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Encephalitis 2023

Poster Booklet

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Amanda is a biologist with a Masters and Doctorate in Science. Amanda completed her doctoral studies in Immunology at the Sao Paulo University, Brazil, studying the mechanisms involved in the modulating activity exerted by toxins isolated from the venom of the Brazilian snake on the generation of innate and adaptive immunity mediated by dendritic cells. During this period, she acquired experience in basic and translational academic research, including immunology, cellular biology, molecular biology and toxinology. In 2019 she took up a postdoctoral researcher position in the Umeå University, Sweden, where she worked on chemical compounds capable of inhibiting adenoviral infection acting on proteins present in host cells. In 2021, Amanda was hired as a Research Specialist at the Nencki Institute to investigate the chromatin regulation in response to epigenetic treatments in paediatric high-grade gliomas (pHGGs). In parallel, she has studied the chromatin alterations, particularly the role of histone modifications induced by hypoxia as well as evaluate the effect of epigenetic drugs in combination with low oxygenation to find way to improve therapy response in H3K27Mexpressing HGGs. She came back to Sweden in 2023 and took up her postdoctoral position in Jakob Theorell group, studying autoimmune neurology, with a special focus on autoimmune encephalitis.

Neuronal autoantibodies identified among patients undergoing dementia investigations: a cohort study in Stockholm

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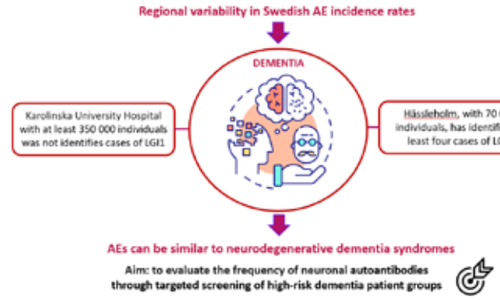
INTRODUCTION

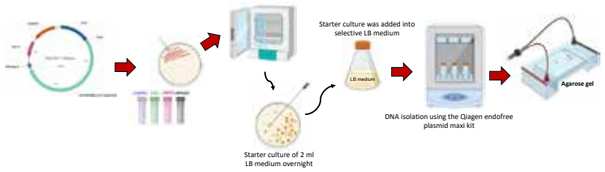
Autoimmune encephalitides (AEs) are a group of disorders in which the host immune system targets self-antigens expressed in the central nervous system (CNS), altering the function of the neuron and often causes deterioration of cognitive function. AEs can thus in certain scenarios resemble neurodegenerative dementia syndromes. Some of the best-characterised diseases are associated with autoantibodies that target neuroglial antigens which include cell surface synaptic antigens (NMDAR) and proteins that stabilize voltage-gated potassium channel complex into the membrane (LGI1 and CASPR2).

OBJECTIVE

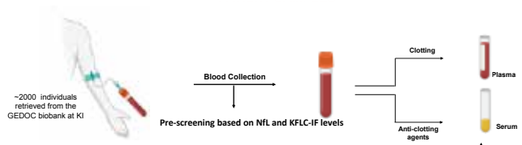


METHODOLOGY

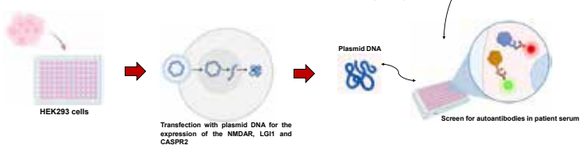
PLASMID DNA



PATIENT SAMPLES



LIVE CELL-BASED ASSAYS (CBA)



GRAPHICAL ABSTRACT

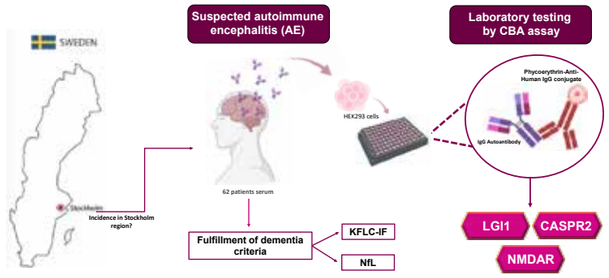


FIGURE 1. Serum samples from 62 individuals, were retrieved from the GEDOC biobank at Karolinska Universitetssjukhuset in Stockholm region for a pilot test. Hypothesising that autoantibody-positive patients should show some signs of inflammation in the CSF, data on NfL and KFLC-IF levels, as well as CSF cell numbers were retrieved for all included individuals. Serum samples from ~2000 such individuals, consented between 2019 and 2023 are been used to detect neuronal autoantibodies by CBA. Methodologically, CBAs expressing CASPR2, NMDAR and LGI1 were used to screen for autoantibodies in patients serum.

PRELIMINARY RESULTS

NfL and KFLC-IF as potential biomarkers to search for AE

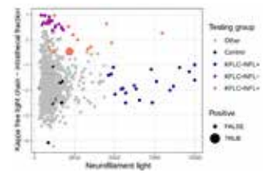


FIGURE 2. Distribution of neurofilament light-chain (NfL) and kappa free light-chain intrathecal fraction (KFLC-IF). Data are based on samples from 859 patients undergoing dementia investigations

Agarose gel images of different types of plasmid DNA

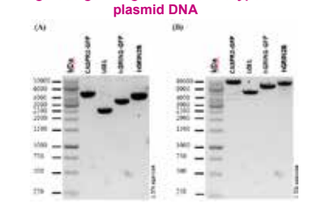


FIGURE 3. Agarose gel electrophoresis of plasmid DNA in standard strains or by restriction enzymes digests. 15µL aliquots of plasmid DNA were analyzed on a 1.5 % agarose gel in a undigested condition **(A)** or after restriction digest with Eam 1105I for CASPR2, LG1 and GRIN1 or KspAI for GRIN2B **(B)**.

Binding of IgG antibodies to CASPR2 clustered by EGFP on HEK293 cell surface

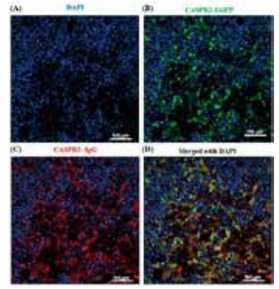


FIGURE 4. Verification of autoantigen specific live cell-based assays for CASPR2 using positive serum control for the optimization of the protocol. Human embryonic kidney 293 (HEK293) cells were plated in 96-well plates in complete medium at the optimum density of 50-70% confluence at the time of transfection. Cells were transfected with a GFP-CASPR2 plasmid using Lipofectamine 2000 and then incubated overnight at 37ºC. Then, HEK293 cells were immunostained with serum from an encephalitis patient with CASPR2 autoantibodies at a dilution of 1:100, followed by fluorescently conjugated secondary antibodies. GFP and CASPR2 expression were monitored 24h after transfection by fluorescence microscopy. **(A)** Nuclei stained with DAPI, **(B)** HEK293T cells transfected with EGFP-labeled CASPR2 plasmid merged images of GFP/DAPI, **(C)** The patient's IgG bound to CASPR2-transfected cells and showed red fluorescence merged with DAPI as a positive control and **(D)** representative merged images of CASPR-2 IgG/GFP/DAPI.

CASPR2 autoantibodies was identified in a an 80-year-old man with elevated levels of NfL and KFLC-IF biomarkers

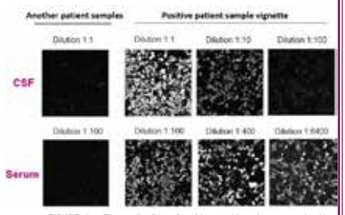


FIGURE 5. The antibodies of a 80-year-old patient reacted with HEK293 cells transfected with plasmid encoding human CASPR2 gene. Diagnosis of CASPR2 encephalitis was based on clinical assessment and demonstration of autoantibodies in cerebrospinal fluid (CSF) and serum. Confirmatory screening performed in the lab after this project's commencement established an end-point dilution in serum at 1/25,400, and an end-point dilution in CSF at 1/1,000, which are typical values for bona fide CASPR2 encephalitis cases. A sample from the control patient shows the negative labeling for both CSF and serum in the smallest serial dilutions.

CONCLUSION

- The positive patient sample indicates that AE should be included as a differential diagnosis during dementia investigations and that NfL and KFLC-IF can likely be used as screening markers. Therefore, these data will provide practical support for future diagnostic procedures for memory clinics.

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Miss Anahat Kaur Kalra is a medical student going into her final year at Cardiff University, after having completed an intercalated BSc in Population Medicine and graduating with first class honours. Her interests are centred around population health, public health, infections and immunology, and health research. These interests led her to choose the Population Medicine BSc at Cardiff University and complete a systematic review about autoimmune encephalitis as her dissertation. She has also previously completed a service improvement project on validating cards for dried blood spot testing to improve hepatitis C diagnosis in Wales, which she was able to present at the Federation of Infection Societies Conference 2021. Alongside her studies she has been highly involved with Students for Global Health UK for three years, including as president of her local branch and as part of the national committee. This has allowed her to expand her knowledge greatly about global health issues, health inequalities and health education. She is passionate about tackling health inequalities and improving access to healthcare. With these goals in mind, she has organised multiple short courses and have also organised and run a two-day national conference for Students for Global Health, dedicated to providing education and information regarding refugee health and welfare.

Does the use of intravenous immunoglobulin (IVIG) improve clinical outcomes in adults with autoimmune encephalitis? A systematic review

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Introduction

- Autoimmune encephalitis is a condition involving inflammation of the brain tissues caused by the body's own immune system. It is thought to be the cause of 1 in 5 cases of encephalitis. There are around 1,000 cases per year being diagnosed in the UK. (1)
- There are serious long term affects associated with the diagnosis, including seizures, cognitive impairment, and psychiatric symptoms (2). Understanding what the most effective treatments are could improve short and long-term outcomes.
- IVIG is effective in treating other autoimmune conditions such as Guillain-Barré syndrome and is used first-line. (3) Using IVIG as a first line immunotherapy for this condition could be beneficial.
- IVIG is a blood product, and dependent on donors and prone to shortages. It is rationed for specific diseases by a commissioning group. Currently IVIG is permitted to use in autoimmune encephalitis if treatment with corticosteroids is not effective. (4)
- IVIG is associated with serious adverse effects which must be considered when deciding the most suitable treatments fore patients. (5)

If there is evidence available regarding the effectiveness or ineffectiveness of IVIG, this should be reviewed so that there can be recommendations made regarding future research and guidelines on using IVIG.

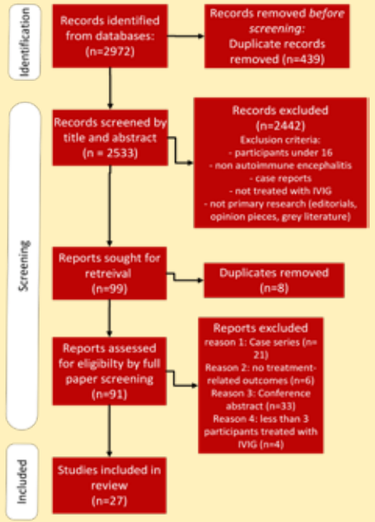
Aims

This systematic review was carried out as part of the wider ENCEPH-IG Trial – a randomised control trial to determine if early treatment with IVIG changes time to recovery.
The aims of this review are:

- To gain a comprehensive understanding of the clinical outcomes when using IVIG as a treatment in adults with autoimmune encephalitis
- To produce a narrative synthesis of the current evidence available surrounding the use of IVIG in autoimmune encephalitis.

Methodology

- Protocol registered on PROSPERO: CRD42023395827
- 4 databases searched: MEDLINE, The Cochrane Library, Web Of Science, Embase
- 20% of titles and abstracts and 20% of full texts assessed by a second reviewer independently
- Quality assessment (using Newcastle-Ottawa scale, ROBINS and ROB2 checklists) and data extraction assessed independently by one reviewer into predefined forms.



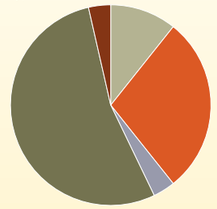
Inclusion criteria:
Population: Adult patients (16 years and older) with clinical or laboratory diagnosis of autoimmune encephalitis.
Intervention: Some or all participants were treated with intravenous immunoglobulin.
Comparator: None specified.
Outcomes: Any clinical outcome concerning treatment.

Study designs included: Randomised controlled trials (RCT), non-randomised clinical trials, retrospective and prospective cohort studies, cross-sectional studies, case-control studies.

Study designs excluded: non-primary research (reviews, opinion pieces, editorials), animal studies, case series, case reports, grey literature, and conference abstracts where there was no full paper available.

Results & Discussion

Types of studies included in review



Quality Assessment
Out of 23 observational studies, one study was deemed to be a high quality study (due to propensity score matching). The rest were deemed as high risk of bias.

There was only one RCT found in the literature, which was deemed to have a low risk of bias.

There were 3 non-randomised trials that had a moderate/serious risk of bias score.

Outcomes	Is there evidence to suggest this?
Reduction of seizures	☑
Cognitive and neurological improvement	☑
Better efficacy as a combination therapy	☑

How does IVIG affect additional treatments
Additional therapies might provide further improvement. Good quality evidence that adding corticosteroid therapy is better.

Adverse events
Adverse events were mostly mild and transient. 2 cohort studies suggesting IVIG carries a lower risk of adverse events than Methylprenisolone.

Does IVIG improve seizure frequency in autoimmune encephalitis?
1 RCT and 6 cohort studies suggest reduced seizure frequency after treatment with IVIG.

How does IVIG affect long and short term neurological outcomes?
IVIG is associated with improved neurological outcomes, both in the short and long term.

How does the use of IVIG impact mortality/discharge rates
Not enough information presented in studies to infer about these.

The quality and diversity of the evidence makes it complicated to compare across the studies.

In observational cohort studies where patients have been treated with various different immunotherapies, it is difficult to statistically compare treatment effect, as severity of the disease or other factors could have impacted the physician's decisions regarding treatment.

There is an urgent need for more clinical trials and good quality observational studies in this area to assess evidence surrounding this intervention

Acknowledgements

I would like to thank my supervisors Dr Emma Thomas-Jones and Dr Paula Foscarini-Craggs for their guidance, as well as Professor Tom Solomon and the wider ENCEPH-IG team and trial, with thanks to the NIHR for funding ENCEPH-IG.

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Dr Antonio Farina is an academic neurologist in Florence, and he spent, as a research fellow in the French Reference Centre of Paraneoplastic Neurological Syndromes and Autoimmune Encephalitis, two years to develop his PhD thesis project, centred on the neurological side effects of immune checkpoint inhibitors.

DIAGNOSTIC AND PROGNOSTIC BIOMARKERS IN IMMUNE CHECKPOINT INHIBITOR-RELATED ENCEPHALITIS

Antonio Farina[1,2], Macarena Villagrán-García[1], Marie Benaiteau[1,3], Cristina Birzu[4], Virginie Desestret[1,3], Anthony Fourier[3], Géraldine Picard[1], Anne-Laurie Pinto[1], Dimitri Psimaras[4], Isabelle Quadrio[3], Noémie Timestit[1], Jérôme Honnorat[1,3], Bastien Joubert MD[1,3]

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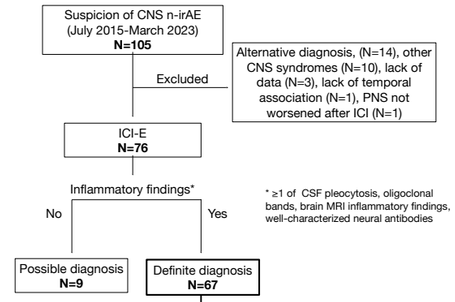
Fig. 1



INTRODUCTION

Encephalitis is the most frequent central nervous system (CNS) neurotoxicity of immune checkpoint inhibitors (ICI). We aimed **1) to assess the clinical presentation, outcomes, and prognostic factors**, and **2) to explore the role of S100B, NfL and GFAP as diagnostic and prognostic biomarkers in ICI-related encephalitis (ICI-E)**.

METHODOLOGY

Retrospective study; patients tested for neural antibodies at the French reference center for paraneoplastic neurological syndromes (PNS) with a definite diagnosis of ICI-E were included (**Fig. 1**). S100B, NfL, and GFAP were analyzed by ELISA in the serum/CSF collected <3 months since onset or relapse of ICI-E patients and in the sera of matched cancer controls. Outcomes were assessed in patients with >3 months of follow-up and defined as good if residual CTCAE grade was <3.

RESULTS

Fig. 2

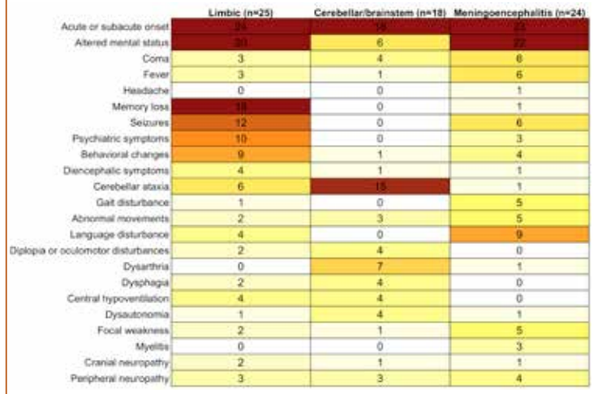


Fig. 3

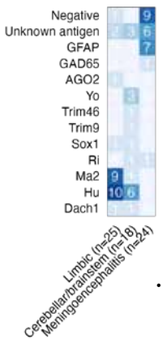


Table 1

Variable, N (%)	Limbic (N=25)	Cerebellar/ brainstem (N=18)	Meningo-encephalitis (N=24)	p
Neuroendocrine tumor	12 (48)	10 (56)	1 (4)	<0.001
PNS-related antibodies	21 (84)	15 (83)	1 (4)	<0.001
Negative antibodies	1 (4)	0 (0)	9 (37)	<0.001
CSF pleocytosis (WBC> 5/ mm3)	12/22 (55)	14/18 (78)	24/24 (100)	<0.001
CSF protein level >60 mg/dl	16/24 (67)	8/18 (44)	20/22 (91)	0.005
Abnormal brain MRI	20/25 (80)	6/17 (35)	8/24 (33)	0.001
Second-line treatment	10/23 (43)	8/18 (44)	2/24 (8)	0.009

- Limbic and cerebellar/brainstem encephalitis had cancer and antibodies associations, and paraclinical findings distinct from meningoencephalitis (**Table 1, Fig. 3**)
- Good outcomes in 19/57 patients (33%), less likely with PNS-related antibodies (**Fig. 4**)

Biomakers analyzed N=27

Fig. 5. Diagnostic role: encephalitis *vs* controls (n=16)

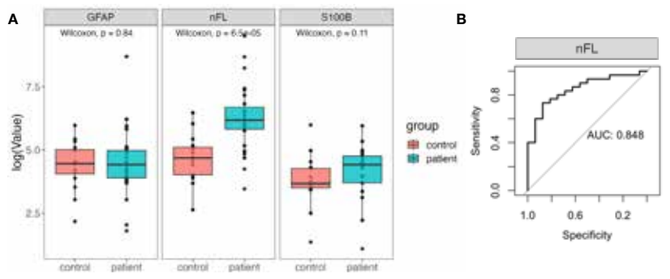


Fig. 6. Outcome correlations

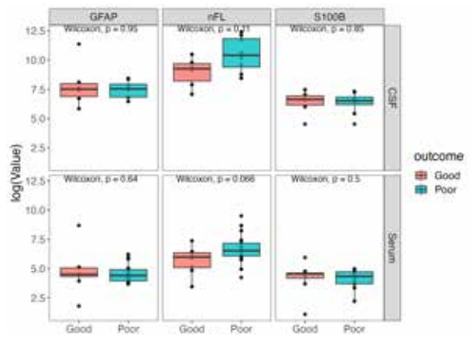


Fig. 4. Multivariate logistic regression exploring potential associations with good outcome (n=57)

Variable		N	Odds ratio (OR)	OR (95% confidence interval)	P
Age		57		0.96 (0.90; 1.03)	0.3
Sex	Male	20			
	Female	37		0.68 (0.15; 2.88)	0.6
CTCAE m0	CTCAE 3	47			
	CTCAE 4	10		0.68 (0.03; 7.28)	0.8
PNS antibodies	No	25			
	Yes	32		0.05 (0.01; 0.21)	<0.001

DISCUSSION/CONCLUSIONS

- The association of limbic and cerebellar/brainstem encephalitis with neuroendocrine cancers and PNS-related antibodies suggest pathogenic mechanisms distinct from meningoencephalitis. PNS-related antibodies are strong predictors of poor prognosis.
- The determination of NfL in serum may be useful for ICI-E diagnosis; serum NfL may provide prognostic information.

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Dr Antonio Malvaso is a young Neurology Resident attending the first year of specialization at IRCCS Mondino Foundation - National Neurological Institute, University of Pavia, Italy. As a student, he has been an intern for three years in the Neurology Department at IRCCS San Raffaele Hospital, Neuroimaging Research Unit, Milan, Italy. He has been involved in neurological clinical and pre-clinical research for four years. In particular, he made an internship at the "Neuroimmunology Unit" at Vita-Salute San Raffaele University (INSPE Milan, Italy). His main interests are neuroimmunology, pre-clinical research, and artificial intelligence (AI) applied to neuroimmunology. He has been very passionate about neuroscience since he was a young child. Indeed, he competed for three consecutive years in the Italian Neuroscience Olympics Games. He is a Resident and Researcher Member and a member of the Neuroimmunology panel in the European Academy of Neurology (EAN) and American Academy of Neurology (AAN). He also made an internship at the Computational, Cognitive and Clinical Neuroimaging Laboratory, Division of Brain Sciences, Department of Medicine at Imperial College London (2021-2022). Now, his main interest is to develop new laboratory techniques for the early diagnosis of autoimmune encephalitis and demyelinating diseases. In the future, one of his challenges is to create a system that integrates clinical reasoning with patient-centered neurologic care, generating a solid link between pre-clinical research and clinical research, in order to obtain early diagnostic strategies that could lead to patient-centered treatments.

Isolated retrograde amnesia in the spectrum of anti-CASPR2/LGI1 encephalitis memory impairment: a case series and systematic review of the literature

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Introduction

Anti-leucine-rich glioma inactivated 1 (LGI1) and anti-contactin-associated protein-like 2 (CASPR2) limbic autoimmune encephalitis (LAE) typically manifest as anterograde amnesia, behavioral disorders and seizures. Retrograde amnesia has also been rarely described. It usually follows a temporal gradient, with memories closer to the event being affected earlier according to Ribot's law **(temporally graded).** Conversely, **temporally ungraded** retrograde amnesia can affect random time frames. We report for the first time in the literature two patients with CASPR2 antibodies and **pure episodic-autobiographical retrograde amnesia** as a manifestation of LAE.

Materials and Methods

Figure 2. Identification, screening and inclusion of studies via databases, in accordance with the PRISMA 2020 statement (Page *et al.*, 2020), using PICOs portal automation tool. We searched on the Pubmed, Scopus and Embase databases using MeSH words. PRISMA flow-chart includes inclusion and exclusion criteria. NIH Quality Assessment Tool for Case Report/Series was used.

Clinical history

Case 1: A **72-year-old male** patient presented with seizures, behavioral changes and ***temporally graded*** retrograde amnesia involving the 6 months preceding the onset of LAE. Brain MRI revealed bilateral temporomesial abnormalities. High titres of anti-CASPR2 antibodies were found in both cerebrospinal-fluid (CSF) and serum.

Case 2: A **52-year-old male** patient presented seizures, behavioral changes, and ***temporally ungraded*** retrograde amnesia dating back more than 12 months to the onset of LAE. Brain MRI showed blurred hyperintensity of limbic structures. Low titres of anti-CASPR2 antibodies were found only in serum.

Antibody testing: CASPR2 antibodies were confirmed in both patients using a fixed and live cell based assay (F-CBA/L-CBA) and immunohistochemistry (IHC) on rat brain

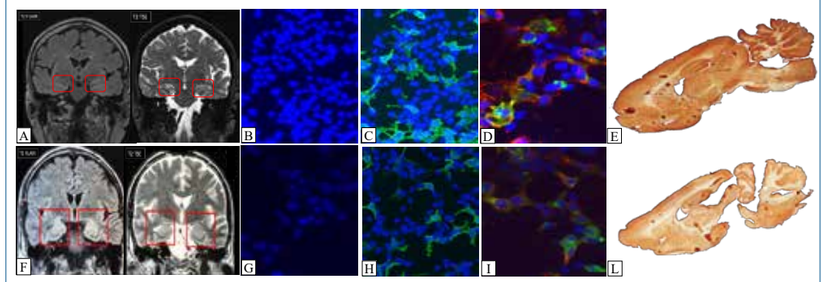

Figure 1. Brain Magnetic Resonance Imaging (MRI) and CASPR2 antibodies detection assays (B-E, G-L) in case 1 (A-E) and case 2 (F-L). Brain MRI coronal sections showed bilateral hippocampal hyperintensity and swelling in Fluid-attenuated-inversion-recovery (FLAIR) and T2 sequences (respectively left and right images) in both patients. Patients' serum did not bind to untrasfected HEK293 cells (B,G) but provided a surface staining on fixed cell-based assay (CBA, green: human IgG) and on live CBA (D,I, red: human IgG; green EGFP tag) with HEK293 cells transfected with CASPR2. Blue: DAPI. The presence of CASPR2 antibodies was confirmed in both patients using immunohistochemistry (IHC) on lightly fixed rat brain slices that, after incubation with patients' serum, showed a neuropilar staining pattern compatible with CASPR2 (E, L, brown: human IgG). Staining intensity in both CBAs and IHC was more intense in case 1 compared to case 2.

Systematic review findings and our results

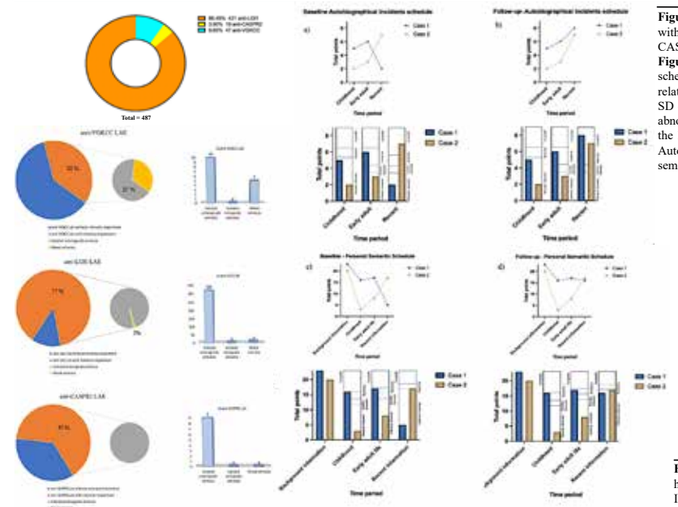

Figure 3 (Left). Proportion of anti-VGKCC, anti-CASPR2 and anti-LGI1 LAE patients with *vs* without memory impairment. Pie graphs describe all included patients. Anti-VGKCC/anti-LGI1/anti-CASPR2 LAE patients with anterograde, retrograde or mixed amnesia (*from top to bottom*). **Figure 4 (Right).** The autobiographical memory interview (AMI). (a) Autobiographical incidents schedule at baseline-individual patient data. CASPR2-Abs LAE patients scores are represented relative to the cutoff points for healthy controls cited in Kopelman et al. (1990): "Acceptable": ±1 SD of the control mean; "borderline": Between 1 SD and 2 SD below the control mean; "probably abnormal": > 2 SD below the control mean; "definitely impaired": Scores at or below which none of the healthy controls scored; (b) Personal semantic schedule at baseline-individual patient data; (c) Autobiographical incidents schedule **after 2 years of follow-up**-individual patient data; (d) Personal semantic schedule **after 2 years of follow-up**-individual patient data.

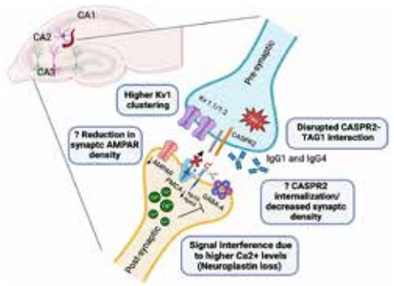

Figure 5. Biological mechanisms of disrupted presynaptic and postsynaptic signal, neuronal hyperexcitability, and decreased plasticity in the Cornu Ammonis 3 (CA3) hippocampal region. Image created with *BioRender.com*

Conclusion and future directions

Retrograde amnesia extremely rare in LGI1/CASPR2 LAE, and commonly associated with anterograde amnesia. Exploring the effects of anti-CASPR2 antibodies on CA3 might provide insight into the pathophysiology of retrograde amnesia.

Contacts

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Dr Venkata Ravi Kumar Banda is a physician-scientist, founder CEO of XCyton Diagnostics Pvt. Ltd. Bangalore and had been developing diagnostics products/platforms for infections. He and his team have developed a molecular diagnostic platform called Syndrome Evaluation System (SES) for the diagnosis of life-threatening and maiming infections such as sepsis, encephalitis, meningitis etc. SES is driven by a purpose “of saving lives, prevent disabilities and reduce health care costs to the patients”. SES allows simultaneous detection of all probable pathogens, which can cause a syndrome - viruses, bacteria, fungi and parasites in a single clinical specimen and in a single test, along with markers for antibiotic resistance.

Dr Ravi Kumar serves in scientific committees of various Government bodies and is a mentor for many biotech startups.

APPLICATION OF SYNDROME EVALUATION SYSTEM (A MULTIPLEX PCR) IN DIAGNOSIS OF CNS INFECTIONS *DO WE NEED TO CHANGE ANY DOGMAS?*

Dr. VENKATA RAVI KUMAR BANDA

Dr. Balasubramanian Natarajan, Bharath N & Anand Lakshmanan

XCyton Diagnostics Pvt. Ltd., Bengaluru, India &

SIRPI products and services Pvt. Ltd., Bengaluru, India

METHODOLOGY

1. 6774 CSF samples were sent to XCyton Diagnostics Pvt. Ltd. between 2014 to 2018 by 11 tertiary care neurological centers, situated across India for molecular diagnostic investigations pertaining to infections of the nervous system.
2. The Samples were accompanied by TRF (Test Requisition Form) where in details such as clinical presentations, CSF cells, CSF protein, CSF sugar, Imaging data such as MRI and CT data, Co-morbidities and other relevant history were asked for. Information in the TRF was not complete in all cases and sketchy in most of the cases.
3. Information additional to that provided in TRF was collected at the time of reporting from the clinicians.
4. 700 samples were not analysed as the data provided in TRF was considered grossly inadequate and hence only 6074 data points were considered and presented in this study.
5. All patients received anti-infectives >3 days before Lumbar puncture was done.
6. The Follow up data were not considered for analysis.
7. The data was tabulated using Microsoft Excel and the tidy data was analysed and visuals were created using R- programming.

KEY FINDINGS

1. We observed detection rate of 38.93% among in Rule-In cases and 2.37 % in among Rule- out cases. Our detection rate was 51.59% in menigitis cases. While, among encephalitis cases rate was at 23.78%.
2. Streptococcus pneumoniae and Mycobacterium tuberculosis presented in as encephalitis in 55.9% (160/286) and 38.2 % (99/259) respectively. These cases were primarily presented with seizures and altered sensorium very much similar to Herpes Simplex Virus 1 & 2 and Varicella Zoster virus encephalitis.
3. Patients with HIV, transplant, Sepsis, Malignancies, prolonged steroid therapy, craniotomy, Ventricular shunts, head injury and being on a ventilator are the risk factors that contributed to 68% of polymicrobial cases. Hospital acquired bacterial and candidal infections were also predisposed by the same risk factors.
4. MRI picture of basal exudates, hydrocephalus, cortical infarcts, granuloma or suspected tuberculomas, thought to be characteristic of infections with Mycobacterium tuberculosis, were observed in other bacterial, fungal and cysticercal lesions.

No.	Particulars	Rule-In	Rule-Out
1	Clinician suspected an infection	✓	X
2	CSF examination (>10cells and Raised protein)	✓	X
3	MRI/CT imaging Normal	X	✓
4	Pre-morbid conditions (Transplantation, Cancer chemotherapy, RTA, Craniotomy etc.)	✓	X
5	ADEM/Metabolic encephalopathy/Autoimmune disease	X	✓
6	Other Diagnostic tests suggestive of Infection	✓	X

Table 1: Infection Rule-In and Rule-out criteria for data analysis

SES SPORADIC ENCEPHALITIS	SES MENINGITIS	SES ACUTE ENCEPHALITIS SYNDROME
Herpes Simplex Virus 1 & 2	Streptococcus pneumoniae	Japanese Encephalitis Virus
Cytomegalo Virus	Mycobacterium tuberculosis	Dengue Virus (1-4
Varicella Zoster Virus	Haemophilus influenzae	West Nile Virus
Human Herpes Virus 6	Neisseria meningitidis	Measles Virus
John Cunningham Virus	Staphylococcus aureus	Mumps Virus
Toxoplasma gondii	Group B Streptococcus	Nipah Virus
Mycobacterium tuberculosis	Enterococcus species	Rubella Virus
Streptococcus pneumoniae	Klebsiella pneumoniae	Enteroviruses
Haemophilus influenzae	Escherichia coli	Rabies Virus
Neisseria meningitidis	Enterobacter aerogenes	Chandipura Virus
Cryptococcus neoformans	Pseudomonas aeruginosa	Chikungunya Virus
	Acinetobacter baumannii	Herpes Simplex Virus 1 & 2
	Bacteroides fragilis	Cytomegalo Virus
	Leptospira species	Varicella Zoster Virus
	Aspergillus species	Human Herpes Virus 6
	Candida species	John Cunningham Virus
	Cryptococcus neoformans	Toxoplasma gondii
		Mycobacterium tuberculosis
		Streptococcus pneumoniae
		Haemophilus influenzae
		Neisseria meningitidis
		Cryptococcus neoformans

Table 2: SES CNS infection Diagnostic Test Panels

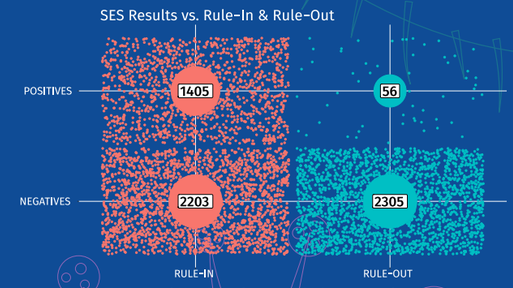

Fig 1. Total Number of cases, which were analyzed retrospectively, SES comprehensively Ruled-In Infections

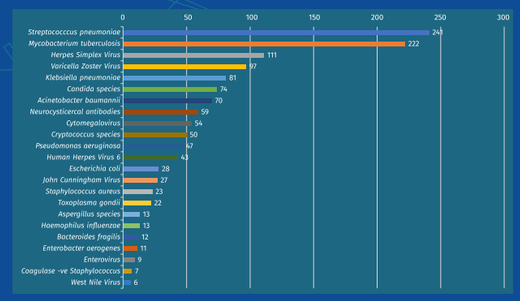

Fig 2. Rank order of organisms detected by SES

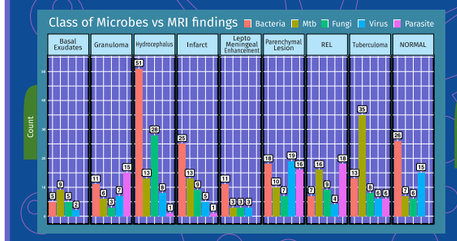

Fig 3. SES results discriminate the infection aetiology better and add value to MRI findings

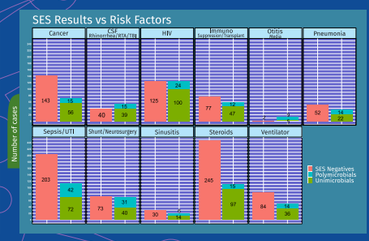

Fig 4. SES detects polymicrobial infections, frequently associated with risk factors

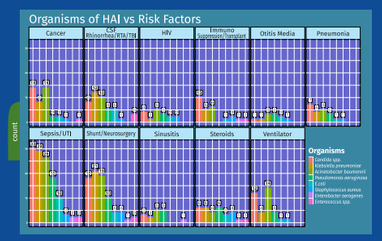

Fig 5. Role of Risk factors in hospital acquired CNS infections

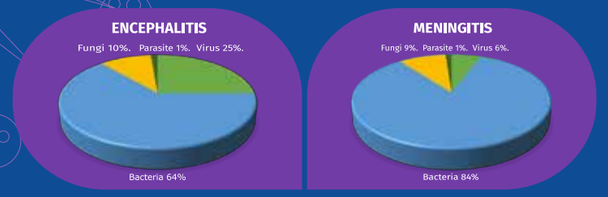

Fig 6. Preponderance of bacterial infections among patients with encephalitis symptoms

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Dr Bernard Liem is a Neurology Registrar from Auckland, New Zealand, currently in his penultimate year of core neurology training. He has an interest in autoimmune neurology, including encephalitis, neuromyelitis optica spectrum disorders and multiple sclerosis. He has been awarded a sought-after ANZAN Overseas Training Position post and is currently working as a Specialty Registrar at the John Radcliffe Hospital in Oxford, United Kingdom.

‘Mimics of Encephalitis’.

Bernard Liem[1], Michelle Liem[1] and Neil E Anderson[1]

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Te Whatu Ora Health New Zealand Te Toka Tumai Auckland

INTRODUCTION

Despite the rapidly growing field of discovery of neuronal autoantibodies in the past 10 years, patients with encephalitis without an identified autoantibody remain the largest group of those hospitalised with encephalitis.[1] These are a heterogenous group of patients, with regards to clinical features, pathologic mechanisms, treatment and outcomes.[2] We aim to provide further characterisation of our cohort of patients in this study. Several patients initially thought to have encephalitis ultimately have a final non-encephalitis diagnosis - a ‘mimic’. We also aim to characterise this subset of patients and look for comparisons.

METHODS

- Retrospective analysis.
- Patients 15 years or older presenting with encephalitis to hospitals in Auckland and Northland between 2009 and 2018 were identified from three overlapping laboratory databases.
- Patients without an identified neuronal autoantibody fulfilling criteria set out by Graus and colleagues (2016)[3] were included.
- Patients subsequently found to have an alternative diagnosis were characterised as ‘mimics’ of encephalitis.
- Demographic, hospitalisation-related data, clinical features, investigations, treatment and outcome data were obtained by the investigators.
- Statistical analysis was with SPSS (2017, *ver* 25.0. *Armonk, NY. IBM Corp*).

RESULTS

- Of 166 patients presenting with suspected encephalitis, excluding those with infectious causes, or known neuroimmunological causes (including those with detected known neuronal auto-antibodies), 63 patients had no detectable neuronal autoantibody.
- 7 fulfilled criteria for ‘definite’, 10 ‘probable’ and 27 ‘possible’ autoimmune encephalitis.
- 15 patients had a final alternative, non-encephalitis diagnosis - a ‘mimic’.

Time to diagnosis

- One patient with neuro-syphilis had symptoms for 1 year prior to seeking medical advice.
- Two patients were diagnosed within 9 days of presentation – due to neuro-rheumatologic aetiologies.
- The longest time to diagnosis (3 years) was in a 16 year old woman with CNS vasculitis. A brain biopsy was required, securing the diagnosis.

Symptoms

- Altered mental status and focal neurological symptoms were the most common presenting symptoms.
- Two patients developed epilepsy – 1 with CNS neoplasm, and 1 with CNS vasculitis.
- Two patients were admitted to intensive care and required intubation.

Outcomes

- Half treated with acyclovir on presentation due to suspicion of possible infectious (viral) encephalitis.
- Dependent on final diagnosis.
- On discharge, similar proportion discharged with good outcome (mRS 0-2, 8 patients) and poor outcome (mRS 3-6, 7 patients).
- To date, only two patients (with neuro-SLE and HaNDL), remained asymptomatic.

DISCUSSION

- Our study reinforces points in recent publications regarding misdiagnosis of autoimmune encephalitis with consequent mimic diagnoses.
- Autoimmune encephalitis is a rare diagnosis.
- Stringent application of the Graus’ criteria for ‘possible’ autoimmune encephalitis is required to ensure true sensitivity of the criteria.
- The detection of low titres of antibodies, especially in certain diseases such as anti-GAD65, and moreso if the clinical picture is not in fitting with a well known syndrome, should prompt clinicians to look for alternative diagnoses.
- It is important for clinicians to be aware of classic presentations of mimics of encephalitis, including some pathognomonic features such as that seen in neuro-rheumatologic conditions.

CONCLUSIONS

In the last few years there has been increased recognition of autoimmune encephalitis as an important differential diagnosis in patients presenting with altered neurological status with associated neurological symptoms and signs. Our study shows the importance of consideration of a broad differential in these patients, as there are many diagnoses that mimic an encephalitis presentation. Broadly, the detection of focal neurological symptoms may alert the clinician to consider alternative differentials, but presentations are otherwise heterogenous. Being familiar with the pathognomonic clinical, laboratory and radiologic findings for these ‘mimic’ diagnoses will help ensure they are not missed across a patient’s diagnostic journey.

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ENCEPHALITIS ‘MIMICS’

There were 10 different diagnoses:

- 4 with CNS vasculitis;
- 2 patients with primary CNS neoplasia;
- 2 with rheumatologic causes - 1 with relapsing polychondritis with encephalitis and 1 with neuro-systemic lupus erythematosus (SLE);
- 2 with neuro-syphilis;
- 2 with neurodegenerative dementia;
- 1 patient with auto-inflammatory syndrome related encephalitis;
- 1 patient with Korsakoff’s psychosis;
- 1 with the syndrome of headaches with altered neurological deficits and CSF lymphocytosis (HaNDL).

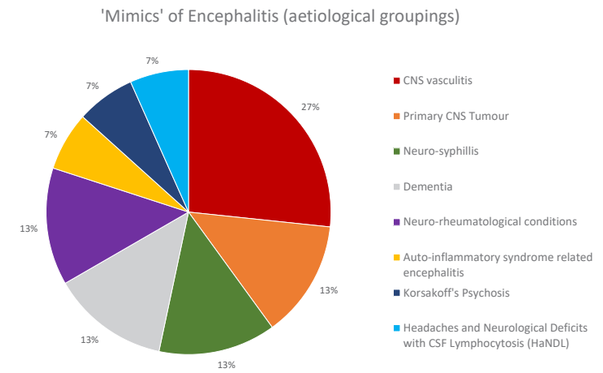

Table 2. Diagnostic clues to mimic diagnoses

Patient	Diagnosis	Diagnostic Clue
1	High grade diffuse glial astrocytoma	Brain biopsy.
2	Glioblastoma multiforme	Tumour appearances on follow up MRI.
3	Probable small vessel vasculitis	Progressive encephalopathy syndrome, PR3 antibody positive, progressive infarction on MRI brain.
4	CNS vasculitis	Progressive encephalopathy syndrome, new left parieto-occipital infarct. Vasculitic changes on digital subtraction angiography – multifocal segmental arterial narrowing.
5	CNS vasculitis	Brain biopsy.
6	CNS vasculitis	Progressive encephalopathy syndrome, MRI brain with meningeal enhancement and progressive vasculopathy of the right middle cerebral artery.
7	Auto-inflammatory syndrome related encephalitis	Clinical syndrome.
8	Relapsing polychondritis with autoimmune encephalitis	Synovitis and ear chondritis.
9	Neuro-systemic lupus erythematosus	Malar rash in a young female.
10	Neuro-syphilis	Positive Rapid Plasma Reagin test (and subsequent confirmatory tests).
11	Neuro-syphilis	Positive Rapid Plasma Reagin test (and subsequent confirmatory tests).
12	Headaches and altered neurological deficits with CSF lymphocytosis	CSF lymphocytosis and clinical syndrome.
13	Korsakoff’s Psychosis	History of significant alcohol use, diagnosis of exclusion.
14	Alzheimer’s disease	In setting of diagnosis of moderate cognitive impairment 6 months prior, diagnosis of exclusion.
15	Vascular dementia	MRI abnormalities, diagnosis of exclusion.

The authors have no disclosures.

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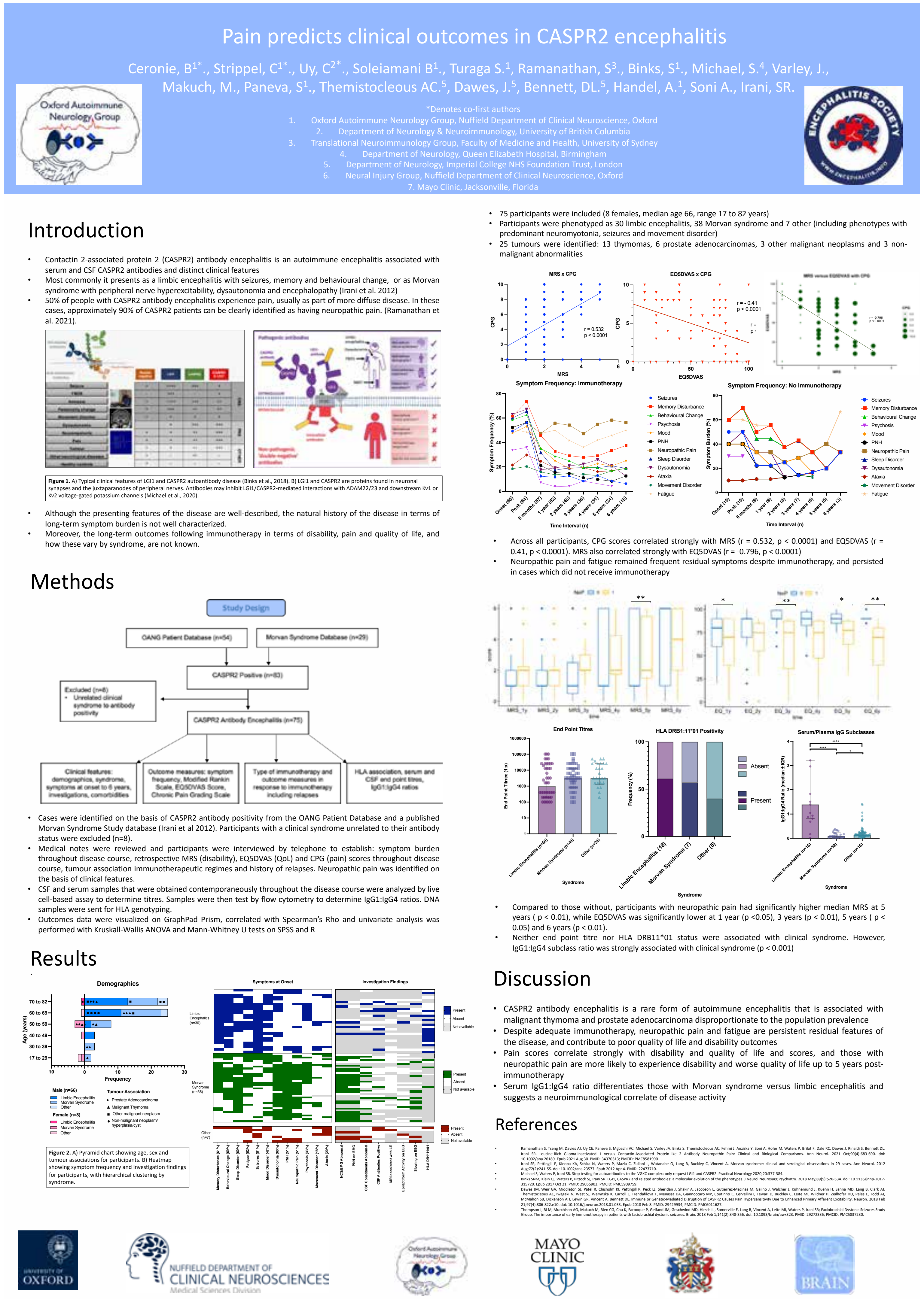
Dr Ceronie is a neurology registrar and clinical research fellow in the Oxford Autoimmune Neurology Group. Following previous work in transcranial magnetic stimulation in epilepsy and the role of B cells in multiple sclerosis, his main interests are in the role of B cell checkpoints in autoimmune encephalitis. In 2022 he was awarded a Guarantors of Brain/Association of British Neurologists Clinical Research Training Fellowship to study the mechanisms of B cell tolerance and checkpoint dysfunction in LGI1 and CASPR2 autoimmune encephalitis. He is currently pursuing this research as a DPhil candidate at the University of Oxford.



Dr Claire Hetherington

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Claire Hetherington is a postdoctoral research associate in Professor Benedict Michael's Infection Neuroscience lab. Claire began her scientific research career with a MBiolSci in Biochemistry & Molecular Cell Biology at the University of Sheffield, completing her master's research on optimisation of iPSC neuronal differentiation with Pfizer and Astrazeneca. She then completed her doctoral research in Translational Neuroscience at the University of Aberdeen, under the supervision of Dr Guy Bewick researching in vitro modelling of motor neurone disease using induced pluripotent stem cells (iPSCs) to form an 'NMJ on a chip' microfluidic model. In 2022, she moved to the University of Liverpool to undertake postdoctoral research on in vitro modelling of CNS infections including SARS-CoV-2 as part of the COVID-CNS study. Her research interests include in vitro models of neurological disease, iPSCs/stem cells, microfluidics and electrophysiology.

Impact of inactivated SARS-CoV-2 on brain endothelial cells and parenchymal cells: insights into cytokine secretion and gene expression

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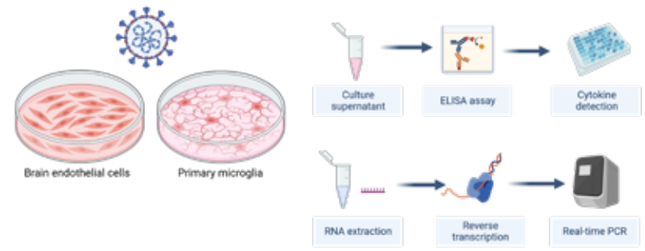
INTRODUCTION

During the early stages of the COVID-19 pandemic, neurological complications were observed in approximately one-third of hospitalized patients (Mao et al., 2020). Exaggerated host inflammatory responses appear to be a key driver of COVID-19 neurological complications (Needham et al., 2022). Rather than a direct effect of endothelial cell infection, this inflammatory response may be driven by the presence of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) that result in pro-inflammatory cytokine secretion by endothelial cells into the parenchyma. SARS-CoV-2 viraemia is detectable in 30.1% of patients admitted to hospital and is associated with higher mortality and more severe disease (Giacomelli et al., 2023). In this study, we are investigating the impact of inactivated SARS-CoV-2 on brain endothelial cells and other brain cells (microglia, astrocytes and neurons) *in vitro*.

METHODOLOGY

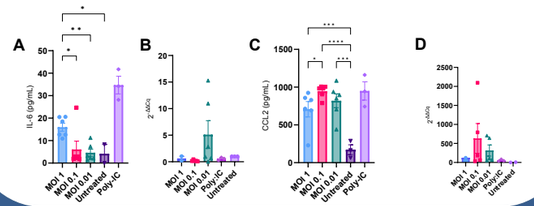
Brain endothelial cells (bEnd.3, ECACC) and primary mouse microglia (ScienCell) were exposed to heat-inactivated SARS-CoV-2 (NISBC, Porton Down) at incremental doses of virus for 24 hours, with media only ('untreated') as a negative control and poly I:C as positive control. After 24 hours the supernatant was harvested and RNA extracted using Trizol. Secretion profiles of IL-6, IFNγ, IL-17RA and CCL2 of bEnd.3 and primary microglia were analyzed by ELISA to assess the response of these cells to inactivated virus, negative control (media) and positive control (20 µg/mL poly I:C). Additionally, microglia were exposed to virus-free (20 nm filtered) supernatant from bEnd.3 previously exposed to inactivated virus, and cytokine expression measured by ELISA. Microglia were also immunostained for CD45 and Iba1 and imaged using confocal microscopy to assess their morphology and activation state.



RESULTS

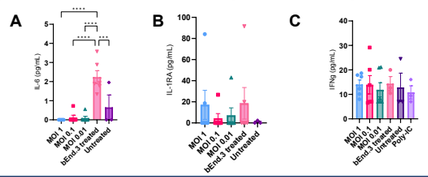
bEnd.3 cytokine expression

bEnd.3 cells exposed to a high dose of inactivated virus (MOI 1) over 24 hours showed a significant increase in IL-6 secretion compared to low dose and untreated (Figure 1a). Transcript levels of IL-6 mRNA were not significantly different (Fig 1b). CCL2 secretion was significantly increased at all doses of virus (Figure 1c) but there was no significant difference in fold expression (Fig 1d). IL-1RA and IFNγ are not produced by bEnd.3 in response to inactivated virus or positive control (data not shown).



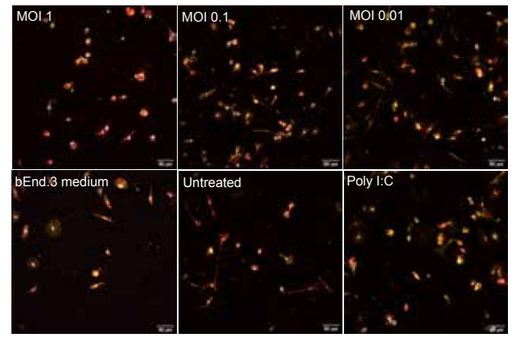
Microglia cytokine secretion

Microglia exposed to filtered supernatant from SARS-CoV-2 exposed bEnd.3 showed a significant increase in IL-6 secretion, which was not seen in response to direct virus exposure (Fig 2.a). There was no significant difference in IL-1RA (Fig 2b) or IFNγ (Figure 2c) secretion.



Microglia activation

Mouse microglia were immunostained for CD45 (yellow) and Iba1 (red). Microglia exposed to a higher dose of virus and activated bEnd.3 medium displayed a more amoeboid ('activated') morphology whereas lower doses and untreated cultures displayed a more ramified ('resting') morphology.



Further work

The methodology presented will be used to determine cytokine expression of mouse astrocytes in response to inactivated virus and bEnd.3 conditioned supernatant, and the effects of bEnd.3, microglia and astrocyte supernatant on neuronal viability. Additionally this methodology will be used in humanised *in vitro* models using the hCMEC/D3 line and iPSC-derived cortical neurons as well as human primary microglia and astrocytes.

CONCLUSIONS

Exposure of brain endothelial cells to inactivated SARS-CoV-2 significantly increases CCL2 secretion, whereas only a high viral load significantly increases IL-6 secretion. Microglia exposed to a high dose of virus or conditioned endothelial medium undergo a morphological change to an activated state, however secretion of IL-1RA and IFNg is not induced, and IL-6 is only induced by treatment with conditioned endothelial medium. Further studies are required to interrogate the precise signalling pathways induced by systemic PAMPs and DAMPs.

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Cell lines were provided by ECACC and ScienCell.

ENCEPHALITIS 2023

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POSTER PRESENTATION

Biomarkers of neurological complications of COVID-19

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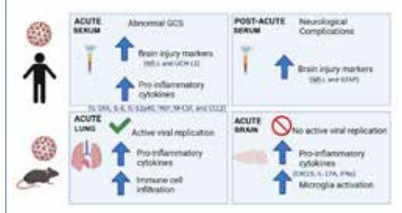
ABSTRACT

Although SARS-CoV-2 causes a respiratory viral infection, there is a significant incidence of neurological complications, including headache, seizure, and stroke, occurring in COVID-19 patients. Little is known about the likely diverse mechanisms causing these pathologies and there is a dire need to understand them and learn how to prevent and treat them. We measured brain injury markers and cytokines in the serum of study participants who had COVID-19. Brain injury markers (Nfl-L, UCH-L1, Tau, and GFAP) were measured by Quanterix Simoa and 48 cytokines were measured by Bio-rad Bioplex-200. We found that two brain injury markers and several cytokines/receptors were significantly associated with an abnormal Glasgow Coma Score in acute COVID-19 patients. Interestingly, when the COVID-CNS participants with neurological complications were studied (weeks to months post initial positive COVID test), the brain injury marker, Nfl-L remained elevated. For the mouse model of parainfectious effects on brain, 2-4 mo heterozygous K18-hACE2 transgenic C57BL/6 male and female mice were infected with $1x10^3$ PFU SARS-CoV-2. On day 5 post-infection, mice were euthanized and serum, brain, and lungs collected for RNA and protein analysis. We observed microglia activation in the brain of low dose-infected mice —interestingly, this was in the absence of active viral replication. Overall, we highlight two findings: (1) SARS-CoV-2 does not directly invade the brain in the majority of cases and so the associated neurological complications might arise from indirect effects, such as immune activation (2) although the immune system plays a critical role in controlling the virus, its dysregulation can cause pathology.



METHODS

We measured brain injury markers and cytokines in the serum of participants who had COVID-19 from two different studies—ISARIC and COVID-CNS. For the ISARIC participants, we examined those participants with a normal vs. abnormal Glasgow Coma Score. In the COVID-CNS study, we could stratify by participants with neurological complications and specific pathologies. Brain injury markers (Nfl-L, UCH-L1, Tau, and GFAP) were measured by Quanterix Simoa and 48 cytokines were measured by Bio-rad Bioplex-200.

For the mouse model of parainfectious effects on brain, 2-4 mo heterozygous K18-hACE2 transgenic C57BL/6 male and female mice were infected with $1x10^3$ PFU SARS-CoV-2. On day 5 post-infection, mice were euthanized and serum, brain, and lungs collected for RNA and protein analysis.

RESULTS

We found that two brain injury markers and six cytokines/receptors were significantly associated with an abnormal Glasgow Coma Score in acute COVID-19 patients. Nfl-L, UCH-L1, IL-6, IL-12p40, CCL2, M-CSF, HGF, and IL-1RA were all elevated with abnormal Glasgow Coma Score (Figure 1). Interestingly, when the COVID-CNS participants with neurological complications were studied (median 4.4 months post initial positive COVID test), the brain injury marker, Nfl-L remained elevated (Figure 2). The highest levels of Nfl-L were seen in the cerebrovascular cases (Figure 2). We observed that COVID-19 infection caused an increase in IgG autoantibodies and these remained elevated post-acutely in neurological complications (Figure 3).

We established a mouse model of parainfectious effects on the brain using a low dose of SARS-CoV-2. This dose still caused lung pathology and elevation of pro-inflammatory cytokines. In the brain of low dose-infected mice, we observed microglia activation in the absence of active viral replication and increased CXCL9 expression (Figure 4).

FIGURE 1: ACUTE BIOMARKERS

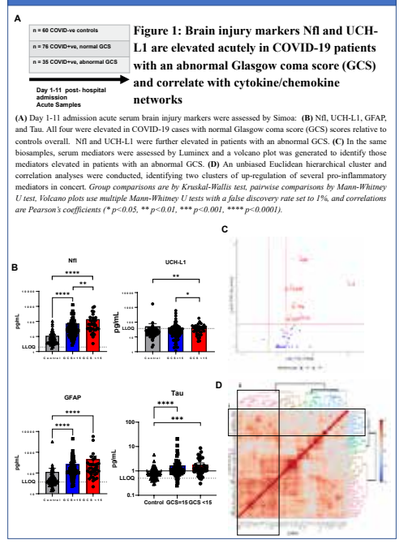


Figure 1: Brain injury markers Nfl and UCH-L1 are elevated acutely in COVID-19 patients with an abnormal Glasgow coma score (GCS) and correlate with cytokine/chemokine networks

(A) Day 1-11 admission acute serum brain injury markers were assessed by Simoa: **(B)** Nfl, UCH-L1, GFAP, and Tau. All four were elevated in COVID-19 cases with normal Glasgow coma score (GCS) scores relative to controls overall. Nfl and UCH-L1 were further elevated in patients with an abnormal GCS. **(C)** In the same biosamples, serum mediators were assessed by Luminex and a volcano plot was generated to identify those mediators elevated in patients with an abnormal GCS. **(D)** An unbiased Euclidean hierarchical cluster and correlation analyses were conducted, identifying two clusters of up-regulation of several pro-inflammatory mediators in concert. *Group comparisons are by Kruskal-Wallis test, pairwise comparisons by Mann-Whitney U test, Volcano plots use multiple Mann-Whitney U tests with a false discovery rate set to 1%, and correlations are Pearson's coefficients (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).*

FIGURE 2: POST-ACUTE BIOMARKERS

Figure 2: In the sub-acute/convalescent phase Nfl remains elevated in those who have suffered a CNS complication, especially stroke and CNS inflammation, despite down-regulation of proinflammatory mediators.

(A-B) Brain injury markers were measured by Simoa in serum from the subacute (<6 weeks from positive SARS-CoV-2 test) and convalescent phase (>6weeksB-E) . **(C)** Within COVID-19 neurological cases Nfl levels were most significantly elevated in patients who had suffered a cerebrovascular or inflammatory neurological complication at the time of SARS-CoV-2 infection **(D)** In subacute and convalescent serum inflammatory mediators were measured by Luminex and displayed by Volcano plot and only one cytokine, IL-18 was different between controls and cases. **(E)** A heatmap of the correlations between mediators did not demonstrate the same interconnectedness which had been identified in acute samples. *Group comparisons are by Kruskal-Wallis test, pairwise comparisons by Mann-Whitney U test, Volcano plots use multiple Mann-Whitney U tests with a false discovery rate set to 1%, and correlations are Pearson's coefficients (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).*

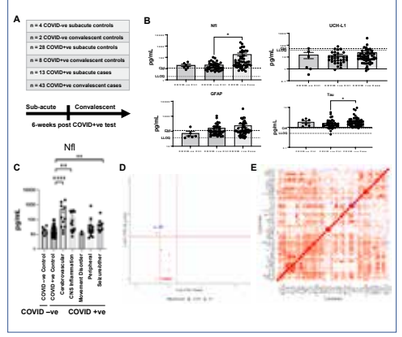


FIGURE 3: AUTOANTIBODIES

Figure 3: Acutely there is a polyclonal antibody response in patients with COVID-19 directed at SARS-CoV-2 spike protein and several CNS epitopes, including UCH-L1, and some correlate with Nfl levels.

(A) Acute serum samples were tested for IgG antibodies by protein microarray and COVID-19 patients showed considerably more binding 'hits' than controls (fluorescence with a Z-score of 3 or above compared to controls), although overall there was no difference in the acute samples between patients with normal or an abnormal GCS. **(B)** Convalescent serum samples revealed that COVID-19+ve neurological cases had elevated numbers of self-reactive antibodies. *Group comparisons are by Kruskal-Wallis test, pairwise comparisons by Mann-Whitney U test, and correlations are Pearson's coefficients (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).*

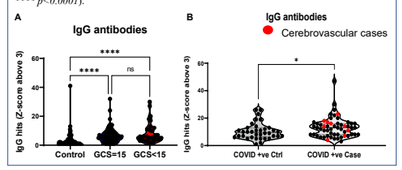
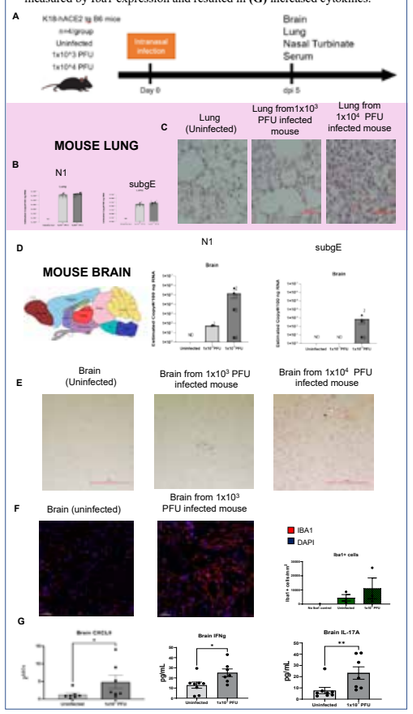


FIGURE 4: PARAINFECTIOUS MOUSE MODEL

Figure 5: Low dose intranasal SARS-CoV-2 infection in human ACE-II transgenic C57BL/6 mice does not cause viral replication in the brain parenchyma, but does induce clustering of mononuclear cells, and microglial activation reflecting clinical disease

(A) Schema of K18 human-ACEII transgenic C57BL/6 mouse study randomised to no infection, 'low dose' infection at $1x10^3$ plaque-forming units (PFU) and 'high dose' infection at $1x10^4$ PFU, with sacrifice, intracardiac perfusion and harvest at day 5 post-infection. **(B-C)** Lung tissue showed presence of viral genes N1 and subgenomic and dose-responsive pathology by H&E. **(D)** At low dose of infection, no subgenomic E was present in brain tissue. **(E)** However, at both low and high doses of infection, clusters of mononuclear cells were observed by H&E. **(F)** Low dose infection increased microglia activation as measured by Iba1 expression and resulted in **(G)** increased cytokines.



CONCLUSIONS

- Two brain injury markers (NFL-L and UCH-L1) are elevated acutely following COVID-19 accompanied by an abnormal GCS
- Six cytokines/receptor (IL-1RA, IL-6, IL-12p40, HGF, M-CSF, and CCL2) are elevated acutely following COVID-19 accompanied by abnormal GCS
- Two brain injury markers (NFL-L and GFAP) remain elevated weeks to months after COVID-19 in cases of neurological complications
  The highest elevations of brain injury markers are seen in cerebrovascular pathologies
- We have established a mouse model of para-infectious SARS-CoV-2 effects on the brain– in the absence of viral replication in the brain, we observe increased cytokines (CXCL9, IFN, IL-17A) and microglia activation (by increased Iba1 expression). This model will be useful for evaluating therapeutic strategies

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Daniela Esser is a bioinformatician by training and works as a postdoc in the Neuroimmunology group at the Institute of Clinical Chemistry in Kiel. Her research focuses on the analysis of next-generation sequencing data in the context of neuroimmunological diseases with the aim to transfer scientific results into clinical application. Her main interest is to reveal novel insights into molecular patterns underlying autoimmune encephalitis to increase the understanding of the pathomechanisms and to improve the diagnostic and therapeutic possibilities. After working as a project manager for a sequencing service provider, Daniela Esser started to investigate molecular patterns underlying inflammation-associated colorectal cancer, for which she received her PhD from the Kiel University. Prior working in the neuroimmunology, she gained experience as a bioinformatican in the Medical Systems Biology group at the Institute of Experimental Biology in Kiel and at the Leibniz Lung Centre in Borstel.

Compartmentalized, clonally expanded plasma cells drive anti-LGI1 and anti-CASPR2 autoimmune encephalitis

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Introduction: Autoimmune encephalitis (AIE) denotes a group of rare inflammatory brain diseases in which autoimmunity targets neuronal or synaptic structures. Although the diseases are clinically heterogeneous, it remains unclear whether underlying immune effector mechanisms are shared or differ between AIE subtypes. In general, the knowledge about the molecular background of AIE is quite limited so far, although this would be the key to better understand and treat the disease.

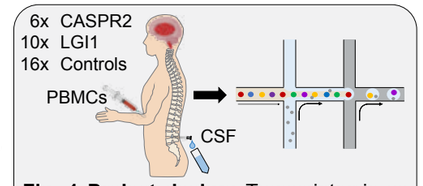


Fig. 1 Project design: Transcriptomic as well as B- and T-cell receptor sequence profiles were investigated for peripheral blood mononuclear cells (PBMCs) and cerebrospinal fluid (CSF) samples from 16 untreated AIE patients and 16 controls using single cell sequencing.

Conclusion: Our study investigated for the first time anti-LGI1 and anti-CASPR2 AIE patients using a large scale unbiased RNA-Seq approach with confirmation assays. We revealed transcriptomic changes and immune profiles reflecting subtle, but highly relevant pathomechanisms in AIE subtypes. The novel insights into underlying molecular processes clearly help to better understand antibody-mediated diseases and provide the basis to improve the possibilities to diagnose and treat AIE in future.

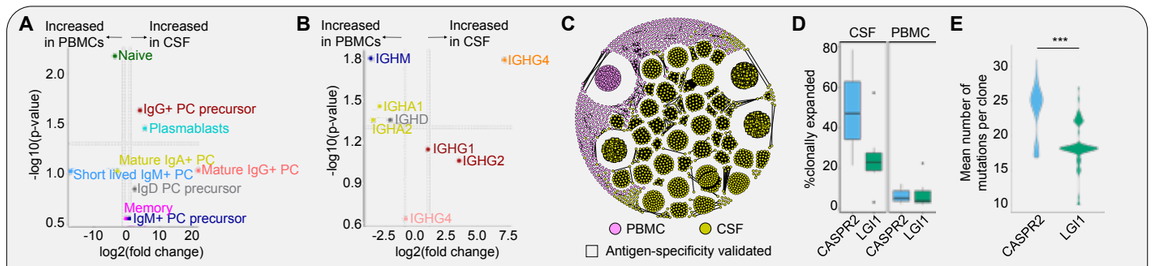


Fig. 2 Analyses of B-cells: Characteristics of relevant B-cells were identified with a comparison between CSF and PBMCs due to the low number of B-cells in the controls. (A) Comparison of cell types. (B) Comparison of immunoglobulin types. (C) Network based on BCR sequences from one CASPR2 patient: Connected cells have identical VDJ genes and the same CDR3 region. These network types were created and analyzed for all samples. (D) Percentage of clonally expanded cells. (E) Mean number of mutations per clone.

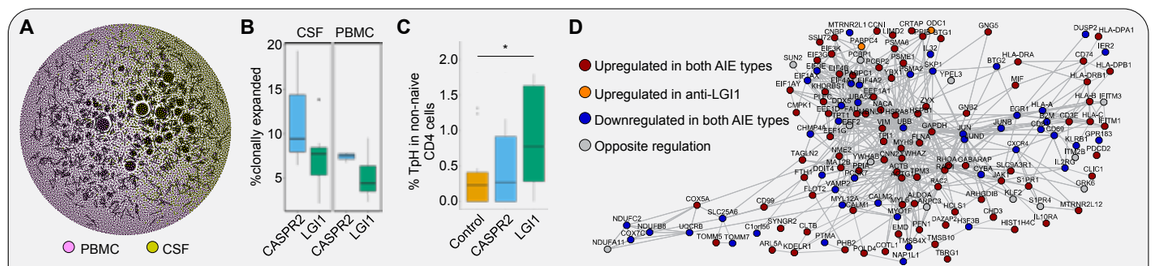
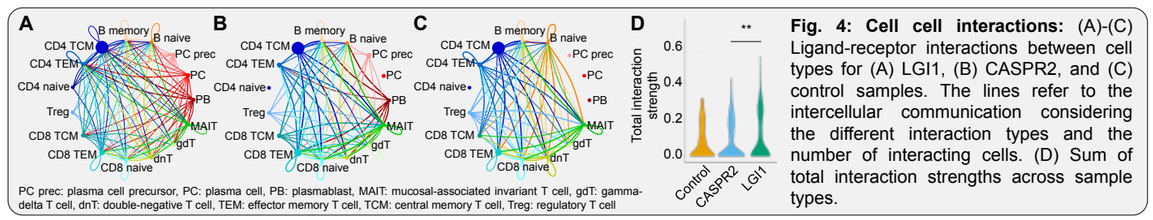


Fig. 3 Analyses of T-cells: (A) Network based on TCR sequences from one CASPR2 patient: Connected cells have identical VDJ genes and the same CDR3 region. (B) Percentage of clonally expanded cells. (C) Increased number of TpH cells in non-naive CD4 cells in AIE samples. (D) Protein-protein interaction network based on differentially expressed genes (DEGs) in non-naive CD4 T-cells. For the figure, the top 150 DEGs in the comparisons between LGI1 and controls and between CASPR2 and controls were considered, respectively.



PC prec: plasma cell precursor, PC: plasma cell, PB: plasmablast, MAIT: mucosal-associated invariant T cell, gdT: gamma-delta T cell, dnT: double-negative T cell, TEM: effector memory T cell, TCM: central memory T cell, Treg: regulatory T cell

Fig. 4: Cell cell interactions: (A)-(C) Ligand-receptor interactions between cell types for (A) LGI1, (B) CASPR2, and (C) control samples. The lines refer to the intercellular communication considering the different interaction types and the number of interacting cells. (D) Sum of total interaction strengths across sample types.

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A passion for understanding the needs of rare disease patients and caregivers, as well as delivering solutions to address their needs has guided Danielle's 20+ year career in biopharmaceutical patient engagement and patient marketing. Danielle is a community builder, with strong skills in patient advocacy, insight generation, strategic planning, disease awareness and education, content development, digital solutions, clinical trial design and enrolment, as well as patient support program development. Utilizing these skills to provide patient-centred consulting to rare disease non-profits has brought inspiration and purpose to Danielle and helped to improve the lives of the rare disease communities she serves. Career highlights include strategic development and execution of multi-channel disease awareness campaigns including Television, Radio, Digital, Social and Experiential Marketing, and Disease Awareness Public Service Announcement (TV and Radio). Quarterly magazine development in partnership with three non-profit patient organizations to bring education, solutions, tips, and tricks to families living with childhood onset epilepsies. Assisting patient organizations in their organizational growth, community expansion, content development and research through consultation, connection and partnership rooted in trust. Finally, design and development of patient experience solutions including patient support programs and injection training.

The patient journey in leucine-rich, glioma inactivated 1 (LGI1) autoimmune encephalitis: collaborative insights for patient-centric support materials

Please use this QR code to download a PDF of the poster.

The Encephalitis Society Conference, London, UK, 4–5 December 2023

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Objective

- To understand the biggest challenges along the LGI1 autoimmune encephalitis (AIE) patient journey and co-develop lay-friendly insight and guidance content with patient organizations to support the community and broader awareness

Background

- AIE is a collection of related conditions where the immune system causes inflammation of the brain, leading to debilitating neurological and psychiatric symptoms[1]
  - LGI1 AIE is the second most common form of AIE, with an estimated annual incidence of 0.83 per 1 million persons, and it represents the most common cause of autoimmune encephalitis of adults older than 40 years[2,3]
  - There are currently no approved treatments for LGI1 AIE[4]
- Rare diseases such as LGI1 AIE present unique challenges in terms of diagnosis and long-term management. Consequently, increased understanding of the patient journey in LGI1 AIE may help to improve diagnosis and clinical outcomes[5]

Methods

- People with clinician-confirmed LGI1 AIE (and/or their caregivers) and neurologists provided their perspectives on LGI1 AIE diagnostic journeys, treatment pathways and needs through a 15-minute online survey and 60-minute telephone interviews. A sample of payers also contributed their perspectives through interviews, but data related to these interviews are not included here
- The uncovered insights were shared with three patient organizations for input and collaboration, to co-create lay-friendly content in the form of an infographic, social media posts, and an engaging PowerPoint presentation

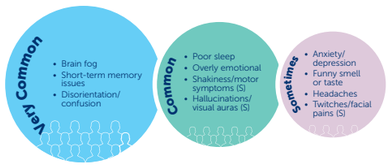
Results

- A total of 13 patients and 10 caregivers (from US and UK), capturing 18 unique patient experiences in all, plus 32 neurologists (from US, UK, Spain, Germany, Japan) were surveyed and interviewed
  - 72% of the patient sample were male
  - Patients shared that their earliest signs and symptoms often began in mid/late adulthood (range 40–71 years, 54 years on average)

Signs & symptoms

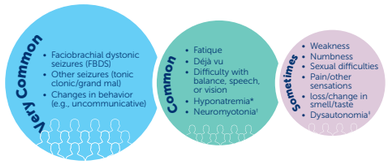
- Patients/caregivers reported that LGI1 AIE initial symptoms were often difficult to define and crept in quietly, taking days to weeks (sometimes longer) to identify (**Figure 1**)
  - Misdiagnosis as epilepsy, depression/anxiety or Alzheimer's are common (**Diagnosis journey**)
- Over the next 2–6 months, symptoms became more numerous, severe, and frequent (**Figure 2**). Patients and caregivers reported confusion and worry as symptoms developed, commonly including seizures and changes in behavior
- Faciobrachial dystonic seizures (FBDS), seen as very sudden movements of the face and/or arm/leg, are a hallmark symptom of LGI1 AIE but are difficult to identify as seizures by patients and caregivers and infrequently occur in the presence of specialist HCPs

Figure 1 Symptoms identified as early signs (by patients/caregivers)



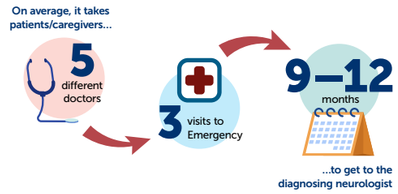
(S) It is suspected that these symptoms are connected to undiagnosed seizure activity.

Figure 2 Further symptoms identified as condition progresses



*low levels of sodium in the blood. †Constant muscle activity due to nerve impulses. ‡Malfunctions of the nerves that regulate involuntary function such as heart rate, breathing, blood pressure.

Figure 3 Journey to diagnosis



Patients tended to wait days to weeks for an antibody test to be administered, and 1–2 weeks to receive test results, although further delays were common

Treatment and recovery

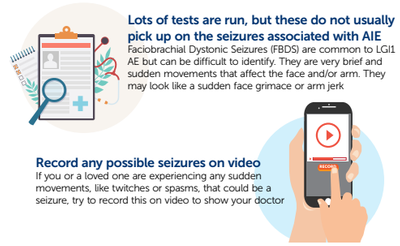
- At diagnosis, patient symptoms tended to be very severe and urgent, requiring in-hospital pharmaceutical care (**Figure 3**). Patients were almost always given intravenous (IV) steroids while waiting for their antibody test results, with further treatment added once diagnosis was confirmed
- Patients were generally discharged from hospital after a couple of weeks and treatment continued with oral steroids and intravenous immunoglobulin (IVIg) through outpatient visits. Immunosuppressive therapy was added as needed
- Patients and caregivers found three types of rehabilitative therapies beneficial to support recovery: 1. physical & occupational therapy, 2. speech & cognitive therapy, and 3. psychological & emotional therapy (**Figure 4**)
  - Patients and caregivers noted that HCPs did not often suggest these therapies

Development of lay-friendly materials

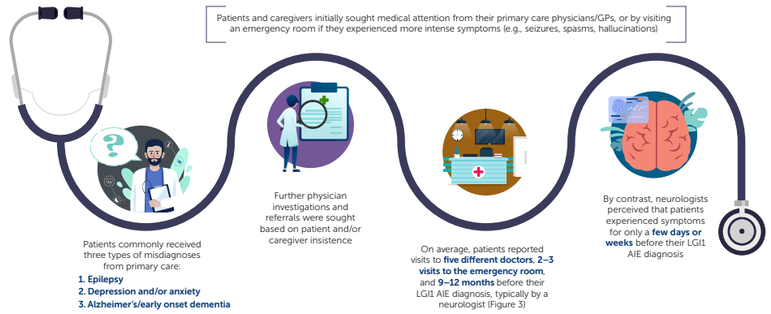
- The output team collaborated with three partner patient organizations to evolve the insights into lay-friendly materials to share with the AIE patient community and wider public
- This led to the creation of three lay-friendly outputs: a visual presentation deck for multi-purpose use, an illustrated infographic for patients/caregivers, and three social media posts for awareness raising
  - Each of these outputs were developed iteratively with partner patient organizations to ensure their story and design would reflect and engage the patient community
- The infographic output developed for patients and caregivers included information and tips to support patients and caregivers with significant challenges identified in the patient journey, such as how to identify and confirm seizures (**Figure 5**)
- Based on further feedback from the patient community the team are developing an animated film version of the patient journey to meet the needs of patients who find textual formats less accessible than audio-visual formats

Figure 4 Three most important therapy types identified by patients/caregivers

Figure 5 Advice on how to identify and record seizures



Diagnosis journey



Summary

Patients with LG1 AIE face significant challenges at diagnosis and treatment stage. The complex and diverse array of symptoms can confound medical professionals, leading to incorrect initial diagnoses and delays in referrals to specialist neurologists

Once diagnosed, patients would benefit from more comprehensive support to include access to rehabilitative therapies alongside pharmaceutical treatments

Each patient's experience is unique and tailored approaches are required to address the various impacts of AIE on individual patients to improve their quality of life

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For questions about this poster, please contact Danielle Hartigh (Danielle.Hartigh@ucb.com)

Disclosures

D. Hartigh is an employee and stockholder, and S. Razdan an employee of UCB Pharma. M. Poe is Director of Patient Support, Programs and Administration of the Autoimmune Encephalitis Alliance. D. Muir is the Medical Advisor & Liaison of International Autoimmune Encephalitis Society. E. Wu is an employee of Fulcrum Research Group, a healthcare research agency. R. Gervais is an employee of Real Chemistry, a health innovation agency.

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Dr Deepti Yagnik is a Neurology Trainee in her final year, currently based in Perth, Western Australia. She completed MBBS and MD in General Medicine from India and migrated to Australia in 2014. She has completed basic physician training and pursuing a fellowship in Autoimmune Neurology.

Tale of two anti-N-methyl-D-aspartate receptor (NMDAR) antibody-positive cases – Is it Autoimmune Encephalitis?

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Introduction

Anti-NMDA receptor encephalitis is an autoimmune disorder targeting the NMDA (N-methyl-D-aspartate)-type glutamate receptors. A retrospective study found that ~1% of all ICU admissions in patients between 18-35 years had NMDAR encephalitis. The presentation is characterized by neuropsychiatric symptoms including seizures, movement disorders, autonomic instability and psychiatric manifestations. The presentation often has a predictable course with distinct phases (Fig.1). The diagnostic investigations include electroencephalogram (EEG,) MRI Brain and detection of IgG antibodies against NR1 subunit of NMDA receptors in cerebrospinal fluid/serum.

We discuss two cases referred for NMDAR encephalitis that posed a dilemma regarding management of isolated psychiatric symptoms.

CASE 1

A 53-year-old lady with three-year history of refractory depression and delusional disorder nonresponsive to multiple neuroleptics was referred by the community mental health team. Initial workup revealed serum anti-N-methyl-D-aspartate receptor (NMDAR) positive. She was on venlafaxine and olanzapine depot. The neurological examination was unremarkable except for blunted affect and delusional thinking. Electroencephalogram (EEG), magnetic resonance imaging (MRI) brain and pelvis, computed tomography (CT) chest, and positron emission tomography CT whole body and brain were normal. Cerebrospinal fluid (CSF) examination was bland with negative oligoclonal bands and +1 anti-NMDAR antibody, although the indirect immunofluorescence returned negative. She was trialled with 5-day intravenous methylprednisolone (1gram) and immunoglobulins (IVIg).

She reported ongoing anxiety and depression on follow-up review as she had self-ceased neuroleptics.

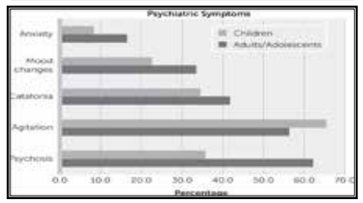


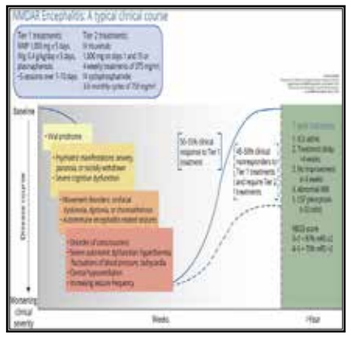
Fig1 – Psychiatric symptom manifestation in NMDAr patients

CASE 2

A 39-year-old lady relocated interstate to Western Australia with an established diagnosis of NMDAR encephalitis in 2010. In 2010 she presented with subacute behavioural changes and seizures. EEG demonstrated generalised slowing with no epileptiform abnormalities. CSF examination revealed pleocytosis (41 cells, predominantly mononuclear) with positive oligoclonal bands. The NMDAR antibodies were positive in the CSF and serum. She was treated with prednisolone, IVIg, antiseizure medications (phenytoin, valproate, and clonazepam), quetiapine and haloperidol. The pelvic imaging (CT and ultrasonography) was negative for ovarian teratoma. She recovered well from this episode; however, had a presumed relapse in 2014, presenting with isolated behavioural changes without recurrence of seizures. She was commenced on 6 monthly intravenous Rituximab (500mg) due to ongoing serum NMDAR positivity. In 2021 it was briefly paused for invitro fertilisation and pregnancy. She underwent an emergency caesarean section at 33 weeks for worsening mental state and recommenced Rituximab 3 days postpartum, which was continued until January 2023.

She was found to be B cell deplete and serum NMDAR antibody negative at the time of clinic review in April 2023 The CSF NMDAR was detected on cell-based assay, however, the confirmatory IIF was negative. She is currently awaiting cerebral PET CT for completion of testing.

She currently suffers from a complex post-traumatic stress disorder and anxiety disorder but otherwise has remained seizure-free.



Discussion

There have been case reports for isolated psychiatric manifestations in NMDAR-related AE, although, most patients develop autonomic dysfunction, seizures, or movement disorders with abnormalities detected on imaging, EEG or serum/CSF NMDA antibodies[1]. In a retrospective study of 23 patients with isolated psychiatric symptoms in anti-NMDA encephalitis; 5 patients presented during an initial episode, whereas 18 patients had symptoms on relapse. Relapse was defined as the new onset or worsening of symptoms at least 2 months after improvement or stabilisation and persistent detection of NMDAR antibodies.

In our patients, there was no associated inflammation on the MRI brain or PET scan, raising questions about AE diagnosis. The case 2 patient had a definite initial presentation consistent with NMDAR encephalitis, however, the following presentation was isolated psychiatric symptoms. The serum NMDAR was positive in both cases with weak positive CBA NMDAR, however, the confirmatory IIF was negative.

The cases depict pitfalls in the diagnosis of autoimmune encephalitis. It is important to cautiously consider clinical and paraclinical parameters when diagnosing AE[2]. We elected to cease case 2 patient's Rituximab therapy as despite B cell repletion there was no evidence of positive NMDAR on CSF or brain imaging.

Further studies are required to look into a biomarker for predicting and diagnosing relapses.

Conclusions

Isolated psychiatric episodes are rare but can occur as initial onset or relapse of anti-NMDAR encephalitis.

For patients with a history of anti-NMDAR encephalitis, any behavioural change might represent relapse. Serum and CSF antibody testing should be obtained, if possible, to support the diagnosis of relapse.

No specific guidelines exist for the treatment of isolated psychiatric symptoms. If found to have positive antibodies in the CSF, the patients should be treated aggressively with immunotherapy and symptomatic management of psychiatric symptoms.

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3. Image courtesy - https://autoimmune-encephalitis.org/anti-nmdar-encephalitis-2

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Dr Deepti Yagnik is a Neurology Trainee in her final year, currently based in Perth, Western Australia. She completed MBBS and MD in General Medicine from India and migrated to Australia in 2014. She has completed basic physician training and pursuing a fellowship in Autoimmune Neurology.

New onset refractory epilepsy (NORSE) secondary to anti Glutamic acid decarboxylase (anti-GAD) and anti-Hu antibody related paraneoplastic encephalitis from lymphocyte rich thymoma

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Introduction

New-onset refractory status epilepticus (NORSE) is a rare clinical diagnosis most often secondary to autoimmune or paraneoplastic encephalitis, with infectious causes less common.

NORSE carries a 16-27% mortality rate in adults and significant long-term neurological sequelae. A cause is only found in approximately half of the cases. Early intervention, seizure management and institution of immunotherapy can significantly reduce morbidity and mortality in NORSE.

CASE

A 33yr old previously well mother of two presented to a regional hospital with three witnessed generalised tonic-clonic seizures preceded by headache and vomiting. She reported a preceding SARS-CoV2 negative flu-like illness. She was loaded with intravenous (IV) levetiracetam (2gm), dexamethasone, ceftriaxone, acyclovir and transferred to a tertiary hospital. She was afebrile with no signs of meningism. She was unable to follow multistage commands. The magnetic resonance imaging (MRI) brain demonstrated T2/ Fluid attenuated inversion recovery (FLAIR) enhancing lesion in the left temporal lobe. A routine electroencephalogram (EEG) demonstrated highly frequent multifocal independent focal epileptiform abnormalities maximal in the right posterior quadrant with electrographic seizures of right posterior quadrant origin. A FDG-PET scan revealed a high uptake mediastinal mass closely related to the right atrium. The serum anti-GAD (>2000) and anti-Hu antibodies returned positive (indeterminate pattern on IIF). The CSF examination was normal with negative antineuronal antibodies. The antiviral therapy ceased following negative viral polymerase chain reaction (PCR) in CSF. Multiple antiseizure medications (ASMs) were added sequentially - clobazam, lacosamide, phenytoin and levetiracetam (figure 1) and commenced on 5-day course of IV methylprednisolone followed by IVIg and Rituximab due to ongoing clinical and electrographic seizures. The cardiothoracic surgeons resected the mediastinal mass as the initial CT-guided core biopsies were indeterminate. The histopathology was consistent with lymphocyte-rich thymoma (figure 2).

Due to ongoing focal status epilepticus, she required intubation and induced coma with propofol and midazolam. A cEEG monitoring in the intensive care unit (ICU) showed highly frequent focal seizures of left hemispheric origin consistent with super refractory status epilepticus. The therapy was escalated to subsequent treatment including thiopentone infusion titrated to burst suppression and sequential addition of valproate, phenobarbital, topiramate and perampanel. She received plasma exchange, 5 exchanges on alternate days. Following a discussion with immunology, the patient's family consented to the commencement of cyclophosphamide. She was seizure-free on day 4 intensive care (ICU) admission. IV anaesthetic agents were gradually weaned and ASMs were rationalised. She was stepped down to the ward after a 41-day ICU admission. She had a period of rehabilitation before returning home following 3-month inpatient admission and continues to remain seizure-free 12 months since admission.

Investigations

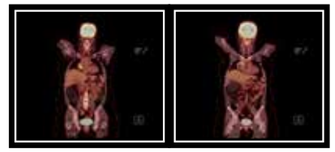


Fig 1. 18F-FDG PET whole body demonstrating 73mm mediastinal mass closely related to the R atrium with high uptake

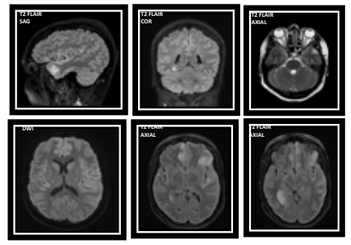


Fig 2- MRI Brain with Gadolinium (at the initial presentation) – Cortical-based FLAIR/T2 hyperintense, involving the bilateral frontal lobes, left insula, the left inferior frontal gyrus, left superior temporal gyrus, the inferior aspect of the right temporal lobe with interval enlargement of the small lesion in the right lingual. None of the lesions demonstrate diffusion restriction or contrast enhancement.

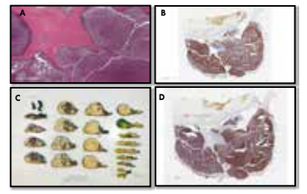
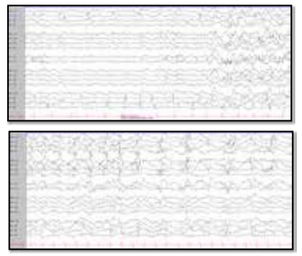
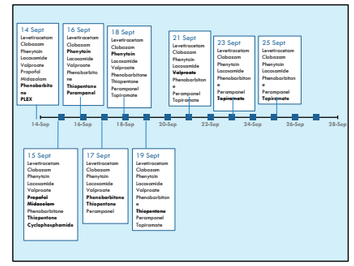


Fig 3- Anatomical Pathology of Thymoma Specimen
A - The tumour typically shows jigsaw puzzle–like lobulation. Pale-staining medullary islands are scattered in a dark-staining thymic cortex–like background.
B - TdT stain labelling densely packed immature T cells in the cortical region and rare cells in the medullary island
C - Cut surface of specimen showing typical encapsulation and large lobules delineated by fibrous septa
D - AE1/3 stain labelling a delicate epithelial cell meshwork in the light-staining medullary islands and darker-staining cortical regions.



Interictal EEG abnormal and demonstrated multifocal epileptiform abnormalities (left anterior temporal, right anterior temporal, right posterior temporal) as well as two focal electrographic seizures of left temporal origin

ANA/ENA/dsDNA- negative	CSF paraneoplastic Ab
Celiac serology	NMDA- CSF/Serum
IgG subclass serum – normal (at admission)	Serum TPO ab <1
MOG Ab negative	Serum AMPA, ANNA-2, anti purkinje, CRMP5, anti-amphiphysin, anti-Ma – not detected
Anti-Hu detected with compatible corresponding IIF pattern	Serum AQP4 – not detected
Serum anti GAD >2000	GABA B serum - not detected



Discussion

The case highlights the importance of aggressive multimodal immunotherapy in the setting of super refractory status epilepticus (SRSE). NORSE although a well-defined syndrome is not a diagnosis on its own. The most common identifiable etiology of NORSE are autoimmune and paraneoplastic encephalitis, out of which a small proportion of patients have GAD-65 related epilepsy.

Thymoma can present with paraneoplastic neurological syndromes[1], however these syndromes are not commonly seen with anti-GAD/anti-Hu antibodies. The anti-Hu paraneoplastic syndromes can be diverse and present as encephalomyelitis. In rare cases, anti-Hu encephalitis can present as seizures, memory issues or behavioural changes when temporal lobes are involved[2]. There is no clear evidence that GAD-65/anti-Hu antibodies are directly pathogenic in the associated syndromes[3].

No specific imaging or laboratory abnormalities have been identified so far that allow for an early diagnosis of NORSE[4]. We recognize that early recognition and diagnosis is key to starting treatment, however, more often than not the patients need to be treated concomitantly during diagnostic workup as earlier treatment leads to favourable outcomes.

Follow Up

The patient has done remarkably well since discharge. She was discharged on clobazam 10mg BD, Phenytoin 100mg daily, Prednisolone 30mg daily, Levetiracetam 1.5gm BD, Phenobarbital 90mg BD, Topiramate 200mg BD. She remains seizure-free 12 months following discharge and we gradually weaned prednisolone and remaining antiseizure medications. She is currently on Topiramate 200mg BD and clobazam 10mg BD (weaning). She has successfully weaned off prednisolone.

In retrospect the patient mentioned having subacute behavioural changes up to 6 months prior to onset of SRSE.

She remains under follow-up with immunology and cardiothoracic team. She completed 6 cycles of cyclophosphamide and was re-dosed with Rituximab recently.

Ongoing CT Chest/Abdo/Pelvis and MRI Brain have been reassuring with no new lesions.

Contact

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Federico Iovino studied Human Biology and Biomedical Sciences at the University of Pavia, Italy (2003-2008), and then moved to Groningen, Netherlands, for his PhD in which he investigated bacterial interactions with the blood brain barrier. After his PhD in Medical Microbiology in November 2013, he immediately started his postdoc in Infection Biology at the Department of Microbiology, Tumor and Cell Biology at the Karolinska Institute in Stockholm, Sweden, where he investigated new therapeutic strategies to prevent bacterial invasion of the brain in vivo. Federicos studies were published in the leading medical journals Journal of Clinical Investigation (2016) and Journal of Experimental Medicine (2017). He became Assistant Professor in Medical Science at Karolinska Institute at the end of 2018, and in 2021 he started his own independent laboratory of neuro-infections and neuroinflammation at the Department of Neuroscience at Karolinska Institute. Federico became Associate Professor in Medical Microbiology in 2022. Federico Iovino's group investigates molecular mechanisms of bacterial interaction with brain cells during meningoencephalitis pathogenesis. The molecular interaction between *Streptococcus pneumoniae* (the pneumococcus), leading cause globally of bacterial meningoencephalitis, and neurons leading to neuronal death was discovered and characterized for the first time in literature by Federico Iovino's group in a recent study published in PLOS Pathogens (https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1009432). In a more recent study published in mBio, Federico's group has also newly observed that bacterial accumulation in the brain severely impairs cognitive functions in vivo (https://journals.asm.org/doi/10.1128/mbio.01886-22).

Modulation of bacterial interaction with brain cells: New therapeutic approaches for bacterial meningoencephalitis

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Background

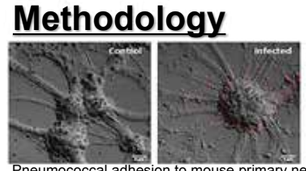
The incidence of bacterial meningoencephalitis remains high (2.5 million cases/year globally), and most of the cases are caused by *Streptococcus pneumoniae* (the pneumococcus). Even if the infection is successfully treated, up to 70% of survivors experience long-term neurological disabilities (sequelae) caused by neuronal damage inflicted by the infection.

Aims

Our aims are to establish new therapeutic approaches to prevent neuronal damage by:

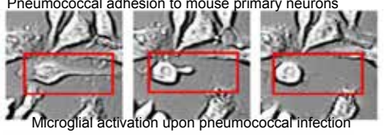
1) Making neurons able to defend themselves by exploiting autophagy to eliminate intracellular bacteria.
2) Blocking bacteria-neuron interaction to prevent neuronal damage.
3) Enhancing microglial phagocytic activity for an efficient elimination of bacteria attacking the brain.

Methodology

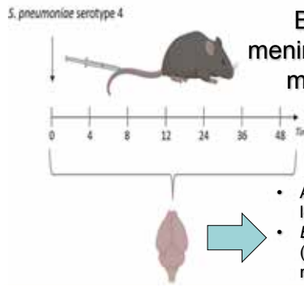


In vitro infection models with neurons and microglia

Pneumococcal adhesion to mouse primary neurons



Microglial activation upon pneumococcal infection



Bacteremia-meningoencephalitis mouse model

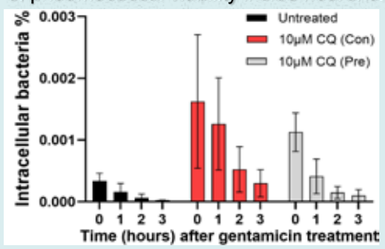
- Assessment of bacterial load in the brain.
- *Ex vivo* analysis (neuroinflammation, neuronal damage).

Conclusions

- ✓Neuronal cells use autophagy to eliminate intracellular bacteria. Therefore, stimulation of autophagy can boost the capability of neurons to eliminate bacteria.
- ✓EV can efficiently block bacteria-neuron interaction, and therefore have the incredible potential to protect neurons during bacterial meningoencephalitis pathogenesis.
- ✓Pneumococcal PspC and NanA trigger microglial phagocytosis and therefore can act as immunostumulatory agents to boost microglial phagocytic activity.

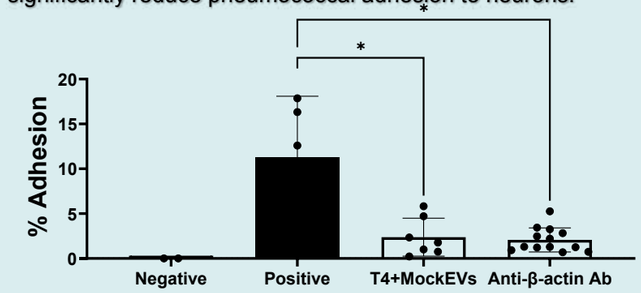
Results

Pneumococcal viability inside human neurons decreases when neuronal autophagy is inhibited. Gentamicin-protection assays allow the study of bacterial survival inside phagocytic cells over time after gentamicin treatment that eliminates all extracellular bacteria , Chloroquine (CQ) added in the culture medium of neurons for only one hour before the infection (Pre) or constantly present in the medium for the entire duration of the experiments (Con) led to an increase of pneumococcal viability inside neurons.

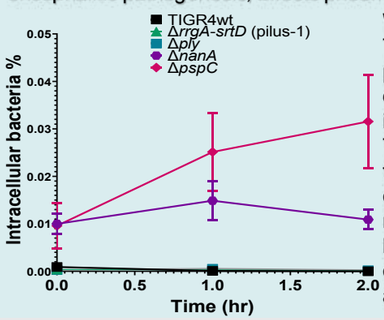


The increase of bacterial viability inside neurons was evident at the earlier time-points after the gentamicin treatment, indicating that at this time-point, inhibition of autophagy was more pronounced than in the later time-points after the gentamicin treatment.

Blocking exposed β-actin impairs pneumococcal adhesion to neurons. We recently discovered that *S. pneumoniae* binds to β-actin exposed on neuronal plasma membrane to invade neurons causing neuronal death (Tabusi,…, Iovino, *PLOS Pathogens*, 2021). Extracellular vesicles (EV), excellent nano-molecules for therapeutic delivery into the brain, can block exposed β-actin on neurons and significantly reduce pneumococcal adhesion to neurons.



Pneumococcal PspC and NanA trigger microglial phagocytosis. Using the gentamicin-protection assays, we have investigated how the absence of certain virulence factors, known to be involved in meningo-encephalitis pathogenesis, affects pneumococcal survival inside microglia.



While TIGR4, TIGR4Δpilus-1, TIGR4Δ*ply* are efficiently killed, pneumococci lacking either pspC or nanA genes are surviving well inside microglia.

Therefore, **PspC** and **NanA** seem to be key proteins that trigger elimination of pneumococci by microglial phagocytosis that can be used as immunostimulants to enhance microglial phagocytic activity.

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Prof Gary Alvarez Bravo is a consultant neurologist with clinical and laboratory experiences in the field of autoantibody mediated diseases of the nervous system at the University Hospital Dr Josep Trueta of Girona, Spain. He is also associate professor at the University of Girona. His focus is on patients with autoimmune encephalitis, neuromyelitis optica spectrum disorders, MOG antibody-mediated demyelinating disease, multiple sclerosis, and other autoimmune systemic diseases with central nervous system involvement. His work includes identification of antibody associated disease phenotypes, the clinical response to treatments, and outcome correlates. He has a specific interest in the development of clinical tools to assess the risk of disability after relapses in immune-mediated disorders, in order to improve patient care.

Assessment of clinical prognosis in autoimmune encephalitis: Girona Score

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Background

Autoimmune encephalitides (AE) are a heterogeneous group of immune-mediated disorders that affect the central, peripheral and autonomic nervous systems. Tools for evaluating risk of having disability after the acute attack are lacking in the assessment of AE. We propose the Assessment of clinical prognosis in autoimmune encephalitis: Girona Score (ACPE-Gi score) to evaluate the severity of AE at diagnosis and predict the risk of disability after either the disease-onset or relapse. Items such as: seizures, memory dysfunction, psychiatric symptoms, consciousness, language problems, movement disorders, ataxia, brainstem dysfunction, pyramidal dysfunction, neuro-imaging autonomic dysfunction, cancer association and recurrences were considered for developing the scale. Every item was scored from 0 to 3 based on their severity. Unlike other clinical scales we have scored the neuroimaging, autonomic function, cancer association and recurrences since we consider these items as important characteristics for the comprehensive evaluation of AE.

Objective To evaluate the severity of AE at diagnosis and predict the risk of disability after either the disease-onset or relapse

Methods

Patients strictly diagnosed with AE according to the current criteria between January 1, 2009 to March 31, 2023 at the University Hospital Dr. Josep Trueta of Girona, Catalonia-Spain. ACPE-Gi score included 14 items and every item was scored from 0 to 3 depending on their severity with a sum ranging from 0 to 41.

ACPE-Gi Score:

Table 1 Detailed Clinical Characteristics of the Score

Seizure	None	0	Gait instability and ataxia	Normal	0
	Controlled seizures	1		Mild, able to walk unassisted	1
	Intractable seizures*	2		Moderate, assisted walking	2
	Status epilepticus	3		Severe, unable to walk	3
Memory dysfunction	None	0	Brainstem dysfunction (number of symptoms)	None	0
	Mild (does not affect daily activities)	1		Gaze paresis	1
	Moderate (interferes with daily activities)	2		Tube feeding	2
	Severe (no recent memory or unable to communicate)	3		Ventilator care due to central hypoventilation	3
Psychiatric symptoms	None	0	Muscle weakness	Normal (Grade V)	0
	Mild (no need for medical intervention because it does not affect daily activities)	1		Mild (Grade IV)	1
	Moderate (need for medical intervention because it interferes with daily activities)	2		Moderate (Grade III)	2
	Severe (needs continuous care or admission because of psychiatric symptom) or unable to check	3		Severe (≤ Grade II)	3
Consciousness	Alert (opens eyes spontaneously)	0	Brain MRI and other ancillary test abnormalities	Normal	0
	Drowsy (opens eyes to voice)	1		Limbic encephalitis (uni or bilateral)	1
	Stupor (opens eyes to pain)	2		focal alterations non LE	2
	Comatose (does not open eyes)	3		Multifocal or diffuse alterations	3
Language problems	None	0	Autonomic dysfunction(Non epileptic)	Normal	0
	Mild (slow but able to express sentences)	1		Isolated symptoms of liability autonomic (LA)	1
	Moderate (unable to express full sentences)	2		More than 1 symptom suggestive of LA	2
	Severe (unable to communicate)	3		Paroxysmal sympathetic hyperactivity	3
Dyskinesia/dystonia	None	0	Cancer association	None	0
	Mild dyskinesia (does not affect daily activities)	1		Low-Risk Antibodies	1
	Moderate dyskinesia (interferes with daily activities)	2		Intermediate-Risk Antibodies	2
	Severe dyskinesia causing secondary medical problems	3		High-Risk Antibodies	3
Rapid Progression	No	0	Recurrences*	First episode	0
	Yes	1		Recurrences*	1

Results:

ACPE-Gi measured the severity in the acute phase and grouped the patients into three groups: mild <8 (32%), moderate (8 to 15) (60%), and severe > 15 (8%). We found the third group had a higher risk of disability compared with the first group (p: 0.035). We identified the mean initial score was significantly higher in the group of patients who had higher mRS at three months compared to the group of patients who had a mild to moderate disability level (mRS≤2) at three months (p: 0.023).

In addition, autonomic symptoms and mental status impairment demonstrated to be an independent risk factors to predict disability (p: <0.05).

Table 2: Comparison of mean with standard deviation for the presence of each variable of the Gi-Score

	NO	Yes	p value
SEIZURE	2,92 ± 1,68	2,77 ± 1,69	0,83
MEMORY	3,38 ± 2,26	2,59 ± 1,28	0,38
PSYCHIATRIC	3,08 ± 2,02	2,62 ± 1,26	0,50
CONSCIOUSNESS	2,15 ± 0,80	3,58 ± 2,02	0,038
LANGUAGE	2,70 ± 1,49	2,93 ± 1,79	0,74
DISKINESIA/DYSTONIA	2,85 ± 1,63	2,80 ± 1,92	0,95
GAIT INSTABILITY	2,42 ± 1,44	3,23 ± 1,79	0,22
BRAINSTEAM	2,53 ± 1,50	3,50 ± 1,85	0,97
WEAKNESS	3,11 ± 1,74	2,14 ± 1,21	0,19
MRI	2,00 ± 1,00	2,95 ± 1,70	0,36
AUTONOMIC	2,35 ± 1,27	3,88 ± 1,96	0,028
RISK CANCER	2,00 ± 0,87	3,31 ± 1,81	0,054
RECURRENCE	2,70 ± 1,58	4,50 ± 2,12	0,14
RAPID PROGRESSION	2,75 ± 1,71	2,92 ± 1,66	0,80

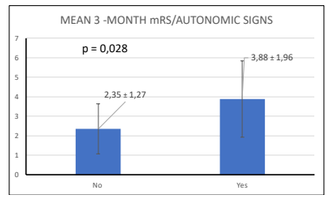

Figure 1: T-Test Comparison of Mean 3-Month mRS Scores by Autonomic Signs

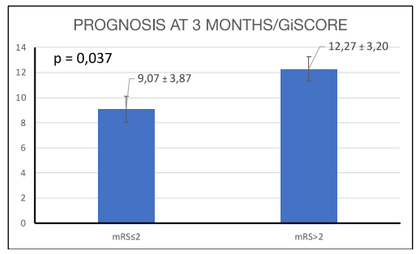

Figure 2: T-Test Comparison of Mean GiSCORE at 3 Months Based on mRS Categories

Conclusion:

The ACPE-Gi score seems to be a reliable scale for comprehensively evaluating the severity of AE in the acute phase and predicting the risk of disability at three months. Dysautonomia and altered mental status predict a poorer prognosis in patients with AE.

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Giuseppe Guglielmini: has nothing to disclose.
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Mr Jack O'Brien-Cairney is currently a PhD student at Aston University, under the primary supervision of Dr Sukhvir Wright, working on developing computational models of pathophysiology in autoimmune encephalitides. Previously, he studied neuroscience at the University of Nottingham where he completed his BSc and MRes degrees. The latter of which focused on studying opioidergic signalling in cranial and spinal astrocytes derived from P2 Wistar rats and the effect that mu-opioid receptor agonists had on signalling parameters and astrocyte morphology. Throughout his PhD, he has developed his skills with regards to programming (principally in MATLAB), and in analysing time series data in the form of continuous video-EEG data. He developed a number of skills relating to microscopy and primary cell culture during his MRes.

Anti-LGI1 antibodies increase sleep fragmentation in a novel rodent model of anti-LGI1 encephalitis

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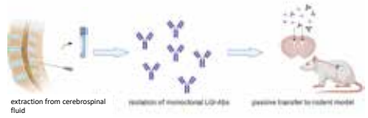
Background & Methods

- **Anti-LGI1 and anti-CASPR2 encephalitis** are inflammatory, neurological diseases that are associated with seizures, cognitive declines, and changes in **sleep behaviour and architecture**

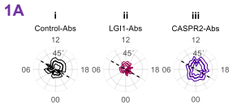


- Here, we sought to develop a model of behavioural changes in anti-LGI1 and anti-CASPR2 encephalitis based on continuous video-EEG recordings of **P21 Wistar rats** used in **passive transfer models of autoimmune encephalitides**
- We constructed a **support vector machine (SVM)** algorithm to:
  - Quantify **abnormal interictal frequency dynamics**
  - Predict **behavioural changes across time**

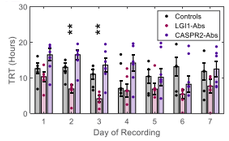
Results

LGI1-Abs significantly reduce predicted total resting times

1A



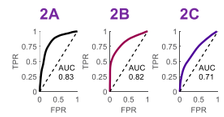
1B



- LGI1-Ab-treated rats rested less than controls:
  - During the day (**1Aii**) (**p = 0.0025**)
  - Overall (**1B**) (**p = 0.0023**)
  - Seizures paired with the biggest decline (**1B**) (**p= 0.0079**)
- CASPR2-Ab-treated rats rested more than controls during the dark phase (**1Aiii**) (**p = 0.0154**)
- The periodicity of predicted rest and wakefulness did not vary across cohorts

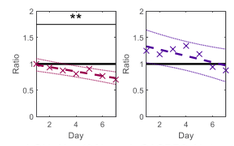
- On average, our SVM model was **81.3% accurate**
- The accuracies of single cohort tests are shown below:
  - Controls (**2A**)
  - LGI1-Ab-treated rats (**2B**)
  - CASPR2-Ab-treated rats (**2C**)

2A **2B** **2C**



Antibody infusion increases discontinuity among periods of rest

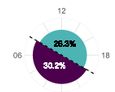
3A **3B**



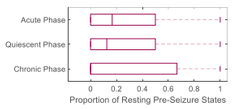
- LGI1-Ab- (**3A**) and CASPR2-Ab-treated rats (**3B**) have increasingly discontinuous periods of rest $R^2_{LGI1} = 0.77$; $R^2_{CASPR2} = 0.47$
- This trend was significant among LGI1-Ab-treated rats (**p = 0.006**) (**3A**)

Light/dark cycle phases and disease progression do not significantly affect pre-seizure behavioural states

4A



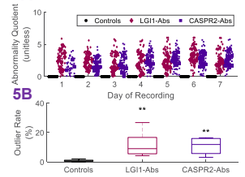
4B



- Seizures arising from rest accounted for:
  - **26.3 ± 7.7%** of daytime seizures (**4A**)
  - **30.2 ± 6.8%** of night-time seizures (**4A**)
- Seizures from rest also did not differ across the acute (n=114), quiescent (n=50), and chronic (n=80) phases (**p =0.9502**) (**4B**)
- Seizure duration had no significant association with pre-seizure state

Frequency dynamics across resting epochs are increasingly abnormal in LGI1-Ab- and CASPR2-Ab-treated rats

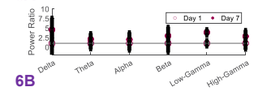
5A



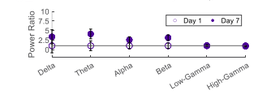
5B

- Mean abnormality of resting epochs is significantly higher in both disease groups compared to controls (**p = 0.0013**) (**5A-B**)
- Overall frequency dynamics among abnormal resting epochs vary between groups (**p= 0.0019**), especially across the 50-70Hz range (**p = 0.0295**) (**6A-B**)

6A



6B



Discussion

Our results have shown that supervised classification of video-EEG data from novel passive transfer models can be used to predict the behavioural state of the rat accurately in the absence of EMG or other data types. This shows that it is possible to create a model of how behaviour is expected to change throughout the progression of anti-LGI1 and anti-CASPR2 encephalitis. Next, we aim to apply these methods to other disease models and explore non-linear dynamics measurements to further validate these findings.

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Following completion of his neurology residency in Lyon, France, Professor Joubert joined the Lyon research group on paraneoplastic neurological disorders in 2015 as a clinical fellow. Combining research with clinical duties, he completed his PhD in 2019 on the clinical-immunological characterization of CASPR2 antibody-associated syndromes. He joined Prof Dalmau's lab in Barcelona (2018-2020) and contributed to an animal model of CASPR2 encephalitis. Back in Lyon, he joined the French Reference Centre for Autoimmune and Paraneoplastic Neurological Syndromes in 2020 and continued working on various antibody-mediated neurological disorders, with a focus on the emerging neurotoxicities caused by cancer immunotherapies and the evaluation of treatment strategies in autoimmune encephalitis. He is currently a Professor of Neurology in the University of Lyon. Using a translational approach combining large clinical cohorts and the identification of biomarkers, his aim is to provide evidence-based treatment strategies in autoimmune encephalitis.

Morvan Syndrome :
Natural history and effect of Rituximab

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Background

Morvan syndrome (MoS)
- Poorly defined entity combining :
  - **peripheral nerve hyperexcitability**
  - ***agrypnia excitata***
  - **positive anti-CASPR2 antibodies**
- Clinical overlap with CASPR2 limbic encephalitis ?
- Remarkable efficacy of rituximab in several case reports

Objectives

- Characterize the natural history of Morvan syndrome
- Characterize the overlap with CASPR2 limbic encephalitis
- Assess the effect of Rituximab

Methods

INCLUSION CRITERIA: MORVAN syndrome
- **A. Positive CASPR2-Abs**
- **B. Peripheral nerve excitability** (PNH)
- ***C. Agrypnia excitata***
  - Insomnia AND nocturnal hallucinations or dream-enactment behavior
  - OR severe insomnia not explained otherwise
- ***C. No reported limbic symptom***
  - Anterograde amnesia
  - Temporal lobe seizure

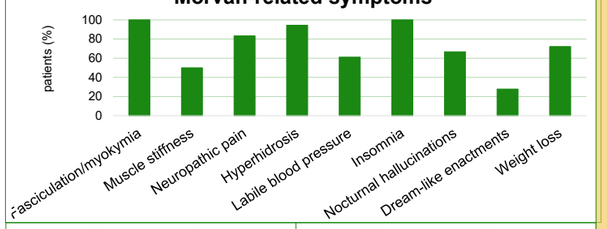
CONTROL POPULATION: limbic /PNH overlap
positive CASPR2 antibodies, limbic symptoms, and signs of PNH

French reference centre 01/01/2006 → 01/06/2023

Results

18 patients | 51.5 yo (17-78) | 33.3% women

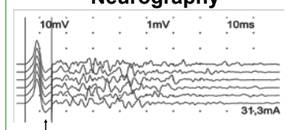
Morvan-related symptoms



Comparison with limbic/PNH overlap

	limbic encephalitis + PNH n=30	Morvan n=18	p
Demographics			
Age, median (range)	67 (53-83)	51.5 (17-78)	<0.001
Female sex, n (%)	0 (0)	6 (33.3)	<0.001
Tumor, n (%)	3 (10)	16 (88.9)	<0.001
Thymoma, n/N (%)	0/3 (0)	16/16 (100)	<0.001
Symptoms, n (%)			
Anterograde amnesia	29 (96.7)	0 (0)	<0.001
Temporal lobe seizures	27 (90)	0 (0)	<0.001
Neuropathic pain	23 (76.7)	15 (83.3)	0.854
Motor PNH	19 (63.3)	18 (100)	0.010
Dysautonomia	16 (53.3)	18 (100)	0.002
Insomnia	14 (46.7)	18 (100)	0.001
Diagnostic tests, n (%)			
CSF CASPR2 Abs	29/29 (100)	0/16 (0)	<0.001
Serum CASPR2 Abs titer, median (range)	40960 (10240-40960)	2560 (10-20480)	<0.001
Serum LGI1 Abs	0 (0)	12 (66.7)	<0.001
HLA DRB1*11:01	13/18 (72.2)	1/8 (12.5)	0.017
Outcome			
time to max mRS in month, mean (SD)	30.8 (38.0)	2.9 (2.3)	<0.001
maximal mRS, mean (SD)	2.82 (0.80)	4.11 (1.13)	<0.001
Death, n (%)	4 (13.3)	8 (44.4)	0.036

Neurography



Afterdischarges : 100%
Myokimic discharges : 85.7%
Neuromyotonic discharges 14.3%

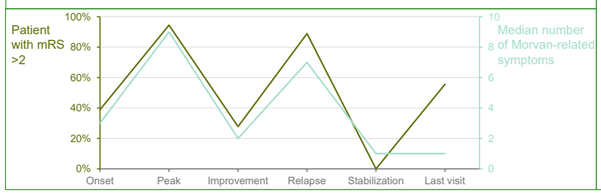
Sleep recordings

Total sleep time : 231min (52-376)
Slow-wave sleep : 4% (0-9)
REM sleep : 3% (0-12)
Arousal index : 39/h (12-56)

24h polysomnographic record of a MoS patient. Presence of N1 sleep only ; total sleep time of 56 min.

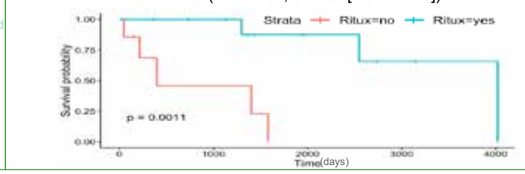
Natural history

- Median mRS at peak : 4 (2-6)
- 16/18 improvement after immunoactive therapy
- **9/16 relapses (56,3%)**
- 8/18 deaths (44.4%)
  - 3 during active disease, time from onset : 1, 6 and 7 months
  - 5 from comorbidity, time from onset : median 52 months (43-132)
- Among survivors, at last visit : mRS ≤ 2 in 8/10 patients (80%)



Treatments

Effect of Rituximab (11/18 patients)
- 11/11 stabilizations with mRS ≤2
- 2 relapses
- Increased survival (HR 0.038, 95%CI [0.033-0.45])



Conclusions

The Morvan syndrome is a severe, life-threatening disorder with a high rate of relapses. Clinical and immunological features differ from CASPR2 limbic encephalitis overlapping with PNH, suggesting distinct immunopathogeneses. Key diagnostic features include neurographic and polysomnographic findings, while rituximab seems both safe and efficient.

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Muñiz-Castrillo S, Joubert B, Elsensohn M-H, Pinto A-L, Saint-Martin M, Vogrig A, Picard G, Rogemond V, Dubois V, Tamouza R, et al.: Anti-CASPR2 clinical phenotypes correlate with HLA and immunological features. J Neurol Neurosurg Psychiatry 2020, 91:1076–1084

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Juliette Brenner is a medical doctor (graduated in 2018), in training to be a neurologist in the Netherlands. She combines this with a PhD trajectory in the group of Dr Maarten Titulaer, on assessing and predicting treatment effects and outcomes of autoimmune encephalitis. In the context of these projects, she set up an extensive structured follow-up schedule and online database for the Dutch nationwide autoimmune encephalitis cohort. She also combined the data of five national anti-NMDAR encephalitis cohorts into a large international database, creating the possibility to do robust statistics for this rare disease. Working on long-term sequelae of autoimmune encephalitis, she realized the need for patient advocacy in the Netherlands and set up the contact between Dutch patients and their families and the UK Encephalitis Society. The resulting Dutch patient advocacy counts over 100 members today.

Long-term follow-up after anti-NMDAR encephalitis: A focus on cognitive sequelae and quality of life.

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Introduction

Anti-NMDARE is a brain inflammation caused by NMDAR antibodies. Symptoms include cognitive and behavioural changes, seizures and autonomic dysregulation. It is a severe disease, many patients end up at the intensive care, but fortunately treatable: over 80% improve to a 'good outcome' on the modified Rankin Scale (mRS≤2) within 18 months.[1] Nevertheless, many patients experience persistent cognitive and emotional sequelae, and a decreased quality of life years after treatment.[2-4] We describe the scope of potential sequelae of anti-NMDAR encephalitis.

Methodology

We have invited all patients diagnosed in the Netherlands with anti-NMDAR encephalitis who were able to self-report and/or participate in cognitive testing, to cross-sectionally assess long-term outcomes, including comprehensive neuropsychological testing and Patient-Reported Outcome Measures (PROM's) of daily activities, participation, mood, fatigue and quality of life. The tests and PROM's have been carefully selected after systematic literature xreviews and focus groups with patients, and span all relevant cognitive domains and constructs.
Patients diagnosed within two years of the study initiation were included in a prospective follow-up schedule.x

Results

92 anti-NMDAR encephalitis patients (79% female, mean age disease onset 29 years) participated in the study, twelve were included in a prospective follow-up schedule.
A year after diagnosis, 75% of patients had a good outcome on the modified Rankin Scale. Nevertheless 46% of patients had not resumed work or school after one year and 14% had resumed work or school at a lower level or with adjustments compared to the situation before the illness.

Cognitive outcomes show persistent deficits

48% of patients assessed *at least* one year after diagnosis (on average 5 years, standard deviation (SD) 6 months) had a cognitive impairment (≤ -1.5 SD below the norm population) in at least one of the four major cognitive domains. The average cognitive outcome after one year was 1 SD below the norm population.

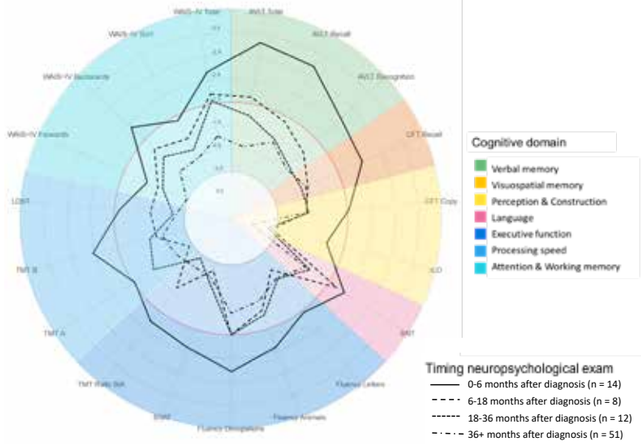

Figure 1: Cognitive outcomes at test level per time frame since diagnosis (cross-sectional assessments). The colours represent cognitive (sub)domains, the light circle in the middle the mean of the norm population. The further outward on the circle, the more deviant scores are from the norm.

Cognitive recovery can take 3 years

Cognitive outcomes improved over time, up till 3 years after diagnosis: from impairments (at group level) on *memory, language* and *executive functions* in the first six months, to scores on average not different from the norm population after 18-36 months. At 36 months, 70% of patients had resumed work (18 % at a lower level or with adjustments).

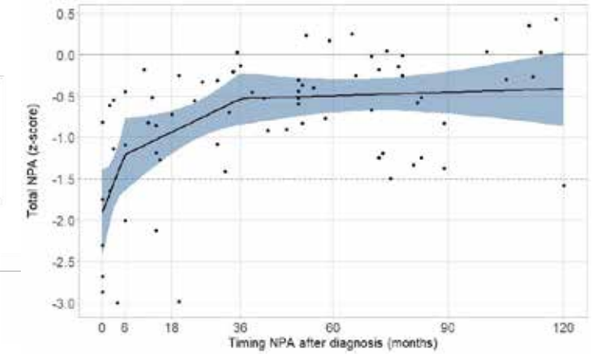

Figure 2: Cognitive outcomes of the complete cohort plotted against time after diagnosis. Scores on average increase up to 36 months (3 years) after diagnosis.

Patient-Reported Outcomes provide detailed information

Independent of recovery time after diagnosis, the cohort on average reported significant difficulties with the *role of physical and emotional sequelae* in daily life (SF36), *vitality* (SF36), *social functioning* (SF36), *participation* (WHO-DAS-II) and *quality of life* (EQ-5D-5L) compared to normative data. These problems were most prominent in the first year after diagnosis.

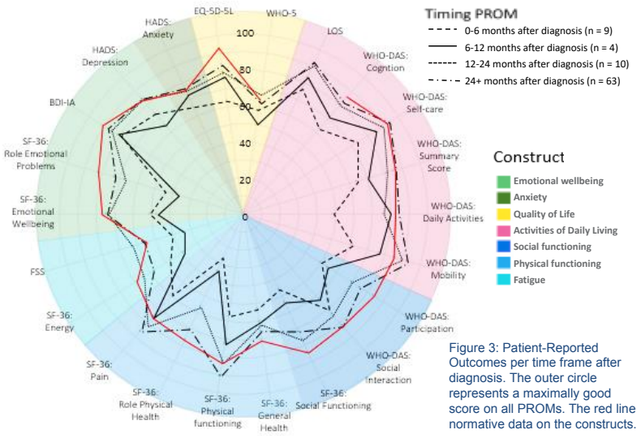

Figure 3: Patient-Reported Outcomes per time frame after diagnosis. The outer circle represents a maximally good score on all PROMs. The red line normative data on the constructs.

Conclusions

- Cognitive deficits and a diminished well-being may persist years after anti-NMDAR encephalitis.
- Cognitive recovery extends beyond 18 months after diagnosis, up to at least 36 months.
- The consequences of encephalitis may have a long-term impact on work activities or education, participation in society and quality of life.

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Evaluation of Neurofilament light as biomarker for disease monitoring and long-term outcome in patients with anti-Lgi1 autoimmune encephalitis

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Introduction

Anti-Lgi1 antibody-associated autoimmune encephalitis is the second most common antibody-positive autoimmune encephalitis, affecting mostly elderly people. The most common clinical manifestations are seizures and limbic encephalitis. Patients respond well to first-line therapy with steroids and second-line therapy such as rituximab, but only about 1/3 of patients return to their cognitive baseline (see Figure 1).

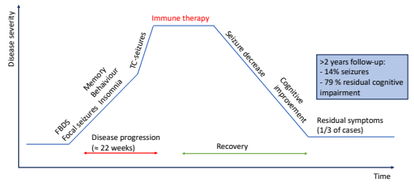

Figure 1 *Course of Anti-Lgi1 autoimmune encephalitis. This figure is adapted from Van Sonderen Neurology 2016*

The main **objective** of this project was to evaluate **Neurofilament light** (neuron specific structural protein) as a possible biomarker for:

- neurocognitive outcome
- seizure cessation

in a well clinically characterised multicentric anti-Lgi1 encephalitis cohort.

Methods

- We recruited 55 patients with anti-Lgi1 encephalitis from the Generate (GErman NEtwork for Reaserch on AuToimmune Encephalitis) network.
- Antibodies were detected in serum and/or CSF by cell-based assays and confirmed by tissue-based assays (see Figure 2).
- Pre-treatment and follow-up samples were tested for NfL levels using a single-molecule-array-based (SIMOA) platform. Associations between (a) clinical manifestations, (b) treatment response, (c) long-term neurocognitive outcome and (d) MRI findings were investigated. In addition, healthy controls were used to compare biomarker levels.

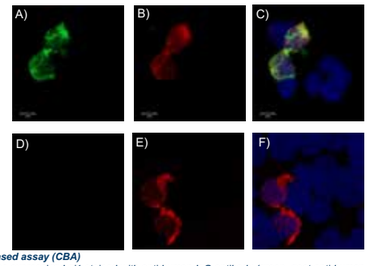

Figure 2 cell-based assay (CBA)
HEK292 cell lines expressing Lgi1 stained with anti-human IgG antibody (green, goat anti-human, Alexa 488 IgG, Life technologies #A11013), secondary anti-human Lgi1 antibody (red, Sigma #G9269) and nuclear staining (blue, DAPI) incubated with anti-Lgi1 positive CSF sample (A-C) and antibody negative control (D-F). A) and D) show staining with anti-human IgG antibody; B) and E) show staining with anti-human Lgi1 antibody; C) and F) merged figures.

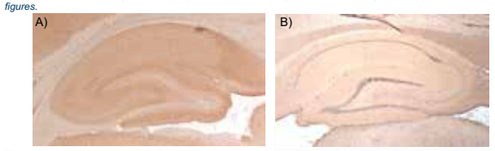

Figure 3 tissue-based-assay (TBA)
Screening for neuropil antibodies using secondary immunohistochemistry on fixed sagittal rat brain slices. A) Positive CSF sample for anti-Lgi1 antibody with reactivity in the hippocampal region; B) Negative CSF sample as control.

Results

- We determined NfL levels in sera and CSF of 55 anti-Lgi1 encephalitis patients (29 (52%) female) with available clinical follow-up data (min-max 24-222 months).
- The mean age at disease onset was 62±10 years (min-max 21-81).
- We found higher serum and CSF levels in anti-Lgi1 patients compared to healthy controls and observed a decrease in NfL concentration after cessation of seizures and induction of immune therapy (see Figure 4).
- Higher CSF NfL levels from the first available sample were associated with the development of persistent neurocognitive deficits at follow-up (see Figure 5).
- No significant differences in serum and CSF NfL concentrations were observed between patients with pathological vs. normal MRI findings and high (>2) vs. low (≤2) mRS at disease onset and last follow-up.

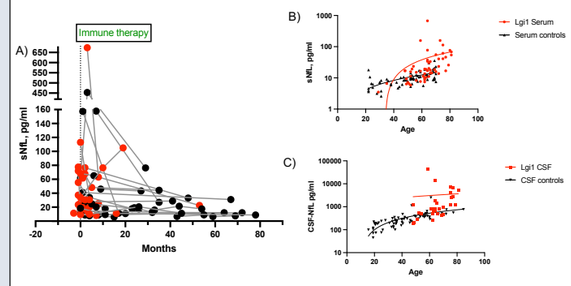

Figure 4 Serum and CSF NfL levels
A) Serum NfL (sNfL) concentration over the course of the disease, vertical puncture line represents the start of immunotherapy, measurements belonging to the same patient are connected by grey lines, red dots indicate attack activity during sNfL measurement; B) sNfL levels in anti-Lgi1 patients (red dots and line) and healthy controls (black dots and line); C) Cerebrospinal fluid NfL (CSF-NfL) levels in anti-Lgi1 patients (red dots and line) and healthy controls (black dots and line).

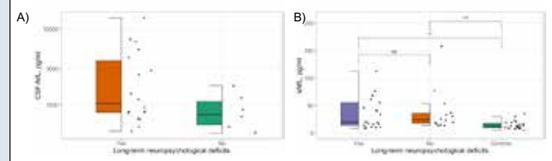

Figure 5 NfL levels and cognitive impairment in patients >60 years old.
A) CSF levels at the first available sample and long-term neuropsychological deficits, showing higher CSF NfL concentrations in patients with long-term neuropsychological deficits; B) sNfL levels and long-term neuropsychological deficits, showing a tendency towards higher levels in patients with long-term neuropsychological deficits without reaching a statistically significant level.

Conclusions and Discussion

Our study shows two key observations supporting futher evaluation of serum and CSF NfL as potential biomarker for monitoring treatment response and predicting long-term outcome in ant-Lgi1 encephalitis:

1) the reduction in serum NfL concentration after cessation of seizures and initiation of immunotherapy;

2) the association of higher CSF NfL levels at disease onset with persistent neurocognitive deficits at long-term follow-up.

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Wissen schafft Gesundheit

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Katharina Wurdack is a clinical neurologist and researcher at the Department of Neurology at Charité – University Clinic in Berlin, Germany. She started her clinical training in 2021 and joined the Cognitive Neurology Lab Berlin (PI Prof. Carsten Finke) in 2022. Katharina's research focus lies on investigating the relationship between cognitive function and (micro-)structural imaging markers in autoimmune-mediated encephalitis. She is also interested in combining different MRI modalities, notably functional and microstructural imaging, to gain a better understanding of how cognitive symptoms arise in neurological diseases. In her free time, she likes to clear her head while cooking, knitting, or practicing aikido.

Long-term Microstructural Integrity Changes in Anti-NMDAR Encephalitis

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Background

Anti-NMDAR encephalitis is associated with **altered microstructural integrity of white matter and** subcortical gray matter structures, notably of the **hippocampus**. The **long-term trajectory of these changes** over the course of anti-NMDAR encephalitis is not known. *Finke et al. 2013, Ann Neurol; Finke et al. 2016, Biol Psychiatry*

Methods

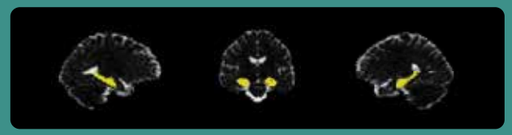
- ✓ 3T-MRI data: T1-w MPRAGE, single-shell DWI sequence
- ✓ Fitting of diffusion tensor model for FA and MD maps
- ✓ Tract-Based Spatial Statistics for central white matter integrity (FA and MD)
- ✓ FreeSurfer recon-all to obtain hippocampal MD maps

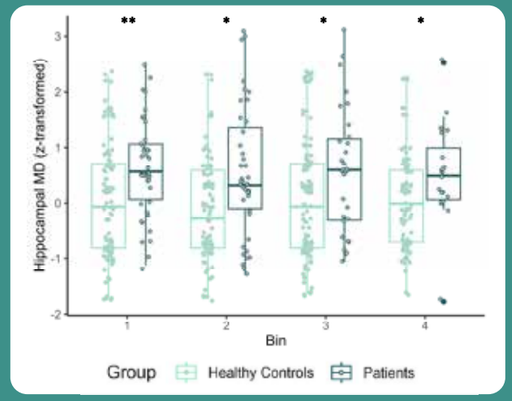
N = 84 anti-NMDAR encephalitis patients with up to 3 follow-ups over up to 10 years

Bin 1 (0-2 years)	**Bin 2** (2-4 years)	**Bin 3** (4-6 years)	**Bin 4** (6-10 years)
48 (38 f)	46 (38 f)	33 (29 f)	26 (23 f)

Healthy control groups matched bin-wise for age and sex

Persistent Impairment of Hippocampal Microstructural Integrity

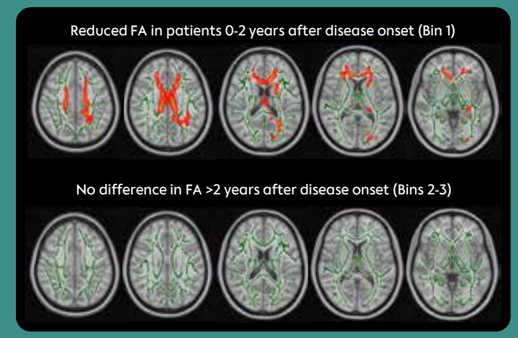


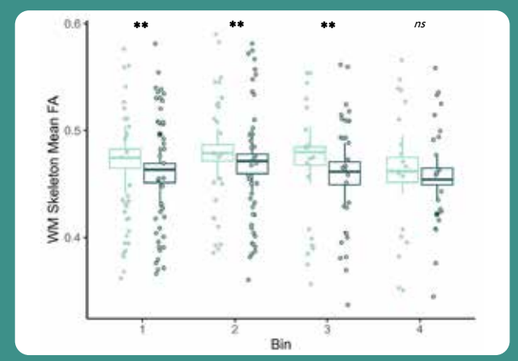


All Wilcoxon's tests: *ns*: not significant * : $p < .05$ ** : $p < .01$

Increased hippocampal MD persists in patients until 10 years after onset of anti-NMDAR encephalitis. A decrease in central white matter FA is only present until 2-6 years after onset.

Long-term Recovery of White Matter Microstructural Integrity





Conclusion

Hippocampal microstructural damage persists for up to ten years after onset of anti-NMDAR encephalitis, while **central white matter integrity recovers** within this timeframe. Microstructural integrity of subcortical structures may play a role in long-lasting cognitive deficits of patients with anti-NMDAR encephalitis.

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Kia Gilani is a final year Adult Neurology resident at the University of Toronto and will be pursuing further training in Clinical Neurophysiology and Epilepsy at The Mount Sinai Hospital in New York City in 2024. His clinical and research interests include autoimmune epilepsy and encephalitis.

Electrodermal Scalp Changes Ipsilateral to Pilomotor Seizures in Autoimmune Encephalitis

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Introduction

- Autonomic involvement is common in both focal and generalized seizures. Rarely, pure autonomic seizures are seen, such as pilomotor seizures, which are frequently associated with autoimmune encephalitis (AE) [1]
- Autonomic discharges in seizures are hypothesized to originate from the hypothalamus, as the result of ictal spread through limbic-hypothalamic connections [2]
- Electrodermal activity (EDA) is a measurable change in skin conductance as a result of autonomic discharge onto eccrine skin glands, usually measured on the surface of the hands, which can take the form of a phasic response to external or internal stimuli [3]
- The neuroanatomical basis of EDA is well studied, originating in the posterior hypothalamus and following descending autonomic pathways through the brainstem and spinal cord onto eccrine glands [4]
- We present a novel high amplitude, infraslow waveform representing scalp phasic EDA, which can be reliably seen in AE associated focal seizures (s-EDA) or can occur spontaneously (ns-EDA)

Methods

- Epilepsy Monitoring Unit (EMU) recordings were screened for EDA with a low frequency filter (LFF) setting of 0.05 Hz, and the features of identified EDA were compared between two groups:
  1. "**AE-EDA Group**": 5 previously identified patients with autoimmune encephalitis, of whom 2 also had seizures captured on simultaneous MEG-EEG (Figure 1A)
  2. "**Control Group**": 12 consecutive EMU and 5 simultaneous scalp-sEEG patients
- To determine the intracranial versus extracranial site of origin of the EEG-recorded infraslow waveform, EDA events captured on scalp EEG were compared to simultaneous MEG, and sEEG recordings in an additional s-EDA patient not included in the AE-EDA group (Figure 1A&B). In a separate patient with ns-EDA the effects of local scalp abrasion on the EEG waveform were studied (Figure 1C&D). An additional patient had simultaneous scalp and palmar electrodes (Figure 3B)
- An additional patient (not in either group) was studied with scalp EEG during experimental adjustment of hypothalamic deep brain stimulation (DBS) parameters as part of a clinical trial (Figure 3A)

Results: Demonstrating Skin Localization

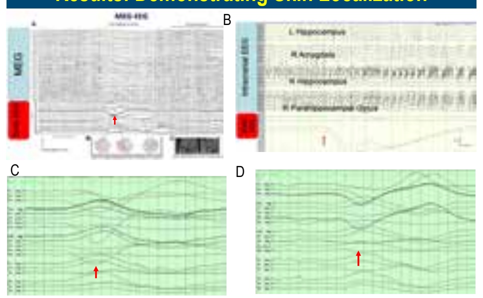


Figure 1: EDA (red arrows) is not associated with a magnetic field correlate on MEG (A) or with intracranial correlate on sEEG (B). One patient with frequent bilaterally symmetrical ns-EDA (C) underwent scraping of the skin underlying the P4 electrode. EDA events captured after the P4 skin abrasion no longer appeared at the P4 position (D), with other EEG activity recorded at P4 unchanged from baseline, confirming the skin as the source of the waveform. The P4 EDA waveform gradually returned over several days as the abrasion healed.

Results: EDA Waveforms

EDA characteristics:

- Infraslow waveform typically lasting >20 seconds, consisting of an initial electropositive deflection followed by a slower electronegative deflection with slow return to the isoelectric line
- Visible typically with LFF set to <0.1 Hz
- No morphological difference between s-EDA and ns-EDA

Categorization scheme:

Axis 1: Onset: Unilateral (Figure 2A), bilateral (Figure 1C)

Axis 2: Hemispheric spread: Present (Figure 2B) or absent (Figure 2A)

Axis 3: Synchrony: Synchronous or asynchronous anatomical regions

Axis 4: Phases: Monophasic (Figure 2A) or multi-phasic waveforms, up to Battle Rope Morphology (Figure 2C)

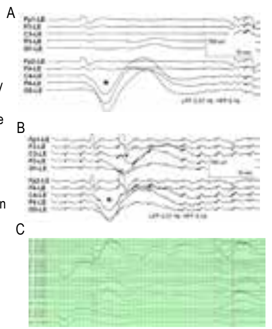


Figure 2: Examples of various EDA morphologies

Modifier: Topographical distribution: Most commonly posterior quadrant (parietal-occipital > central electrodes (Figure 2A), less frequently temporal-zygomatic regions (Figure 2C), and rarely frontal-central regions.

Results: S-EDA vs. Control Group

AE-EDA Group Characteristics

Age at AE onset	Age At EMU	Days of EMU Recording	Etiology	Antiepileptic medications	Immunotherapy History	EMU Ictal Findings	MEG
48	49	12	LGI-1	Topiramate Phenytoin Acetazolamide	IVIG, steroids mycophenolate mofetil, rituximab	R and L anterior temporal	Yes- no seizure captured
62	68	16	Seronegative AE	Levetiracetam and clobazam (weaned) Acetazolamide initiated	Steroids, IVIG, mycophenolate mofetil	R and L anterior Temporal	Yes- no seizure captured
43	43	13	LGI-1	Levetiracetam and clobazam (weaned) Acetazolamide and phenytoin initiated	Steroids	R Anterior Temporal	Yes- seizure captured
33	36	12	LGI-1	Phenytoin Acetazolamide	Steroids, IVIG, mycophenolate mofetil	R and L anterior Temporal	Yes- seizures captured
65	66	11	Seronegative AE	Lacosamide Keppra Clobazam	Steroids	R Temporal seizures	No

Control Group Characteristics

	Age At EMU	Days of EMU Recording	Etiology	Antiepileptic Medications	EMU Ictal Findings	EMU Interictal Findings	EDAS	EDAs with Seizures
Scalp EEG	32	3	PNES	None	None	None	0	None
Scalp EEG	56	3	Left TLE	Lamotrigine Phenytoin Primidone Levetiracetam	None	Left mesial temporal	3	None
Scalp EEG	54	3	PNES	Gabapentin, Carbamazepine	None	None	16	None
Scalp EEG	33	3	Left MTS	Carbamazepine Clobazam	None	Left mesial temporal	0	None
Scalp EEG	35	3	Bilateral TLE	Carbamazepine Keppra	Bilateral temporal	Right mesial temporal, left posterior temporal	3	None
Scalp EEG	30	3	Left TLE	Lamotrigine	Left posterior temporal	Left posterior temporal	0	None
Scalp EEG	28	3	Left TLE	Topiramate Lamotrigine	Left mesial temporal	Left mesial temporal	0	None
Scalp EEG	46	3	Right TLE	Levitiracetam	Bilateral Temporal	Right anterior temporal	5	None
Scalp EEG	33	3	Left TLE	Lamotrigine	none	posterior temporal	18	None
Scalp EEG	40	3	PNES	Topiramate	none	none	23	None
Scalp EEG	38	3	Left TLE, glioma	Levetiracetam Phenytoin	Left temporal	Left mesial temporal	0	None
Scalp EEG	62	3	Right MTS	Clobazam Lacosamide Brivaracetam	None	Left basolateral temporal	1	None
Intracranial + scalp EEG	25	3	Left TLE, right occipital periventricular heterotopia	Lacosamide Levetiracetam	None	Right and left mesial temporal	15	None
Intracranial + scalp EEG	30	3	Right TLE	Lamotrigine Carbamazepine Clobazam	Right mesial temporal	Right mesial temporal	0	None
Intracranial + scalp EEG	33	3	Right TLE	Brivaracetam Lamotrigine Carbamazepine	None	Right insular	2	None
Intracranial + scalp EEG	45	3	Right Parietal epilepsy	Carbamazepine, Perampanel	None	Right parietal	1	None
Intracranial + scalp EEG	38	3	Right TLE	Lacosamide Lamotrigine Brivaracetam Clobazam	None	Right temporal	23	None

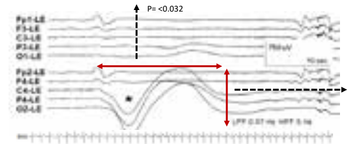
EDA Occurrence

	AE-EDA Group	Control Group
Total days of EMU recording	63	36
EDA Count	Total (5/5 patients, Avg 5.68/day) • **182 with seizure** • 176 without seizure EDA **BEFORE** ictal change on EEG: **100%** (average delay 19.21 ± 8.7 seconds)	**106 total** (11/17 patients, avg 2.1/day, range 0-14) • None with seizure

S-EDA Onset Lateralization	# of occurrences	% Total Occurrence
Ipsilateral to Seizure	69	79.3%
Contralateral to Seizure	0	0
Bilateral	18	20.7%

Group	EDA Duration (seconds)
S-EDA	22.08 ± 9.44
Control	21.3 ± 9.8

P= <0.032



Group	EDA Amplitude (uV)
S-EDA	381.1 ± 360
Control	521.8 ± 540

P= 0.001

Group	HR before EDA onset	HR After EDA onset	
S-EDA	78.87	88.37	P<0.0001
Control	73.19	71.84	NS

Results: Physiologic Correlates

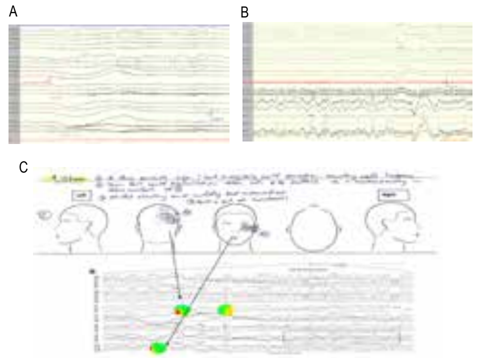


Figure 3: (A) Onset of high frequency bipolar DBS of the hypothalamus (red arrow) resulted in an instantaneous ipsilateral EDA event and the patient describing a sensation of warmth. The EKG showed an associated increase in heart rate. (B) Scalp EDA is not associated with any correlate on palmar electrodes; typical palmar EDA is independent of scalp EDA. (C) Example from patient 4's hand drawn accounts of anatomic regions where piloerection was felt during one seizure, with numbers denoting sequential spread. The discrete, sequential areas of spreading "chills" correspond to anatomical regions where phasic EDA was seen in association with previously captured seizures. Many permutations of laterality and anatomical distribution were described by this patient in dozens of recorded seizures.

Discussion

- We have demonstrated that this novel infraslow scalp EEG waveform is a phasic electrical potential generated in the skin, as no correlate was seen on MEG or intracranial EEG recordings (Figure 1). Abrading the skin caused the waveform to disappear only in the area that was scraped, further confirming the skin as the generator of the signal (Figure 1D)
- Unilateral scalp EDA triggered by high frequency hypothalamic DBS implicates the hypothalamus as the final common pathway for generation of the scalp EDA, under unilateral control
- Stimulation of limbic structures has been shown to trigger typical palmar EDA in humans [5] and hypothalamic stimulation in cats has produced bilateral corporeal EDA, [6] however, this is the first demonstrated instance of human hypothalamic stimulation triggering a phasic EDA response
- The remarkable similarity of the DBS-triggered EDA and s-EDA provides evidence for the hypothesis that autonomic seizures are a product of ictal propagation to the hypothalamus [2]
- ns-EDA was seen in 11/17 randomly selected controls, almost exclusively during sleep, indicating scalp EDA events to be a relatively common, normal physiologic finding
- s-EDA onset is **always** ipsilateral to the side of seizure onset (if the EDA is not simultaneously bilateral at onset), and always precedes seizures
- Prior attempts at correlating EDA with seizures have used hand EDA monitors, with most studies showing EDA emerging after onset of seizure activity, [7] unlike the scalp EDA described here. Thus, s-EDA is a physiological entity distinct from the general autonomic surges seen in seizures
- Scalp EDA was not associated with extremity EDA (Figure 3B), which implies that a distinct physiologic pathway could be responsible for scalp EDA relative to EDA elsewhere in the body [8]
- The detailed phenomenological drawings by patient 4 from the AE-EDA group precisely matched the anatomical regions where EDA was seen in the same patient's prior EEG recordings. These same anatomical regions are also activated discretely in ns-EDA
- The anatomical distribution of EDA is remarkably consistent in s-EDA, ns-EDA and hypothalamic DBS-induced EDA, affecting stereotyped, discrete anatomical regions on the head. This suggests the existence of autonomic dermatomes within the scalp that have not previously been described

Conclusions

- Sporadic scalp EDA events are a normal entity in humans, usually occurring during sleep
- Seizure-related scalp EDA events are a rare occurrence, highly suggestive of pilomotor seizures in AE. A diagnosis of underlying AE should be considered in those detected to have s-EDA preceding ictal onset at EEG
- Unilateral onset s-EDA accurately localizes the hemisphere of seizure onset (100% specificity)
- Scalp EDA is physiologically distinct from extremity EDA, but also initiated in the hypothalamus
- Scalp EDA corresponds to areas where patients feel piloerection, and is likely a physiologic correlate of the seizure itself
- Scalp EDA can be a helpful biomarker for seizures. If seen with clinical events suspicious for seizure, the EDA may signify ipsilateral temporolimbic ictal activity, even if no definite typical seizure pattern is seen on scalp EEG
- There are likely not yet delineated autonomic dermatomes in the scalp

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POSTER PRESENTATION

Successful Treatment of Pilomotor Seizures in Autoimmune Encephalitis with Acetazolamide

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Introduction

- Pilomotor seizures are strongly associated with Autoimmune Encephalitis (AE), particularly with anti-LGI1 encephalitis [1]
- Seizures with AE can occur in the setting of active neuroinflammation, termed "symptomatic seizures secondary to autoimmune encephalitis", and chronically after resolution of neuroinflammation, termed "autoimmune associated epilepsy" [2]
- Acetazolamide has been known to be sometimes effective in treating focal and generalized epilepsy since the 1950s [3,4]
- Inhibition of carbonic anhydrase II by acetazolamide results in build up of CO_2 and subsequent decrease of extracellular pH [4]
- Acetazolamide's antiseizure mechanism may be multifactorial, but is hypothesized to be related to the effects of decreased pH on neuronal ion transport [5,6]
- We present evidence from 6 patients with definite AE (5 anti-LGI1,1 seronegative) that responded to acetazolamide therapy

Methods

- Six patients with definite AE and pilomotor seizures were identified over 5 years
  - Epilepsy monitoring unit (EMU) video-EEG recordings were acquired in four cases (patients 1-4), with seizure frequency and treatment response systematically assessed
  - Patients 5 and 6 were followed solely as outpatients
  - Patient 1 (anti-LGI1) had maintained a 3-year daily seizure diary pre-admission while receiving cycling acetazolamide therapy

Patient Characteristics

Participant	Encephalitis Etiology	Hyperventilation Induced Seizures	Age at onset	Age at Time of Diamox Trial	Clinical Setting at Time of Acetazolamide Exposure	Immunotherapy at Time of Acetazolamide Initiation	Other Antiepileptics at Time of Acetazolamide Use
Patient 1	LGI1	Yes	33	EMU admission: 42	EMU; in context of seropositive relapse* (Figure 3)	None	None
				Seizure diary: 39-42	Remission of encephalitis, ongoing pilomotor seizures (Figure 2)	None	One of phenytoin, lacosamide or carbamazepine
Patient 2	LGI1	Yes	48	49	EMU during initial presentation (cognitive decline, seizures) (Figure 4)	IVIG	Phenytoin, Topiramate
Patient 3	Seronegative	Yes	62	68	EMU during remission of encephalitis, ongoing pilomotor seizures (Figure 4)	Mycophenolate Mofetil	Clobazam and Levetiracetam (weaned and discontinued)
Patient 4	LGI1	Yes	43	43	EMU during initial presentation (pilomotor seizures) (Figure 4)	None	Clobazam and Levetiracetam (weaned and discontinued)
Patient 5	LGI1	Yes	43	44	Outpatient, initial presentation	Steroids	Levetiracetam
Patient 6	LGI1	No	68	68	Outpatient, initial presentation	Steroids	Phenytoin

*AE relapse over the year preceding EMU admission; worsening cognition, mood lability, increased pilomotor seizure frequency. Repeat LGI1 autoantibody serology strongly positive (previously negative). Treated with pulse steroids, mycophenolate mofetil, no improvement. Immunosuppression was discontinued and patient admitted to EMU to better characterize seizures and consider transition to Rituximab.

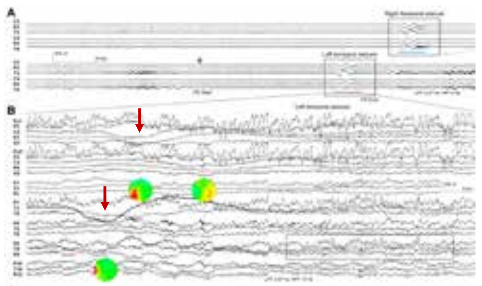


Figure 1: Patient 1. Independent right and left temporal lobe seizures (boxes, A), the hyperventilation induced seizure associated with ipsilateral infraslow electrodermal activity over left temporal-central-parietal region (red arrows, B).

Results

Patient 1

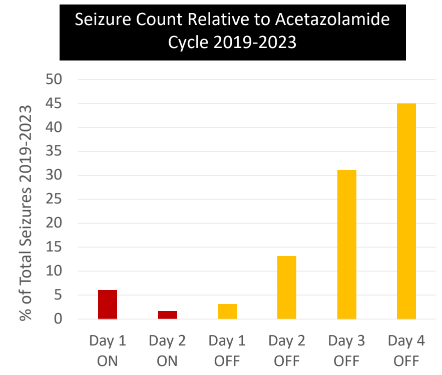


Figure 2: Patient 1 Pre-admission seizure diary of 1079 days while cycling acetazolamide 2 days on, 4 days off. 1203 pilomotor seizures were documented. Seizure occurrence correlated strongly with the 6-day cycle: the fewest seizures were seen while on Acetazolamide, with a gradual increase in seizure frequency during each discontinuation period.

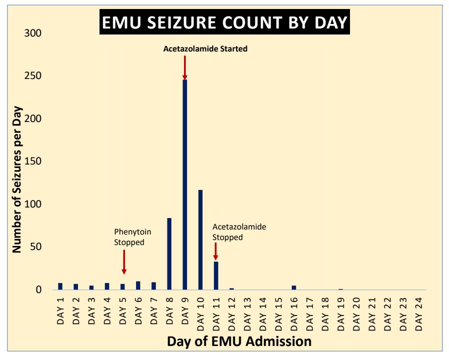
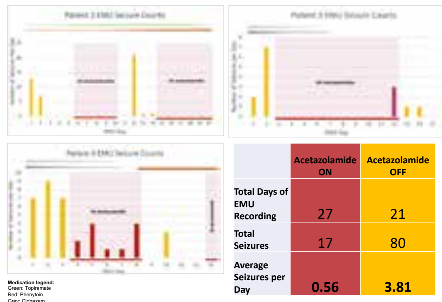


Figure 3: Patient 1 2023 EMU admission during relapse of anti-LGI1 encephalitis. Acetazolamide was stopped on day 1; phenytoin was weaned and stopped on day 5, serum level undetectable by day 7. A dramatic rise in seizure frequency (focal aware pilomotor) occurred on day 8, reaching 250 seizures a day on day 9 with a corresponding right temporal EEG status epilepticus. Acetazolamide monotherapy (500 mg bid) was started with an evening dose on day 9 and continued for a total of 5 doses with near complete resolution of seizures over 2 days, the response unexpectedly sustained.

Patients 2-4



Medication legend:
Green: Topiramate
Red: Phenytoin
Grey: Clobazam
Blue: Levetiracetam

	Acetazolamide ON	Acetazolamide OFF
Total Days of EMU Recording	27	21
Total Seizures	17	80
Average Seizures per Day	0.56	3.81

Figure 4: Patients 2-4 EMU daily seizure counts. Patient 2 cycled acetazolamide while on escalating doses of phenytoin and topiramate (the latter reduced during the last 6 days of admission). Patients 3 and 4 were weaned from levetiracetam and clobazam as acetazolamide was administered; patient 4 started oral phenytoin without loading after initial 5-day course of acetazolamide. The average seizure count on acetazolamide ON days was 0.56, and OFF was 3.81 (P=0.004).

Patients 5 and 6

	Pilomotor seizure frequency before acetazolamide	Pilomotor seizure frequency after acetazolamide
Patient 5	3-5 daily	2 weekly
Patient 6	15-20 daily	None

Discussion

- 5/6 patients had hyperventilation induced seizures, an example from patient 1 is shown in Figure 1. Studies at our institution have revealed that hyperventilation induced focal seizures are highly suggestive of an underlying AE, typically targeting the voltage-gated potassium channel (VGKC) complex [7,8]
- Hyperventilation results in systemic and cerebral hypocapnia and alkalosis, which is associated with increased epileptogenicity [4,9]
- Conversely, acetazolamide leads to cerebral hypercapnia and acidosis, and has been shown to decrease limbic epileptiform discharges and high frequency oscillations in animal models [6,10]
- Given the rarity of hyperventilation induced focal temporolimbic seizures [11,8] and their specific association with AE, seizures of this type may be more susceptible to changes in pH than in other focal epilepsies
- Prominent responses to acetazolamide have been described in a family with autosomal dominant nocturnal frontal lobe epilepsy and in patients with Glut1 deficiency epilepsy, with as yet unidentified, channel-specific mechanistic links suspected for both entities [12,13]
- 5/6 patients in this cohort were diagnosed at some point in their illness with anti-LGI1 encephalitis; the seronegative case did not have autoantibody testing performed at initial presentation 4 years earlier, and could possibly have initially had anti-LGI1 encephalitis as well
- Temporal lobe epileptogenicity in anti-LGI1 encephalitis is fairly well described and relates to reduced KV1 currents and increased excitability of dentate, CA1 and CA3 neurons [14, 15]
- Pilomotor seizures have been described in other forms of AE (anti-NMDAR and anti-GAD65) [17] and in paraneoplastic encephalitis (anti-Ma2, anti-Hu), which have mechanisms of epileptogenicity different from anti-LGI1 encephalitis [16, 17]
- It is not clear whether the pH sensitivity of our cohort is seen only in patients with anti-LGI1 encephalitis, in seizures secondary to AE or autoimmune epilepsy of any etiology, or more broadly in pilomotor/autonomic seizures of any etiology
- As most, if not all, of these patients had anti-LGI1 encephalitis, it is more likely that acetazolamide responsiveness is specific to this particular type of VGKC complex AE. Further study is required to make this determination

Conclusions

- Acetazolamide is highly effective in controlling pilomotor seizures in AE, both for acute symptomatic seizures secondary to autoimmune encephalitis, and autoimmune associated epilepsy
- A short course of acetazolamide was extraordinarily effective as rescue monotherapy in focal temporal lobe status epilepticus
- Hyperventilation sensitivity and the exquisite antiseizure effect of acetazolamide suggests that cerebral pH changes play an important role in the mechanism underlying pilomotor seizures in VGKC-type AE patients
- Acetazolamide should be considered as a mechanism-specific treatment for pilomotor seizures in AE, which can be refractory to typical antiseizure medications and immunotherapy

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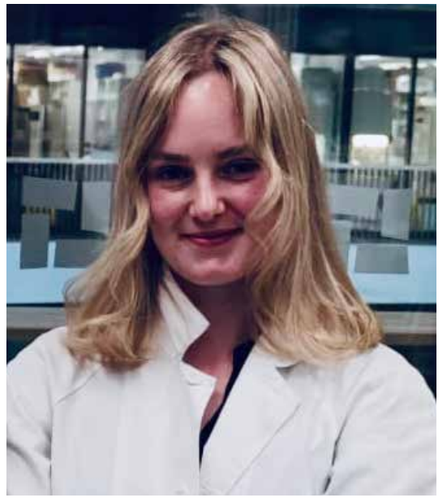

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Ms Kristine Farmen finished her bachelor's degree in molecular biology from the University of Oslo, Norway and Nottingham University, UK in 2018. Then she pursued a master's degree in molecular medicine at Aarhus University, Denmark. Here the focus of her research and thesis was the early Immune response in Parkinson´s disease. In 2021 she started her current employment as a PhD student working in the research group of Federico Iovino investigating bacterial meningoencephalitis. Her main line of research is in vivo modelling of the disease and investigating the cellular changes in the brain during brain infection and the long-term impact on neurological function.

Tempo-spatial invasion pattern of *Streptococcus pneumoniae* and dynamic changes in the cellular brain environment during pneumococcal meningoencephalitis

Kristine Farmen, Miguel Tofiño Vian, Katrin Wellfelt, Lars Olsson, Federico Iovino

Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

Relevance

Pneumococcal meningoencephalitis is a life-threatening condition. Even if the infection is successfully treated, up to 50% of survivors experience long-term neuronal sequelae, such as cognitive decline, motor impairments and hearing loss. It is therefore crucial to gain a better understanding of the pathological process to develop new therapeutic strategies.

Introduction

Streptococcus pneumoniae cause direct damage to neurons through interaction and release of virulence factors, and indirect damage due to the vast inflammatory response in the brain. Little is known about the invasion pattern of the bacteria into the brain and its correlation to pathological processes.

The aim of this project was therefore to describe 1) the spatial temporal localization of *S. pneumoniae* in the brain and 2) its consequence on cortical neuronal damage, neurogenesis, and neuroinflammation in the hippocampus.

Method

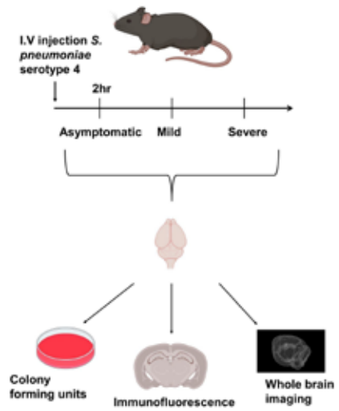

Figure 1. Summary of methodology

Results

S. pneumoniae infiltrates in a temporal but not spatial pattern

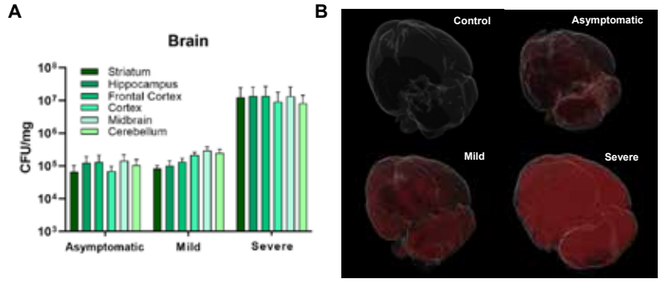

Figure 2. Spatio-temporal pneumococcal invasion of the brain. 2A) Bacterial titres in the brain quantitative assessed n=5 and 2B) Qualitative assessed through 3D whole brain imaging and light-sheet microscopy, bacterial signal in red. n=1

Invasion of *S. pneumoniae* causes microglial activation and loss of neuroblasts in the hippocampus

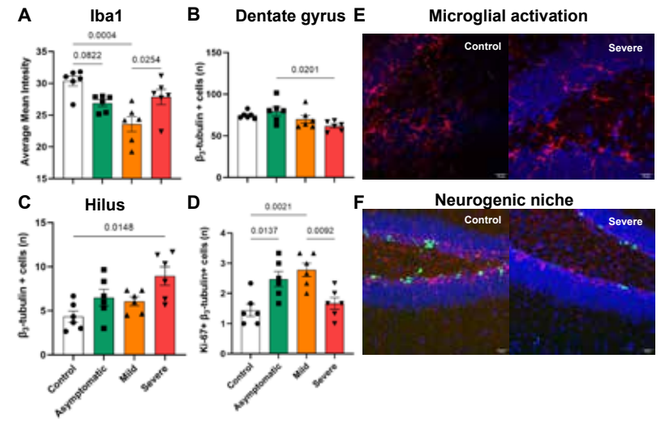

Figure 3. Microglial activation and changes in the neuroblast population upon pneumococcal brain invasion. The quantification of average mean intensity of the Iba1 staining during disease progression are shown in 3A), representative images shown in 3E) Iba1 in red, DAPI in blue. Neuroblasts (β3-tubulin) in the dentate gyrus decreased during disease 3B), increased in hilus indicating migration of this population 3C) The proliferation of neuroblast (Ki-67+ β3-tubulin+ double positive increased in early stages of disease, before decreasing in severe symptomatic mice 3D) representative images shown in 3F) β3-tubulin in red, Ki-67 in green, DAPI in blue. n=3

Conclusion

Our results indicate that invasion of *S. pneumoniae* into the brain cause loss of neuroblasts, neurons and alteration in the brain inflammatory environment which potentially leads to the onset of neurological sequelae.

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The Strategic Research Area Neuroscience (StratNeuro)

ÅHLÉN-STIFTELSEN

THERAKIND

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BERGVALLS STIFTELSE

TORE NILSONS STIFTELSE FÖR MEDICINSK FORSKNING

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Performance of the Granillo Risk Score for Autoimmune Encephalitis: A Nationwide Danish Cohort Study

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Background

Differentiating Autoimmune Encephalitis (AE) from Viral Encephalitis (VE) is often challenging and causes significant delays in treatment initiation.

The aim of this study was to investigate the performance of the clinical risk score for AE, developed by Granillo and coworkers[1], in a nationwide Danish cohort of definite AE and VE.

Materials & Methods

The Danish National Autoimmune Encephalitis Database n=90

The Study Group for Infections in the Brain Database n=529

Excluded n=242
Not fulfilling criteria for encephalitis n=101
Competing risk factors for encephalopathy n=7
Other diagnoses n=4
Missing data n=5

Definite AE cases n=90

Definite VE cases n=287

Total included n=377

The Granillo Risk Score for AE	
Score Items	**Score value** Yes = 1 point No = 0 point
Charlson comorbidity index <2	0-1
Symptom onset >6 days	0-1
Psychiatric or memory complaints	0-1
Absence of robust inflammation in CSF	0-1
Total	**0-4**

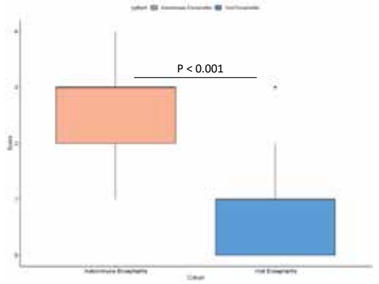
Risk of AE	Score
Low risk	0-1
Intermediate risk	2-3
High risk	4

Results

Baseline characteristics

	AE cohort n=90	VE cohort n=287	p-values
Age Median (IQR)	50 (22,67)	72 (61,80)	<0.001
Female n (%)	47 (52)	149 (52)	>0.9
Diagnoses n (%)			
Autoimmune			
NMDAR*	58 (64)	-	-
LGI1	23 (26)	-	-
GABA-B	6 (7)	-	-
AMPAR	3 (3)	-	-
Viral			
HSV1	-	126 (44)	-
VZV	-	122 (43)	-
TBE	-	20 (7)	-
HSV2	-	12 (4)	-
HIV	-	5 (2)	-
EBV	-	2 (1)	-
Characteristics n (%)			
CCI<2	50 (56)	47 (16)	0.004
Subacute onset <6 days	67 (74)	33 (11)	<0.001
Psychiatric and/or memory complaints	87 (97)	48 (17)	<0.001
Absence of robust inflammation in CSF	52 (58)	90 (31)	0.005

Overall

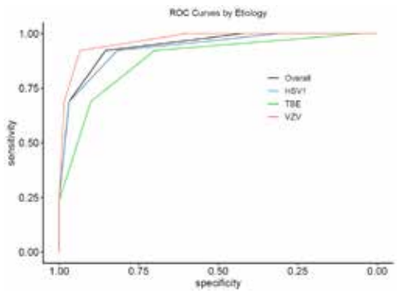


Boxplot of the score distribution in the two cohorts. Medians are marked with a bold horizontal lines. Whiskers indicate maximum and minimum values and the upper and lower borders of each boxplot represent the 75% quartile and the 25% quartile respectively. The cohorts are on the X-axis and the Granillo score value is on the Y-axis. The p-value was calculated on the medians using the χ^2-test.

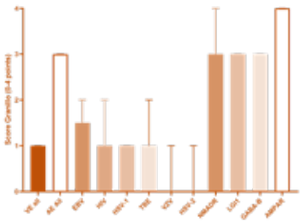
Score	Sensitivity	Specificity	PPV	NPV
0	0%	58%	0%	65%
1	8%	57%	5%	66%
2	23%	89%	39%	79%
3	46%	97%	82%	85%
4	23%	100%	100%	81%
≥3	69%	97%	87%	91%

Abbreviations: PPV ; positive predictive value ((true positives)/(true positives + false positives))*100, NPV ; negative predictive value ((true negatives)/(false negatives + true negatives))*100. Sensitivities, specificities, PPVs and NPVs are calculated separately for each score value with cases of autoimmune encephalitis (AE) as reference.

Subgroups



ROC curves for the overall comparison and three selected diagnostic subgroups in the VE cohort (VZV, HSV1 and TBE. The plot illustrates the variation in score performance by etiology.



Barplot with median score values of the AE and VE cohorts and for all diagnostic subgroups. The Granillo score is on the Y-axis and the diagnostic groups are on the X-axis. The height of the bars indicate median score values and the whiskers indicate maximum score values.

Conclusion

The risk score developed by Granillo and co-workers performed well in a Danish setting. Testing for AE should be considered with score of ≥3. We suggest implementing the score in clinical practice.

1. Granillo A et al "Development and validation of a risk score to differentiate viral and autoimmune encephalitis in adults" Clin Infect Dis. 2023

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Dr Laura Atkinson is a Senior Clinical Scientist in Molecular Microbiology at Great Ormond Street Hospital for Children, where Laura oversees the laboratory for our metagenomics pathogen detection service. This service was initially designed to identify the infectious cause of encephalitis in patients with neurological infection but has since expanded to a range of severe illness of suspected infectious cause. Most notably, their service was instrumental in the investigation into the outbreak of severe hepatitis of unknown aetiology in children in 2022.

NHS Great Ormond Street Hospital for Children NHS Foundation Trust

UCL

Translating metagenomics into clinical practice for complex paediatric neurological presentations

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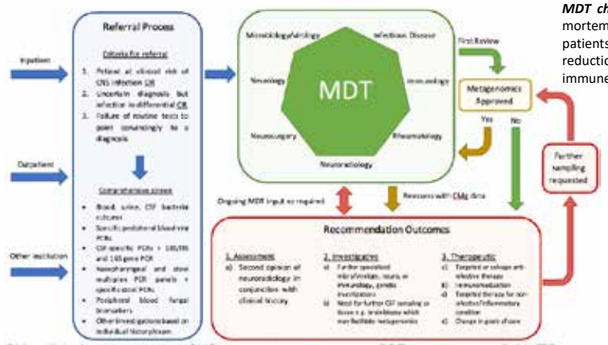
Introduction

Paediatric meningoencephalitis is a complex diagnosis with multiple infective and non-infective aetiologies[1]. Immunocompromised paediatric patients in particular are at increased risk of complex neurological infection[1]. To address this, we implemented a routine clinical metagenomics (CMg) service for children with complex neurological presentations. In parallel with the laboratory, a neuro-infection multidisciplinary team (MDT) was established to discuss the investigation and management of children with undiagnosed CNS pathology.

Here, we show the impact a CMg service integrated with a neuro-infection MDT has made on a case-series of 60 paediatric patients at a tertiary paediatric hospital in London, United Kingdom.



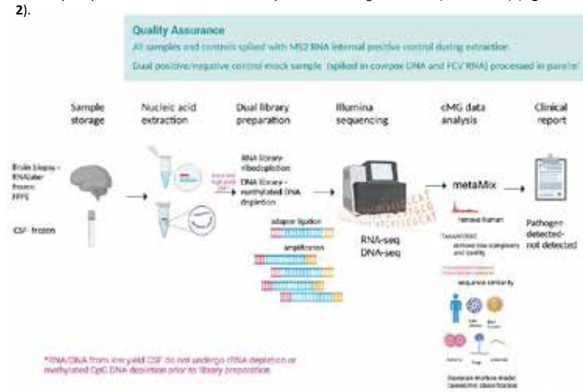
CMg: clinical metagenomics; CNS: central nervous system; CSF: cerebrospinal fluid; ITS: internal transcribed spacer; MDT: multidisciplinary team; PCR: polymerase chain reaction

Figure 1: The Great Ormond Street neuro-infection MDT clinical pathway.

Methods

Inclusion in the current study: CMg referral was decided based on the following three criteria: 1) Patient at clinical risk of CNS infection 2) Uncertain neurological diagnosis 3) Failure of routine tests to point convincingly to a diagnosis (**Figure 1**). Data were extracted from electronic medical records (EPIC®). MDT outcomes were assessed and categorised independently by two reviewers.

Metagenomics: Nucleic acid was purified from each specimen, followed by preparation of DNA and RNA libraries for sequencing[2]. DNA and RNA-seq were performed on the Illumina NextSeq. Sequence data were taxonomically classified using metaMix[3] (version 0.4) (**Figure 2**).



CSF: cerebrospinal fluid; CMg: clinical metagenomics; FFPE: formalin-fixed paraffin-embedded tissue. Figure created with BioRender.

Figure 2: Clinical metagenomics pipeline from sample collection through to clinical reporting.

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Results

Patients CMg results: Between 2014 and 2022, CMg detected a causative pathogen in 14/60 patients (23%). Rates of pathogen detection were highest for brain biopsy material compared to CSF, and microbes of unknown clinical significance were more frequent in CSF compared to brain (**Figure 3A**). 36/60 patients were immunocompromised; 12/36 were positive for a pathogen, in contrast to 2/24 immunocompetent patients (**Figure 3B**). Two pathogens were identified in brain tissue that had not previously been known to cause encephalitis: Coronavirus OC43[4] and Mumps vaccine strain[5]. In a further two patients, CMg detected pathogens which had only been detected in cases of encephalitis once before: Avian Orthoavulavirus and Astrovirus VA1/HMO-C[6,7,8]. A full breakdown of pathogens detected by CMg can be found in **Table 1**.

MDT change in management: Results for 3/60 cases referred for CMg were only available post-mortem. The MDT recommended changes to clinical management in 74% (n=42) of the remaining patients based on the CMg results. Interventions included initiation of targeted anti-infective therapy, reduction in immunosuppressive therapy due to pathogen discovery by metagenomics or increasing immune-mediated therapy due to a negative metagenomics result.

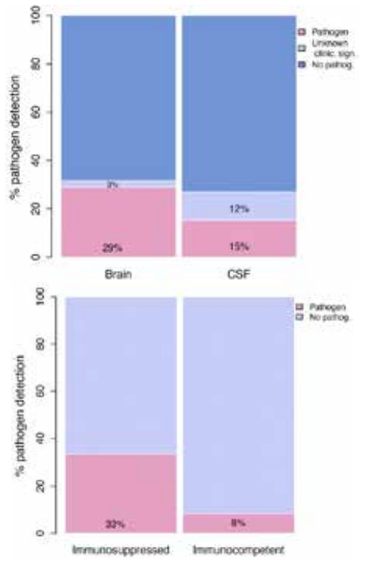

Figure 3: Pathogen detection rate by metagenomics per **(A)** clinical specimen and **(B)** patients' immune system status.

Sample	Pathogens detected	Number of cases	Pathogen read counts
Brain	Astrovirus VA1/HMO-C	2	46 (case 1) & 4,900,000 (case 10)
	Coronavirus OC43	1	997,453 (case 4)
	Mumps virus (vaccine strain)	1	77,624 (case 45)
	Aspergillus fumigatus	2	34 (Case 9) & 3135 (case 49)
	Mycobacterium Tuberculosis	1	26 (case 33)
	Avian orthoavulavirus	1	1,113,387 (case 41)
	HHV6B	1	25,122 (case 59)
	EBV	1	38,200 (case 3)
	HSV	1	2 (case 29)
CSF	Adenovirus	2	4,300,000 (case 11) & 14 (case 14)
	CMV	1	25,766 (case 39)
	HHV6B	1	10 (case 59)

Table 1: Pathogens detected by metagenomics in 14 patients and their respective read counts.

Conclusions

We present a neuro-infection MDT that has successfully integrated CMg into the diagnostic pathway for complex undiagnosed CNS presentations. Our data demonstrate that both positive and negative CMg results can be of great utility in clinical decision making particularly in immunocompromised individuals.

***** Nisha Ranganathan, Alexander Lennon, Jack C D Lee, Dimitrios Champsas, Angelika Kopec, Divya Shah, Cristina Venturini, Garth Dixon, Surjo De, James Hatcher, Kathryn Harris, Kristian Aquilina, Maaike A. Kusters, Karyn Moshal, Delane Shingadia, Austen JJ Worth, Giovanna Lucchini, Ashirwad Merve, Thomas S Jacques, Alasdair Bamford, Marios Kaliakatsos.

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Laura Khatib, MSc is a neurology resident at the Montpellier University Hospital (Montpellier, France). She focuses on the management of patients with autoimmune and inflammatory disorders of the nervous system, including multiple sclerosis and other central nervous system demyelinating diseases, central nervous system involvements in multisystem inflammatory disorders, and autoimmune encephalitis. She recently spent 18 months as a research fellow in the French National Center for Autoimmune Encephalitis and Paraneoplastic Neurological Syndromes in Lyon, France (head: Prof J Honnorat).

Brain MRI lesions in anti-NMDA receptor encephalitis: Clinical and prognostic implications

Laura Khatib, Nicolas Lundahl Ciano-Petersen, Julie Pique, Romain Marignier, François Cotton, Clarisse Carra-Dallière, Xavier Ayrignac, Pierre Labauge, Jérôme Honnorat, Bastien Joubert

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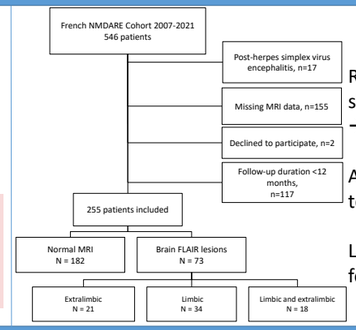
INTRODUCTION

Anti-NMDA receptor encephalitis (NMDARE) :

- Highly sterotyped neurological syndrome mostly affecting young women
- **20% are dependent** for life activities 2 years after onset
- Baseline brain MRI : unremarkable = 45%
  limbic lesions = 22%
  extralimbic lesions = 23-30%

<u>Objective</u> - To characterize NMDARE brain MRI lesions
Morphological characteristics ?
Associated clinical features?
Impact on the 2-years clinical outcomes ?

METHODS



Review of all available brain MRIs showing extralimbic lesions
→ LK , JP [blinded]

Anti-MOG / AQP4 antibody testing (166 sera)

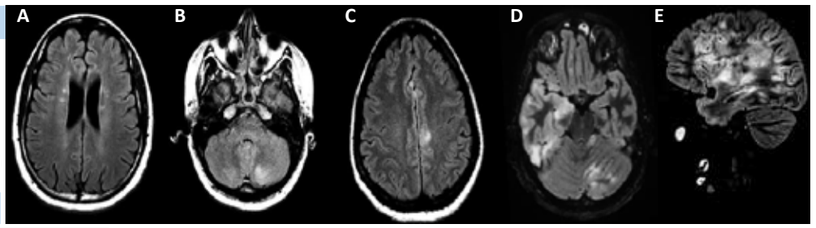
Longitudinal analysis of clinical features

RESULTS

1. Extra-limbic lesions (39/255 patients, **15.3%**)

A. Morphology

A. **MS-like** (14/39, 36%)
B. **Cerebellar** (5/39, 12.8%)
C. **Cortical (**6/39, 15.4%)
D. **Multifocal** (14/39, 36%)
E. ≥2 lobes (**multifocal/extensive,** 4/14, 29%)



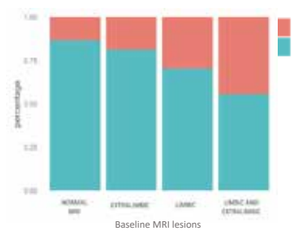
B. Associated clinical features

Patients	Controls N= 216	extralimbic lesions N = 39	p-value
Male, n (%)	42 (19.4)	12 (30.8)	0.168
Age, mean (SD)	22.08 (13.51)	26.31 (19.16)	0.095
Tumor, n (%)	63/208 (30.3)	6/36 (16.7)	0.140
Non-typical NMDARE symptoms, n (%)	22 (10.2)	23 (59.0)	**<0.001**
Cranial nerve palsies	10 (4.6)	8 (20.5)	**<0.001**
Oculomotor disorders	3/214 (1.4)	5 (12.8)	**0.001**
Cerebellar ataxia	14 (6.5)	16 (41.0)	**<0.001**
Sensorimotor impairment	19 (8.8)	16 (41)	**0.001**
Myelitis	1 (0.5)	5 (12.8)	**<0.001**
Typical symptoms, n (%)	216 (100.0)	39 (100.0)	1.000
Behavioral disorder	208 (96.3)	37 (94.9)	1.000
Cognitive impairments	199 (92.1)	37 (94.9)	0.788
Seizures	186 (86.1)	27 (69.2)	**0.017**
Dizorganisation of speech	152 (70.4)	24 (61.5)	0.363
Hyperkinetic movement disorders	169 (78.2)	25 (64.1)	0.089
Dysautonomia	113/215 (52.6)	16 (41.0)	0.250
Sleep disorder	82 (38.0)	11 (28.2)	0.325
Impaired consciousness	136/215 (63.3)	23 (59.0)	0.743
Limbic MRI lesions, n (%)	37/216 (17.1)	18 (46.2)	**<0.001**
AQP4/MOG antibodies, n (%)	0/166	5/39 (15.3%)	**<0.001**
Second-line treatments, n (%)	174 (86.6)	28 (71.8)	**0.038**

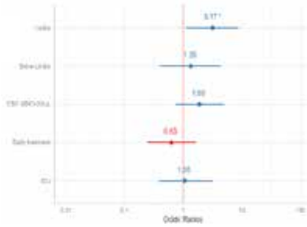
C. Overlap with demyelinating disorders

Criteria for demyelinating disorders (MS, MOGAD, NMOSD)	**Fulfilled** 10 patients	MS (n=4), MOGAD (n=4), NMOSD (n=2) **Non-typical symptoms** : 9/9 (100%) **Clinical episodes separated from NMDARE** in 5 patients (MS, 3; MOGAD, 1; NMOSD, 1) **Extralimbic lesions**: MS-like (n=7) or multifocal extensive (n=3) **Concomitant limbic lesions** : 5/10 (50%) patients **2-years mRS>2**: 3/8 (37.5%) patients
	Not fulfilled 29 patients	**Non-typical symptoms** (51.7%), **all simultaneous to NMDARE** onset or relapse **Extralimbic lesions**: MS-like (n=7), cerebellar/cortical (n=11), multifocal (n=11, 1 extensive) **Concomitant limbic lesions** : 13/29 (44.8%) patients **2-years mRS>2**: 8/26 (30.8%) patients

2. Clinical outcomes 2 years after disease onset



A. Depending on MRI findings at baseline
linear trend (chi-square): p<0.001



B. Multivariate analysis : effect of baseline parameters

CONCLUSIONS

Limbic and extralimbic lesions often coexist in NMDARE. **Extralimbic lesions** associate with **higher frequencies of non-typical symptoms,** but do not always correspond to overlapping demyelinating disorders. While only limbic lesions independently associate with poor outcomes, a cumulative effect of limbic and extralimbic lesions is likely, suggesting that **patients with abnormal baseline MRI may need more aggressive treatment**.

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Titulaer MJ, Höftberger R, Iizuka T, Leypoldt F, McCracken L, Cellucci T, Benson LA, Shu H, Irioka T, Hirano M, Singh G, Cobo Calvo A, Kaida K, Morales PS, Wirtz PW, Yamamoto T, Reindl M, Rosenfeld MR, Graus F, Saiz A, Dalmau J. Overlapping demyelinating syndromes and anti–N-methyl-D-aspartate receptor encephalitis. Ann Neurol. 2014 Mar;75(3):411-28. doi: 10.1002/ana.24117. PMID: 24700511; PMCID: PMC4016175.

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Loana Penner was born in the South of Germany, graduated from High School in the State of New York and obtained a bachelor's degree in biomedical engineering before enrolling in medicine at Ulm University. In 2019 she started her doctoral thesis project in the group of Jan Lewerenz, for which she organized the recruitment of the patients with NMDA receptor encephalitis and Multiple Sclerosis overlap syndromes as well as the controls with NMDAR receptor encephalitis only from the patients enrolled at the various study sites in the registry of the German Network for Research on Autoimmune Encephalitis (GENERATE) in 2019 as well as collection of the biosamples. Within her doctoral, she analyzed the cerebrospinal fluid data that are described in this abstract, while in imaging characteristics are analyzed in a parallel project at the Charité in Berlin (see Abstract Kuchling et al.). Loana Penner will graduate from medical school in November. She wants to start her training as a paediatrician in 2024.

NMDA Receptor Encephalitis and Multiple Sclerosis Overlap Syndrome - Cerebrospinal Fluid Characteristics and Genetic Findings in Comparison to Patients with NMDA Receptor Encephalitis

Loana Penner[1], Anja Tietz[2], Joseph Kuchling[3], Leonie Maier[3], Daniel Rapp[1], Catharina Gross[4], Stjepana Kovac[4], Kurt-Wolfram Sühs[5], Felix Konen[5], Philipp Schwenkenbecher[5], Dominic Bertram[6], Thomas Korn[6], Christian Geis[7], Frank Leypoldt[8], Klaus-Peter Wandinger[8], Klemens Angstwurm[9], Jonathan Wickel[7], Anna Katharina Mundorf[10], Nico Melzer[10], Amei Ludwig[10], Marie Madlener[11], Katrin Doppler[12], Franziska Thaler[13], Marie Süße[14], Brigitte Wildemann[15], Christian Bien[16], Tania Kümpfel[13], Harald Prüss[3], Jens Dreyhaupt[17], Carsten Finke[3], Gregor Kuhlenbäumer[2], Jan Lewerenz[1], on behalf of the German Network for Research on Autoimmune Encephalitis (GENERATE).

[1]University Hospital Ulm, [2]University Hospital Schleswig-Holstein, [3]Charité - University Medicine Berlin, [4]University Hospital Münster, [5]Hannover Medical School, [6]Technical University Munich, [7]University Hospital Jena, [8]University Hospital Schleswig-Holstein and Kiel University, [9]University Hospital Regensburg, [10]Heinrich Heine University Duesseldorf, [11]University of Cologne, Faculty of Medicine and University Hospital, [12]University Hospital Würzburg, [13]Ludwig-Maximilians University Munich, [14]University Hospital Greifswald, [15]University Hospital Heidelberg, [16]Krankenhaus Mara and University Medicine Oldenburg, [17]Ulm University

Introduction:
NMDA receptor encephalitis (NMDAR-E) is a subacute non-chronic antibody-mediated disease of the brain, where antibodies against the NMDA receptor induce severe but transient neuronal dysfunction. Overlaps (OV) of NMDAR-E with demyelinating diseases, neuromyelitis optica spectrum disorders (NMOSD) and MOG antibody-associated diseases (MOGAD), have been described (1). However, detailed cerebrospinal fluid (CSF) findings in NMDAR-E closely resemble those of patients with multiple sclerosis (MS)(2). We investigated the frequency of MS/NMDAR-E-OV patients among NMDAR-E enrolled in the GENERATE registry and characterized the neurochemical and genetic characteristics of these patients. Clinical and imaging characteristics were analyzed in a parallel project (talk by Kuchling et al.).

Methods:
Patients with definite NMDAR-E (Ctrl-NMDAR-E) and NMDAR-E with concomitant MS (NMDAR-E/MS-OV) were identified in the GENERATE registry. Serum samples of NMDAR/MS-OV patients were tested for Aquaporin 4 (AQ4) and MOG antibodies. For analysis of CSF data propensity matching with a 1:3 ratio (NMDARE-MS/NMDARE) was performed and a sex- and age matched MS control cohort generated. Detailed CSF findings including cell count, CSF/serum ratios (Q) for albumin (Alb), IgG, IgA and IgM, oligoclonal bands (OCB) and MRZ reaction were obtained from the database. Q_{Alb}, Q_{IgG}, Q_{IgA} and Q_{IgM} were converted to z scores using a reference cohort of 17,811 non-inflammatory CSF results. NMDAR-E/MS-OV CSF findings were compared to Ctrl-NMDAR-E following propensity score matching according to age and disease duration. The polygenic risk score PGS001831 (http://www.pgscatalog.org/) for multiple sclerosis (MS-PGRS) was calculated from Infinium Global Screening Array (GSA; Illumina) data in comparison to 1,203 control DNAs.

Results

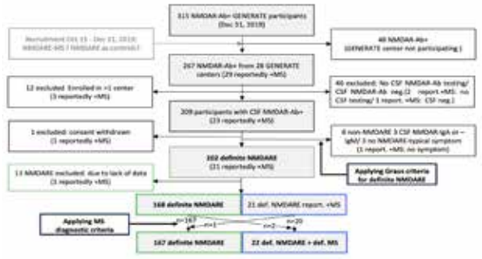

Figure 1: Flow chard of the study recruitment. Ab=Antibody, MS=Multiple Sclerosis, def. = definite

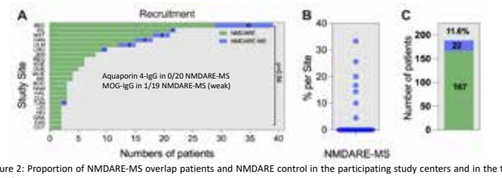

Figure 2: Proportion of NMDARE-MS overlap patients and NMDARE control in the participating study centers and in the total cohort

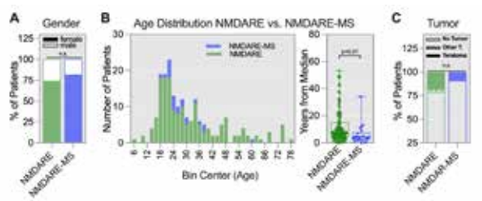

Figure 3: Demographic characteristics and tumor frequency in NMDARE and NMDARE-MS patients

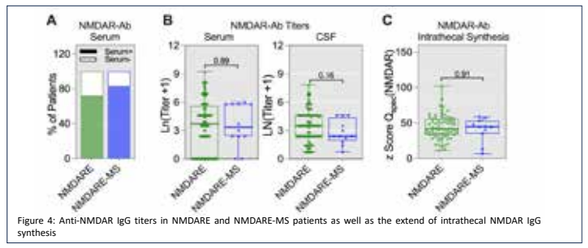

Figure 4: Anti-NMDAR IgG titers in NMDARE and NMDARE-MS patients as well as the extend of intrathecal NMDAR IgG synthesis

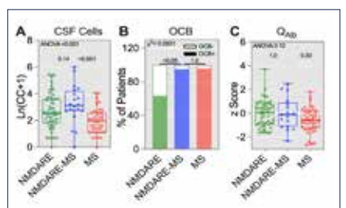

Figure 5: CSF Leukocyte count, presence of oligoclonal IgG as well as blood-CSF barrier function in NMDARE, NMDARE-MS and a cohort of 44 MS patients matched by gender and age to the NMDARE-MS cohort

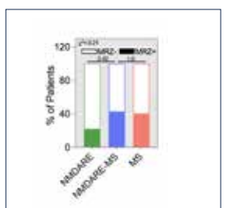

Figure 6: Frequency of a positive MRZ reaction in NMDARE, NMDARE-MS patients and the matched MS cohort

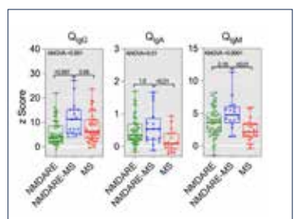

Figure 7: NMDAR-MS patients share high IgG synthesis with MS patients but high intrathecal IgM synthesis with NMDARE patients

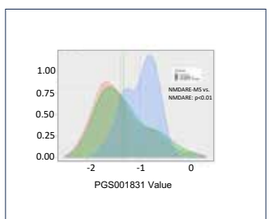

Figure 8: A polygenic risk score for MS is higher in NMDARE-MS compared to NMDARE and controls

Conclusion

MS is frequent demyelinating disease in NMDAR-E patients. Anti-NMDAR IgG titers as well as intrathecal synthesis of anti-NMDAR IgG in NMDAR-MS as well as CSF pleocytosis are indistinguishable from patients with NMDARE only. As in MS, presence of oligoclonal IgG occurs more often in NMDAR-MS compared to NMADARE only. In addition, NMDARE with concomitant MS is characterized by more pronounced intrathecal IgG synthesis, while it shares more robust intrathecal IgM synthesis with NMDARE. Patients with NMDARE-MS show a higher MS-PGRS than those with NMDARE only. Possibly, the chronic neuroinflammation in MS is a risk factor for the development of NMDAR-E.

References:
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CIELO: A Randomised, Double-blind, Placebo-controlled, Phase 3 Basket Study of Satralizumab in Patients with NMDAR- or LGI1-antibody Encephalitis

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Introduction and objectives

- Autoimmune encephalitis (AIE) is a group of rare, severe, antibody-mediated neurological diseases characterised by prominent neuropsychiatric symptoms[1,2]
- The most common subtypes of AIE are those with antibodies targeting the N-methyl-D-aspartic acid receptor (NMDAR) or leucine-rich glioma-inactivated 1 (LGI1)[2]
- There are currently no approved treatments for AIE, and evidence-based treatments that reduce long-term cognitive and physical disability, as well as persistent seizures and disabling symptomology, are needed[3,4]
  - A recent randomised trial suggested the benefit of intravenous immunoglobulin (IVIG) in reducing seizure frequency in certain AIE subtypes[5]
- People with AIE have elevated levels of the multifunctional cytokine interleukin-6 (IL-6)[6]
- Processes regulated by IL-6 signalling, such as B- and T-cell differentiation, B-cell proliferation, survival and functioning of autoantibody producing plasma cells, and blood−brain barrier regulation, are thought to have a role in AIE pathogenesis[6–10]
- Anecdotal reports of IL-6 receptor (IL-6R) inhibition in AIE have described clinical benefits; hence, IL-6R is a therapeutic target of interest[11]
- Satralizumab is a humanised, monoclonal recycling antibody that targets the soluble and membrane-bound forms of the IL-6R, blocking IL-6 signalling[12]
- CIELO (NCT05503264) is the first study of satralizumab in patients with AIE

Methods

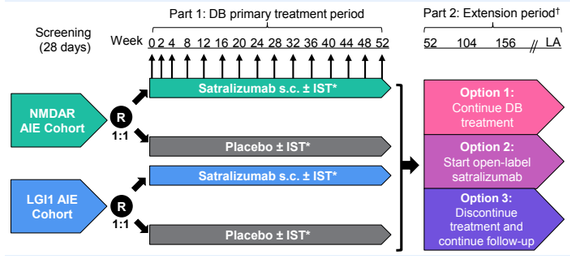
- CIELO will enrol ~102 patients aged ≥12 years with a diagnosis of probable or definite NMDAR AIE and ~50 patients aged ≥18 years with LGI1 AIE who have:
  - Onset of AIE symptoms ≤9 months prior to randomisation
  - Modified Rankin Scale (mRS) score ≥2 at randomisation
- Patients will be stratified as "new onset" or "incomplete responder" (**Table 1**)
- CIELO (**Figure 1**) includes a 52-week double-blind primary treatment period (Part 1), followed by an optional extension period (Part 2)
- In Part 2, participants can either continue double-blind treatment, receive open-label satralizumab, or discontinue treatment and continue follow-up assessments

Table 1. Definitions of "New onset" and "Incomplete responder" for inclusion criteria

	New onset	Incomplete responder
Acute first-line therapy	≤6 weeks before randomisation	>6 weeks before randomisation
Prior treatment	No immunotherapy additional to acute first-line therapy	Treatment with other immunotherapy in addition to acute first-line therapy*

*RTX initiated ≥2 months before screening (last dose ≥4 weeks before randomisation), IST treatment ≥2 months before screening, OCS, or repeated pulse therapy; patients should have no improvement in mRS score within 4 weeks before randomisation with prior immunotherapy, and patients who have received repeated courses of acute first-line therapy must have completed treatment ≥2 weeks before randomisation. IST, immunosuppressive therapy; mRS, modified Rankin Scale; OCS, oral corticosteroids; RTX, rituximab.

Figure 1. CIELO study design summary



†Treatment administered. *Incomplete responders may continue to receive the following background IST treatments: AZA, MMF, and intravenous cyclophosphamide. Patients may receive baseline OCS, which must be tapered from Week 4. All patients are permitted to receive symptomatic AIE medications. ‡The extension period lasts ~2 years from when the last patient enters the extension period. AIE, autoimmune encephalitis; AZA, azathioprine; DB, double-blind; IST, immunosuppressive therapy; LA, last administration; LGI1, leucine-rich glioma-inactivated 1; MMF, mycophenolate mofetil; NMDAR, N-methyl-D-aspartic acid receptor; OCS, oral corticosteroids; R, randomised; s.c., subcutaneous.

Study endpoints

Primary endpoint

- Proportion of patients with a mRS score improvement ≥1 from baseline without the use of rescue therapy at Week 24

Secondary endpoints

Not in hierarchical order, and will be tailored to the individual cohort

- Time to mRS score improvement ≥1 from baseline without the use of rescue therapy
- Time to rescue therapy
- Time to seizure freedom or cessation of status epilepticus without the use of rescue therapy
- Change in Clinical Assessment Scale of Encephalitis (CASE) score from baseline at Week 24
- Montreal Cognitive Assessment (MOCA) total score at Week 24
- Rey Auditory Verbal Learning Test (RAVLT) score for LGI1 AIE cohort at Week 24
- mRS score for NMDAR AIE cohort at Week 24, as measured on a 7-point scale

Safety

- Incidence, seriousness, and severity of adverse events
- Change from baseline in targeted vital signs, electrocardiogram parameters, and clinical laboratory test results

Pharmacokinetics and Pharmacodynamics

- Serum IL-6 and soluble IL-6R
- Serum and/or cerebrospinal fluid concentrations of satralizumab

Exploratory endpoints

- Degree of disability, clinical severity, mood, quality of life, and functional living
- Additional exploratory biomarker assessments including longitudinal assessments

Conclusions

- Randomised evidence to guide treatment decisions is urgently required in AIE
- CIELO will assess the efficacy, safety, pharmacokinetics, and pharmacodynamics of IL-6 inhibition with satralizumab in patients with NMDAR AIE and LGI1 AIE
- CIELO will recruit participants from approximately 85 sites across 16 countries, with 22 sites across European countries including Austria, Czech Republic, France, Italy, the Netherlands, Poland, and Denmark

To find recruiting sites near you, scan this QR code

Miss Lottie Rayburn
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References

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Dr Manoj Upadhya has been working as a Postdoctoral Research Associate since 2020 on autoimmune encephalitis (AE) with Dr Sukhvir Wright at Aston University, Birmingham, UK. The work has progressed very well over recent years, leading to significant publications in the field and Manoj has been an integral part of that success, performing neurosurgery and making in vivo and in vitro recordings from the hippocampi of rats infused with monoclonal antibodies from children with anti-NMDA receptor, anti-GABAA receptor and anti-LGi1 encephalitis. Manoj finished his Ph.D. program from the Department of Pharmaceutical Sciences of the Nagpur University, India and moved to industry as Chief Executive Scientist and was soon appointed as Director for treatment for Parkinson's disease Program. After successful completion of this challenge Manoj moved back to academia and eventually moved to Aston University. Manoj's excellent academic record speaks for itself with 26 publications in his name as first author and co-author, 638 citations and a h-index of 16, as well as holding a molecular patent for the treatment of Parkinson's disease with Sunpharma Advanced Research Company Ltd., and molecule is currently in phase 2 clinical trials and is expected to be in phase 3 trials.

Effects of neurosteroid Pregnenolone sulphate in a NMDA receptor antibody-mediated seizure rat model

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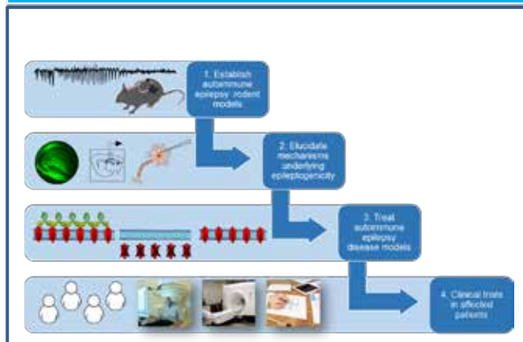
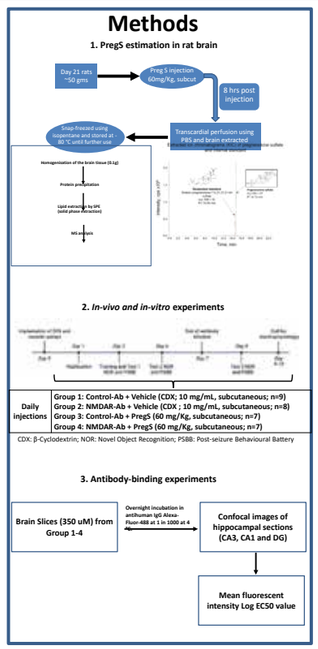


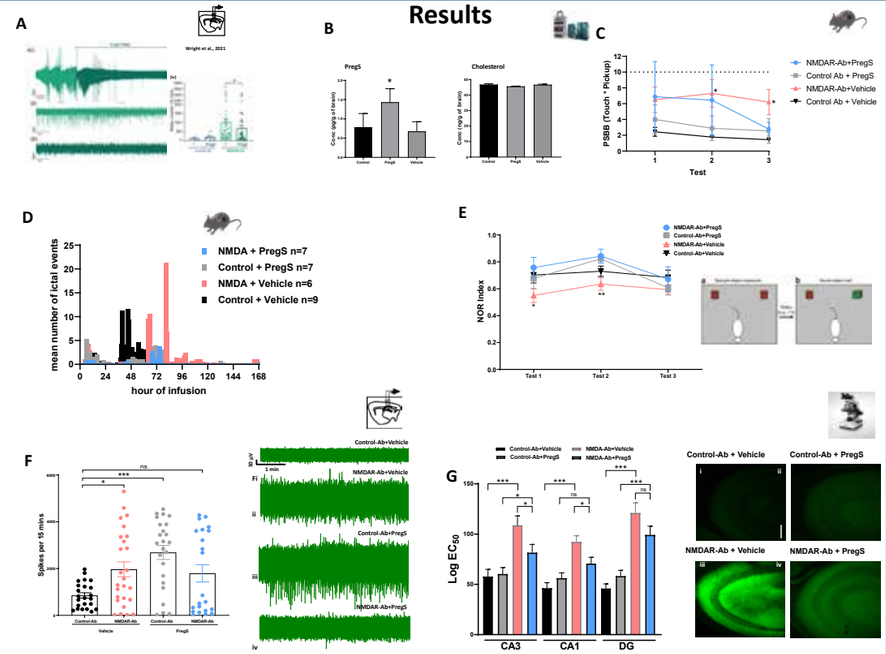
Background

N-methyl-D-aspartate receptor antibody (NMDAR-Ab) mediated encephalitis is a neuro-immunological disorder that presents with seizures. NMDAR-Abs cause internalisation of NMDARs while Pregnenolone sulfate (PregS) a neurosteroid upregulates NMDARs in the brain. Our previous in vitro studies have shown that PregS can reduce established ictal activity caused by NMDAR-Abs (Wright et al., 2021). Herein, we aimed to determine baseline brain PregS levels in vivo and following subcutaneous PregS injections using an in-house modified Liquid chromatography-Mass spectrometry (LC-MS) method, and in vivo effects of PregS injections on ictal activity and behaviour in our NMDAR-Ab-mediated seizure rodent model.

Methods



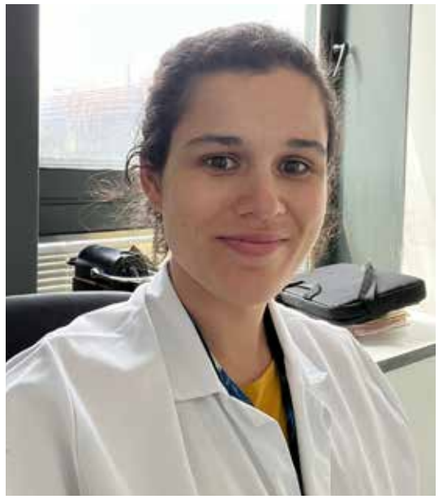
Results



Local field potential (LFP) discharges in vitro before and after addition of PregS to brain slices from animals treated with NMDAR antibody for 7 days **(ai)**. The number of interictal/spike events in NMDAR antibody treated slices was **significantly reduced** after application of PregS 100 μM **(aiv)**. Scale bar 10μV vs. 5 mins. Subpanels **(aii)** and **(aiii)** represent the magnification of pre- and post-PregS 100 μM trace area, respectively. Scale bars 50 μV vs. 5 s (Wright et al., 2021) **(A)**. **Increased endogenous PregS levels** observed following subcutaneous injection in rats *P<0.05 vs Control/Vehicle (**B**). Graph representing the Post-seizure behavioural battery (PSBB) scores observed over the period of 9 days. Scores >10 show represents hyperexcitability associated with spontaneous seizures. NMDAR-Ab+Vehicle group was the most hyperactive as it showed higher PSBB scores as compared to control+Vehicle (P<0.05) **(C)**. Graph illustrating mean number of ictal events over time in all experimental groups. NMDA-Ab+PregS group **showed evolution of ictal activity** compared to NMDAR-Ab+Vehicle **(D)**. Novel object recognition (NOR) index calculated over the duration of infusion on days 2, 6 and 9 (Test1, 2 and 3) and data is depicted in mean ± SEM. There was **significant increase and restoration of NOR index** in the NMDAR-Ab+PregS group as compared to NMDAR-Ab+Vehicle group on Days 2 and 6 **(E)**. LFP recordings from Control-Ab and NMDAR-Ab infused rats treated with Vehicle only or PregS over the period of 9 days post-mortem brain slices. NMDAR-Ab+Vehicle (n=24) treated brain slices showed an **increase in spontaneous ictal-like spike activity in the hippocampus (CA3 and CA1) region** as compared to control-Ab+Vehicle slices (n=23) (P=0.04). However, the brain slices from the NMDAR-Ab+PregS (n=22) was not significant compared to that of the Control-Ab+Vehicle slices (n=23) **(F)**. Fluoroscence intensity of confocal images (i-iv) of hippocampus from brain slice of NMDAR-Ab infused rats **shows typical staining pattern with secondary anti-human IgG** (green) as compared to control-Ab. There was significant binding of NMDAR-Ab to the brain regions as compared to control-Ab across the groups (i-iv)**(G)**.

Discussion

NMDAR antibodies disrupt normal brain circuit function and are associated with a range of neurological symptoms in patients, including epileptic seizures. Most of these symptoms are now understood to be directly caused by antibody-induced hypofunction of the excitatory NMDARs. PregS, a sulfated ester of pregnenolone, is a positive NMDAR modulator that increases surface expression of functional NMDARs containing the NMDAR subunit subtype 2A (NR2A) or subtype 2B (NR2B). Increased levels of PregS proves the used LC-MS method to be sensitive and accurate to identify endogenous brain neurosteroid levels. The present work included in vivo treatment with PregS in the NMDAR-Ab mediated seizure rat models. Increased ictal events/spikes count in hippocampus region depicts the seizure like activity and decreased NOR index reflects the compromised cognition in the vehicle treated NMDAR-Ab infused rats, however the daily injection of PregS seems to alleviate the NMDAR-Ab induced symptoms. Further the increased fluorescence intensity in the brain regions confirm the region-specific binding of the antibody. The reduction in the ictal event or restoration of cognitive functions in the PregS injected rats might be attributed to the increased expression of NMDAR subunits. Molecular studies are underway for quantification of NMDARs and associated synaptic proteins to affirm the effect of PregS in NMDAR-Ab induced encephalitis model. **Reference** Wright et al. Communication Biology 2021; 4(1):1106.



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Marie Rafiq is a young neurologist working in the Cognitive Neurology Department at the Toulouse University Hospital. She works particularly on autoimmune encephalitis and cognitive disorders affecting young people. Her areas of interest are biomarkers of cognitive pathologies and the links between neuroinflammation and neurodegeneration.

Anti-IgLON5 encephalitis is associated with anti-retinal reactivity without retinal functional alteration

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Introduction

Anti-IgLON5 encephalitis is a recently defined autoimmune disorder of the nervous system associated with autoantibodies against IgLON5. This progressive disorder combining autoimmunization with neurodegeneration, may have very heterogenous manifestations. The broad clinical spectrum consists of sleep disorder, bulbar symptoms and gait abnormality followed by cognitive dysfunction.

IgLON5 is a cell adhesion protein whose role is not fully understood. In humans, it is mainly expressed in the brain and testis. IgLON5 transcripts are also found in the retina. However, retinal involvement is not classically described in anti-IgLON5 autoimmune encephalitis (AIE).

The aim of this work is to investigate the immunological specificities of anti-IgLON5 antibodies on the retina and compare this with possible functional alterations in affected patients.

Methods

- **Inclusion of patients seen at the Toulouse University Hospital between 2019 and 2023** with clinical symptoms suggestive of anti-IgLON5 AIE and the presence of anti-IgLON5 antibodies confirmed by cell based assay (Euroimmun©) in serum or CSF.
- **Anti-retinal antibodies were detected by an indirect immunofluorescence (IFI) method on monkey retina slices in patient's serum and CSF.**
- **Ophthalmological investigations including :**
  - Macular OCT (optical coherence tomography)
  - Papillary OCT with measurement of peripapillary nerve fiber layer thickness (RNFL)
  - Pattern-ERG
  - Global electroretinogram (global ERG)

Results

1**) 6 patients with AIE with anti-IgLON5 antibodies were included**. Their clinical characteristics are summarized in **Table 1**.

Patient	Sex	Age	Age at onset	Presentation	Time to diagnosis (years)	Disease duration (years)	Onset	History of cancer	Abnormal ocular movements	Brain MRI	CSF analysis	Treatment	Outcome
Patient 1 Bu.M.	M	74	68	Sleep disorder, gait instability	4	6	Chronic	Yes[1]	No	Non specific change	Normal	IgIV, Rtx, CP	No change
Patient 2 L. N.	F	72	67	Gait instability	2	5	Chronic	No	Horizontal-torsional nystagmus	Mild cerebellar atrophy	Normal	IgIV, Rtx, CP	No change
Patient 3 C. F.	F	72	68	Cognitive complaints, gait instability and abdormal movements	2	4	Chronic	No	No	Non specific change	Elevated tau and phospho-tau and decreased Abeta 1-42	IgIV, Rtx	Progressive cognitive decline
Patient 4 Bi.M.	M	81	79	Cognitive complaints, gait instability and abnormal movements	1	1	Subacute	No	No	Non specific change	High protein level	IgIV, Rtx, Plex, CP	Mild Worsening
Patient 5 G.L.	F	71	68	Bulbar symptoms, gait instability, abnormal movements	2	3	Subacute	No	Complex oculomotor palsy and bilateral ptosis	Non specific change	Normal	IgIV, Rtx, Plex, CP	Death (respiratory failure)
Patient 6 M.J.	M	63	61	Abnormal ocular movements and headaches	1	2	Subacute	No	Vertical gaze palsy and right ptosis	Non specific change	Pleocytosis (20 cells) and high protein level	Rtx, CP	Subtle improvement

Table 1: Patient characteristics.

IVIg: intravenous immunoglobulin

Rtix: rituximab

CP: cyclophosphamide

Plex: plasma exchange

[1]melanoma surgically removed in 1992

2) Immunological analysis of anti-retinal antibodies revealed **granulous fixation in the inner grain layer of the retina** in all 5 patients tested (Fig 1).

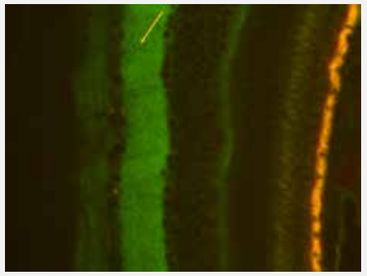

Figure 1: Indirect immunofluorescence of patient serum on monkey retina.
Arrow: inner layer of retinal grains

3) 5 of the 6 patients had neuro-ophthalmological disorders not specific to retinal damage, ranging from diplopia to reduced visual acuity. However, **no common morphological or electrophysiological ophthalmological findings could be identified (Table 2).**

Patient	Macular OCT	Papillary OCT	Pattern-ERG	Global electroretinogram
Patient 1 Bu.M.	Normal foveolar profile on the left and macular dysversion on the right, Macular epiretinal membrane	Severe global optic atrophy on the left and uninterpretable on the right (papillary dysversion)	Unreliable on the right, within the standards of the centre on the left	Normal
Patient 2 L. N.	Normal foveal profile	No RNFL measurement possible	Reduced N95 wave on the right, within the centre norms on the left	Normal
Patient 3 C. F.	Normal foveal profile	Normal	In the centre norms on the right, reduction in P50 and N95 waves on the left	Normal
Patient 4 Bi.M.	Normal foveal profile	Normal	In the standards of the centre on the right, decrease wave N95 on the left	Normal
Patient 5 G.L.	Macular drusen on the right; pigment epithelial detachment and retinal serous detachment on the left	Moderate overall optic atrophy	Impossible to interpret, poor fixation	Normal
Patient 6 M.J.	Normal foveal profile	Normal	P50 wave reduction on the right, within the standards of the centre on the left	Normal

Table 2: Results of neuro-ophthalmological investigations. *RNFL:peripapillary retinal nerve fibers*

Discussion/Conclusion

This study found **strong binding of anti-IgLON5 to the inner grain layer of the retina** using an IFI method, **suggesting a previously undescribed protein expression of IgLON5.** This **finding was not associated with anatomical or functional retinal damage in any of the patients.** It does, however, provide an opportunity to discuss the pathophysiological mechanism of anti-IgLON5 AIE, and in particular the spreading patterns of the antibody in humans.

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Maron Mantwill has studied philosophy / psychology (B.A). and a master at the Berlin School of Mind and Brain called “Mind and Brain“ (M.Sc.). He is currently doing his thesis at Charité-Universitätsmedizin Berlin with a focus on single-subject analysis of the functional connectome and machine-learning based prediction of neurological diseases (mostly multiple sclerosis and NMOSD). At the same time, he is also working on (long-term) functional connectivity in autoimmune encephalitis, and soon will start a project that combines virtual brain modelling, human patient data and animal models of anti-NMDA-Receptor encephalitis to predict disease course. Generally, he is interested in Functional connectivity, its association with behavior and clinical variability as well as machine-learning based single-subject analysis / prediction (of behavior and disease).

Long-term Hippocampal Connectivity Changes in Anti-NMDAR Encephalitis

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Introduction

Anti-NMDAR encephalitis has been associated with decreased connectivity between the hippocampus, default-mode network and medial prefrontal regions.

The longitudinal development of functional connectivity changes is not well understood.

Finke et al. 2013, Ann Neurol, Peer et al. 2017, Lancet Psychiatry

Methods

- ✓ Resting-state fMRI: two protocol with different parameters
- ✓ BIDS-based processing using fmriprep, aCompCor, ComBat for harmonization
- ✓ Hippocampus-seed based functional connectivity using nilearn

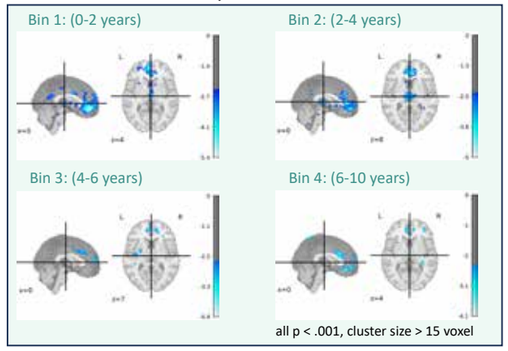
Sample

N = 93 (up to 4 follow-ups; up to 10 years)
Healthy control groups matched bin-wise for age and sex

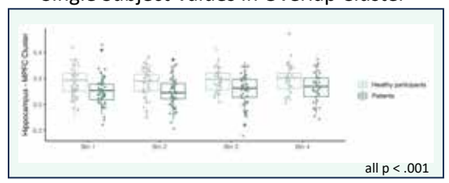
Bin 1 (0-2 years)	**Bin 2** (2-4 years)	**Bin 3** (4-6 years)	**Bin 4** (6-10 years)
51 (42 f)	50 (41 f)	42 (37 f)	36 (32 f)

Results

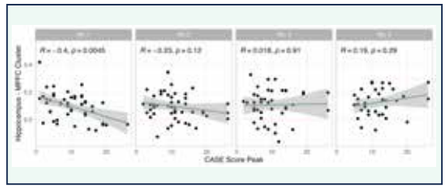
Group Differences



Single Subject Values in Overlap Cluster



Clinical Correlation



Conclusion

Hippocampus-based functional connectivity **changes persist over 10 years**.

While functional connectivity differences persist, **cluster sizes decrease** continuously along analysis bins.

Clinical parameters are initially significantly **negatively associated** with functional connectivity.

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Mr Matias Ryding is a cell biologist who focuses on utilizing human induced pluripotent stems to study disease mechanisms. In his early career his main focus was on familial types of Parkinson's disease, but since 2020 his research has been centred around autoimmune encephalitis. He has a master's degree in the field of biomedicine from the University of Southern Denmark, and he will soon hand in his PhD thesis on disease mechanisms of different subtypes of autoimmune encephalitis. He is part of Odense Autoimmune Encephalitis Research group which focuses solely on autoimmune encephalitis and how to improve diagnosis, treatment, and our understanding of disease mechanisms.

ODENSE AUTOIMMUNE ENCEPHALITIS RESEARCH GROUP

IgLON5 disease: A novel treatment target discovered through large scale proteomics

SDU University of Southern Denmark

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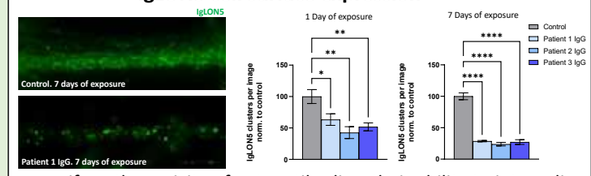
BACKGROUND

IgLON5 disease is a rare subtype of autoimmune encephalitis (AE), and the only type of AE that results in extensive neurodegeneration. The outcome of this type of AE is particularly poor and approximately one third of all cases have ended in death within two years of disease onset. Patients develop autoantibodies against the extracellular protein IgLON5 which is suspected to be involved in synapse formation. Antibody binding results in internalisation of IgLON5 which leads to hyperphosphorylation of tau and neuronal cell death. Deposits of hyperphosphorylated tau have been found in the brains of IgLON5 patients post mortem. To date, it is still unknown how IgLON5 internalisation leads to tau phosphorylation and cell death.

IgLON5 cell surface expression

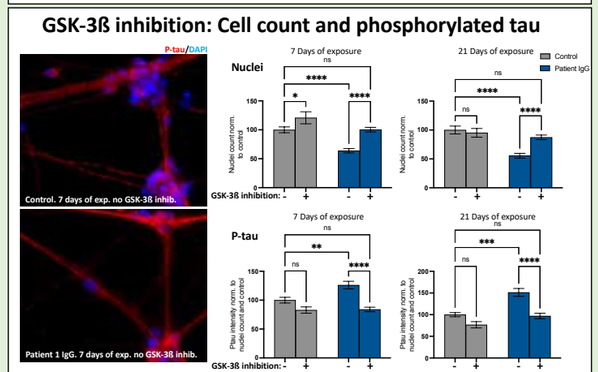


To verify pathogenicity of autoantibodies, their ability to internalise IgLON5 was tested. IgG from all three IgLON5 patients internalised IgLON5 significantly. Data for all patients are combined in the following graphs.

METHODS

Human neural stem cells were differentiated for 30 days into neural cultures containing neurons and astrocytes. Cultures were then exposed to IgG fractions of serum from three IgLON5 patients. Cultures were analysed by various means after different exposure time: Live cell staining of IgLON5 clusters (1 and 7 days of exposure), proteomic analysis (mass spectrometry (MS), 1 day of exposure), phosphorylated tau (p-tau) staining (7 and 21 days of exposure), and lactate dehydrogenase (LDH) release (7 and 21 days of exposure).

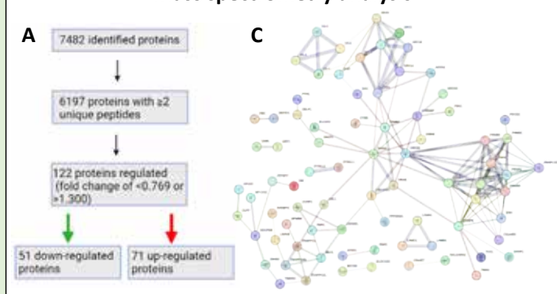
GSK-3ß inhibition: Cell count and phosphorylated tau



Stainings showed decreased nuclei count and increased p-tau in cultures exposed to anti-IgLON5 IgG (7 and 21 days). GSK-3ß inhibition normalised both parameters.

RESULTS

Mass spectrometry analysis

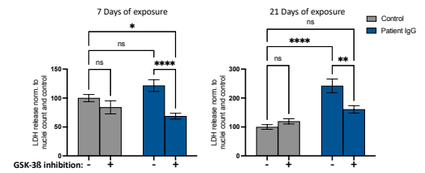


B

Molecular and cellular functions	P-value range	# Molecules
Cellular development	1.07E-02 – 1.18E-06	34
Cellular growth and proliferation	1.07E-02 – 1.18E-06	37
Cell cycle and survival	1.08E-02 – 2.07E-06	51
Cell cycle	9.65E-03 – 1.87E-05	21
Cellular assembly and organisation	9.65E-03 – 1-87E-05	38

Large-scale quantitative MS analysis identified 122 dysregulated proteins in cultures exposed to IgLON5-patient IgG compared to untreated controls after 1 day (A). Ingenuity Pathway Analysis (Qiagen) revealed several affected molecular and cellular functions which all related to cellular development and survival (B). With protein-protein interaction analysis (STRING-db.org) we identified a potential therapeutic target: GSK-3ß (not found regulated by MS) (C). GSK-3ß phosphorylates tau and induce apoptosis, and its activity was expected to be increased due to changes of several of it's regulators (ERBB2=0.748 (inhibitor), HRAS=1.318 and KRAS=1.337 (inducers) numbers are fold change of relative protein quantity).

GSK-3ß inhibition: LDH release



Measurements of LDH released to medium revealed increased cell death in cultures exposed to anti-IgLON5 IgG. GSK-3ß inhibition significantly reduced cell death in anti-IgLON5 IgG treated cultures.

Conclusions

Mass spectrometry allowed us to find a possible link between IgLON5 internalisation, and neurodegeneration. One of the main functions of GSK-3ß is to phosphorylate tau. GSK-3ß is currently being investigated as a possible treatment target of Alzheimer's disease, in which increased GSK-3ß activity also seem to play a role. By inhibiting GSK-3ß we were able to attenuate the increased cell death and phosphorylation of Tau induced by antibody exposure. We speculate if treatment with a GSK-3ß inhibitor in combination with standard immune suppression could improve the outcome of IgLON5 disease patients.

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Dr Matteo Gastaldi is a neurologist specialised in the treatment of antibody mediated disorders of the nervous system including Myasthenia Gravis, MOGAD and NMOSD and Autoimmune Encephalitis. He trained in Neurology and obtained a PhD from the University of Pavia. During his PhD he attended as research fellow the Neuroimmunology Laboratory at NDCN in Oxford for a year supervised by Professor Angela Vincent. Later, he spent three months as a research fellow in the neuroimmunology laboratory in IDIBAPS in Barcelona under the supervision of Francesc Graus and Josep Dalmau. During these experiences Matteo acquired skills in the implementation of immunological assays for the detection of neuroglial antibodies. Since 2021, he is the Head of the Neuroimmunology Research Unit in Pavia and the Co-head of the Neuroimmunology Diagnostic Laboratory. He is also involved in patient care and performs once a week a neuroimmunology clinic dedicated to patients with antibody mediated disorders of the nervous system.

Prognostic relevance of serum longitudinal antibody titres and NfL levels in CASPR2 and LGI1 antibody associated autoimmune encephalitis

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Introduction

Autoantibodies against LGI1 and CASPR2 are diagnostic and pathogenic markers of specific forms of autoimmune encephalitis (AE). However, no clear prognostic markers have been identified in this condition. Serum Neurofilament light chain (NfL) have emerged as a marker of axonal injury in other forms of AE such anti-NMDAR AE.

Aim

To study the prognostic relevance of longitudinal serum titers and NfL levels in anti-LGI1/CASPR2 AE.

Methods

Retrospective cohort study including 23 patients with anti-LGI1/CASPR2 definite AE, and at least one available longitudinal sample > 60 days from onset. Titers were measured with endpoint dilution using a live cell-based assay. Twenty-eight/142 samples (20%) were classified as from "acute phase", 15 (10%) as "postacute" and 99 (70%) as "remission". Median number of samples per patient was 4 (range 2-18). Median time of collection of the last follow up sample was 42 months (range 6-70) from onset. NfL were measured using ELLA. Outcome was measured with modified Rankin Scale (mRS) and with Clinical Assessment Scale in AE (CASE).

Patients

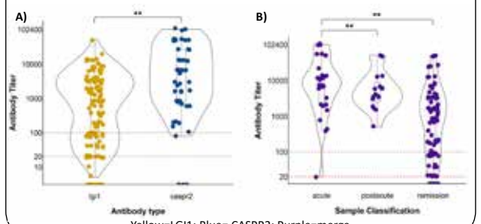
Patients detailed information is reported in **Table 1**

	Total (n=23)	LGI1 (n=15)	CASPR2 (n=8)
Female sex, n (%)	10 (43)	10 (67%)	0 (0%)
Age at onset (years), median (IQR)	63 (59-71)	64 (58,5-71,5)	63 (60,75-70)
Time to diagnosis (days), median, IQR)	92 (27-168)	87 (25,5-132,5)	138,5 (75,25-221,75)
Follow-up (months), median (IQR)	44 (24-54)	42 (23,5-54,5)	49,5 (33-55)
Relapsing course, n (%)	10 (43%)	5 (33%)	5 (62,5)
mRS at diagnosis, median (IQR)	3 (3-3, 2-5)	3 (3-3, 3-5)	3 (2-3, 2-3)
CASE at diagnosis, median (IQR)	6 (4-7)	7 (5,5-8)	5 (2,75-6,25)
Clinical features at diagnosis, n (%)			
memory deficit	15 (65)	10 (67)	5 (62)
seizure	22 (96)	14 (93)	8 (100)
mood/behaviour/psy	15 (65)	11 (73)	4 (50)
Tumor, n (%)	3 (13)	0 (0)	3 (37,5)
Altered MRI at diagnosis, n (%)	16 (70)	12 (80)	4 (50)
Bilateral	8 (35)	6 (40)	2 (25)
Altered EEG at diagnosis, n (%)	21 (91)	14 (93)	7 (87)
CSF altered at diagnosis, n (%)	18 (78)	12 (80)	6 (75)
Hyponatremia, n (%)	7 (30)	7 (46)	0 (0)
First-line immunotherapy, n (%)	23 (100)	15 (100)	8 (100)
Second-line immunotherapy, n (%)	13 (57)	9 (60)	4 (50)
Rituximab	6 (26)	5 (33)	1 (12)
mRS at last follow-up, median (IQR, range)	2 (1-3, 0-5)	1 (1-2,5, 0-3)	3 (1-3)
CASE at last follow-up, median (IQR, range)	1 (1-3, 0-10)	2 (1-3, 0-4)	1 (1-4,25, 0-10)

Results

Titres, antibody specificity and disease phase

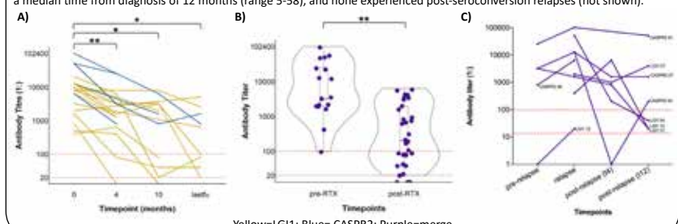
Median anti-CASPR2 serum titers (1:6400, range: 0-1:102400) were higher than median anti-LGI1 (1:1280, range 0-1:51200) (P<0.001) (**panel A**), and correlated with the disease phase, being higher during the acute phase (1:8000, range 1:20-1:102400) compared to post-acute (1:4000, range: 1:500-1:51200) and remission (1:800, range: 0-1:51200) (p<0.001) (**panel B**). This difference remained significant when anti-LGI1 (p<0.001) and anti-CASPR2 (p=0,002) titres were analysed separately (not shown).



Yellow=LGI1; Blue= CASPR2; Purple=merge

Longitudinal titre dynamic, treatments and seroconversion

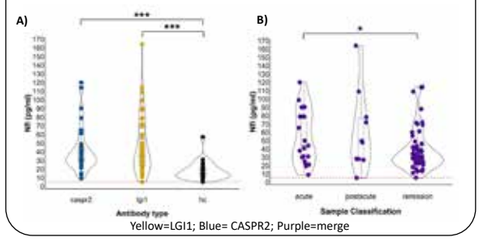
Analysing paired onset-follow up samples, titers reduced at 4 months (n=14 pairs available) with a median reduction of 4,5-fold (range 0,625-800) (p<0.001), at 10 months (n=11 pairs available) with a median reduction of 8-fold (range 1-1024) (p=0.01) and at last follow-up (n=10 pairs available) with a median reduction of 40-fold (range 0,39-640) (p=0.01)(**panel A**). Titres were affected by the administration of immunosuppression. Median titers in samples collected before rituximab administration were higher (median 1:3200 range: 100-1:102400) compared to samples collected afterwards (median 1:160 range: 0-1:6400) (p<0.001) **(panel B)**. Relapses always occurred with a positive sample (n=11), and in 4/5 patients with pre-relapse samples available titres increased at relapse (**panel C**). Seven patients became seronegative (2 with anti-CASPR2, 4 after rituximab) after a median time from diagnosis of 12 months (range 5-58), and none experienced post-seroconversion relapses (not shown).



Yellow=LGI1; Blue= CASPR2; Purple=merge

NfL levels, antibody target and disease phase

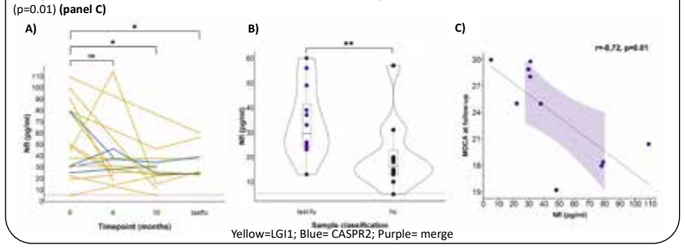
Nfl levels in samples from patients with anti-LGI1 (median: 35pg/mL, range: 5-164) and anti-CASPR2 (median: 31 pg/mL, range: 9-120) AE were higher (p<0.001) compared to those collected from a group of age and sex matched controls. Within patients with AE, NfL levels in samples collected during the acute phase (median: 40, range:9,11-120) were higher compared to levels in samples from the remission phase (median:31 pg/mL; range:5,3-114) (p=0.05) **(panel B)**.



Yellow=LGI1; Blue= CASPR2; Purple=merge

NfL levels during follow-up and correlation with disability

Analysing paired onset-follow up samples, onset NfL levels (median 31 pg/mL, range 5,3-99) reduced at 10 months (n=10 pairs available), with a median reduction of 7,1 pg/ml (range=-6,8-88,77) (p=0,03) and at last follow-up (n=9 pairs available) with a median reduction of 17,9 pg/ml (range: -9,3-54) (p=0.05) **(panel A).** Interestingly, anti-LGI1/CASPR2 AE NfL levels at last follow-up were higher compared to NfL levels in a group of age and sex matched controls (p=0.002, **panel B**). NfL levels in patients did not correlate with disease severity scales (mRS and CASE) or any other clinical feature (not shown). However, higher NfL levels during the acute phase of the disease correlated with a more severe cognitive impairment at follow-up measured with MoCA (p=0.01) **(panel C)**



Yellow=LGI1; Blue= CASPR2; Purple= merge

Conclusion

Anti-CASPR2/LGI1 titres correlate with disease phase, decrease over time and after the administration of immunosuppression. Seroconversion to negative associate with reduced relapse risk. NfL levels correlate with disease phase and higher levels at onset associate with long term cognitive impairment. These preliminary data suggest that both titres and NfL longitudinal monitoring could be a useful biomarker in LGI1 and CASPR2 AE, and warrant further studies

Bibliography: Binks et al. LGI1, CASPR2 and related antibodies: a molecular evolution of the phenotypes. J Neurol Neurosurg Psychiatry. 2018 May;89(5):526-534; Brenner J et al. Predictive Value of Serum Neurofilament Light Chain Levels in Anti-NMDA Receptor Encephalitis. Neurology. 2023 May 23;100(21):e2204-e2213.

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Dr Miguel Tofiño-Vian studied a BSc and MSc in Biotechnology in the Polytechnic University of Valencia, then he deepened his understanding of research approaches to medicine through a MSc in Biomedical Biotechnology by the Polytechnic University of Valencia. He defended his PhD in Biomedicine and Pharmacy in 2018, focused on the anti-inflammatory and regenerative properties of mesenchymal stem cell derived extracellular vesicles in chronic inflammatory conditions. Since then, he has worked as a postdoctoral researcher in Federico Iovino's group, focused on bacterial meningoencephalitis.

Extracellular vesicles as a novel therapy approach in bacterial meningoencephalitis

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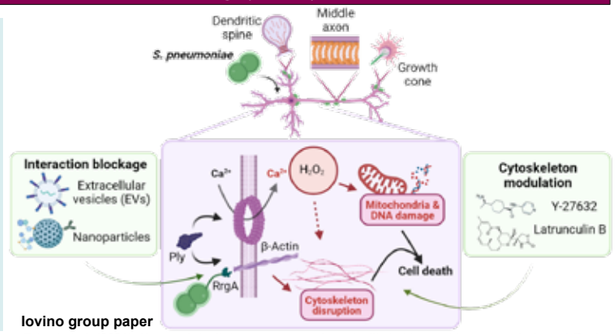
Relevance

Neurological damage is a major consequence of bacterial meningoencephalitis, but little is known about mechanisms of bacterial interaction with neurons leading to neuronal cell death, and how to prevent them. Our group has recently shown that serotype 4 *S. pneumoniae* uses pilus-1 to interact with exposed neuronal β-actin, leading to attachment and invasion. This work aimed to test if the blockage of the β-actin-mediated host-pathogen interaction through extracellular vesicles (EVs) prevented invasion and neuronal damage.



Iovino group paper

Tabusi et al. (2021). Neuronal death in pneumococcal meningitis is triggered by pneumolysin and RrgA interactions with β-actin. *PLoS Pathog*, Mar 24;17(3).

Methods

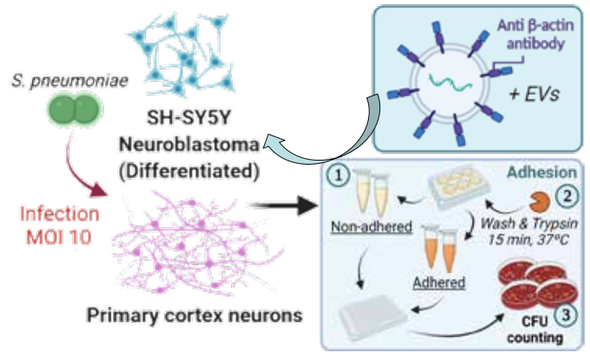


Figure 1. Summary of the methodology

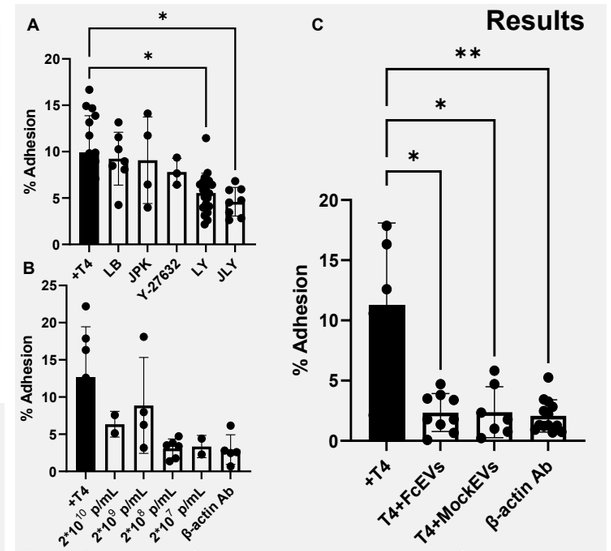


Figure 2. Adhesion after treatment with mock and anti-β-actin-conjugated (Fc)EVs. (A) Effect of actin-modulating drugs on adhesion, (B) Titration with FcEVs and (C) EVs influence on adhesion after 2h infection at MOI 10.

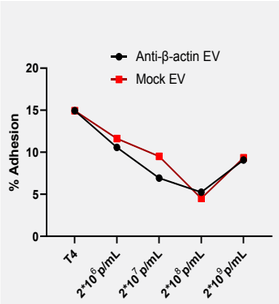


Figure 3. Dose-response curves of anti-β-actin conjugated EVs and mock EVs after 2h infection with TIGR4 at MOI 10.

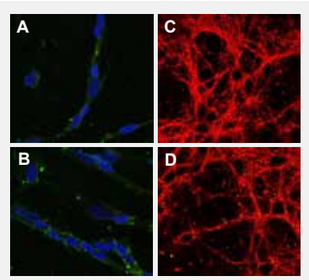
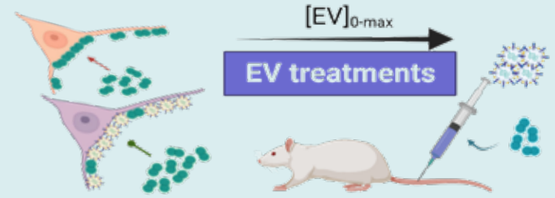


Figure 4. Confocal microscopy of SH-SY5Y (A-B) and mouse primary cortex neurons (C-D) infected (B,D) or not (A,C) with MOI 50 TIGR4. (A-B) Green: β-actin, DAPI. (B-D) Red: L1CAM; green: β-actin.

Work hypothesis & future experiments



Conclusion

EVs proved an efficient in vitro tool to prevent neuronal damage caused by *S. pneumoniae*. Further insights into the mechanisms of action involved may prove EVs as an effective tool to prevent neurological sequelae in patients of bacterial meningoencephalitis.

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Dr Nadia Savino has been a medical doctor since June 2021. During university she taught younger medical students in anatomy and physiology and worked also as a research assistant in the paediatric department, where she also wrote her thesis. Her first year as a doctor she worked for six months in an internal medicine department and thereafter for six months as a general practitioner. Since September 2022 she has worked in the neurology department as a resident doctor where her interest in neurology and especially autoimmune encephalitis started. She is therefore a part of Odense Autoimmune Encephalitis Research group which focuses solely on autoimmune encephalitis and how to improve diagnosis, treatment,

Isolated sensory neuropathy and neuropathic pain: a rare phenotype of anti-Caspr2 disease

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Introduction

- Contactin-associated protein-like 2 (Caspr2)- antibodies are known to cause a variety of symptoms affecting both the central and peripheral nervous system and most patients have multiple symptoms.
- Neuropathic pain is a frequent symptom and has been proposed to be attributed to small fiber neuropathy (SFN) by previous studies.
- This study presents a case of anti-Caspr2 with isolated small fiber neuropathy and neuropathic pain and a systematic review of similar cases in the literature.

Methods

- For the systematic review a PubMed search was performed. Abstracts were screened to select relevant articles.
- Studies reporting cases with Caspr2-antibody in serum and/or cerebrospinal fluid and isolated pain and/or sensory neuropathy was included in the study.

Results

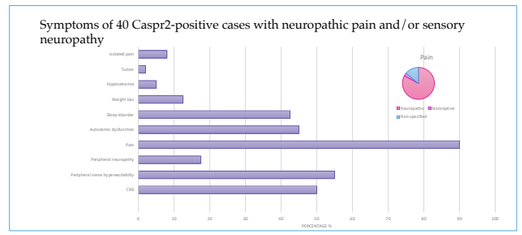
Case study:

- A 77-year-old male presented with neuropathic pain in the lower extremities for 3 months, a partly intended weight loss, and minor balance difficulties
- QST and QSART revealed a small fiber neuropathy
- Common causes of sensory neuropathy were ruled out with anamnesis and standard diagnostic blood tests.
- Serum and cerebrospinal fluid tested strongly positive for anti-Caspr2 antibodies and the patient was diagnosed with Caspr2-antibody disease.
- Treatment with 1 g intravenous methyl prednisone (IVMP) for five days lead to a complete remission of neuropathic pain.

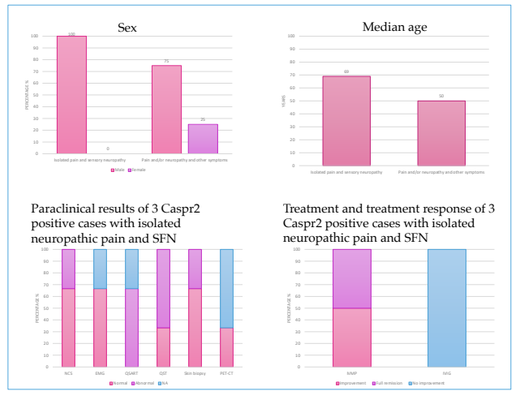
Results

Systematic review

- 24 papers with sufficient available data describing a total of 48 cases with neuropathic pain and/or sensory neuropathy were initially included
- 8 cases also had positive leucine-rich glioma-inactivated 1 (LGI1) antibodies.



- Ultimately, only 5 papers describing a total of 11 cases with isolated pain and/or sensory neuropathy was discovered
- Only 3 cases including our current case had available data for further analysis
- All 3 cases had isolated neuropathic pain and SFN



Conclusions

Anti-Caspr2 antibodies as the cause of isolated neuropathic pain and/or sensory neuropathy is most likely underreported.
We propose that Caspr2-antibody autoimmunity should be considered as a potential cause of cryptogenic sensory neuropathy or neuropathic pain and thus should be included as a part of the diagnostic tests in these patients.

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ENCEPHALITIS SOCIETY
The brain inflammation charity

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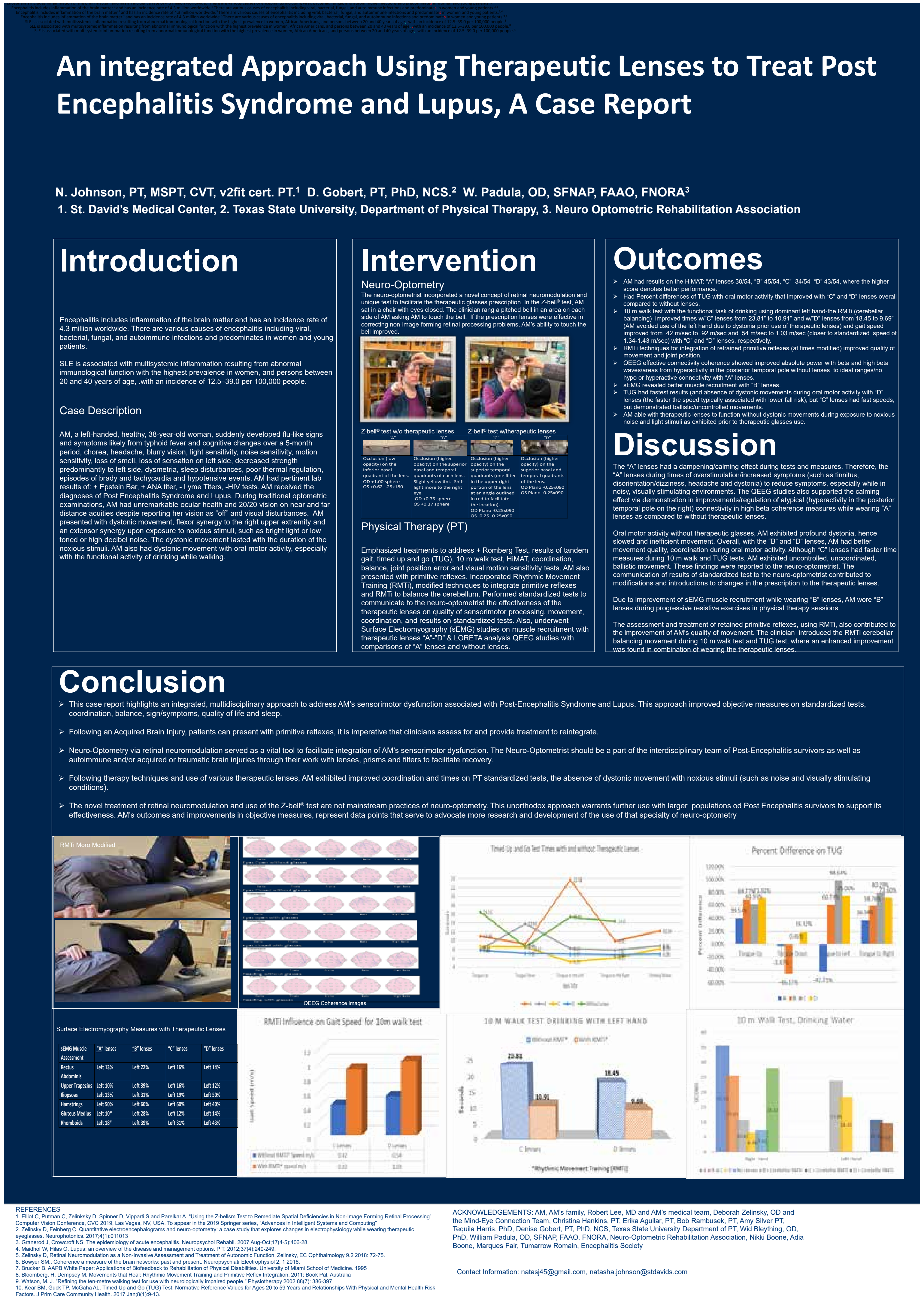
Natasha Johnson Fair received her undergraduate degree from University of Texas at San Antonio, Magna Cum Laude with a Bachelor's of Science in Biology in 2001. She completed her Master's of Science in Physical Therapy degree in 2003. She is certified Competent in the Evaluation and Treatment of Vestibular Disorders from Emory University. She has completed the Specialization Certification in treatment of Post-Concussion Syndrome/Mild Traumatic Brain Injury. She is actively completing a fellowship in the Neuro-Optometric Rehabilitation Association (NORA) for the assessment and treatment of visual dysfunctions following Neurological Injury/Insult. She has worked as a Senior Physical therapist, at St. David's Rehabilitation for 20 years (and approximately 8 years at Rehab Without Walls), specializing in Vestibular, Vision, Neurological, Post-Concussion Syndrome/Mild Traumatic Brain Injury and Brain Injury Rehabilitation and Aquatic Treatments. She serves as a Clinical Instructor for physical therapy students at St. Davids Rehabilitation. Senior leadership at St. David's Medical Centre selected her to participate in the Neal Kocurek Scholarship Program as a mentor to positively influence future generations of healthcare professionals based on her work ethic and practices in her areas of expertise. She has served as a mentor in the program for the past 12 years. She also serves on the education staff for an international vestibular certification course through the 360 Neuro Health Institute.



An integrated Approach Using Therapeutic Lenses to Treat Post Encephalitis Syndrome and Lupus, A Case Report

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Introduction

Encephalitis includes inflammation of the brain matter and has an incidence rate of 4.3 million worldwide. There are various causes of encephalitis including viral, bacterial, fungal, and autoimmune infections and predominates in women and young patients.

SLE is associated with multisystemic inflammation resulting from abnormal immunological function with the highest prevalence in women, and persons between 20 and 40 years of age, .with an incidence of 12.5–39.0 per 100,000 people.

Case Description

AM, a left-handed, healthy, 38-year-old woman, suddenly developed flu-like signs and symptoms likely from typhoid fever and cognitive changes over a 5-month period, chorea, headache, blurry vision, light sensitivity, noise sensitivity, motion sensitivity, loss of smell, loss of sensation on left side, decreased strength predominantly to left side, dysmetria, sleep disturbances, poor thermal regulation, episodes of brady and tachycardia and hypotensive events. AM had pertinent lab results of: + Epstein Bar, + ANA titer, - Lyme Titers, -HIV tests. AM received the diagnoses of Post Encephalitis Syndrome and Lupus. During traditional optometric examinations, AM had unremarkable ocular health and 20/20 vision on near and far distance acuities despite reporting her vision as "off" and visual disturbances. AM presented with dystonic movement, flexor synergy to the right upper extremity and an extensor synergy upon exposure to noxious stimuli, such as bright light or low toned or high decibel noise. The dystonic movement lasted with the duration of the noxious stimuli. AM also had dystonic movement with oral motor activity, especially with the functional activity of drinking while walking.

Intervention

Neuro-Optometry

The neuro-optometrist incorporated a novel concept of retinal neuromodulation and unique test to facilitate the therapeutic glasses prescription. In the Z-bell® test, AM sat in a chair with eyes closed. The clinician rang a pitched bell in an area on each side of AM asking AM to touch the bell. If the prescription lenses were effective in correcting non-image-forming retinal processing problems, AM's ability to touch the bell improved.

Z-bell® test w/o therapeutic lenses — Z-bell® test w/therapeutic lenses

"A"	"B"	"C"	"D"
Occlusion (low opacity) on the inferior nasal quadrant of the lens. OD +1.00 sphere OS +0.62 -.25x180	Occlusion (higher opacity) on the superior nasal and temporal quadrants of each lens. Slight yellow tint. Shift light more to the right eye. OD +0.75 sphere OS +0.37 sphere	Occlusion (higher opacity) on the superior temporal quadrants (one filter in the upper right portion of the lens at an angle outlined in red to facilitate the location). OD Plano -0.25x090 OS -0.25 -0.25x090	Occlusion (higher opacity) on the superior nasal and temporal quadrants of the lens. OD Plano -0.25x090 OS Plano -0.25x090

Physical Therapy (PT)

Emphasized treatments to address + Romberg Test, results of tandem gait, timed up and go (TUG), 10 m walk test, HiMAT, coordination, balance, joint position error and visual motion sensitivity tests. AM also presented with primitive reflexes. Incorporated Rhythmic Movement Training (RMTi), modified techniques to integrate primitive reflexes and RMTi to balance the cerebellum. Performed standardized tests to communicate to the neuro-optometrist the effectiveness of the therapeutic lenses on quality of sensorimotor processing, movement, coordination, and results on standardized tests. Also, underwent Surface Electromyography (sEMG) studies on muscle recruitment with therapeutic lenses "A"-"D" & LORETA analysis QEEG studies with comparisons of "A" lenses and without lenses.

Outcomes

- AM had results on the HiMAT: "A" lenses 30/54, "B" 45/54, "C" 34/54 "D" 43/54, where the higher score denotes better performance.
- Had Percent differences of TUG with oral motor activity that improved with "C" and "D" lenses overall compared to without lenses.
- 10 m walk test with the functional task of drinking using dominant left hand-the RMTi (cerebellar balancing) improved times w/"C" lenses from 23.81" to 10.91" and w/"D" lenses from 18.45 to 9.69" (AM avoided use of the left hand due to dystonia prior use of therapeutic lenses) and gait speed improved from .42 m/sec to .92 m/sec and .54 m/sec to 1.03 m/sec (closer to standardized speed of 1.34-1.43 m/sec) with "C" and "D" lenses, respectively.
- RMTi techniques for integration of retrained primitive reflexes (at times modified) improved quality of movement and joint position.
- QEEG effective connectivity coherence showed improved absolute power with beta and high beta waves/areas from hyperactivity in the posterior temporal pole without lenses to ideal ranges/no hypo or hyperactive connectivity with "A" lenses.
- sEMG revealed better muscle recruitment with "B" lenses.
- TUG had fastest results (and absence of dystonic movements during oral motor activity with "D" lenses (the faster the speed typically associated with lower fall risk), but "C" lenses had fast speeds, but demonstrated ballistic/uncontrolled movements.
- AM able with therapeutic lenses to function without dystonic movements during exposure to noxious noise and light stimuli as exhibited prior to therapeutic glasses use.

Discussion

The "A" lenses had a dampening/calming effect during tests and measures. Therefore, the "A" lenses during times of overstimulation/increased symptoms (such as tinnitus, disorientation/dizziness, headache and dystonia) to reduce symptoms, especially while in noisy, visually stimulating environments. The QEEG studies also supported the calming effect via demonstration in improvements/regulation of atypical (hyperactivity in the posterior temporal pole on the right) connectivity in high beta coherence measures while wearing "A" lenses as compared to without therapeutic lenses.

Oral motor activity without therapeutic glasses, AM exhibited profound dystonia, hence slowed and inefficient movement. Overall, with the "B" and "D" lenses, AM had better movement quality, coordination during oral motor activity. Although "C" lenses had faster time measures during 10 m walk and TUG tests, AM exhibited uncontrolled, uncoordinated, ballistic movement. These findings were reported to the neuro-optometrist. The communication of results of standardized test to the neuro-optometrist contributed to modifications and introductions to changes in the prescription to the therapeutic lenses.

Due to improvement of sEMG muscle recruitment while wearing "B" lenses, AM wore "B" lenses during progressive resistive exercises in physical therapy sessions.

The assessment and treatment of retained primitive reflexes, using RMTi, also contributed to the improvement of AM's quality of movement. The clinician introduced the RMTi cerebellar balancing movement during 10 m walk test and TUG test, where an enhanced improvement was found in combination of wearing the therapeutic lenses.

Conclusion

- This case report highlights an integrated, multidisciplinary approach to address AM's sensorimotor dysfunction associated with Post-Encephalitis Syndrome and Lupus. This approach improved objective measures on standardized tests, coordination, balance, sign/symptoms, quality of life and sleep.
- Following an Acquired Brain Injury, patients can present with primitive reflexes, it is imperative that clinicians assess for and provide treatment to reintegrate.
- Neuro-Optometry via retinal neuromodulation served as a vital tool to facilitate integration of AM's sensorimotor dysfunction. The Neuro-Optometrist should be a part of the interdisciplinary team of Post-Encephalitis survivors as well as autoimmune and/or acquired or traumatic brain injuries through their work with lenses, prisms and filters to facilitate recovery.
- Following therapy techniques and use of various therapeutic lenses, AM exhibited improved coordination and times on PT standardized tests, the absence of dystonic movement with noxious stimuli (such as noise and visually stimulating conditions).
- The novel treatment of retinal neuromodulation and use of the Z-bell® test are not mainstream practices of neuro-optometry. This unorthodox approach warrants further use with larger populations od Post Encephalitis survivors to support its effectiveness. AM's outcomes and improvements in objective measures, represent data points that serve to advocate more research and development of the use of that specialty of neuro-optometry

RMTi Moro Modified

QEEG Coherence Images

Timed Up and Go Test Times with and without Therapeutic Lenses

Percent Difference on TUG

Surface Electromyography Measures with Therapeutic Lenses

sEMG Muscle Assessment	"A" lenses	"B" lenses	"C" lenses	"D" lenses
Rectus Abdominis	Left 13%	Left 22%	Left 16%	Left 14%
Upper Trapezius	Left 10%	Left 39%	Left 16%	Left 12%
Iliopsoas	Left 13%	Left 31%	Left 19%	Left 50%
Hamstrings	Left 50%	Left 60%	Left 60%	Left 40%
Gluteus Medius	Left 10*	Left 28%	Left 12%	Left 14%
Rhomboids	Left 18*	Left 39%	Left 31%	Left 43%

RMTi Influence on Gait Speed for 10m walk test

10 M WALK TEST DRINKING WITH LEFT HAND

*Rhythmic Movement Training (RMTi)

10 m Walk Test, Drinking Water

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Dr Nicolas Chiriboga is an early career paediatric neuro intensivist. He was previously awarded a Children's Miracle Network Grant to study T Cell Receptor Sequencing in patients with NID. He was awarded a fellow travel scholarship to present this project at the Paediatric Neurocritical Care Research Group. He attended medical school at Universidad San Francisco de Quito. After medical school, he participated in a one-year research fellowship at Boston Children's Hospital (through the Harvard University Principles of Practice of Clinical Research Program). After starting residency at the University of Florida College of Medicine-Jacksonville, he continued his research trajectory in projects in the NICU and PICU. He then completed a Paediatric Critical Care Medicine fellowship at the University of Florida in Gainesville, Florida and a Paediatric Neurocritical Care Fellowship at Northwestern University/Lurie Children's Hospital. For the last 8 years he has been a co- investigator, and now co-PI in the Neuroimmune Disorders Adaptive Immunity Study. As junior faculty in the University of Tennessee Health Sciences Center, he plans to continue his academic endeavours in project related to Neuroimmunity and Neurocritical Care.

Regularly Dysregulated: the role of T Cell dysregulation in neuroimmune disorders, including autoimmune encephalitis

Nicolas Chiriboga, MD[1]; Ahmad Alkhasawneh, MD[2]; Guillaume Labiloy, PhD[3]; Juan Cardenas, MD[4]; Yuksel Aydin, MD[4]; Jose Irazuzta, MD[4]

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Background

Over the last decade, there has been an almost continuous discovery of cell and antibody mediated nervous system disorders. A misguided auto-reactive injury has been suspected to be the base pathophysiological principle in Neuroimmune Disorders (NID); including autoimmune encephalitis (AE). We propose that, in some of these conditions, the adaptive immune system may have a common cellular immune pathogenesis, driven predominantly by a dysregulation in the T cell response, despite variability on the phenotypical clinical presentation. We further propose that biomarkers of this dysregulation could serve as a basis to narrow down a diagnosis of autoimmune encephalitis in patients presenting with seronegative encephalitis.

Methods

We have characterized the adaptive immune response on pediatric patients presenting with clinical symptoms compatible with NID. Flow cytometry with deep immunophenotyping of T cells was performed on peripheral blood obtained during the acute clinical phase and in a group of age matched controls (Cohort). The percentage of T cell subsets (naïve, central memory, effector memory, effector memory) were compared between NID cases and age matched controls.

Results

We included 7 patients with confirmed NID and 14 Cohorts as controls. Patients with confirmed NID exhibit a pattern of dysregulation of CD4+ lineages associated with autoimmune processes. Specifically, this dysregulation was related to Teff and Treg cells. A CD4 % of Th1 + Th17 cells of ≥ 86% was found to have a sensitivity of 71% and specificity of 92%. Also, CCR4+ Treg cells <1% of the Treg population was a marker of patients affected with NID.

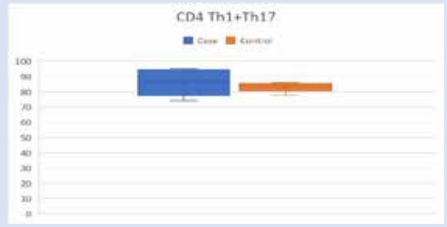

Figure 1: Graph showing elevated Th1 + Th17 populations on NID cases

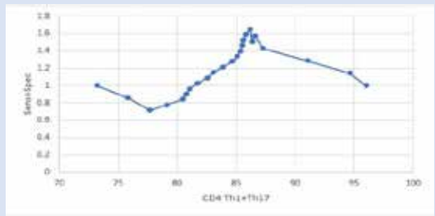

Figure 2: Graph showing ideal sensitivity and specificty for Th1 + Th17 cells at 86%

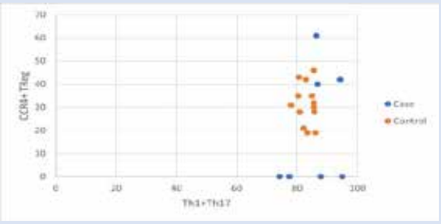

Figure3: Graph showing correlations between Th1 + Th17 cells and CCR4+Treg cells

Discussion

We found a characteristic CD4+ dysregulation associated with NID (including AE), as compared to healthy controls. In some subjects in our cohort with NID, their Th1+Th17 (effector cells) to CCR4 + (T regulator cells) ratio was unbalanced. These findings, warrant further study of whether a marked increase in Th1+ Th17 and a reduction on the CCR4+ Treg lineage can serve as a screening tool for the detection of difficult to diagnose NID; such as seronegative cases. An improved understanding of T cell-based immune dysregulation could be the basis for enhanced diagnostic and therapeutic modalities.

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Patients with neuropsychiatric complications of COVID-19 have worse long-term functional outcomes: COVID-CNS- A multicentre case-control study

Dr. Shil R, Dr. Seed A, Dr. Wood G, Dr. Huang Y, Dr. Ellul M, Prof. Solomon T, Prof. Michael BD; On Behalf of COVID clinical neurosciences study

The Walton Centre for Neurosurgery and Neurology, University of Liverpool, Liverpool, United Kingdom

Introduction

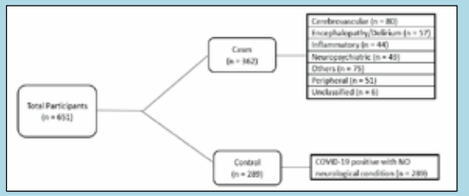
It has been well-established that patients who have been hospitalised with COVID-19 often have morbidity on discharge affecting activity of daily living and employment, and mental health. However, little is known about these outcomes in patients who were hospitalised with neurological and psychiatric complications due to of COVID-19.

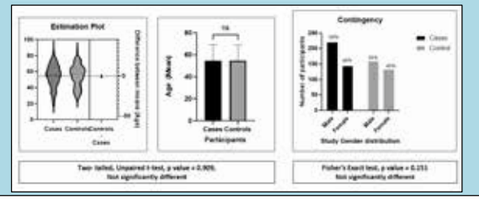
Background

It has been well-established that patients who have been hospitalised with COVID-19 often have morbidity on discharge affecting activity of daily living and employment, and mental health. However, little is known about these outcomes in patients who were hospitalised with neurological and psychiatric complications due to of COVID-19.

Objectives

To determine the functional outcomes of patients who were hospitalised with neurological and psychiatric complications of COVID-19 relative to those hospitalised with isolated respiratory COVID-19, in terms of activities of daily living and employment, and the impact of mental health on these outcomes.





Methods and Design

Prospective multi-centre Case-Control Study

We analysed 651 patients who had been hospitalised with COVID-19: 362 cases (COVID-19 related neurological or psychiatric complication), and 289 controls (COVID-19 respiratory disease with no neurological complications).

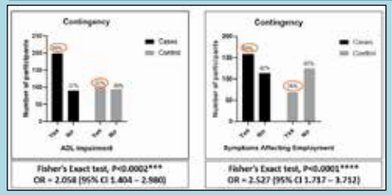
Participants completed baseline questionnaires at median intervals between 12-15 months post-discharge. We assessed Activities of daily living, symptoms impacting occupation, and anxiety and depression. A multivariable logistic regression analysis was performed to identify risk factors associated with the outcome measures.

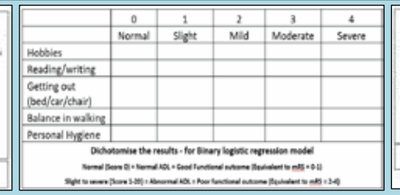
Results

At follow-up, 69% (n = 199) of cases had impairment of activities of daily living, compared to 52% (n = 101) of controls (**OR 2.01 [95% CI 1.40-2.98], p <0.0002**). Among the cases, 58% (n = 159) had persistent symptoms impacting their occupation, compared to 35% (n = 69) controls (**OR 2.52 [95% CI 1.71 – 3.71], p <0.0001**). There was no significant difference in the proportion of cases and control with anxiety and depression at follow-up. Cases were more likely to have required intensive care admission compared to controls (**32% vs 19% [OR 2.00, [95% CI 1.39-2.85], p < 0.0002**). Controls were more likely than cases to have severe WHO-grade COVID-19 on admission (**46% vs 25%, p<0.0001**) and to have severe WHO-grade COVID-19 at peak of admission (**42% vs 17%, p<0.0001**).

Within specific diagnostic groups, the greatest proportion who had impairment in ADLs relative to controls were those who had had neuropsychiatric complications (**OR 2.4 [95%CI 1.14 – 4.85], p=0.01**). The greatest proportions with symptoms impacting employment were those with neuropsychiatric or inflammatory complications (**OR 4.18, [95%CI 2.00 – 8.24], p<0.0001 and 3.26 [95%CI 1.40 – 7.30], p0.006 respectively**). However, there were no significant differences in the proportions with abnormal GAD-7 or PHQ-9 scores between any of the diagnostic groups of cases and controls.

Within Cases, multivariate logistic regression analysis identified that age>50 years, female gender, and hypertension were associated with impairment in activities of daily living, and use of angiotensin inhibitors and statins were associated with no impairment of the ADLs, AUROC curve **0.793 (95% CI 0.7131-0.8746)**.



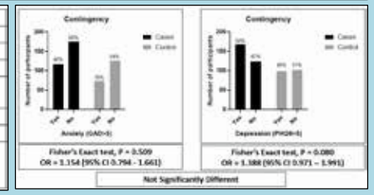


	0	1	2	3	4
	Normal	Slight	Mild	Moderate	Severe
Hobbies					
Reading/writing					
Getting out (bed/car/chair)					
Balance in walking					
Personal Hygiene					

Dichotomise the results - for Binary logistic regression model

Normal (Score 0) = Normal ADL = Good Functional outcome (Equivalent to mRS = 0-1)

Slight to severe (Score 1-20) = Abnormal ADL = Poor functional outcome (Equivalent to mRS = 1-4)



Discussion

Several reports have shown that patients with neurological and psychiatric complications associated with COVID-19 are likely to perform worse while in the hospital, post-discharge and also in longer-term follow-up **[1-2]**. On a larger prospective cohort study of 52759 patients, Beretta et al, reported that patients with neurological complications associated with COVID-19 were found to have a favourable long-term functional outcome as the incidence of the neurological complications had declined over the course of the pandemic and the treatment modality has evolved for better patient care in different settings along with the use of dexamethasone and remdesivir **[3]**. However, in our cohort, the outcome measure was based specifically on the impairment of the ADLs, rather than persisting symptoms as was performed in the study by Beretta et al, and this could be an explanation for poor functional outcome in our cohort.

Our participants also reported having persistent symptoms which are having an impact on their occupation, which is significantly a concerning issue. This would mean that patients with neuropsychiatric complications due to COVID-19 would need multidisciplinary team support by the healthcare professionals, occupational health workers, and social care team post-recovery, to prevent occupational impact in the long run.

Mental health outcome was not significantly different in both groups in our cohort. An explanation is that both groups of participants had similar impacts on mental health due to respiratory illness, neuropsychiatric illness, and both due to COVID-19, during the pandemic.

Limitations

Firstly, due to the missingness of important data variables, the total number of participants, whose functional outcome was analysed had to be reduced from 651 to 484. Secondly, due to the missingness of the mRS score in the database to assess clinicians' perspective of patients' outcomes, an adjusted ADL scoring tool based on the data availability was used in the study, and although this is not an externally validated tool, it was adapted based on the questions from an approved ADL outcome measuring tool, which is UPDRS. Nevertheless, this adjusted ADL tool was synchronized with the mRS score, with anyone having normal ADLs, were grouped into mRS scores 0-1, a good functional outcome, and those with impaired ADLs, were grouped into mRS scores 2-4, a poor functional outcome.

Conclusion

In this large prospective, multi-centre study, it has been shown that patients with neurological and psychiatric complications associated with COVID-19 were at higher risk of having impairment in their activities of daily living and are more prone to have persisting symptoms affecting their occupation. Being female, aged more than 50 years old, pre-existing hypertension was associated with poor functional outcome, and being on ACEI/ARB and statins are protective factors, leading to good functional outcomes. These findings indicate a prompt and specific management approach to prevent short and long-term morbidities in these vulnerable populations. Poor functional outcome was not associated with depression or anxiety.

Future Prospects

Healthcare workers should be aware of the functional impact of neurological and psychiatric complications of COVID-19, beyond depression and anxiety, on both independence and ability to return to employment, so that management can be targeted as MDT approach to provide rehabilitation and ongoing support following discharge.

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Enterovirus encephalitis in adult patients taking Ocrelizumab

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Introduction

The modern era of Multiple Sclerosis (MS) therapy includes highly potent immune suppressors. These have been shown to be highly effective at limiting MS disease activity and are largely well tolerated. Ocrelizumab is an anti-CD20 monoclonal antibody which selectively depletes CD20 expressing B-cells and T-cells.[1] Herpes virus infections have been reported more frequently in clinical trials compared to placebo or interferon but not other serious infections . However, the use of anti-CD20 agents in MS is still relatively new and post-marketing surveillance is crucial for safety monitoring.

Case 1

30 year old man diagnosed with RRMS in 2015, treated with Tecfidera for 2 years before starting Ocrelizumab in 2017, relapse free for 6 years.
In December 2022 he had short diarrhoeal illness, followed by constant headaches, evolving in January 2023 to diplopia, fever, weight loss, and then confusion.
He was admitted with rhombencephalitis (see fig 1) and treated acutely with antivirals and antibiotics. He was subsequently treated with regular IVIg, fluoxetine (for antiviral properties), favipravir, nitazoxanide and ribavirin. Pocapavir was also considered.
He continues to undergo rehabilitation

Reference lab viral PCR
Enterovirus 5' UTR RNA PCR: Enterovirus RNA detected
Enterovirus VP1 genotype: Coxsackie B virus type 5 detected
Reference Lab Comment: Coxsackie B5 (CV-B5) confirmed by partial sequencing of the gene encoding the Enterovirus VP1.

Immunoglobulin levels	Sep 22	Feb 23
IgG (6-16g/L)	6.7	25.1
IgA (0.8-2.8g/L)	0.80	0.53
IgM (0.5-1.9g/L)	0.10	0.10

Table 1 Immunoglobulin levels. September 2022 was during ocrelizumab treatment, while the February and August levels were post-IVIg

	Jan 23	June 23
WCC	<3	17
Differential	n/a	n/a
Culture	No growth	No growth
Protein	0.94	1.52
Glucose (CSF/Serum)	3.0/6.2	2.9/na

Table 2 CSF results from lumbar punctures in month 1 and 6 of admission. The LP was heavily blood stained

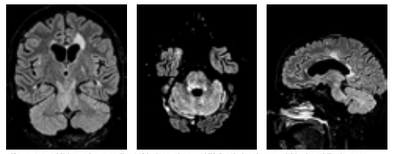

Figure 1: In addition to longstanding MS lesions, abnormal T2 FLAIR signal areas within the midbrain, pons, middle cerebellar peduncles and peri-dentate regions appeared at the onset of the encephalitis and persists

Case 2

38 year old woman diagnosed with RRMS in 2012, started ocrelizumab therapy in 2017, suspended in 2022 for pregnancy and resuming in November 2022.
She presented in June 2023 with five days of headache then gradual lethargy, irritability, meningism and incomprehensible speech over 24 hours and low grade fever (37.9C).
Her initial LP was indicative of an infectious meningoencephalitis. The second LP showed reduced WCC with increased protein but this was post-IVIg (table 4).
She was also treated empirically with broad spectrum antimicrobials and one dose of oral dexamethasone, followed by with one course of 1g/kg IVIg. Remdesevir was considered but not given. She made a rapid and complete recovery.

Reference lab viral PCR
Enterovirus 5' UTR RNA PCR: DETECTED
Enterovirus VP1 genotype: **Untypable**
Reference Lab Comment: The presence of Enterovirus RNA was detected in a 5'UTR-specific pan-enterovirus RT-PCR.
Enterovirus genogroup A,B,C, VP1-specific PCRs were negative, and we were unable to characterise this strain.

	D1	D13
WCC	100	41
Differential	70% PMN	n/a
Culture	No growth	No growth
Protein	0.82	1.62
Glucose (CSF/Serum)	2.5/6.2	2.3/na

Table 4 Lumbar puncture results from day of admission and shortly before discharge.

Immunoglobulin levels	May 23	Jun 23
IgG (6-16g/L)	8.1	5.6
IgA (0.8-2.8g/L)		0.82
IgM (0.5-1.9g/L)	0.91	0.6

Table 3 Serum immunoglobulin levels. The May 2023 levels were immediately prior to Ocrelizumab dosing, and the June 2023 levels were pre-IVIg.

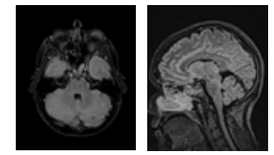

Figure 2 Patient 2 had no MRI changes other than the pre-existing MS-related plaques which showed no change. Due to her agitation she had limited sequences available.

Conclusions

- Enterovirus encephalitis is a rare complication of long-term treatment with anti-CD20 therapies
- Patients on Ocrelizumab may be more susceptible to enterovirus encephalitis
  - The mechanism is likely to be similar to that reported in other anti-CD20 monoclonal antibodies: hypogammaglobulinemia and narrowed Ig repertoire blunting responses to infection.[2,3]
  - We hypothesise that the clinical difference between the two patients may be due to the IgG levels
- IVIg should be considered early and other anti-viral agents can discussed on a case-by-case basis by the multi-disciplinary team.[4]

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Sander Raymaekers was very keen on life sciences since childhood and always wanted to work in healthcare. He graduated as a Master in Bioscience engineering from KU Leuven (Belgium) in 2011 and finished an additional master's in molecular biology at the Free University of Brussels (Belgium) in 2012, focusing on human health, genetics, and virology. He completed a PhD in biotechnology working on songbird brain development in 2017 and made the switch to the industry. He was employed in various roles in the pharma industry before shifting focus to diagnostics in 2019. Since then, he has expressed his passion for science at miDiagnostics by designing and optimising assay for respiratory and CNS infectious diseases, working on sample prep, assay design, microscopy and bridging biochemistry and engineering towards Point-of-care diagnostics.

Development of a fast and sensitive HSV-1/2 test for Meningoencephalitis diagnosis

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Introduction and clinical need

Meningitis and encephalitis are highly urgent and life-threatening infections of the Central Nervous System (CNS). Approximately 2 out of 5 cases of infectious encephalitis are caused by Herpes Simplex Virus (HSV) infection [1]. The key to surviving encephalitis is early detection and effective treatment of the underlying cause [2]. However, the diagnosis of HSV CNS infection is in many cases still impaired by insufficient sensitivity, long turnaround times or excessive manual steps, putting a patient's life and wellbeing at risk. In response to this clinical need, we are currently developing a real time PCR test to enable a quick and reliable diagnosis of CNS infections caused by HSV in cerebrospinal fluid (CSF).

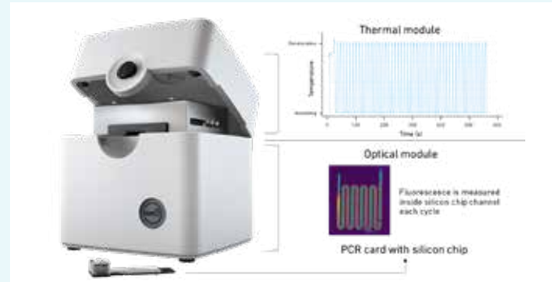
HSV-1&2 test component development

Ultra-fast qPCR platform

The proprietary platform used for this assay was designed and developed for maximal qPCR speed (50 cycles in < 10 minutes) and ease of use, using the following subcomponents:

- Fast thermal cycling using a compact thermoelectric cooler
- Silicon chip reaction chamber in a molded PCR card, which miniaturizes reaction volume and maximizes heat transfer and reaction speed
- Compact optical module without moving parts to maximize cycling speed

The main specification for speed was met after a single iteration: a full 50 cycle qPCR run takes ± 9 minutes, 20 seconds.



Simple sample preparation workflow

A simple sample process workflow was developed to complement the fast qPCR, keep turnaround time low and reduce potential for error. The final workflow includes:

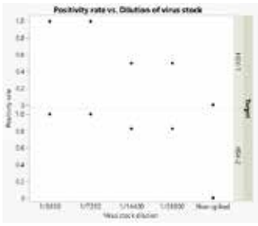
- Less than 10 minutes hands-on time
- A proprietary dilution buffer for chemical sample inactivation and pre-processing
- Freeze-dried proprietary detection mix with room temperature storage of at least 6 months and compatible with ultra-fast qPCR cycling
- Molecular weight cutoff filter (Merck) for quick virus concentration

Performance: sensitivity, specificity and sample compatibility

Assay performance was evaluated as follows:

- Clinical human CSF from non-symptomatic patients was used for all testing, either pooled or individual.
- Inactivated NATtrol HSV-1 (MacIntyre strain) and HSV-2 (MS strain) (Zeptometrix, USA) were used as viral models and spiked into human CSF.
- Samples were run according to the sample processing workflow detailed above.
- Detection was determined on the miDiagnostics PCR platform. A sample was called positive for HSV-1 or HSV-2 if the following conditions were met: (1) Cq of the respective PCR curve was ≤ 46, (2) delta of Relative Fluorescent Units of the respective PCR curve was ≥ 400 and (3) 5-parameter curve fit $R^2 \geq 0.98$.

Sensitivity/Limit of Detection (LOD)



The Limit of Detection was determined to be < 500 copies/ml (N = 6 per dilution). The exact number will be allocated during follow-up studies using multiple strains.

Specificity and compatibility

Non-spiked individual samples were tested, incl. samples indicative of illness, to evaluate Limit of Blank (LOB)

Sample type	HSV-1/2 Detected
Clear (frozen)	0/10
Bloody, yellowish or symptomatic (frozen)	0/9

Low concentration of both viral models (< 500 copies/ml) was tested on several samples

Sample type	HSV-1 & 2 Detected
Clear (frozen, pooled)	30/30
Bloody, yellowish or symptomatic (frozen, individual)	8/9
Clear (fresh, individual)	6/6

Conclusion

Based on the above developments and results, we can conclude that an ultra-fast qPCR assay was successfully developed for the detection of HSV-1 and HSV-2 in CSF. The components allow for very fast cycling with a minor laboratory footprint. A simple workflow should allow easy integration in clinical laboratory routine. Finally, the performance results suggest that the final assay can meet a high standard of sensitivity and is compatible with the sample material expected in the clinical setting. In short, the assay in development is a strong contender to fill a clinical need in the battle against meningitis and encephalitis. Further studies are required to confirm analytical & clinical sensitivity and specificity.

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miDiagnostics

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Dr Sara Cornacchini is a dedicated physician specializing in Neurology, with a strong focus on Neuroimmunology. In 2020, she graduated with honours in Medicine and Surgery from the University of Ferrara with a thesis centred around multiple sclerosis and its diverse presentation across various ethnicities. Throughout her academic journey, she sought opportunities to broaden her knowledge and gain practical experience in participating in an enriching exchange program at New York University, where she undertook a rigorous gross anatomy course. She then travelled to Brazil to undergo a training in neurosurgery. Committed to making a positive impact, she consistently engaged in various charity and public health projects. These ranged from providing healthcare services to underserved communities to conducting medical education programs in schools. After her graduation, she worked in primary care, including family medicine and first-aid services, as well as assisting in the management of the COVID-19 pandemic within the public health department. In 2021, she began her neurology residency at the University of Florence. Here, she is dedicating her research and publications to advancing our understanding of the complex relationship between the immune system and the nervous system, with a particular focus on neuroimmunology. She is deeply immersed in the study of antibody-mediated diseases, with a specific focus on autoimmune encephalitis and myasthenia gravis.

The clinical value of brain FDG-PET in the diagnostic criteria for autoimmune limbic encephalitis

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BACKGROUND

Paraclinical exams play a crucial role in the diagnosis of autoimmune encephalitis (AE). Among them, brain magnetic resonance imaging (MRI) is the main neuroimaging analysis based on the 2016 criteria proposed by Graus et al. [1]. However, a significant number of AE do not show any MRI abnormalities [2]. In such cases, brain 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) could reveal metabolic abnormalities even when the results of all other ancillary tests (MRI, cerebrospinal fluid (CSF), or electroencephalogram, (EEG)) are normal [3]. In this study, we explored the diagnostic performance of brain FDG-PET in patients with AE and its correlation with clinical improvement.

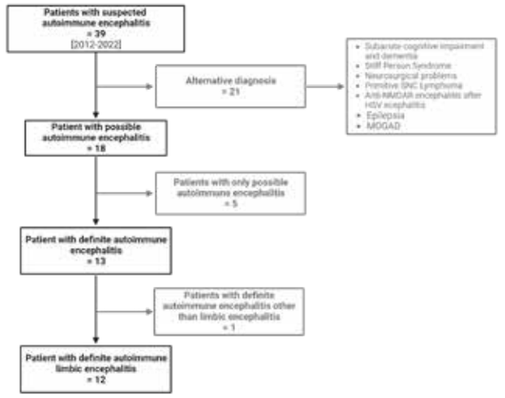
MATERIALS AND METHODS

In this single-centre, observational, retrospective study, all patients who fulfilled the Graus criteria for definite limbic encephalitis [1] and underwent brain FDG-PET were included. Clinical and paraclinical data were extracted from all the available medical charts.

RESULTS

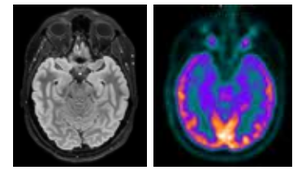
Of 39 patients who presented to our clinic with a suspected diagnosis of autoimmune or paraneoplastic encephalitis (years 2012- 2022), 12 patients with definite limbic encephalitis were included. The MRI criteria for AE were not met in 6/12 patients (50%), because of the presence of unilateral mesial-temporal lobe T2 abnormalities (n=3/6) or normal brain MRI (n=3/6). In these patients the fulfilment of the Graus criteria relied on the presence of neural antibodies (n=3/6) and/or brain FDG-PET abnormalities (n=6/6). Moreover, FDG-PET revealed abnormalities in 7/12 patients who did not have CSF pleocytosis and in all the seronegative cases (n=8/12).

Overall, FDG-PET was abnormal in all but 1 patient, who underwent FDG-PET one month after immunotherapy. In the acute phase of disease, brain FDG-PET detected bilateral (n=4/12) or unilateral (n=3/12) mesial temporal lobes hypermetabolism, especially involving hippocampus or amygdala, while during the chronic phase a bilateral hypometabolism of the mesial temporal lobes was found in the majority of cases (n=6/12). In the 4 patients who performed a follow-up FDG-PET (median 11 months from the first FDG-PET) after the acute phase, an evolution from temporo-mesial hypermetabolism to normal or reduced metabolism was observed in the same regions. Clinical improvement was observed in these patients in accordance with the FDG-PET changes (median mRS 2 ➞1).



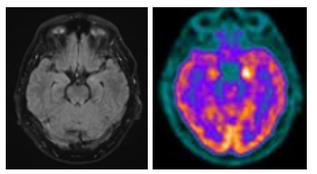
Sex, age	F, 26	F, 55	F, 82	F, 65	M, 67	F, 74	F, 62	M,74	M, 76	F, 70	F, 62	M, 57
Antibodies	**Hu**	**SN**	**SN**	**SN**	**CASPR2**	**SN**	**SN**	**SN**	**LGI1**	**SN**	**SN**	**GABA-B**
(1) Subacute onset of working memory deficits, seizures, or psychiatric symptoms suggesting involvement of the limbic system	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
(2) Bilateral brain abnormalities on T2-weighted fluid-attenuated inversion recovery MRI highly restricted to the medial temporal lobes	×	✓	✓	unilateral	×	unilateral	✓	✓	×	✓	✓	unilateral
FDG-PET medial temporal lobes abnormalities	✓	✓	✓	unilateral	unilateral	unilateral	✓	✓	✓	✓	✓	×
(3a) CSF pleocytosis	×	×	×	✓	✓	✓	✓	×	×	✓	×	×
(3b) EEG with epileptic or slow-wave activity involving the temporal lobes	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
(4) Reasonable exclusion of alternative causes	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

A.



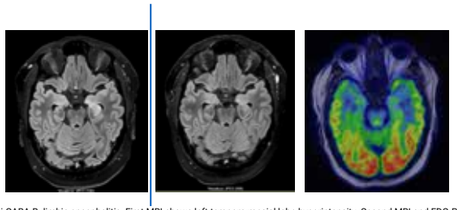
F, 26 y. Anti-Hu limbic encephalitis. MRI and FDG-PET at the same timepoint (chronic phase of disease). FDG-PET shows a hypometabolic temporo-mesial pattern.

B.



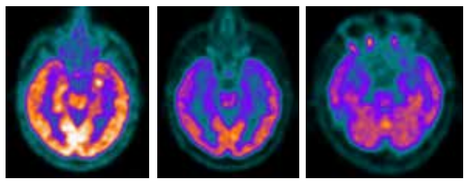
M, 67 y.Anti-CAPSR2 limbic encephalitis. MRI and FDG-PET at the same timepoint (acute phase of the disease). FDG-PET shows a hypermetabolic left temporo-mesial pattern.

C.



M, 57 y. Anti-GABA-B limbic encephalitis. First MRI shows left temporo-mesial lobe hyperintensity. Second MRI and FDG-PET done at the same time after immunotherapy result negative for tempo-mesial lobes abnormalities.

D.



F, 62 y. Seronegative limbic encephalitis. Evolution of the FDG-PET showing a progressive reduction of temporo-mesial lobes hypermetabolism from the acute phase to disease remission.

DISCUSSION AND CONCLUSION

In this study, as previously demonstrated [4-5], brain FDG-PET was more sensitive than brain MRI in confirming the diagnosis of limbic AE, especially in seronegative patients [3]. In addition, a correlation between regression of FDG-PET hypermetabolism and clinical improvement was observed longitudinally in single patients. Our data suggest that brain FDG-PET is a valuable tool for the diagnosis and management of autoimmune encephalitis and should be included in the diagnostic work-up of patients with a clinical suspicion of autoimmune limbic encephalitis.

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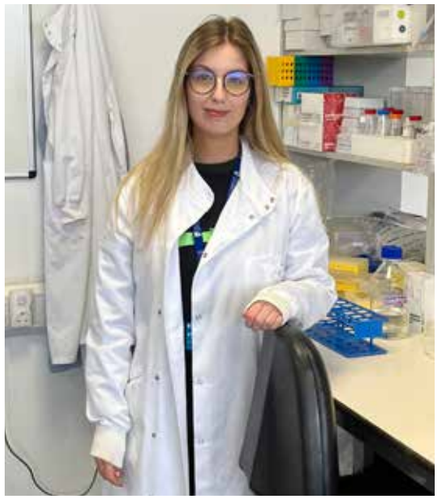

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Sarah is a PhD student at the University of Liverpool and is a member of the Infection Neuroscience Laboratory directed by Professor Benedict D. Michael. Her research focuses on using in vitro modelling, specifically microfluidic chips, to model the blood brain barrier. Using this model, she aims to look at blood brain barrier damage and neuronal damage caused by infection, such as HSV-1 and VZV. As well as inhibiting key chemoattractant protein pathways to assess if any are suitable for future work to develop therapeutics that prevent infection causing damage to the brain.

Investigating direct and indirect effects of viral infections on the brain and prevention via immune suppression

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UNIVERSITY OF LIVERPOOL
The Infection Neuroscience Lab

INTRODUCTION

Viral infections may cause life-changing neurological complications, such as encephalitis and seizures[1].
The brain is protected from toxic substances and microorganisms by the blood-brain barrier (BBB), a highly regulated interface between the blood vessels and the brain (Figure 1). The components that form the BBB (endothelial cells, basement membrane, pericytes, astrocytes, microglia) all play important roles allowing the barrier to function. Molecular complexes between endothelial cells, known as tight junctions (Claudin, Occludin, Junctional Adhesion Molecules (JAMs)) and adherens junctions, are key components for BBB integrity and greatly contribute to its selective permeability[2].
Viruses can damage the BBB and increase its permeability. This can allow microorganisms to cross the BBB, as well as neutrophils, causing a large immune response and inflammation in the brain. The mechanisms by which these pathogens cross the BBB and enter the brain are an area of active research due to the lack of treatment options currently available for affected patients[3]. Two proposed methods by which infections cause neurological inflammation are either a direct attack on neural cells in the brain after crossing the BBB, or an indirect parainfectious route by which infection of endothelial cells of the BBB can cause inflammation in the brain.
Animal models, such as mouse and rat, can be expensive, require ethical approval and have inter-species diversities. *In vitro* models, such transwell assays, offer a cheaper alternative but do not replicate haemodynamic shear stress. A newer *in vitro* model known as a microfluidic chip offers an alternative option that accounts for these characteristics that are critical for understanding the BBB microenvironment[4].
This project aims to investigate the effects of Herpes Simplex Virus-1 (HSV-1) and Varicella Zoster Virus (VZV) on the BBB. Using a microfluidic device to model a "brain-on-a-chip" during infection will allow identification of key factors of BBB damage and neuronal death. Additionally, inhibiting different chemoattractant pathways will demonstrate if these detrimental effects on brain cells can be prevented. Thus, increasing our understanding of viral disease mechanisms and possible targets for the development of new therapeutics.

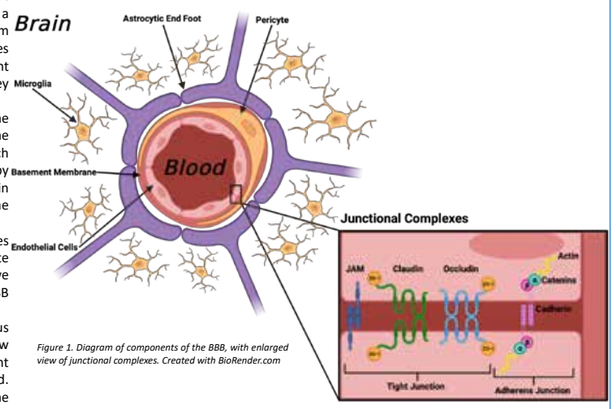

Figure 1. Diagram of components of the BBB, with enlarged view of junctional complexes. Created with BioRender.com

MICROFLUIDIC CHIP MODEL

Using microfluidic chips to model the BBB offers a new alterative to animal models allowing strikingly similar BBB interactions.

This project will use co-culturing and microfluidics to grow a "brain-on-a-chip" using endothelial cells, astrocytes, microglia and neurons. The design of the chips allows chemical and cellular communication between endothelial cells and neurons and glia, as found *in vivo*.

Benefits of microfluidic chips:

- Ability to accurately model haemodynamically relevant shear stress & perfusion
- Spatial separation of specialised cell types recapitulating *in vivo* physiology
  - Allows optimisation of culture conditions for each cell type – biologically relevant
  - More accurately model's effects of infection/drugs as found with *in vivo*

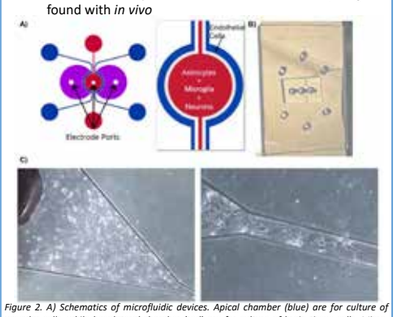

Figure 2. A) Schematics of microfluidic devices. Apical chamber (blue) are for culture of vascular cells, while basolateral chamber (red) are for culture of brain tissue cells. Micro-channels enable communication of cells between chambers, B) Image of microfluidic chip, C) Microscopy image of bEnd.3 mouse endothelial cells growing in apical chamber of chip. Figure 2A taken from Synvivo website: https://www.synvivobio.com/synbbb/

VIRAL INFECTION

Two viruses, from the family *Herpesviridae*, with potential different mechanisms of causing neurological complications will be investigated:

- HSV-1 → Neural infection = 'Direct'
- VZV → Endothelial infection = 'Indirect'/parainfectious

Aim to identify key factors of BBB damage and neuronal death.

Previous work by the Infection Neuroscience Lab has identified cytokines/chemokines and brain-injury proteins of interest. The microfluidic co-culture model will be exposed to both HSV-1 & VZV. Immunofluorescent staining, TEER measurements, live imagine of infection and neutrophil behaviour will be access after infection to detect changes to the BBB.

Additionally, different chemoattractant pathways will be inhibited to investigate their effect on damage to the BBB after viral infection. Data analysed will isolate pathways that have potential therapeutic use to prevent detrimental effects on the BBB and neural cells that result in patients developing neurological complications.

TRANSENDOTHELIAL ELECTRICAL RESISTANCE (TEER)

TEER measurements provide a quantitative method to validate the integrity and permeability of the endothelial monolayer[5]. Using electrodes either side of the monolayer, the electrical resistance between the two is measured (Figure 3). The two electrodes are connected to an analyser that produces a direct current voltage and measures the resulting current through the monolayer. Ohm's law can then be applied to the output values. A high impedance (KΩ) indicate an integral monolayer, and any decreases to this value are used to show an increase in the permeability of the barrier formed.

Pilot data for TEER has been collected and is shown in Figure 4. An increase in the integrity of the endothelial monolayer was found from day 0 (initial seed) to day 4. Flow rate was introduced on day 1 and indicates that shear stress provides a large benefit in improving the strength of the barrier and thus reducing its permeability.

A value of ≥50kΩ has been used to identify an integral endothelial monolayer in transwell models[6]. Currently, these data shows values of at least 50kΩ after 4 days of growth, with some channels showing values of upward of 400kΩ. To increase these values, endothelial cells will now be grown under flow conditions for longer, and co-culturing with other cell populations.

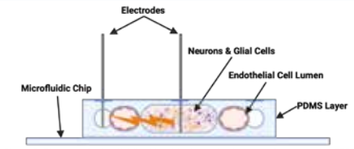

Figure 3. Diagram of microfluidic chip with electrode placement for measuring TEER. Created with BioRender.com

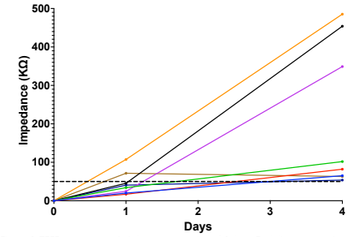

Figure 4. TEER measurements taken over a period of 4 days. Each line represents an individual chamber where hCMEC/D3 have been seeded. Black dotted line represents 50KΩ.

IMMUNOFLUORESCENCE STAINING

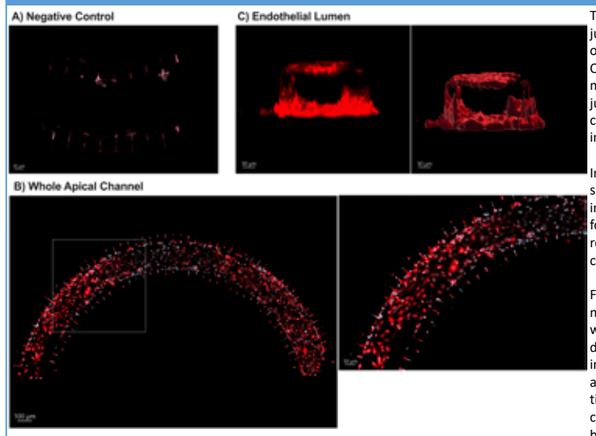

Figure 5. Expression of the tight junction protein ZO-1 (red) and DAPI (nuclei, blue) in hCMEC/D3. A) Negative control, B) Whole apical channel showing high expression of ZO-1, closer view displayed on the right, C) Cross-sectional view of endothelial cell forming an inner lumen in apical chamber (Left: Maximum Intensity Projection view; Right: 3D computer rendered modelled surface).

To confirm the expression of tight junctions, immunofluorescent staining of tight junction proteins (ZO-1 and Claudin-5) are imaged using fluorescent microscopy. A high expression of tight junction proteins show the endothelial cells can form a strong integral barrier inside the apical chamber.

Images of ZO-1 (Red) expression is shown in Figure 5B. Also shown are images (Figure 5C) displaying the formation of a lumen. This is done to replicate the formation of endothelial cells found in the blood vessel.

Future work will establish a co-culture model using neurons and glial cells and will be imaged to characterise the different cultures on the chips. Live-imaging of viral infection and neutrophil adhesion and migration over a length of time generates rendered movies that can be analysed for neutrophil behaviour before and after viral infection, and during chemoattractant inhibition.

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NIHR Health Protection Research Unit in Emerging and Zoonotic Infections at University of Liverpool

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Dr Scarlett Harris completed her Bachelor of Medicine and Surgery at the University of Oxford. She intercalated a DPhil during her medical degree, working at the Sir William Dunn School of Pathology. Her research focused on immunological responses to glycated proteins and the roles of scavenger receptors in obesity. During the final years of her medical degree, she developed an interest in neuropsychiatry and has explored this through additional placements and research. From August 2023 she will be undertaking an academic foundation programme at Imperial College London with a focus on early phase clinical trials and translational medicine.

NMDAR-Antibody Encephalitis in Pregnancy: A Systematic Review of Case Reports

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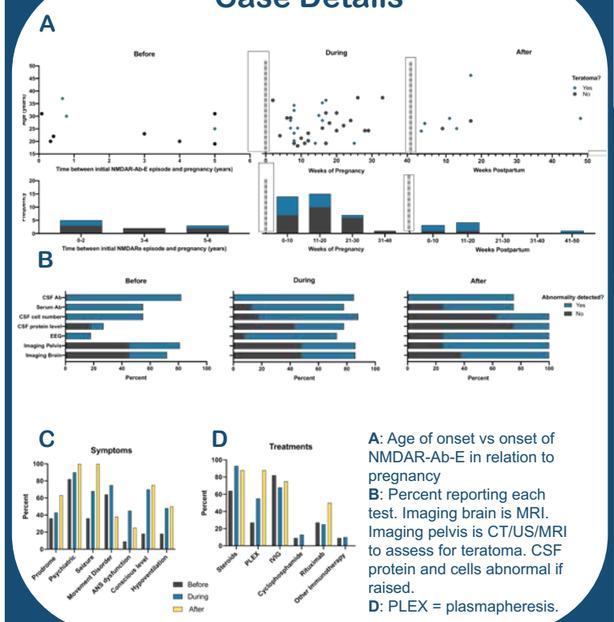
Introduction

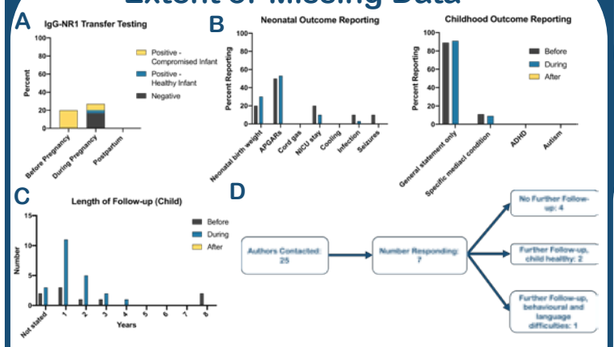
- NMDAR antibody encephalitis (NMDAR-Ab-E) is mediated by IgG autoantibodies against the NMDA receptor NR1 subunit[1].
- It largely affects females of childbearing age[2].
- Animal models of NR1-IgG transplacental transfer have shown reduced survival and neurodevelopmental abnormalities in offspring[3].
- The available clinical data is more reassuring, but systematic reviews focused on neonatal outcomes are lacking[4].

Case Details



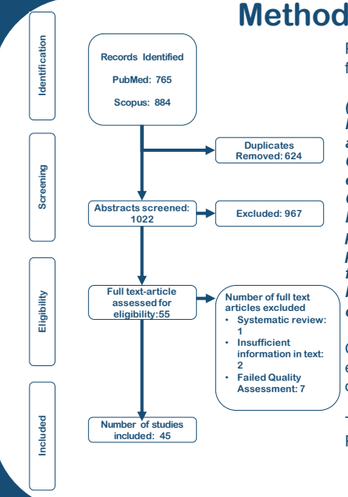
A: Age of onset vs onset of NMDAR-Ab-E in relation to pregnancy
B: Percent reporting each test. Imaging brain is MRI. Imaging pelvis is CT/US/MRI to assess for teratoma. CSF protein and cells abnormal if raised.
D: PLEX = plasmapheresis.

Extent of Missing Data



A: Percentage of cases testing neonate for IgG-NR1.
B: General statement examples: "healthy", "met all developmental milestones", "developing normally"
D: We attempted to contact authors for further childhood follow-up, number responding and outcome shown.

Methods



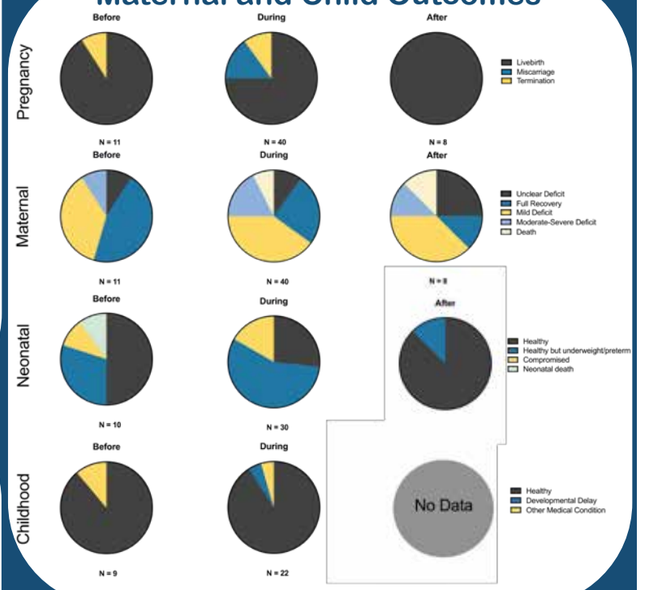
PubMed and Scopus searched using following terms:

("anti-NMDA receptor" OR "anti-NMDAR" OR "anti-N-methyl-D-aspartate receptor encephalitis" OR "NMDAR-antibody encephalitis" OR "NMDAR-Ab-E" OR "NMDAR encephalitis" OR NMDARe) AND (pregnancy OR postpartum OR post-partum OR puerperal OR puerperium OR foetus OR fetus OR gestation OR birth OR neonate OR infant OR child OR perinatal)

Quality assessed using the tool for evaluating the methodological quality of case reports and case series[5].

The review was preregistered on Prospero[6].

Maternal and Child Outcomes



Conclusions

- **Mothers with pregnancy complicated by NMDAR-Ab-E have generally good outcomes**
- **We find no evidence of increased incidence of neurodevelopmental disorders in children born to above**
- **There is lack of long-term follow-up, detailed neonatal and childhood health outcomes and antibody transfer testing**
- **The development of registries would provide consistent follow-up**

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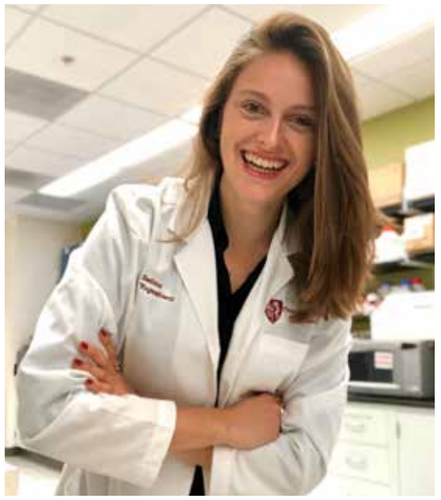

Ms Selina Yogeshwar

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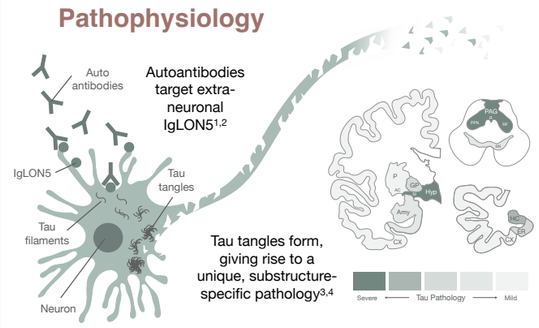
Selina is a PhD student, completing her research in the labs of Carsten Finke (Charité Universitätsmedizin Berlin, Germany) and Emmanuel Mignot (Stanford University, USA). The title of her thesis is Anti-IgLON5 disease - from genetics to neurodegeneration, in which she investigates HLA-association and autoimmune onset of this condition, as well as pathological atrophy progression over the disease course. She holds a Human Sciences BSc and Neuroscience MSc from University College London (UCL) and is a fellow at the Einstein Centre for Neurosciences

Cross-Sectional and Longitudinal Brain Atrophy Patterns in Anti-IgLON5 Disease

S Yogeshwar[1,2,3], F Bartels[1], G Picard[4,5], S Muñiz-Castrillo[3], T Grüter[6], J Lewerenz[7], A McKeon[8,9], M Titulaer[10], EJM Mignot[3], I Ayzenberg[6], J Honnorat[4,5], C Finke[1] & anti-IgLON5 disease collaborators*

Background

Pathophysiology



Anti-IgLON5 Disease

Type of autoimmune encephalitis

First described in 2014[1]

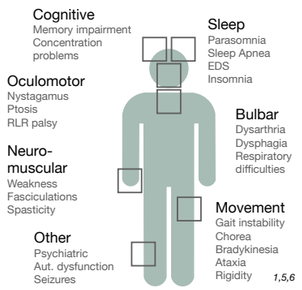
Mean age of onset >60[5,6]

Not sex specific

Immunotherapy has limited success[5]

Neurodegenerative nature remains elusive[2,3,4]

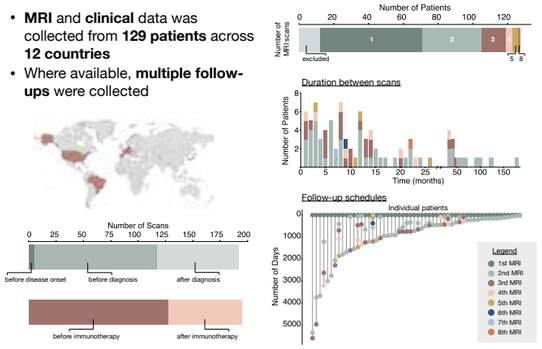
Clinical Presentation



Methodology

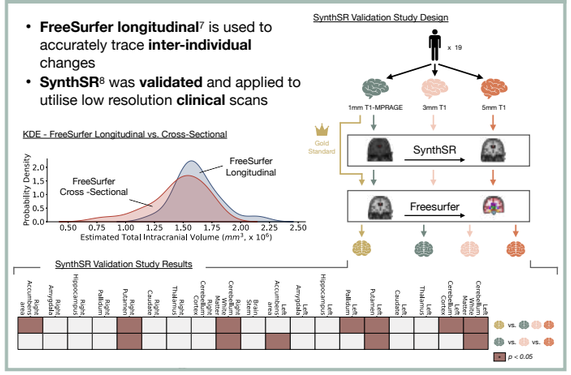
Patient Recruitment

- **MRI** and **clinical** data was collected from **129 patients** across **12 countries**
- Where available, **multiple follow-ups** were collected



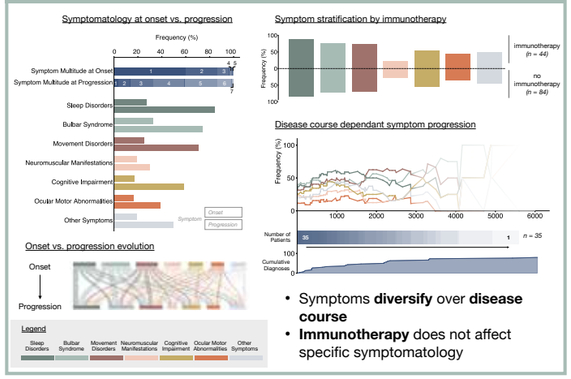
Subcortical Segmentation

- **FreeSurfer longitudinal**[7] is used to accurately trace **inter-individual** changes
- **SynthSR**[8] was **validated** and applied to utilise low resolution **clinical** scans



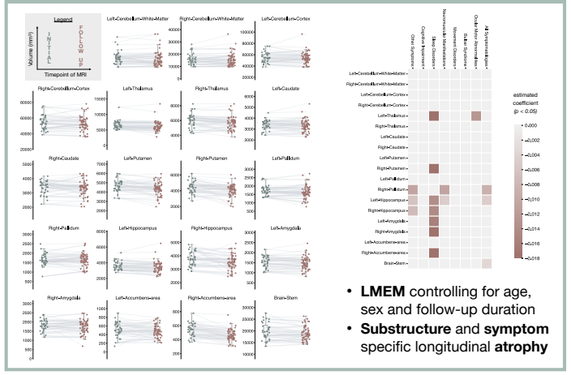
Results

Clinical Trajectory



- Symptoms **diversify** over **disease course**
- **Immunotherapy** does not affect specific symptomatology

Longitudinal Atrophy Patterns



- **LMEM** controlling for age, sex and follow-up duration
- **Substructure** and **symptom** specific longitudinal **atrophy**

References

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* scan for full list of anti-IgLON5 disease collaborators

Ms Simona Serra

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Ms Simona Serra received a B.Sc. with honors in Biotechnologies from the University of Salento (Lecce, Italy) in 2021. During her bachelor studies, she joined the Institute Curie (Paris) as an intern to study the affinity and toxicity between PDC derivatives and G4 structures. In 2023 she graduated with honors in M.Sc. in Molecular and Cell Biology at the University of Bologna, after spending a semester as a visiting student at the Faculty of Pharmacy of Paris-Citè University (Paris, France) and another one joining the lab of L. Tjernberg at the department of Neurobiology, Care Science and Society at Karolinska Institute, where she investigated the APP localization in hippocampal primary culture, using super resolution microscopy. Currently she is a PhD student in Dr. Iovino's group, where she is investigating neuro-infection and neuroinflammation field.

Quantification of released Pneumolysin from different pneumococcal strains and its association with neuronal damage

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Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden
Department of Microbiology and Immunology, Loyola University, Chicago, USA
Department of Molecular Genetics and Infection Biology, Greifswald University, Germany

Relevance

Pneumolysin (PLY) is a pore-forming toxin released by Streptococcus pneumoniae (the pneumococcus), major pathogen globally causing bacterial meningoencephalitis. Its neurotoxicity is well-described, but it is unknown if it has an isoform-specificity or if it is harmful in a dose-dependent way. Our previous work has shown that PLY facilitates pneumococcal interaction with neurons (Tabusi,..., Iovino, *PLOS Pathogens*, 2021). This study is aimed to test Ply obtained from different strains and assess their toxic effects and their mechanisms of action.

Methods

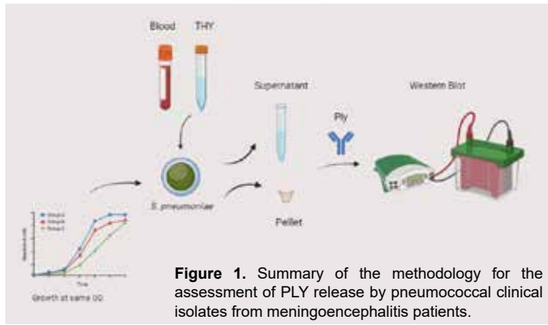


Figure 1. Summary of the methodology for the assessment of PLY release by pneumococcal clinical isolates from meningoencephalitis patients.

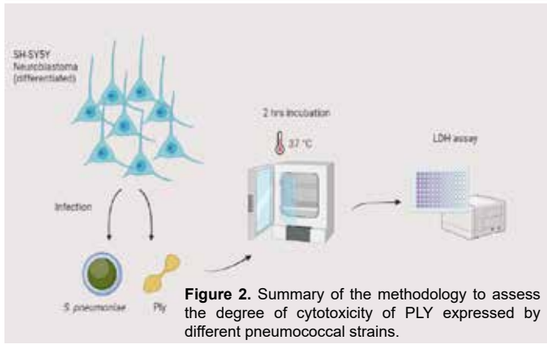


Figure 2. Summary of the methodology to assess the degree of cytotoxicity of PLY expressed by different pneumococcal strains.

- Infections have been carried out with both laboratory strains and clinical isolates of *S. pneumoniae*.
- Cytotoxic assays and analysis of pore formation is carried out with purified recombinant Ply using the gene sequences of the *ply* genes of the different pneumococcal strains.

Results

Fig.3

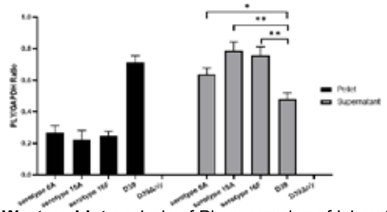


Figure 3. Western blot analysis of Ply expression of laboratory strain D39 and meningoencephalitis clinical serotypes 6A, 15A and 16F.

Fig.4

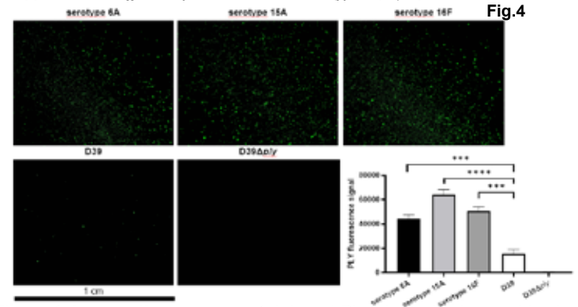
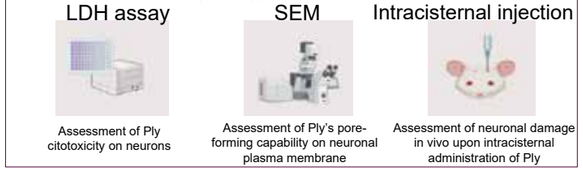


Figure 4. Immunofluorescence staining of Ply released in human blood by the laboratory strain D39 strain and clinical serotypes 6A, 15A and 16F. D39Δ*ply* (lacking Ply) is used as control.

Fig.5

Figure 5. A) Gene sequencing of serotypes 6A, 15A and 16F *ply* genes. Difference in nucleotides compared to the original *ply* gene sequence of the laboratory strain D39 is underlined in blue. **B) *In silico* Ply structure** from *S. pneumoniae* (amino acid substitution is represented by red spheres). **C)** Nucleotide differences cause one single amino acid replacement of D379 by N379, leading to a different D4 morphology (the D4 domain anchors to the plasma membrane to initiate the formation of the pore).

Ongoing Experiments



Conclusions

Our data suggest that Ply is not present in a unique isoform and clinical isolates of meningoencephalitis release significantly higher amounts of Ply than laboratory strains.

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A Specialised Foundation Year 2 doctor working at Aintree University Hospital in Liverpool. Thomas is working with the neuroscience research lab for his academic block, with a focus on encephalitis. Alongside his study on validating the SEIZURE score, he is involved in an update on the UK Encephalitis Guidelines and an epidemiology study on brain abscesses at the Walton. He is hoping to progress into the Acute Care Common Stem programme with an interest in neuro-critical care after taking a year out in Australia.

Preliminary findings in an international validation of the SEIZURE score in encephalitis patients

Thomas Hughes[1,2], Greta Wood[2,3], Tan Hui Jan[4], Jamil Kahwagi[5], Susana Arias Rivas[6], Jesus Garcia de Soto[6], Tom Solomon[2,3], Benedict Michael[2,3]

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Introduction

- Seizure risk in encephalitis has been poorly predicted historically.
- Giving prophylactic anti-epileptics to all patients with encephalitis is not recommended due to inadequate evidence.[1]
- The SEIZURE score was developed in the UK to stratify inpatient seizure risk for encephalitis patients in 2022.[2]

SEIZURE Score Variable	AUROC (95% CI)
Age	0.716 (0.634-0.798)
Presence of fever	
GCS on admission	
Probable aetiology *	0.761 (0.684-0.839)

*Table 1- A summary of the SEIZURE score components and Area Under Receiver Operating Curve. * Represents the additional criteria for the inpatient SEIZURE score.*

Aims and Methods

- This study aims to validate the SEIZURE scoring system internationally amongst different populations.
- Retrospective data was transferred by interested parties to a central database.
- The scoring systems were applied separately on preliminary cohorts from Senegal, Spain, and Malaysia, and together as a global cohort.

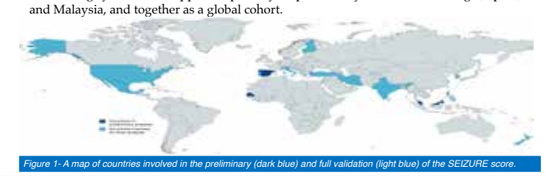

Figure 1- A map of countries involved in the preliminary (dark blue) and full validation (light blue) of the SEIZURE score.

Results

- 156 patients were included in preliminary analysis.
- The scoring system had the best discriminatory ability in Malaysia and the worst in Senegal.
- The aetiology was varied in Spain, mostly herpes simplex and other human herpesvirus strains in Senegal, and mostly suspected or unknown infections in Malaysia.

Country	Number of patients (n)	Age, years, median (IQR)	Male, n (%)	Antibody-associated, n (%)	Bacterial, n (%)	Herpes simplex, n (%)	Mycobacterium tuberculosis, n (%)	Varicella zoster, n (%)	Infection- other, n (%)	ADEM/ immune, n (%)	Unknown aetiology, n (%)	Inpatient seizure, n (%)
Senegal	52	43 (28-51.5)	29 (55.8)	0 (0)	0 (0)	23 (44.2)	4 (7.69)	4 (7.69)	21 (40.4)	0 (0)	0 (0)	24 (46.2)
Spain	52	68 (51.5-75)	31 (59.6)	0 (0)	16 (30.8)	7 (13.5)	1 (1.92)	8 (15.4)	7 (13.5)	7 (13.5)	6 (11.5)	12 (23.1)
Malaysia	54	40 (28-56.5)	32 (59.3)	1 (1.85)	0 (0)	0 (0)	9 (16.7)	1 (1.85)	19 (35.2)	5 (9.26)	19 (35.2)	25 (46.3)

Table 2- An overview of the demographic and aetiological data, and the proportion of patients that seized during their admission for each country involved in the preliminary analysis. Abbreviations- ADEM (acute disseminating encephalomyelitis), IQR (interquartile range).

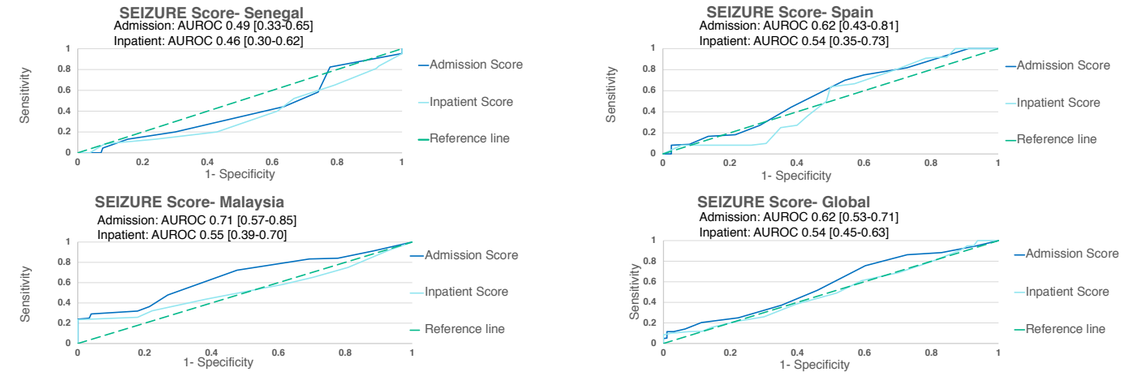

Figure 2- Receiver operating curves for individual countries (Senegal, Spain and Malaysia) and together as a global cohort, showing area under the curve for the SEIZURE score on admission and as an inpatient.

Conclusions

- The scoring system showed the most promising discriminatory performance in Malaysia as a point of care scoring test. The inpatient scoring system performed poorly.
- The scoring system performed poorly in Senegal with limited discriminatory performance in Spain and globally.
- The aetiology of encephalitis varied between differing populations with large numbers of opportunistic infections noted, most of which were generalised as "Infection- other".
- The demographics and encephalitis aetiology between different populations were hugely variable and differing levels of healthcare alongside unique sociocultural environments likely affected the utility of the scoring systems.
- All countries involved would benefit from development of their own scoring systems that accounts for unique differences in their populations.

Next steps

- The completed study will comprise of a cohort of over 1500 encephalitis patients from across the globe with a truly international reach.
- Once the international validation is completed, the study will highlight populations for which the current scoring system is a viable, accessible risk stratification tool.
- Populations that perform poorly under the current scoring system will be highlighted as potentially benefitting from the development of a local tool.

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2. Wood GK, Babar R, Ellul MA, *et al*. Acute seizure risk in patients with encephalitis: development and validation of clinical prediction models from two independent prospective multicentre cohorts. BMJ Neurol Open. 2022 Sep 5;4(2):e000323. doi: 10.1136/bmjno-2022-000323.

Acknowledgments

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Tim Hartung is a post-doctoral researcher at the Cognitive Neurology Lab, Berlin (Prof. Carsten Finke) and a physician at the Department of Neurology and Experimental Neurology at Charité – Universitätsmedizin Berlin, Germany. He investigates imaging markers of neuroimmunological diseases using methods such as resting-state and quantitative MRI. Coming from a background in psycho-oncology, he has a keen interest in epidemiology and psychometrics. Tim holds degrees in psychology and philosophy (M.A., University of Cambridge) and medicine (M.D., Leipzig University).

Imaging alterations in autoimmune encephalitis: a systematic review and meta-analysis

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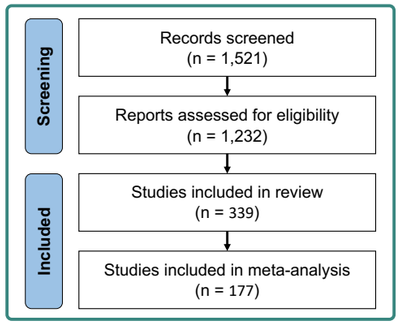
Background

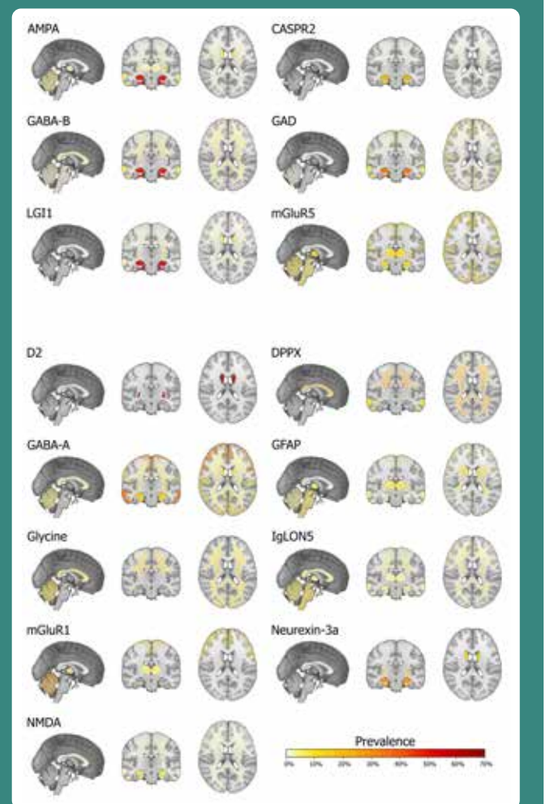
- **Diagnostic Significance:** Understanding typical imaging patterns aids clinicians in recognizing autoimmune encephalitis (AIE), enhancing diagnostic confidence and clinical decision-making.
- **Growing Data Availability:** Increasing reports on various antibody targets provide ample data for statistical analysis, allowing for meta-analytic summaries of imaging abnormalities.
- **Systematic Review and Meta-Analysis** of acute brain imaging in AIE, assessing pooled prevalence of imaging abnormalities, and visualizing affected brain regions, highlighting disease-specific patterns.

Methods

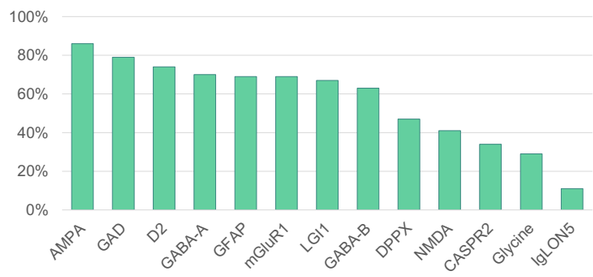
- **Comprehensive Literature Search :** peer-reviewed articles published 2007-2021 in English.
- **Analysis Methods:** Meta-analysis was conducted using a random effects model.
- **Bias Assessment:** Methodological quality was evaluated using the JBI critical appraisal checklist for case reports. Reporting bias was assessed using funnel plots, Doi plots, and the Luis Furuya-Kanamori (LFK) index.



Distinct imaging patterns across autoimmune encephalitides



Prevalence of acute MRI alterations



Conclusions

- **Prevalence Variation:** Prevalence of acute imaging alterations in AIE ranged from 11% to 86%.
- **Limbic encephalitis:** T2/FLAIR hyperintensities in the MTL were typical in encephalitis with antibodies against AMPA-R, CASPR2, GABA-B-R, GAD, LGI1 and mGluR5, rare in GFAP, GABA-A-R, IgLON5, Neurexin-3a and NMDA-R encephalitis, and absent in other AIE types.
- **Distinct Antibody Patterns:** Specific antibodies were linked to characteristic MRI patterns (e.g., unilateral basal ganglia hyperintensity for LGI1 or cortico-subcortical lesions in GABA-B-R encephalitis).
- **Red Flags:** Unilateral lesions or asymmetric MTL involvement, patchy contrast enhancement or ischemia are atypical for AIE, indicating possible alternative diagnoses.
- **Clinical Significance:** Understanding distinct AIE imaging patterns is relevant for accurate diagnosis and guidance of treatment decisions.

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Dr Vasundharaa S Nair (MPhil, PhD) worked with people with various psychiatric and neurological conditions and their families for past 6 years thereby providing individual and family interventions. Her research interests are in the field of mental health, biological disasters like ZIKA, COVID19, Multiple Sclerosis and Acute Brain Infections. She is a passionate academic, psychotherapist and researcher. She is trained in SDG from the HAN university, participated in the Asia Pacific Youth Exchange- Vietnam and India. She has won several accolades names Corona Changemaker, Under 30 emerging industry expert, Youth Icon award, women starter award and young achiever award to name a few. She is an advisory member at Yellow Club, member at OSTEM, campaign specialist at WeMakeChangeIndia and council member at UP mental health council under WICCI India. Academically, she did her PhD under the University Grants Commission from NIMHANS, was an Honorary Fellow under the University of Liverpool and was awarded the National Institute of Health Research Global Health Research Grant 2021. She has published articles across national and international journals and believes that for change to happen, one must be part of the process and contribute their bit.

A qualitative exploration of social factors influencing care for Persons with Brain Infections in Recife, Brazil.

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Acknowledgements : Lorena Cronemberger[a], Walter Fabricio[b], Rebecca Rawlinson[b], Netravathi M[c], Pradeep BS[c], Kasi Sekar[c], Anita Desai[c] , Ravi V[c] , Benedict Michael[b] and Priscilla Rupali[d]

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NIHR Global Health Research PTTA Grant 2021

A collaboration between Fiocruz Pernumbuco in Recife, National Institute of Mental Health and Neurosciences , Bengaluru under the NIHR Global Health Research Group on Brain Infections.
Aiming to explore the social factors influencing care for Persons with Brain Infections in Recife, Brazil.

Objectives:
1. To understand the social factors influencing care for Brain Infections in a Brazilian Hospital setting
2. To understand the difficulties faced by them in tackling these challenging diseases
3. To establish the social factors which could help inform interventions for better care

Methodology

Based on COM-B[1] principles, improve the capability, through optimum opportunities which improves the motivation towards achieving targeted behavior.

Methodology: Qualitative study through Expert Interviews in the field of Brain Infections in Recife, Brazil using a Topic Guide.

Entire research done via Online Platform using a translator through consent and appointments due to COVID-19.

Fig 1: COM-B model (Michie et.al., 2011)

Phases

Phase 1 - Virtual Exploration
Exploration about similarities and differences in Brain Infections care pathway in India and Brazil
Social Scientist: Ms. Lorena

Phase 2 - Joint meeting
Establishment of a Topic Guide
Getting translators and translated consent form

Phase 3 - Key Informant Interviews
Time Period: 10 days
Using Topic Guide with key stakeholders
Recorded Interview: 1-2 hours each

Phase 4 - Analysis
Time Period: 10 days
Transcriber : 3
Fellow herself : 4
Themes were generated

- Passion for being a service provider
- Struggles of delay in seeking care
- Availability, accessibility and adherence concerns
- Levels of care
- Healthcare systemic factors

Findings

Explained the joy and the passion in being a service provider which helped them make this decision to be in this field.
Concerns of delay and sequelae have been observed for the patients coming from rural and urban regions. More of a male preponderance has been noticed with the age groups majorly being the younger children and the elderly.

"The bed space, the investigations, and the treatment, etc. to name a few are in the direct category. Indirect manner the loss of employment when they are admitted, of their caregivers, the continued treatment at home, and the other societal burden constitutes the indirect costs."
(Consultant Neurologist)

As a hospital, they do practice a multidisciplinary team approach with the presence of doctors, nurses, physiotherapists, social workers, and lab members for the care for brain infections.

"People living in remote areas, they might not have very active kind of regional political bodies, and they might not know very much about what they are entitled." (Social Worker)

Conclusion

Training and capacity building through qualitative interviews helped in understanding and sharing of the best practices. The larger project interactions in the background with the grant experience helped in understanding the barriers and facilitators across the places for the care and management of Brain Infections from a LMIC context.

My experience with the group and through PTTA grant has definitely increased my ability to understand the lacunae and the potential areas of work for Brain Infections which has influences not just my research but also my clinical practice.

Further work shall focus on:

Contributing to overall capacity building through learning, research and training ensuring better care for Brain Infections with the help of the larger global team.

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