video recording 1 year after the diagnosis of "anti-CASPR2 encephalitis". The patient is irregularly blinking with her eyes and produces the typical artifacts. With ongoing blinking, she then has one of her habitual seizures with slowed-down responsiveness. The EEG shows an undisturbed alpha rhythm without signs of an epileptic seizure. These findings speak in favor of a non-epileptic event.

REFERENCES

1. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol* (2016) 15(4):391–404. doi:10.1016/S1474-4422(15)00401-9
2. Carvajal-Gonzalez A, Leite MI, Waters P, Woodhall M, Coutinho E, Balint B, et al. Glycine receptor antibodies in PERM and related syndromes: characteristics, clinical features and outcomes. *Brain* (2014) 137(Pt 8):2178–92. doi:10.1093/brain/awu142
3. Meinck HM, Faber L, Morgenthaler N, Seissler J, Maile S, Butler M, et al. Antibodies against glutamic acid decarboxylase: prevalence in neurological diseases. *J Neurol Neurosurg Psychiatry* (2001) 71(1):100–3. doi:10.1136/jnnp.71.1.100
4. Bien CG, Mirzadjanova Z, Baumgartner C, Onugoren MD, Grunwald T, Holtkamp M, et al. Anti-contactin-associated protein-2 encephalitis: relevance of antibody titres, presentation and outcome. *Eur J Neurol* (2017) 24(1):175–86. doi:10.1111/ene.13180
5. Mula M, Pini S, Cassano GB. The neurobiology and clinical significance of depersonalization in mood and anxiety disorders: a critical reappraisal. *J Affect Disord* (2007) 99(1):91–9. doi:10.1016/j.jad.2006.08.025
6. Schwenkenbecher P, Sarikidi A, Wurster U, Bronzlik P, Suhs KW, Raab P, et al. McDonald criteria 2010 and 2005 compared: persistence of high oligoclonal band prevalence despite almost doubled diagnostic sensitivity. *Int J Mol Sci* (2016) 17(9):1592. doi:10.3390/ijms17091592
7. Probst C, Saschenbrecker S, Stoecker W, Komorowski L. Anti-neuronal autoantibodies: current diagnostic challenges. *Mult Scler Relat Disord* (2014) 3(3):303–20. doi:10.1016/j.msard.2013.12.001
8. Benbadis SR, Tatum WO. Overintepretation of EEGs and misdiagnosis of epilepsy. *J Clin Neurophysiol* (2003) 20(1):42–4. doi:10.1097/00004691-200302000-00005
9. Lancaster E, Huijbers MG, Bar V, Boronat A, Wong A, Martinez-Hernandez E, et al. Investigations of Caspr2, an autoantigen of encephalitis and neuromyotonia. *Ann Neurol* (2011) 69(2):303–11. doi:10.1002/ana.22297
10. van Sonderen A, Arino H, Petit-Pedrol M, Leypoldt F, Kortvelyessy P, Wandinger KP, et al. The clinical spectrum of Caspr2 antibody-associated disease. *Neurology* (2016) 87(5):521–8. doi:10.1212/WNL.0000000000002917
11. Joubert B, Saint-Martin M, Noraz N, Picard G, Rogemond V, Ducray F, et al. Characterization of a subtype of autoimmune encephalitis with anti-contactin-associated protein-like 2 antibodies in the cerebrospinal fluid, prominent limbic symptoms, and seizures. *JAMA Neurol* (2016) 73(9):1115–24. doi:10.1001/jamaneurol.2016.1585
12. Benbadis SR. The tragedy of over-read EEGs and wrong diagnoses of epilepsy. *Expert Rev Neurother* (2010) 10(3):343–6. doi:10.1586/ern.09.157
13. Irani SR, Pettingill P, Kleopa KA, Schiza N, Waters P, Mazia C, et al. Morvan syndrome: clinical and serological observations in 29 cases. *Ann Neurol* (2012) 72(2):241–55. doi:10.1002/ana.23577
14. Armangue T, Santamaria J, Dalmau J. When a serum test overrides the clinical assessment. *Neurology* (2015) 84(13):1379–81. doi:10.1212/WNL.0000000000001425
15. Irani SR, Michell AW, Lang B, Pettingill P, Waters P, Johnson MR, et al. Faciobrachial dystonic seizures precede Lgi1 antibody limbic encephalitis. *Ann Neurol* (2011) 69(5):892–900. doi:10.1002/ana.22307

Conflict of Interest Statement: CGB gave scientific advice to UCB Pharma (Monheim, Germany) and obtained honoraria for speaking engagements from Eisai (Frankfurt,

Germany), UCB Pharma (Monheim, Germany), Desitin (Hamburg, Germany), Biogen (Ismaning, Germany), and Euroimmun (Lübeck, Germany). CGB received research support from Deutsche Forschungsgemeinschaft (Bonn, Germany), Gerd-Altenhof-Stiftung (Deutsches Stiftungs-Zentrum, Essen, Germany), Diamed (Köln, Germany), and Fresenius Medical Care (Bad Homburg, Germany). He is a consultant to the Laboratory Krone (Bad Salzuflen, Germany) regarding neural antibodies and therapeutic drug monitoring for antiepileptic drugs.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership