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Approval Page

Brain Magnetic Resonance Imaging and Electroencephalography predictors of outcomes in children with anti-NMDA receptor encephalitis

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Master's of Science

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Abstract Cover Page

Brain Magnetic Resonance Imaging and Electroencephalography predictors of outcomes in children with anti-NMDA receptor encephalitis

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An abstract of
A thesis submitted to the Faculty of the
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Abstract

Brain Magnetic Resonance Imaging and Electroencephalography predictors of outcomes in children with anti-NMDA receptor encephalitis

By Grace Gombolay, MD

Objective:
Anti-NMDA receptor encephalitis (NMDARE) is a neuroinflammatory disorder characterized by neuropsychiatric symptoms. NMDARE can result in up to 10% mortality and up to 25% with poor neuropsychiatric outcomes. Currently, few biomarkers can predict poor outcomes in NMDARE. In this thesis, I examine features from common ancillary tests performed in children with NMDARE, including brain magnetic resonance imaging (MRI) and electroencephalography (EEG) and their associations with outcomes.

Methods:
Retrospective data was collected for this study, including multicenter data for MRI T2 hyperintense lesions and a single center for MRI volumetric data and EEG features. Odds ratios with 95% confidence intervals were used to calculate odds of persistent disability based on neuroimaging abnormalities. Multivariable logistic or linear regression modeling was performed to assess the relationship of T2-hyperintense MRI lesions, MRI volumes, or EEG features with outcomes. Initially complete case analyses were performed, and then for sensitivity analysis, multiple imputation was performed for missing data (SAS 16.0, Cary, NC).

Results:
An abnormal brain MRI correlated with poor one-year outcomes (OR 2.9; 95% CI: 1.2-7.0), particularly lesions located in the frontal (OR 4.2; 95% CI: 1.5-11.6) and occipital lobe (OR 6.8; 95% CI: 1.1-43.3). Even after adjusting for potential confounders including age of onset, improvement in less than four weeks and time-to-treatment, abnormal MRI was a significant factor in predicting poor one-year outcomes as defined by mRS (OR 3.0, 95% CI 1.0-8.8), along with frontal (OR 3.3, 95% CI 1.1-10.5), and occipital lobe lesions (OR 14.5, 95% CI 1.9-113.4). I then performed two pilot studies: for brain volumetric studies, higher brain volumes correlated with poor functional outcomes, including total brain ($p<0.001$), whole brain gray matter ($p=0.004$) and whole brain white matter ($p=0.05$). For EEG characteristics, loss of PDR ($p = 0.013$), loss of sleep architecture ($p = 0.041$), and epileptiform discharges ($p = 0.041$) correlated with mRS at one year.

Conclusions:
T2 hyperintense brain MRI lesions on brain MRI, brain volumes, and certain EEG parameters may predict poor outcomes in NMDARE. Further larger studies are needed to corroborate these findings.

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CHAPTER 1: Introduction

1.1. Background

Identifying biomarkers to predict treatment response and prognosis is critical in patients with anti-NMDA receptor encephalitis (NMDARE). NMDARE is an autoimmune encephalitis (AE) characterized by neuropsychiatric symptoms, including seizures, psychosis, movement disorders, speech regression, autonomic instability, and central hypoventilation.[1,2] NMDARE causes neuropsychiatric morbidity in 20%[3] and mortality in 10% in cohorts that include children and adults.[4-6] The prevalence of AE, including NMDARE, is not well known but prevalence of AE in children and adults is estimated at ~13.7/100,000.[7,8] Patients typically have a variable response to immunotherapy.[1] NMDARE occurs in all ages, but up to 46% are younger than 18 years old.[6] NMDARE is a common cause of encephalitis in children,[1] and occurs four times as often as encephalitis from herpes simplex virus (HSV), varicella zoster virus, and West Nile virus.[9] Children with NMDARE require prolonged hospitalization (average hospitalization is 39 days; range 14-108), including admission to the intensive care unit (ICU) and inpatient rehabilitation.[10] While children have better outcomes than adults,[5] they face prolonged recovery with poor outcomes in 25% at 2 years, and the majority with good outcomes will have residual symptoms, including executive dysfunction and psychiatric diagnoses.[11,12] Identifying predictive biomarkers in NMDARE would allow for accurate prognostication in NMDARE, and allow for personalized interventions to improve outcomes.

1.2. Limited data exists on the natural history, optimal management, or long-term outcomes in pediatric NMDARE.

The pediatric population is important to study independently from adults because of differences in clinical course and outcomes.[5] Children with NMDARE present with higher rates of movement disorders, language dysfunction, and seizures as compared to adults,[13] and children < 2 years old have worse outcomes. While adolescents have better outcomes than adults, adolescent age is associated with relapses.[6] Limited data exists on the typical clinical course, treatment paradigms, and long-term outcomes such as comorbidities in children with NMDARE. Previous, larger studies have not included long term follow up, and the studies that included follow up time had small numbers (i.e., ≤36 patients).

1.3. Barriers to treating NMDARE

NMDARE is rare, difficult to study at a single center, and the disease course and optimal treatment regimens are unknown in children as only consensus guideline-based therapies exist. Furthermore, while NMDARE is caused by a neuroinflammatory response, the full immune cell profile is unknown. The pathophysiology of AE is attributed to an immune response to a trigger, such as tumors and viruses (e.g., HSV).[5,14,15] NMDAR antibodies are pathogenic, disrupting neuronal signaling by downregulating the NMDA receptor.[16] Immunotherapies are used to target these autoantibodies, including corticosteroids, intravenous immunoglobulin (IVIG) and plasma exchange (PLEX). Since B cells secrete antibodies, B cell targeted therapies such as the anti-CD20 antibody rituximab are also used.[17] However, 30% of patients require additional treatments such as T cell targeted treatments, but the roles of T cells and other immune cells are unknown in NMDARE. Despite our improved understanding of NMDARE and treatments, 92% of pediatric and adult patients have residual

symptoms after AE,[18] and 25% have poor outcomes,[19] so improved treatments are vital. With a defined biomarker (the anti-NMDAR antibody), NMDARE provides a unique opportunity to investigate underlying mechanisms of immune dysfunction that could apply to other neuroimmunological diseases.

1.4. **Biomarkers to predict clinical course and treatment response in NMDARE.**

Currently, no biomarkers predict clinical course and treatment response in NMDARE. Disease severity and prognosis is unpredictable at time of presentation. Patients with more severe disease are more likely to require second-line immunotherapy agents and earlier treatment along with earlier treatment response is associated with improved outcomes.[6,20] Since there is no consensus for a standardized treatment approach, immunotherapy treatment approaches vary by provider, including type, timing and duration of treatment.[5] Thus optimal treatment strategies are not well studied. An international consensus panel for treatment of pediatric NMDARE, in which I participated, recommends first-line treatments (steroids, intravenous immunoglobulin (IVIG), and/or plasmapheresis (PLEX)) and then to wait for patient response, which may take weeks to months, prior to administering second-line therapy, as these more aggressive and riskier treatments are not recommended for all.[21] Rituximab is often the initial second-line agent used to target pathogenic CD20+ B cells. Another second-line agent, cyclophosphamide, which targets other immune cells including T cells, is reserved for patients who are refractory to first-line treatments and rituximab. Second line treatments are associated with poor outcomes, but this may reflect disease severity and failure of first-line treatments as opposed to causing poor outcomes. My **goal** is to

identify patients who will require rituximab and/or cyclophosphamide and provide evidence for the benefits of earlier administration of second-line agents to improve outcomes.

Overall impact

Identifying a biomarker at the time of diagnosis to identify those who would benefit from second-line treatment earlier would be paradigm shifting and the first step towards providing *personalized medicine* in NMDARE. Identification of such a biomarker would allow for the initiation of earlier, more aggressive treatment, which could lead to faster and improved long-term outcomes.

1.5. Chapter Preview

This section serves as a preview of each of the following chapters to provide a structure of the thesis.

1.5.1. Chapter 2: Magnetic Resonance Imaging Lesions and their Association with Outcomes in Pediatric NMDARE

Magnetic resonance imaging (MRI) of the brain is commonly performed in patients with NMDARE. However, only 30% of patients will have abnormal MRIs, despite having neurological and psychiatric symptoms. The most common abnormalities are T2 weighted lesions on brain MRIs, but limited studies are available on whether lesion location is associated with outcomes in pediatric NMDARE. Here I will discuss a retrospective, multi-center patient cohort with the largest number of pediatric NMDARE patients than what has been previously published.

1.5.2. Chapter 3: MRI volumetric studies

In addition to MRI lesions, other MRI abnormalities such as volume loss or atrophy has been reported in patients with NMDARE. One prior study examined global atrophy and its association with outcomes in pediatric NMDARE, finding that low brain volumes associated with poor outcomes. However, this study did not examine whether changes in volumes in different parts of the brain, for example gray matter versus white matter, were associated with outcomes in NMDARE. Moreover, this study used a clinical scale that does not capture cognitive deficits, which are highly important clinical markers in NMDARE. In Chapter 3, I will discuss our findings in MRI volumetric studies in pediatric NMDARE patients using a different scale, the WeeFIM, which is the pediatric version of the Functional Independence Measure (FIM) assessment used in inpatient rehabilitation settings. The WeeFIM can be standardized for normal development depending on age (the WeeFIM DFQ – developmental functional quotient), and captures self-care, mobility, and cognition.

1.5.3. Chapter 4: Electroencephalography predictors for outcomes in pediatric NMDARE

Electroencephalography (EEG) is also commonly obtained in patients with NMDARE to measure electrical brain wave activity. EEG is abnormal in a majority of patients, up to 90%, with a myriad of findings. In this chapter I will discuss some initial work on EEG features and their association in outcomes in children with NMDARE.

1.5.4. Chapter 5: Future Work

Chapter 5 will discuss future work that will build upon the projects in this thesis and the next steps.

1.5.5. Chapter 6: Conclusions

Here I will summarize this thesis work.

CHAPTER 2: Magnetic Resonance Imaging Lesions and their Association with Outcomes in Pediatric NMDARE

2.1. Background and prior work by others

While magnetic resonance imaging (MRI) of the brain is a standard component in the evaluation for patients with suspected NMDARE, current methods to define prognosis based on MRI have not been developed. Brain MRIs can provide detailed information about structural abnormalities and are more sensitive to small lesions and inflammation than another commonly used neuroimaging modality, computed tomography. Brain MRI abnormalities associated in NMDARE include lesions on T2-weighted imaging which are often easier to detect on T2 FLAIR (fluid-attenuated inversion recovery) sequencing. Administration of contrast (usually gadolinium, although other contrast agents are beginning to be utilized), is helpful to identify areas of areas increased vascular permeability in the brain parenchyma or leptomeninges, suggestive of active or acute inflammation.

Interestingly, while patients with NMDARE are often neurologically impaired with significant psychiatric symptoms, only approximately one-third of children and adults with NMDARE will have abnormalities on brain MRIs. The most common abnormalities are T2 FLAIR lesions.[22-24] Prior work has indicated that abnormal MRIs are part of a predictive score called NEOS (anti-NMDAR Encephalitis One-Year Functional Status) score to predict poor outcomes at one year.[20] The NEOS is a five point scale, with one point for each of the following criteria: 1) ICU admission, 2) treatment delay of more than four weeks, 3) lack of improvement within 4 weeks, 4) abnormal brain MRI, and 5) CSF white cell count > 20 cell/uL; However, more detailed analyses of lesion locations

and their association with outcomes have been lacking. One prior study examined how specific brain MRI abnormalities predicted outcomes in both children and adults with NMDARE. This study included 53 pediatric and adult subjects with NMDARE, and reported that T2-hyperintense hippocampal lesions and progressive cerebellar atrophy associated with worse outcomes. However, this study included merely 17 children.[23] Here I assessed the association of T2-hyperintense brain MRI lesions with clinical outcomes in a multi-center pediatric NMDARE cohort.

2.2. Approach

Since 2010, Children's Healthcare of Atlanta (CHOA) has had between 4-8 patients with anti-NMDA receptor encephalitis (NMDARE) per year, which has led to a database of 56 patients at CHOA as of December 2021. However, since NMDARE is rare, I have assembled the first and only multi-center central database in the United States (US) for autoimmune encephalitis (AE) and NMDARE patients called CONNECT (CONquering Neuroinflammation and Epilepsies ConsorTium) to begin to understand the clinical course, treatments, and long-term outcomes in AE. An expansion of the repository I set up at Children's Healthcare of Atlanta (CHOA),[25-29] CONNECT is a prospective, multi-center biorepository that collects clinical data, ancillary testing (MRI and EEG) performed as standard of care, immunotherapy, symptomatic treatments, patient reported outcomes, neuropsychological testing, and biospecimens (blood, CSF, tissue, and DNA). CONNECT aims to connect multiple sites and patients with rare neuroinflammatory diseases to improve our understanding, and also to connect clinical symptoms with ancillary testing findings, biomarkers, and underlying pathogenic

pathways. Funded by the Pediatric Epilepsy Research Foundation (PERF) until 2025, CONNECT currently has four sites recruiting patients and will expand to 12 total sites in three years. My ultimate goal is to use magnetic resonance imaging (MRI), electroencephalography (EEG), blood and cerebrospinal fluid (CSF) markers to identify those who would benefit from more aggressive second-line therapy earlier instead of waiting for first-line treatments to take effect.

In parallel with prospective studies, CONNECT has a retrospective data collection arm that will be important in providing early insight. Due to the rarity of NMDARE, it will be a few years before we have recruited a sufficient number of patients to achieve our goals. The findings from the retrospective database will inform the prospective database and provide additional hypotheses in pathogenesis and optimal treatments in these patients.

2.2.1. Data Collection

Retrospective data was collected from 11 institutions which are tertiary pediatric centers across the United States. The 11 institutions included: Boston Children's Hospital, Children's Healthcare of Atlanta, Children's Hospital Colorado, Children's Hospital Los Angeles, Children's National Hospital, Dell Children's Medical Center, New York University, Rady Children's Hospital-San Diego, Seattle Children's Hospital, University of Virginia, and Vanderbilt Children's Hospital.

2.2.2. Patient characteristics

Children who were under 18 years of age at the time of diagnosis of pediatric NMDARE and who were diagnosed between January 1, 2008, and September 1, 2022 were included in this study. Diagnosis of NMDARE was confirmed with positive

cerebrospinal fluid (CSF) anti-NMDA receptor (NMDAr) antibodies and if the child had at least one of six neuropsychiatric symptoms, as per the diagnostic criteria for NMDARE.[30] The six types of symptoms include: seizures, movement disorders, psychiatric symptoms including psychosis, anxiety, depression, and/or hallucinations, autonomic instability, speech changes (which can include nonsensical speech, paucity of speech, or mutism), and central hypoventilation. Patients with positive NMDAR antibodies in the serum only and without confirmed CSF antibodies were excluded as patients without autoimmune encephalitis can have a false positive in the serum.[31,32] Institutional Review Board approval was obtained at each study site.

2.2.3. Clinical data collection

Clinical data was collected for each pediatric NMDARE patient. Demographic data included age of onset, which was calculated by the time from birthdate to date of symptom onset. Gender, ethnicity, and race was also collected. Length of stay was calculated by the difference between date of hospital admission and date of hospital discharge. The initial symptoms at time of presentation was also collected, along with whether the patient required intubation for respiratory support, and/or gastrostomy tube placement as the patient was unable to eat food by mouth long-term. Ancillary testing results included CSF white cell count with CSF pleocytosis being defined as greater than 5 cells per microliter and whether the initial electroencephalography study was normal or abnormal. The NEOS score was also included, with one point for each of the following criteria: 1) ICU admission, 2) treatment delay of more than four weeks, 3) lack of improvement within 4 weeks, 4) abnormal brain MRI, and 5) CSF white cell count > 20 cell/uL.

2.2.4. Magnetic Resonance Imaging (MRI) data

MRI T2-hyperintense lesion data were collected from the initial brain MRI after a neuroradiologist review for clinical purposes and then location was extracted by a neurologist. For an example of a patient with T2 hyperintense lesions, please see Figure 1. Subjects with prior herpes simplex virus (HSV) encephalitis were excluded as a subset of patients with HSV encephalitis can develop NMDARE, and these patients usually have poorer outcomes than those without a history of HSV encephalitis, which would bias the patients towards worse outcomes.[33] Moreover, HSV encephalitis often results in multiple T2 FLAIR lesions,[34] and thus, identifying which T2 lesions are a result of NMDARE versus HSV encephalitis would be challenging.

2.2.5. Primary outcome

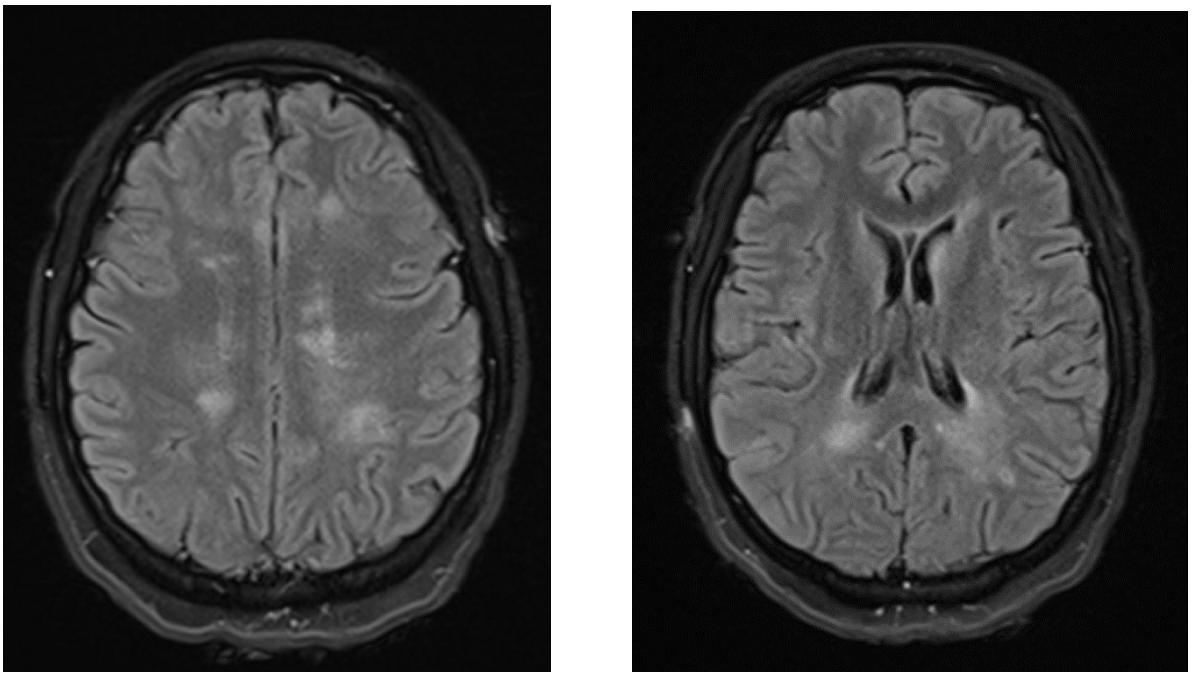
Primary outcome was identified using the modified Rankin Scale (mRS) at one year from diagnosis. The mRS is a measure of neurological disability focused on functional motor status,[35] and has been used as a measure of disability after neurological symptoms, including after stroke.[35] A good outcome was defined as mRS between 0-2 and a poor outcome was defined as mRS 3 or greater.

2.2.6. Statistical Analysis

Descriptive statistics were performed. For continuous data, the mean and standard deviation were reported for populations with normal distribution, whereas the median and interquartile range (IQR) were included for populations with skewed distributions. For discrete data, frequency and percentages were reported. Significance was set at $p < 0.05$ and hypothesis tests including Student's t test or Wilcoxon rank sum test were 2-sided. Odds ratios with 95% confidence intervals were used to calculate

odds of persistent disability based on neuroimaging abnormalities. Multivariable logistic regression modeling was performed to assess the relationship of T2-hyperintense MRI lesions with outcomes. Initially complete case analyses were performed, and then for sensitivity analysis, multiple imputation was performed for missing data (SAS 16.0, Cary, NC).

Figure 1: Representative magnetic resonance images from patients with anti-NMDA receptor encephalitis with T2 FLAIR (fluid-attenuated inversion recovery) brain lesions in the frontal and parietal lobes.



2.3. Results

2.3.1. Initial patient characteristics

We collected data from 192 pediatric NMDARE subjects from 11 institutions. Seventeen subjects were excluded: five subjects had unavailable MRI data, seven subjects did not have confirmed CSF NMDAR antibodies, and five subjects had prior HSV encephalitis (Figure 2). A total of 175 subjects were included, with an average age of 11.6 years (SD: 5.0 years) and 70% were female (Table 1).

Thirty-four percent (60/175) had abnormal brain MRIs with the most common abnormalities being T2-hyperintense frontal (31/60=52%), temporal (28/60=47%), and parietal (21/60=35%) lesions (Table 1). Factors associated with an abnormal brain MRI included need for intubation, ICU admission, higher NEOS score, and poor mRS (mRS ≥ 3) at one year.

Figure 2: Flow diagram of pediatric anti-NMDA receptor encephalitis subjects included and excluded from this study.

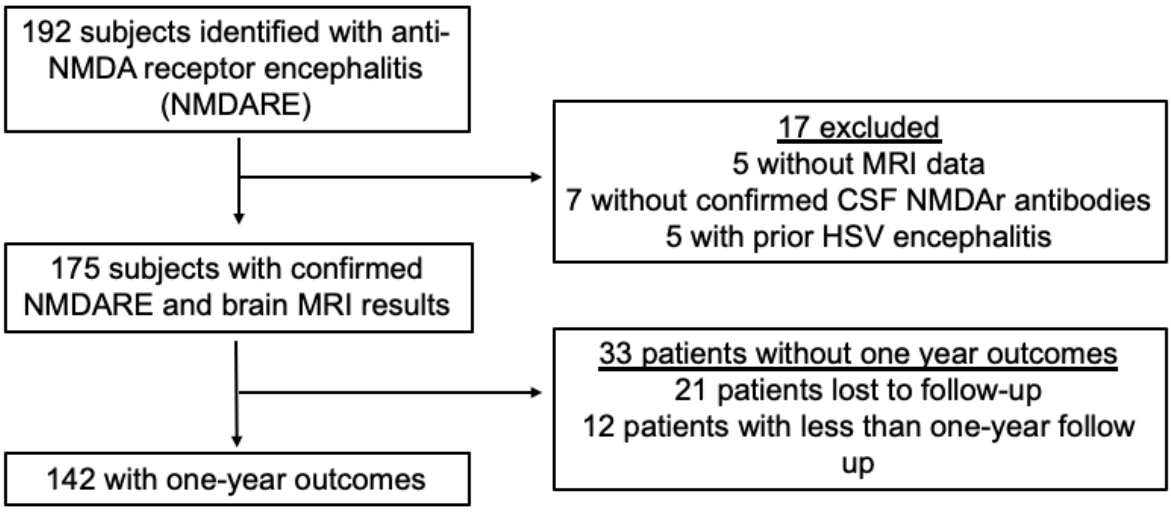


Table 1: Demographic information for the entire cohort of pediatric anti-NMDA receptor encephalitis subjects with MRI data available (N=176).

	Whole cohort (N=175)	Normal MRI (N = 115)	Abnormal MRI (N=60)	p-value
Age in years, Mean (SD)	11.6 (5.0)	11.7 (4.7)	11.4 (5.5)	0.528
Sex, Female:Male, No. (%)	108:47(70:30)[20]	74:34 (69:31)[7]	34:13(72:28)[13]	0.634
CSF WCC, median (IQR)	11 (4, 37)[15]	9 (4, 33)[11]	15.5 (5.5, 38.0)[4]	0.442
CSF WCC > 20, No. (%)	59 (34)	35 (30)	24 (40)	0.204
Hospital LOS days, Median (IQR)	45.5 (14,0, 50.0)[14]	25.0 (13.5, 46.5)[11]	29.0 (14.0, 59.0)[3]	0.236
Intubation, No. (%)	44 (25)	23 (20)	21 (35)	**0.030**
ICU admission, No. (%)	86 (50)[2]	50 (44)[1]	37 (61)[1]	**0.032**
G-tube, No. (%)	61 (36)[5]	36 (33)[5]	25 (41)	0.246
Tumor, No. (%)	28 (16)[1]	18 (16)	10 (16)[1]	0.826
Abnormal EEG, No. (%)	136 (80)[4]	88 (78)[2]	48 (83)[2]	0.453
Symptoms, No. (%)				
Seizure	129 (76)[7]	82 (75)[6]	47 (78)	0.650
Agitation	138 (82)[7]	91 (84)[7]	47 (78)	0.337
Catatonia	60 (36)[7]	38 (35)[7]	22 (37)	0.848
Hallucinations	92 (56)[11]	61 (58)[9]	31 (53)[2]	0.613
Hypoventilation	23 (14)[8]	13 (12)[7]	10 (17)[1]	0.388
Movement disorder	114 (67)[6]	77 (71)[6]	37 (62)	0.233
Speech changes	140 (84)[9]	89 (83)[8]	51 (86)[1]	0.580
Suicidal ideation	13 (8)[14]	8 (8)[10]	5 (9)[4]	0.772
Treatment, No. (%)				
IV steroids	164 (93)	106 (92)	58 (97)	0.245
IVIG	162 (93)[1]	107 (94)[1]	55 (92)	0.754
PLEX	75 (43)[2]	45 (39)[1]	30 (51)[1]	0.152
Second line	132 (76)[2]	87 (77)[2]	45 (75)	0.770
Rituximab	130 (75)[1]	85 (75)[1]	45 (75)	0.950
Cyclophosphamide	22 (13)[1]	13 (11)[1]	9 (15)	0.500
MMF	11 (6)[4]	5 (5)[4]	6 (10)	0.200
Other	6 (4)[8]	4 (4)[7]	2 (3)[1]	1.000
MRI brain T2 lesion location, No. (%)				
Hippocampus	11 (6)	0 (0)	11 (18)	--
Parietal	21 (12)	0 (0)	21 (35)	--
Thalamus	9 (5)	0 (0)	9 (15)	--
Temporal	28 (16)	0 (0)	28 (47)	--

Pons	2 (1)	0 (0)	2 (3)	--
Occipital	7 (4)	0 (0)	7 (12)	--
Midbrain	3 (2)	0 (0)	3 (5)	--
Medulla	3 (2)	0 (0)	3 (5)	--
Frontal	31 (18)	0 (0)	31 (52)	--
Basal ganglia	10 (6)	0 (0)	10 (17)	--
Other MRI findings, No. (%)				
Atrophy present	7 (4)	0 (0)	7 (12)	--
MRI leptomeningeal enhancement	14 (8)	0 (0)	14 (23)	--
MRI parenchymal enhancement	17 (10)	0 (0)	17 (28)	--
Outcomes				
Time to Treatment, median (IQR)	15 (9, 26)[39]	14 (9, 26)[20]	15.5 (8.0, 28.0)[18]	0.158
Treatment before 4 weeks, No. (%)	108 (79)[38]	76 (80)[20]	32 (76)[18]	0.615
Improve < 4 weeks, No. (%)	86 (51)[7]	54 (49)[5]	32 (55)[2]	0.435
Time to improvement, days, median (IQR)	17 (8, 31)[61]	17 (8, 35)[38]	16.5 (7.0, 28.0)[22]	0.162
mRS poor at one year, No. (%)	29 (20)[33]	14 (15)[14]	15 (29)[9]	**0.047**
mRS = 0 at one year, No. (%)	35 (25)[33]	27 (30)[14]	8 (16)[8]	0.064
mRS poor at two years, No. (%)	14 (13)[67]	7 (10)[47]	7 (18)[20]	0.287
mRS = 0 at two years, No. (%)	39 (36)[67]	29 (43)[47]	10 (25)[20]	0.065

SD: standard deviation, [#]Missing data
CSF: cerebrospinal fluid, WCC: white cell count, LOS: length of stay, IQR: interquartile range, ICU: intensive care unit, G-tube: gastrostomy tube, EEG: electroencephalography, MRI: magnetic resonance imaging, mRS: modified Rankin score

2.3.2. One-year outcomes results

For one-year outcomes, data was available in 142 participants, 29 of whom had poor (mRS ≥ 3) and 113 had good (mRS ≤ 2) outcomes (Table 2). An abnormal brain MRI correlated with poor one-year outcomes (OR 2.9; 95% CI: 1.2-7.0). When examining which lesion locations are associated with poor outcomes, frontal (OR 4.2; 95% CI: 1.5-11.6) and occipital lobe T2-hyperintense lesions (OR 6.8; 95% CI: 1.1-43.3) were associated with poor outcomes. Other variables associated with poor outcomes at one-year included intubation, intensive care unit (ICU) admission, gastrostomy placement, prolonged hospital length of stay, plasma exchange and/or second-line treatments (including rituximab and cyclophosphamide), and no improvement in less than four weeks from disease onset (Table 2).

2.3.3. Characteristics of patients without one-year outcomes

For those without one-year outcomes, 18 were not included because one year had not passed since symptom onset. We also assessed those lost to one-year follow up to see if those patients were lost to follow up due to faster recover or milder disease. We assessed these potential reasons by comparing characteristics such as lower mRS scores at 3 and 6 months, or if these patients had a higher proportion of improvement in less than 4 weeks. However, no differences were observed in these characteristics between those included versus excluded at one-year follow-up.

Table 2: Demographic information for the cohort of pediatric anti-NMDA receptor encephalitis subjects with available one-year outcomes assessed by modified Rankin Score (mRS) (N=143).

	Entire Cohort (N=142)	Good (N=113)	Poor (N=29)	p-value
Age in years, Mean (SD)	11.7 (4.9)	11.9 (4.6)	10.4 (5.7)	0.121
Sex, Female:Male, No. (%)	39 (31)[16]	30 (29)[11]	9 (36)[5]	0.522
CSF WCC, median (IQR)	10 (4, 37)[7]	10 (4, 32)[4]	14 (4, 40)[3]	0.631
CSF WCC > 20, No. (%)	46 (32)	34 (30)	12 (41)	0.245
Hospital LOS days, Median (IQR)	24 (13, 49)[10]	21 (12, 46)[7]	43.5 (17, 61)[3]	**0.041**
Intubation, No. (%)	34 (24)	19 (17)	15 (52)	**<0.0001**
ICU admission, No. (%)	69 (49)	46 (41)	23 (79)	**0.0002**
G-tube, No. (%)	48 (35)[4]	27 (25)[3]	21 (75)[1]	**<0.0001**
Tumor, No. (%)	18 (13)[1]	11 (10)[1]	7 (24)	0.058
Abnormal EEG, No. (%)	108 (78)[3]	83 (75)[2]	25 (89)[1]	0.211
Symptoms, No. (%)				
Seizure	107 (78)[4]	84 (76)[3]	23 (82)	0.513
Agitation	110 (80)[5]	85 (78)[4]	25 (89)[1]	0.180
Catatonia	46 (33)[4]	33 (30)[3]	13 (46)[1]	0.100
Hallucinations	74 (55)[7]	63 (59)[6]	11 (39)[1]	0.064
Hypoventilation	18 (13)[6]	14 (13)[4]	4 (15)[2]	0.787
Movement disorder	89 (64)[4]	67 (61)[3]	22 (79)[1]	0.081
Speech changes	117 (85)[5]	109 (79)[4]	25 (89)[1]	0.765
Suicidal ideation	10 (7)[8]	9 (8)[7]	1 (3)[1]	0.687
Treatment, No. (%)				
IV steroids	132 (93)	105 (93)	27 (93)	1.000
IVIG	132(93)	103 (91)	29 (100)	0.214
PLEX	60 (42)	39 (35)	21 (72)	**0.0002**
Second line	109 (77)[1]	81 (72)[1]	28 (97)	**0.006**
Rituximab	107 (75)	79 (70)	28 (97)	**0.003**
Cyclophosphamide	19 (13)	7 (6)	12 (41)	**<0.0001**
MMF	11 (8)[3]	10 (9)[2]	1 (4)[1]	0.463
Other	6 (4)[5]	3 (3)[3]	3 (11)[2]	0.091
MRI brain T2 lesion location, No. (%)				

Abnormal brain MRI	51 (36)	36 (32)	15 (52)	**0.047**
Hippocampus	10 (7)	6 (5)	4 (14)	0.121
Parietal	18 (13)	11 (10)	7 (24)	0.057
Thalamus	8 (6)	5 (4)	3 (10)	0.360
Temporal	23 (16)	17 (15)	6 (21)	0.572
Pons	2 (1)	1 (1)	1 (3)	0.368
Occipital	7 (5)[1]	3 (3)[1]	4 (14)	**0.033**
Midbrain	3 (2)	1 (1)	2 (7)	0.106
Medulla	3 (2)	2 (2)	1 (3)	0.500
Frontal	27 (19)	17 (15)	10 (35)	**0.017**
Basal ganglia	9 (6)	6 (5)	3 (10)	0.389
Other MRI findings, No. (%)				
Atrophy present	7 (5)[1]	3 (3)	4 (14)[1]	**0.029**
MRI leptomeningeal enhancement	12 (8)	9 (8)	3 (10)	0.710
MRI parenchymal enhancement	17 (12)	12 (11)	5 (17)	0.342
Outcomes				
Time to Treatment, median (IQR)	15 (9, 25)[29]	15 (9, 28)[22]	17 (7, 23)[7]	0.120
Treatment before 4 weeks, No. (%)	89 (77)[29]	71 (78)[22]	18 (82)[8]	1.000
Improve < 4 weeks, No. (%)	70 (52)[7]	61 (56)[5]	9 (33)[2]	**0.031**
Time to improvement, days, median (IQR)	6 (8, 34)[47]	14 (7, 27)[37]	34 (13, 66)[10]	0.523
mRS = 0 at one year, No. (%)	35 (25)	35 (31)	0 (0)	---
mRS poor at two years, No. (%)	14 (13)[34]	2 (2)[29]	12 (50)[5]	---
mRS = 0 at two years, No. (%)	39 (36)[34]	39 (46)[29]	0 (0)[5]	---

SD: standard deviation, [#]Missing data
CSF: cerebrospinal fluid, WCC: white cell count, LOS: length of stay, IQR: interquartile range, ICU: intensive care unit, G-tube: gastrostomy tube, EEG: electroencephalography, MRI: magnetic resonance imaging, NEOS: anti-NMDA One-Year Functional Status Score, mRS: modified Rankin score

2.3.4. Multivariable logistic regression modeling to predict poor one-year outcomes

We then performed multivariable logistic regression, by adjusting for factors which are contributory to poor outcomes in NMDARE, including age of onset, improvement in less than four weeks and time-to-treatment. Even after adjusting for these variables, abnormal MRI was a significant factor in predicting poor one-year outcomes as defined by mRS (OR 3.0, 95% CI 1.0-8.8), in addition to age-of-onset (OR 0.90, 95% CI 0.82-0.99) and no improvement in less than 4 weeks (OR 4.1, 95% CI 1.37-12.6). When examining brain lesion locations on MRI, frontal lobe lesions (OR 3.3, 95% CI 1.1-10.5), and occipital lobe lesions (OR 14.5, 95% CI 1.9-113.4) were most predictive for poor outcomes even when adjusting for age of onset, time to treatment and improvement in less than 4 weeks. Sensitivity analyses were performed using multiple imputation to fill in missing data for one-year mRS outcomes. Abnormal MRI still predicted poor outcomes at one year (OR 2.6, 95% CI 1.01-6.8), along with frontal (OR 3.2, 95% CI 1.3-7.9) and occipital lobe lesions (OR 12.9, 95% CI 1.7-96.0), even when adjusting for age, treatment in less than four weeks, and improvement in less than four weeks.

2.4. Discussion

In this pediatric NMDARE multi-center cohort, abnormal MRI was associated with poor one-year outcomes, and particularly if T2-hyperintense lesions were located in the frontal and/or occipital lobes. To our knowledge, this is the first study to examine T2-hyperintense lesion locations and their association with outcomes in pediatric NMDARE. Additionally, this is the largest single pediatric NMDARE cohort and the largest study to

date that examines the associations of MRI lesions with clinical outcomes in children with NMDARE.

Despite multiple neuropsychiatric symptoms, only 34% of children with NMDARE had abnormalities on their brain MRIs, which associated with poorer prognosis at one year. This may be reflective of how T2 lesions may represent cytotoxic injury, which is less likely to be reversible. Thus, having an abnormal MRI may reflect more severe disease in NMDARE, and the underlying mechanisms of which patients have T2 lesions versus those who do not still need to be elucidated.

Interestingly lesion location also predicted poor outcomes in our cohort. The frontal lobe is involved in acquiring, executing and controlling many different functions, anywhere from motor responses to complex decision making. Moreover, disruption of the frontal lobe circuitry contributes to many developmental disorders including attention-deficit/hyperactivity disorder (ADHD), executive dysfunction and impulsivity.[36] As these symptoms (ADHD, executive dysfunction and impulsivity) are some of the most common residual symptoms in NMDARE,[37] T2-hyperintense frontal lobe lesions may help to identify those at higher risk for these residual symptoms.

Memory problems are also common in NMDARE, but T2 hippocampus/mesial temporal lesions did not associate with outcomes in this study. Surprisingly, while rare (7/175=5%), T2-hyperintense occipital lobe lesions associated with poor outcomes, but may be due to widespread brain involvement, as the majority of patients had T2 lesions in more than one brain location, and thus reflective of widespread injury. However, the occipital lobes are responsible for visuospatial processing,[38] which can be altered in NMDARE, especially in the acute phase.[19] Thus, additional studies assessing whether

occipital lobe lesions contribute to residual symptoms versus disruptions of different brain networks.

Limitations include that we performed a descriptive study of MRI lesion location without including lesion volumes as larger lesion volumes would presumably lead to higher disability. Brain networks and disruption of brain networks were also not included in this study, as many neurological processes are not dependent on a single location but through networks in the brain. Though our dataset had missing data, including 19% without one-year mRS scores, many of these subjects had not yet reached one-year follow-up time. Furthermore, the patients that were lost-to-follow-up appeared random and did not seem to involve those with milder disease, as those with milder disease are less likely to follow up in clinic. Compounding this, data was collected from tertiary and quaternary pediatric medical centers and thus severity bias and convenience sampling are present in this dataset. This could impact both the rates of neuroimaging abnormalities and the likelihood of one-year disability. To account for missing data, we performed sensitivity analyses using multiple imputation, and still observed the same associations of MRI lesions with outcomes. Finally, mRS was used as this outcome measure is standardized across institutions, but as the mRS is biased towards motor disability, it may not adequately capture the residual cognitive and/or neuropsychiatric symptoms in NMDARE.[37]

We found that T2-hyperintense frontal and occipital lobe lesions associate with poor outcomes in pediatric NMDARE. Future studies should also explore the association of MRI lesions and locations with residual neuropsychological outcomes.

CHAPTER 3: Brain MRI volumes and their associations with outcomes in NMDARE

3.1. Background and prior work

In addition to T2 lesions, MRIs can also be used to assess brain volumes. Brain volumes can be assessed subjectively by the person reviewing the MRI or by objectively by quantitative software. As discussed in Chapter 2, the most common MRI abnormalities are T2 hyperintense lesions.[22-24] Another imaging feature, "reversible brain atrophy," on MRI has been reported in different cases of NMDARE[39,40] patients. These patients were mostly adults but reports were also included in a 17 year old[39]; 5 patients had reversal of brain atrophy based upon appearance.[41] Moreover, progressive cerebellar atrophy has been associated with worse outcomes in a group of both pediatric and adult NMDARE patients.[23] One prior volumetric analysis in pediatric NMDARE reported that decreased brain volumes in children with NMDARE associated with poor outcomes using the Pediatric Cerebral Performance Category (PCPC), a measure to assess functional status in critically ill children.[42] However, the PCPC may lack precision unless major clinical changes occur and has limited utility in younger children.[43] Correlation of the PCPC has been reported with more detailed neuropsychological measures, with wide variability observed within each PCPC category.[44]

However, MRI volumetric analysis in NMDARE has not been previously performed using a more detailed functional scale, such as the WeeFIM. The WeeFIM (Functional Independence Measure for children) is an 18-item inventory that assess a child's independence across three functional domains of mobility, self-care, and

communication/cognition. These measures are more nuanced than the modified Rankin score (mRS) which is biased towards motor disability, or the PCPC as discussed previously. Associating MRI characteristics with cognitive outcomes may be more clinically relevant and reflective of outcomes in NMDARE since up to 90% of NMDARE patients will report residual cognitive or neuropsychological impairments after recovery from NMDARE.[18] Here we evaluate MRI brain volumes in a group of pediatric NMDARE patients and correlate with functional status scores and one-year outcomes.

3.2. Approach

3.2.1. Study Design and Participants

Retrospective review of patients presenting to Children's Healthcare of Atlanta from 2010 to June 2021 was included in this study. MRIs were included only if high resolution T1 weighted MRI images were available as these are the type of T1 MRI images that are compatible with the FreeSurfer software. FreeSurfer is an open access that is able to analyze and visualize brain structures,[45] and was used to perform volumetric analysis of brain MRIs. Only initial brain MRIs were included if they were obtained prior to treatment, as medications such as steroids and sedation can affect brain volumes. Whole brain volumes, along with total gray matter, total white matter, cerebellum, subcortical gray matter, and lateral ventricle volumes were collected.

WeeFIM scores were obtained upon admission and discharge from inpatient rehabilitation. The WeeFIM Developmental Functional Quotient (DFQ) normalizes raw WeeFIM scores to represent age-appropriate functioning.[46,47] For WeeDFQ, a score of less than 30 was poor or dependent function, a score of 30-84 reflected partial

dependence or moderate, and then 85 or higher marked those who were independent, good, or functioning at an age appropriate level. The pediatric modified Rankin score from 0-6 was calculated at 1 year from disease onset, with 0-2 defined as good and 3-6 defined as poor outcomes.[35]

3.2.2. Statistical analysis

Descriptive statistics were initially used. For continuous variables with normal distributions, the means and standard deviations (SD) were reported versus the median and interquartile range (IQR) for continuous variables with skewed distributions. For discrete variables, the frequency and percentages were reported.

Once the raw volumetric scores from the patients were obtained, the scores were first scaled with age/gender specific means and standard deviations reported from healthy controls in the literature. The percentile for each brain volume segment was then assigned to each value based on the Z score.

Linear regression using Pearson's correlation was applied to assess the correlation between different regional volumes percentiles and outcomes using the WeeFIM and the modified Rankin score (mRS). Then multivariable linear regression was used to assess whether the correlation between brain volume percentiles and outcomes were affected by covariates including time from symptom onset until the MRI was obtained, since MRIs may have been obtained at different times during the disease course.

3.3. Results

3.3.1. Descriptive statistics

While 49 patients with potential NMDARE have presented to CHOA from January 2010 until June 2021, 41 had CSF confirmed antibodies. Ten patients were excluded as the initial brain MRIs were not directly available for analysis and two patients were excluded due to prior herpes simplex virus (HSV) encephalitis (see Figure 3).

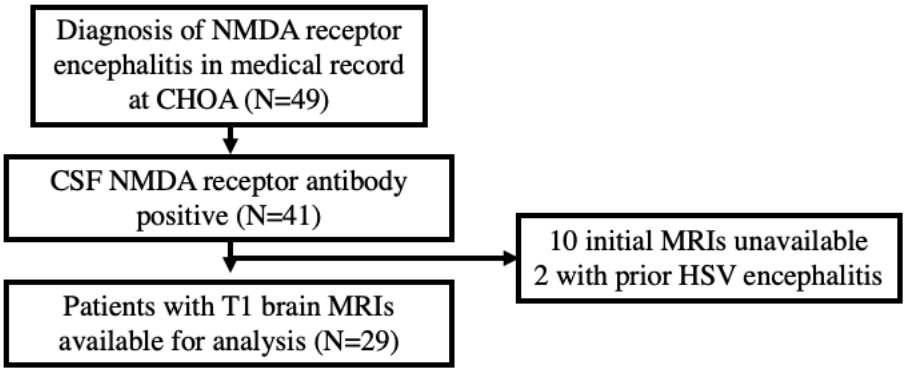


Figure 3. Flow diagram of patients with NMDARE included in the brain MRI volumetric analysis study.

The initial descriptive statistics of this cohort is shown in Table 3. Average age was 11.4 (SD 5.1) years of age, with 22/29 (76%) were female. At admission, 100% of patients had a poor WeeFIM DFQ score of less than 30, whereas at discharge, 16/29 (62%) still had poor/dependent outcomes, versus 8/29 (31%) with moderate/partially dependent, and 2/29 (8%) had good/independent outcomes. For one year outcomes, the modified Rankin Score was used, and found that 9/29 (39%) had poor outcomes at one year.

Table 3. Descriptive statistics for the NMDARE patients included in the brain volumetrics study.

	Mean (SD) or Median (Q1, Q3) or N(%) N = 29
Age (years)	11.38 (5.07)
Female	22 (75.9%)
Hispanic	7 (25.0%)
Length of Hospitalization (days)	23 (16, 51)
ICU admit, N (%)	26 (89.7%)
Length of inpatient rehabilitation stay (days)	42 (21, 49.5)
Time to treatment- first line (days)	16 (10, 24)
Time to treatment- second line (days)	29 (23, 58)
Need for tracheostomy, N (%)	17 (58.6%)
Immunotherapy, N (%)	
Any First-line (Steroids, IVIG, PLEX) N (%)	29 (100.0%)
First-line Steroids (%)	27 (93.1%)
First-line IVIG (%)	28 (96.6%)
First-line PLEX (%)	20 (69.0%)
Any Second-line, N (%)	21 (72.4%)
Second-line Rituximab (%)	21 (72.4%)
Second-line Cyclophosphamide (%)	8 (27.6%)
Ovarian teratoma, N (%)	5 (17.2%)
CSF pleocytosis, N (%)	17 (60.7%)
EEG abnormal, N (%)	25 (86.2%)
DFQ Score at Admission	
Performance in essential daily functional skills	
Poor/Dependent, N (%)	26 (100.0%)
Total DFQ Score	29.14 (15.25)
Self-Care DFQ Score	30.09 (17.87)
Cognition DFQ Score	25.77 (14.21)
Mobility DFQ Score	30.98 (19.78)
DFQ Score at Discharge, N (%)	
Performance in essential daily functional skills	
Poor/Dependent	16 (61.5%)
Moderate/Partially Dependent	8 (30.8%)
Good/Independent	2 (7.7%)
Total DFQ Score	52.4 (27.87)
Self-Care DFQ Score	56.6 (30.67)
Cognition DFQ Score	47.5 (26.85)
Mobility DFQ Score	50.81 (28.67)
Poor outcome (mRS) at 1 year from onset	9 (39.1%)

IVIG: intravenous immunoglobulin, PLEX: plasma exchange, CSF: cerebrospinal fluid, EEG: electroencephalography, DFQ: WeeFIM Developmental Functional Quotient, mRS; modified Rankin score

3.3.2. Associations of brain volumes with outcomes in pediatric NMDARE

First, we examined the associations of brain volumes with outcomes in pediatric NMDARE, including total brain volumes, total gray matter, total white matter, lateral ventricles, and the cerebellum (see Table 4). The higher total WeeFIM DFQ score at admission was associated with lower total brain volume, lower whole-brain grey matter volume, lower whole-brain white matter volume, and lower cerebellum volume. An example of the association of total brain volumes with WeeFIM scores is depicted in Figure 4. The higher total DFQ score at discharge is associated with a lower volume of total brain and whole-brain white matter. As for longer length of stay, the only variable that associated with longer length of stay larger volumes of the lateral ventricles (see Table 4).

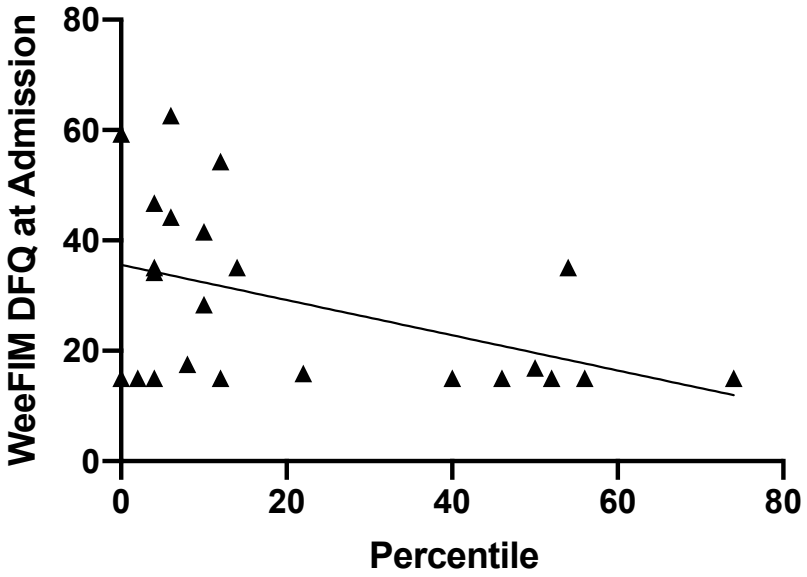


Figure 4. Correlation of WeeFIM DFQ (Developmental Functional Quotient) at admission and percentile of total brain volumes in pediatric NMDARE patients.

Table 4. Correlation between different regional volumes percentile and outcomes without consideration of any other covariates

	Total DFQ at Admission		Total DFQ at Discharge		Length of Stay	
MRI Variables (Percentile)*	Pearson's r	p-value	Pearson's r	p-value	Pearson's r	p-value
Total brain	**-0.67**	**<0.001**	**-0.45**	**0.031**	-0.09	0.678
Whole-Brain GM	**-0.58**	**0.004**	-0.16	0.465	-0.15	0.5
Whole-Brain WM	**-0.41**	**0.050**	**-0.44**	**0.038**	-0.09	0.697
Subcortical GM	-0.26	0.240	-0.03	0.909	-0.1	0.646
Lateral ventricles	-0.12	0.575	-0.18	0.409	**0.42**	**0.044**
Cerebellum	**-0.55**	**0.007**	-0.28	0.192	-0.12	0.599

Note: Estimates with a p-value less than 0.05 were **bolded**. Pearson's correlation was applied to assess the correlation between different regional volumes percentile and outcomes. GM: gray matter, WM: white matter, DFQ: WeeFIM Developmental Functional Quotient

We then assessed whether brain MRI volumes associated with one year modified Rankin scores, by comparing Z scores in the patients with good (mRS 0-2, N = 14) versus the Z scores in the patients with poor outcomes (mRS 3-6, N = 8) at one year. However, no differences were observed in all the brain volume parameters, including whole brain, total gray matter, total white matter, lateral ventricles, and cerebellar volumes.

3.3.3. Multivariable linear regression modeling of association of brain volumes with outcomes in pediatric NMDARE

We then performed multivariable linear regression modeling by adjusting brain volumes with other covariates, including age and sex, as both of these can affect brain volumes. We also included the time from disease onset to time of MRI as this can also vary

between patients to determine whether timing of MRI affects our results. After adjusting for age, sex, and days between symptom onset and timing of MRI, whole brain gray matter and whole brain white matter associated with total WeeFIM DFQ at admission and at discharge, and then cerebellar volumes were associated with WeeFIM discharge scores (see table 5).

Table 5. Regression models between WeeFIM DFQ (Developmental Functional Quotient) and different regional volumes Z scores, adjusted for age, sex, and days between symptom onset and MRI.

Z score	Total DFQ at Admission, Estimate (95% CI)	p-value	Total DFQ at Discharge, Estimate (95% CI)	p-value
Total brain	-5.8 (-13.44, 1.84)	0.214	-16.35 (-28.88, -3.82)	0.037
Whole-Brain GM	-1.64 (-9.93, 6.65)	**0.047**	-16.37 (-29.26, -3.49)	**0.031**
Whole-Brain WM	-7.43 (-17.48, 2.62)	**0.007**	-12.21 (-30.82, 6.39)	**0.092**
Subcortical GM	0 (-6.34, 6.35)	0.398	-5.52 (-16.8, 5.75)	0.254
Lateral ventricles	-3.15 (-9.28, 2.98)	0.409	-4.88 (-16.19, 6.43)	0.320
Cerebellum	-2.44 (-8.5, 3.61)	0.104	-12.35 (-21.83, -2.87)	**0.018**
Brainstem	-5.8 (-13.44, 1.84)	0.214	-9.57 (-25.04, 5.9)	0.086

Note: Estimates with a p-value less than 0.05 were **bolded**. GM: gray matter, WM: white matter, DFQ: WeeFIM Developmental Functional Quotient

3.4. Discussion

Here we assessed whether quantitative volumetric assessments of the brain in pediatric NMDARE were associated with outcomes in NMDARE. Surprisingly, we found an inverse correlation with brain volumes and WeeFIM DFQ scores, which is the

opposite of what has been previously reported of lower brain volumes associated with poor outcomes.[42] We found this inverse association despite adjusting for time to when MRI was obtained and adjusting for sex and age, since these variables may affect brain volumes. One potential reason for observing this inverse relationship could include that patients with more severe outcomes may have had more brain edema at the time of onset so that could be another variable to examine in future studies.

Strengths of this study include that brain volumes were measured quantitatively and not qualitatively, and that the initial brain MRI prior to treatments were used to assess brain volumes. We also used a different metric that includes cognitive outcomes in NMDARE, which is not often used as an outcome metric, when residual deficits in NMDARE are predominantly cognitive and not motor disability. We also have a relatively larger number of subjects included in this study as pediatric NMDARE is a rare disease. Moreover, we included outcomes at a standard time of one year from onset, whereas prior work used outcomes at the last follow up timepoint, which is variable for patients.[42]

3.4.1. Limitations

Limitations of this study include that patients were included from a single center. Moreover, we had some missing data in which patients without brain MRIs available for analysis were excluded, which may also introduce bias. Another limitation is the timing of when the initial MRI is obtained is variable among patients from time from disease onset but we used time between onset and date of first MRI to adjust for this potential confounder. We also used FreeSurfer and age and sex normalized to historical controls,

without using internal controls. This could be mitigated by using NeuroQuant in future studies.

3.4.2. Conclusions

Here I assessed quantitative brain MRI volumes and their associations with outcomes in pediatric NMDARE and found an inverse correlation. Further studies to confirm and to assess why this relationship is observed will be pursued.

CHAPTER 4: Electroencephalography predictors for outcomes in pediatric NMDARE

In this chapter another ancillary test, electroencephalography (EEG) and its features associated with poor outcomes in pediatric NMDARE, will be discussed. This work has been published.[27]

4.1. Background and prior work by others

4.1.1. Introduction to EEG

Before embarking on the discussion of EEG findings in NMDARE, I will first provide a brief introduction to EEG. Neuronal activity is communicated through electrical signaling, which can be captured by electrodes near the brain, usually on the scalp, through EEG. Scalp or surface EEG measures the electrical activity and summation of the synchronization of millions of cortical neurons. EEG is useful as a neurodiagnostic tool and is often used in patients with abnormal movements to assess for seizures or in those with altered mental status. While EEGs are most commonly used to diagnose seizures or epilepsy, many neurophysiological processes can be assessed by EEG even in the absence of seizures. EEG waves can differ depending on whether the brain is awake, drowsy, or asleep; or even if the brain is under sedation.[48]

EEG waves can be divided into the number of oscillations per second, or frequency, which is measured in Hertz (Hz). These frequencies are divided into different categories, including infra-slow oscillations (less than 0.5 Hz), delta (0.5 to 4 Hz), theta (4 to 7 Hz), alpha (8 to 12 Hz), beta (13 to 30 Hz) and high frequency oscillations (greater than 30 Hz). Sleep stages can also be assessed using EEG, including N1, N2, N3 (also known as slow wave sleep), and rapid eye movement (REM) sleep (see Figure 5).

One EEG feature, called the posterior dominant alpha rhythm (PDR), which is 8 to 12 Hz, is usually present in normal awake EEG and most prominently in the occipital EEG electrodes (see Figure 6). This PDR is observed in the normal background of awake individuals, usually starting around 3 years of age and into adulthood. A normal PDR can be absent in patients with neurological pathology and medications.

Figure 5. Representative electroencephalography (EEG) features. EEGs are presented in standard bipolar montage with sensitivity set at 7 microvolts and a time base of 30 mm per second. Part A demonstrates sleep stages: N1 as identified with vertex waves, N2 demonstrating sleep spindles and vertex waves, N3 which is identified by slow wave sleep, and then REM (rapid eye movement) sleep. Each dark green vertical line is 1 second. Note: this figure is published:[27]

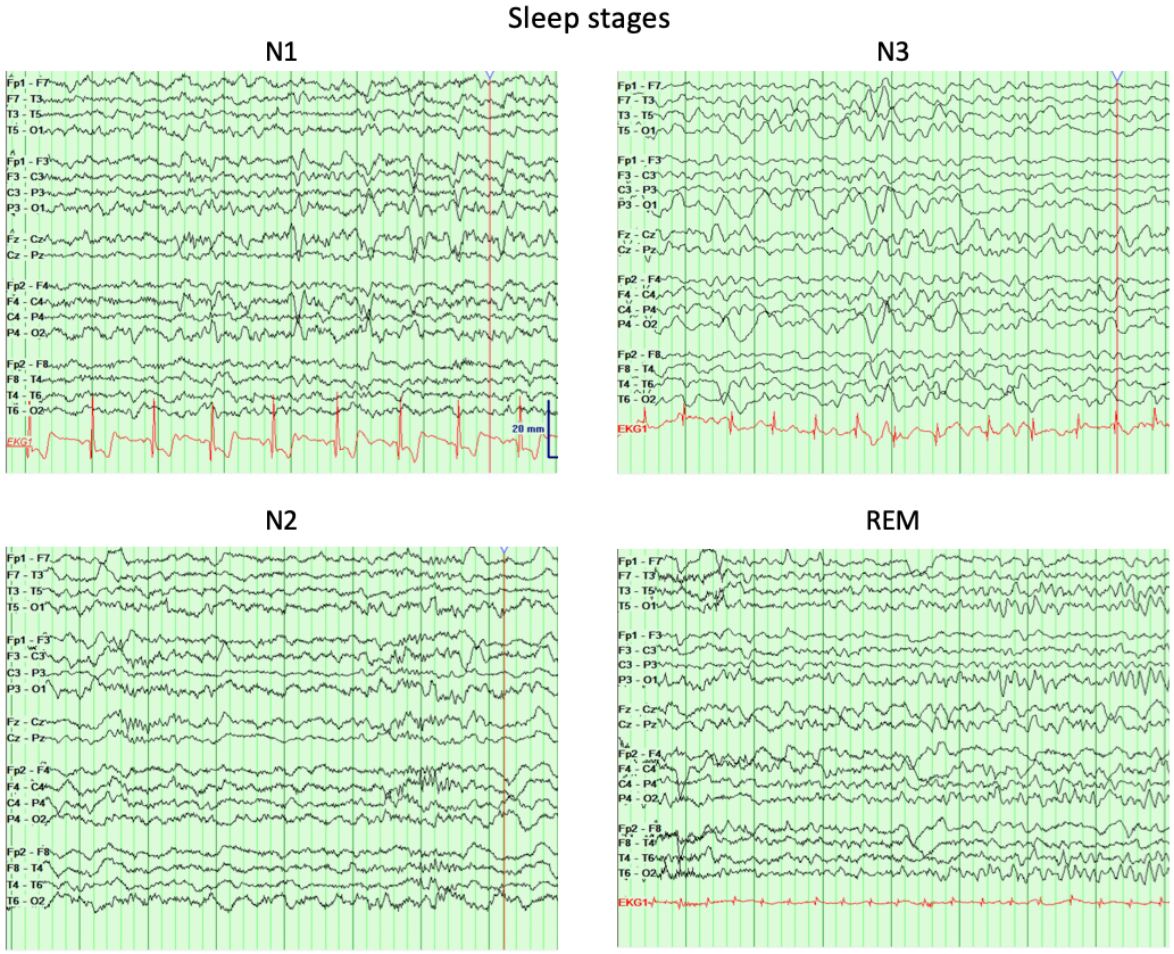


Figure 6. Example of an EEG with normal awake background and a normal posterior dominant rhythm of 8-9 Hertz. Each dark green horizontal line is 1 second. Note: this figure is published:[27]

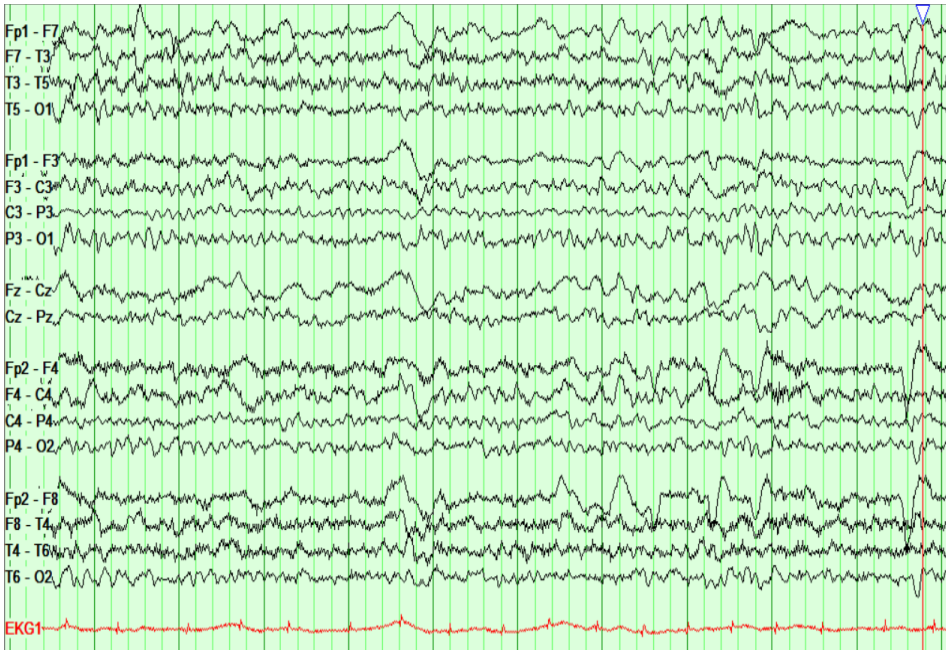
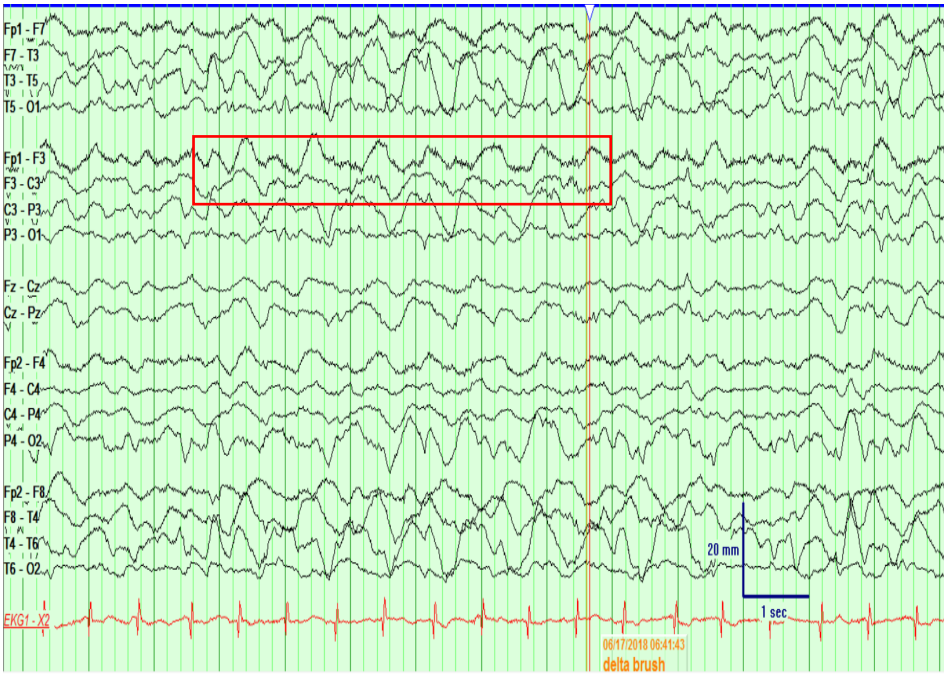


Figure 7. An example of extreme delta brush, with prominent delta waves accompanied by overlying lower amplitude beta activity in the bifrontal leads, which is highlighted by the red box. Each dark green horizontal line is 1 second. Note: this figure is published:[27]



4.1.2. EEG features reported in patients with NMDARE

EEGs are often abnormal in NMDARE, with up to 90% of patients having an abnormal EEG at the time of presentation.[49] However, the types of EEG abnormalities are variable in NMDARE. The most commonly described EEG features pediatric NMDARE include background slowing in 77-80%, rhythmic delta activity in 59-86%, and interictal epileptiform discharges in 56-89%.[2,4,8-10] Another EEG features termed "extreme delta brush" (EDB) has been of interest in NMDARE as this feature has been identified as an electrophysiologic biomarker of NMDARE. Delta brush is characterized by slow delta waves (0.5 to 4 Hz as described in 4.1.1.) with overlying beta activity (13 to 30 Hz, see Figure 7). Delta brush can be normal in premature infants but should not be seen in children or adults. EDB which appears similar to delta brush is identified in 30% of adults with NMDARE and is associated with prolonged hospitalization and potentially worse outcomes.[50] In a meta-analysis of 1550 adult and pediatric NMDARE patients, EDB has been associated with poor outcomes. Rates of EDB in pediatric NMDARE have previously been reported as between 6-53%.[51-53] Reported rates of electrographic seizures range from 11-52% with status epilepticus in 0-50%.[2,4,8-10] Sleep disturbances have also been reported in patients with autoimmune encephalitis with changes often seen in stage III and REM sleep.[5] While changes in sleep architecture have been reported in autoimmune encephalitis [54], whether electrophysiological changes in sleep architecture are associated with outcomes in NMDARE are unknown. Moreover, limited studies are available on whether EDB and other EEG characteristics correlate with clinical course and prognosis particularly in children with NMDARE. Here I

discuss electrophysiological characteristics including sleep architecture in a pediatric NMDARE population and their association with one-year outcomes.

4.2. Study Design and Methods:

4.2.1. Study design

Institutional Review Board approval was obtained for this study. Retrospective chart review and EEGs was performed in children with NMDARE who presented to Children's Healthcare of Atlanta between January 1, 2017 and April 1, 2021. Patients were identified with the diagnostic code for anti-NMDA receptor autoimmune encephalitis (G04.81). NMDARE was diagnosed in patients with a confirmed positive cerebrospinal fluid (CSF) anti-NMDA receptor antibody test and if patients had at least one of six clinical neuropsychiatric symptoms consistent with NMDARE (seizures, psychosis, movement disorders, speech regression, autonomic instability, and/or central hypoventilation) [30]. Patients were included if the initial EEG prior to treatment were available and 1 year follow up were included. All initial EEGs occurred in less than 48 hours of admission to the hospital for NMDARE and prior to treatment. Fourteen NMDARE patients were identified; however, five patients were excluded due to the following reasons: one had no EEGs available for re-review; one had EEGs available but the first EEG obtained within 48 hours of presentation was unavailable for review; one had prior herpes simplex viral encephalitis which would result in the EEG abnormalities, and two patients did not have one year follow up data available. The first EEG was reviewed, which was 24 hours of the full recording in all patients to provide enough time to capture sleep except for one patient with a routine EEG. For the patient

with the routine EEG, despite being shorter than 24 hours, sleep stages were captured and thus was included in this study.

4.2.2. EEG characteristics and EEG review

EEG characteristics included extreme delta brush, absence or presence of a normal posterior dominant rhythm (PDR), generalized rhythmic delta activity (GRDA), epileptiform discharges, electrographic seizures, and presence or absence of sleep architecture (see Figures 5 and 6). Sleep was considered present if N1, N2, N3 and/or REM was present. N1 was defined by the presence of vertex waves; N2 was identified if sleep spindles or K complexes were present; N3 was defined with the presence of slow waves, and REM was defined by the presence of central saw tooth waves (Figure 5). EEGs were independently reviewed by two epileptologists, and a third in the case of disagreement.

4.2.3. *Clinical Outcomes*

Primary clinical outcomes included the modified Rankin scale (mRS), a measure of neurological disability[35], at 1 year from diagnosis. The mRS is a measure of functional motor status and has been used as a measure of disability after neurological symptoms, including after stroke.[35]

A good outcome was defined as mRS between 0-2 and a poor outcome was defined as mRS 3 or greater. Secondary outcomes assessed included hospital length of stay (LOS), inpatient rehabilitation LOS, and number of immunotherapies needed, as longer LOS and higher number of immunotherapies administered are generally associated with more severe NMDARE.

4.2.4. Statistical analysis

Statistical analyses were performed using SAS v.9.4 (Cary, NC). Descriptive statistics were performed to discuss rates of EEG features and other clinical characteristics in our cohort. For continuous variables, means and standard deviations were reported for relatively normally distributed populations. Medians and interquartile ranges were reported for skewed data. Percentages and frequencies were reported for proportions. Differences in characteristics between patients with good and poor outcomes at one year were assessed using the Student's t-test for means for approximately normal distributions, Wilcoxon's rank-sum test for skewed data, and the chi-square test for proportions.

For each EEG characteristic, initially we performed univariate analyses for good versus poor outcomes. We then performed a multivariable logistic regression model with good versus poor outcomes at one year as defined by mRS as the outcome variable and then included a backwards stepwise regression analysis to determine the optimal model of EEG characteristics that predicted poor mRS outcomes at one year. We also performed a multivariable linear regression model of one year mRS outcomes with backwards stepwise regression analyses to identify the optimal EEG characteristics that associated with one year outcomes.

4.3. Results:

Individual and overall patient characteristics are noted in Table 6. Six girls and three boys were included. Median age at presentation was 12.7 years (IQR 8.8, 15.8). EEG characteristics for each patient is listed in Table 7. Loss of PDR was noted in five of nine (56%) patients were noted at the beginning and persistent throughout the 24-hour

recording. EDB was present in six of nine (67%) patients; interestingly, evolution of delta brush (i.e., more prominent) was noted over the 24 hours in two patients who had poor outcomes but for the rest of the patients, the background did not change during the initial 24-hour recording. For one patient with just the routine EEG, all normal features were present, including sleep stages were captured (Patient 7). Seven of nine patients (78%) reported sleep problems, but only three (33%) had loss of sleep architecture on EEG. If sleep architecture were present on EEG, most patients had N1, N2, N3 with or without REM with the exception of the one routine EEG that had N1 and N2, but not N3. Six of nine (67%) patients reported clinical seizures and three of nine (33%) had epileptiform discharges, but no electrographic seizures were captured on initial EEG for all patients. The clinical seizures occurred prior to the EEG recording and did not occur during the EEG. While delta brush, epileptiform discharges, and loss of sleep architecture were observed in three of nine (33%) patients, these characteristics were found in unique patients and not in the same patients. Absence of posterior dominant rhythm (PDR) and presence of generalized rhythmic delta activity (GRDA) were each found in 4 (44%) patients, but also in different patients (see Table 7).

The number of and specific immunotherapies administered, inpatient hospital length of stay (LOS), inpatient rehabilitation LOS, and total hospitalization LOS (inpatient hospital plus inpatient rehabilitation LOS) were examined for each EEG characteristic. Loss of PDR was associated with increased length of inpatient rehabilitation (28.8 versus 10 days, $p = 0.026$) as did loss of sleep architecture (30.3 versus 13.3 days, $p = 0.020$). The number of immunotherapy treatments administered was also increased in

patients with loss of PDR (4.33 vs 3.25, p = 0.014) and loss of sleep architecture (4.67 versus 3.5, p = 0.045; Figure 8).

Table 6: Individual and summary of clinical characteristics of pediatric NMDARE patients. Note: this table is published:[27]

Patient Number	Age (years)	Sex	Hospital LOS (days)	Rehab LOS (days)	Clinical Seizures	Immune tx number	Immune tx given	Sleep Problems
1	8.6	M	15	28	+	4	IVMP, IVIG, PEX, RTX	-
2	14.1	M	11	13	+	3	IVMP, IVIG, RTX	+
3	14.9	M	4	0	+	3	IVMP, IVIG, RTX	-
4	1.9	F	7	8	+	3	IVMP, IVIG, RTX	+
5	9.0	F	6	12	-	4	IVMP, IVIG, PEX, RTX	+
6	11.7	F	24	23	+	5	IVMP, IVIG, PEX, RTX, CYC	+
7	12.7	F	40	19	-	4	IVMP, IVIG, PEX, RTX	+
8	16.7	F	22	33	-	4	IVMP, IVIG, PEX, RTX	+
9	16.7	F	169	35	+	5	IVMP, IVIG, PEX, RTX, CYC	+
Total	Median 12.7 (IQR 8.8, 15.8)	3M: 6F	Median 15 (IQR 6.5, 32)	Mean 19 (SD 11.8)	N= 6 (67%)	Mean 3.9 (SD 0.8)		N = 7 (78%)

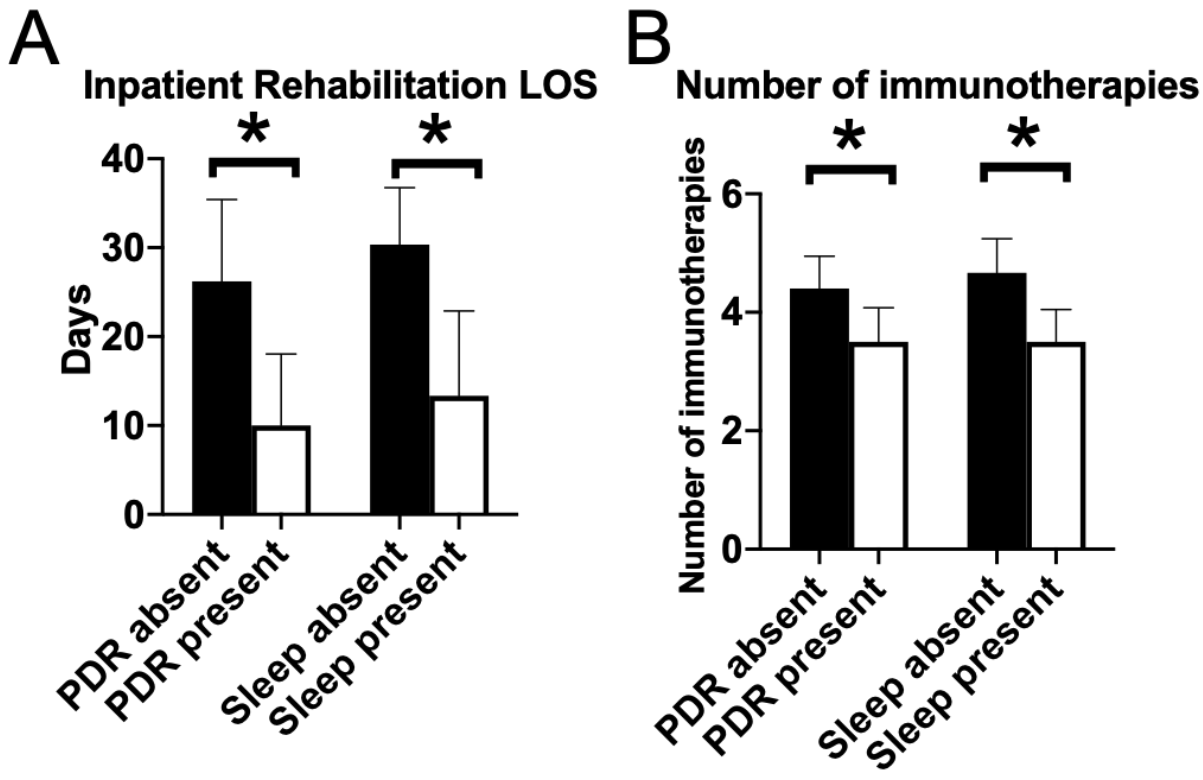
M = male, F= female, LOS: length of stay, tx: treatment, IVMP: intravenous methylprednisolone, IVIG: intravenous immunoglobulin, PEX: plasmapheresis, RTX: rituximab, CYC: cyclophosphamide

Table 7. Individual and summary of EEG characteristics in pediatric NMDARE patients. Note: this table is published:[27]

Patient Number	**EEG patterns present? (+/-)**					**mRS at one year**
	Delta Brush	GRDA	Normal PDR	Epileptiform discharges	Sleep stages	
1	+	+	-	-	N1, N2, N3, REM	3
2	-	-	+	-	N1, N2, N3	0
3	-	-	+	-	N1, N2. N3	0
4	+	-	+	+	N1, N2, N3, REM	0
5	+	-	-	-	N1, N2, N3	4
6	+	+	-	+	-	3
7*	-	-	+	-	N1, N2[#]	2
8	+	+	-	-	-	0
9	+	+	-	+	-	4
Total	N=6 (67%)	N = 4 (44%)	N = 4 (44%)	N = 3 (33%)	N=6 (67%)	Mean 1.8 (SD 1.8)

EEG: electroencephalogram, GRDA: generalized rhythmic delta activity, PDR: posterior dominant rhythm, mRS: modified Rankin score
*Designates routine EEGs.
#N3 not captured on this routine EEG.

Figure 8. The effects of posterior dominant rhythm (PDR) and sleep architecture on inpatient rehabilitation length of stay (LOS, A) and number of immunotherapies administered (B). *indicates $p < 0.05$. Note: this figure is published:[27]



One-year outcomes utilizing the modified Rankin scores (mRS) were assessed by EEG characteristic. Loss of PDR predicted poor outcomes at one year (Fischer's exact test, $p = 0.048$). We then performed a multivariable logistic regression analysis to determine which EEG characteristic predicted poor one-year outcomes, but no EEG characteristics predicted poor one-year outcomes. We then performed a multivariable linear regression model to predict one-year MRS scores. We initially included all EEG variables and then removed a variable in a backwards stepwise fashion. After GRDA and delta brush were removed, loss of PDR ($p = 0.013$), loss of sleep architecture ($p = 0.041$), and epileptiform discharges ($p = 0.041$) correlated with mRS at one year.

4.4. Discussion:

Electrographic characteristics may predict outcomes in NMDARE. EDB, an electrophysiologic NMDARE biomarker, is associated with prolonged hospitalization in adults [50]. Additionally, normal posterior dominant rhythm (PDR) has been associated with better outcomes[52] whereas greater than 50% slow waves have been associated with worse prognosis [55]. In our cohort, loss of PDR and absent sleep architecture were suggestive of more severe clinical course as evidenced by an increased number of immunotherapies administered and longer inpatient rehabilitation. This study may reflect a more severely impacted patient cohort as our patients received a high number of immunotherapies.

While loss of PDR was the only EEG characteristic associated with higher mRS scores at one year, we found that absent sleep architecture and epileptiform discharges also may correlate with worse outcomes at one year. These findings have not been previously reported, to our knowledge. These characteristics were associated with one-year outcomes, even when adjusting for PDR. If sleep was present, then the 24-hour EEG was mainly helpful in capturing slow wave and/or REM sleep, as the one patient without N3 or REM captured was a routine EEG. In patients in which sleep was absent, even having the prolonged recording of 24 hours did not capture sleep. Two patients had more evolving delta brush as the recording continued so the 24-hour recording was helpful to detect prominent delta brush. However, the 24-hour recordings did not alter the results of the study except to capture sleep. Nevertheless, whether evolution of features associates with prognosis would be interesting to examine in a larger cohort.

The EEG changes are attributable to the pathophysiology of NMDARE. Anti-NMDA receptor antibodies are pathogenic and cause receptor downregulation. This downregulation disrupts multiple networks, including loss of inhibition. This loss of inhibitory synapses results in neuronal excitation and subsequent epileptiform discharges and seizures [16]. Additionally, the NMDA receptor is important in sleep circuits [56] and anti-NMDAR antibodies likely disrupt sleep pathways. Interestingly, different EEG features were observed in different patients, which demonstrates the variable electrophysiological phenotypes.

The limitations of this study include a small number of patients at a single institution. Another limitation is that inpatient rehabilitation length of stay (LOS) may be influenced by other factors such as insurance coverage along with functional outcomes. Despite the small sample, we were able to identify potential EEG characteristics that correlate with one-year outcomes. Additionally, we examined outcomes at a set timepoint of one year. This is in contrast to prior studies that examined EEG features with outcomes only did so at variable times after diagnosis, such as greater than 6 months[52] or at time of discharge[55].

In conclusion, loss of normal PDR, absence of sleep architecture, and epileptiform discharges are associated with higher modified Rankin scores at one year in NMDARE. Larger studies are needed to confirm these findings. Understanding the underlying mechanisms in the heterogeneity in clinical presentation and prognosis is important to improve treatments and outcomes in NMDARE

CHAPTER 5: Future Work

In this chapter, I will discuss future work that will build upon the projects in this thesis and the next steps.

5.1. The CONNECT registry

The CONNECT registry (CONquering Neuroinflammation and Epilepsies ConsorTium) is the first United States based multi-center prospective patient registry for autoimmune encephalitis. A goal of CONNECT is to connect patients and families with rare diseases, with institutions and providers; CONNECT also aims to connect clinical symptoms with ancillary testing features, neuropsychological assessments, patient and caregiver reported outcomes, and biospecimens to understand the underlying pathogenic pathways leading to autoimmune encephalitis. CONNECT includes the initial raw EEG and MRI files in a central repository for independent review, which will make this future work feasible. This prospective registry will have 12 sites enrolling patients by 2025.

5.2. Magnetic resonance imaging (MRI) brain lesions and their association with outcomes

In Chapter 2, I discussed how T2 lesions located in the frontal and occipital lobes associated with poor outcomes at one year in pediatric NMDARE. However, the outcome measure was using the modified Rankin Score (mRS). While the mRS is a commonly and easily used score with its ease of calculation, the mRS is skewed

towards motor functioning and less of the cognitive outcomes which are commonly seen in NMDARE. In future studies in CONNECT, I aim to associate T2 lesion location and specific neuropsychological measures using formal neuropsychological tests. Moreover, I will have the capability of calculating lesion volume in a particular location in the brain, and be able to associate those volumes with outcomes in NMDARE.

NeuroQuant and NeuroQuant MS (formerly known as LesionQuant) are imaging software tools able to perform brain volumetric analyses with comparison to age-matched healthy controls and also automatically label and quantify volumes of segmentable brain structures and lesions from MRI brain studies. NeuroQuantMS™ is a module within NeuroQuant® that can identify and monitor lesion changes over time. NeuroQuant was the first FDA approved medical software for fully automated analysis of images. NeuroQuantMS has the ability to automatically label and assess volumetric quantification of T2-weighted lesions from MRI FLAIR (fluid-attenuated inversion recovery) images. Based upon the work presented in Chapter 2 on MRI lesion location and their association with outcomes in NMDARE, NeuroQuantMS would be the next step is assessing whether lesion volumes are associated with outcomes in NMDARE.

5.3. Brain volumetric changes in NMDARE and their association with outcomes

5.3.1. Brain structure volumes and their association with outcomes in pediatric NMDARE

In Chapter 3, I discussed MRI volumes of main brain structures and their associations with outcomes in NMDARE. Future work would be to utilize NeuroQuant will be a more detailed method to assess brain structures including normalization with

age-matched healthy controls to assess whether a particular part of the brain is more predictive of outcomes in NMDARE. For example, using NeuroQuant, we could examine whether hippocampal, basal ganglia, or cerebellar size associate with outcomes. The hippocampus is important for memory and memory is often affected in NMDARE; the basal ganglia are involved in movement disorders, and cerebellar atrophy is associated with poor outcomes in adults with NMDARE. I could also explore thalamic atrophy, as this has been shown to correlate with cognitive dysfunction in adult patients with multiple sclerosis, another type of neuroinflammatory disease.[57] NeuroQuant would be able to further assess specific structures within the brain that my previous analyses were unable to perform.

5.3.2. Brain structure volumes and their association with outcomes in pediatric NMDARE

Moreover, we can examine serial MRIs to see whether changes in brain volumes including overall brain volume and volumes of brain structures are correlated with poor outcomes in NMDARE. One prior study in adults with NMDARE demonstrated that progressive cerebellar atrophy correlated with poor outcomes. We would also examine whether cerebellar atrophy is observed in children with NMDARE and whether this atrophy would also predict poor outcomes in children.

5.4. *Future work for EEG characteristics and their associations with outcomes in pediatric NMDARE*

The work presented in chapter 4 was performed in a small group of patients. We would leverage the prospective multicenter registry connect to validate these findings in a larger cohort. Additionally, this EEG study was performed at a single institution, and having a multi-institutional study could reduce patient ascertainment bias. Moreover, while extreme delta brush did not correlate with poor outcomes in our cohort, a meta-analysis of NMDARE patients suggested that extreme delta brush is predictive of poor outcomes.

We could also examine serial EEGs to see if there any features in subsequent EEG that can predict prognosis. Some of these features could include persistent loss of a posterior dominant rhythm, persistent absence of sleep stages, and/or epileptiform discharges. We could also examine whether time to normalization of EEG is predictive of good outcomes. We could also examine EEG features that predict the risk for long term epilepsy in NMDARE, as long-term epilepsy is rare, and most patients are successfully weaned off of antiseizures medications after one year.

5.5. *Blood and cerebrospinal fluid biomarkers to predict outcomes in pediatric NMDARE*

While not discussed in this thesis, additional future studies include examining blood and cerebrospinal fluid for biomarkers to predict prognosis. We could also examine whether these markers associate with severe or abnormal EEG or MRI features in NMDARE.

CHAPTER 6: CONCLUSIONS

Anti-NMDA receptor encephalitis (NMDARE) is rare in children but is a common cause of encephalitis in this age group. NMDARE can result in morbidity and mortality, and even those with apparently good outcomes, as assessed by the modified Rankin Score, have residual neuropsychiatric symptoms including cognitive impairment. Early treatment response is crucial to improved long term outcomes. However, biomarkers to guide early aggressive treatments are lacking. Aggressive treatments may not be needed in all with NMDARE, and these treatments have risk and side effects that have to be weighed with the potential benefits. My ultimate goal is to identify a treatment and prognostic biomarker to help identify those who would benefit from earlier aggressive therapy.

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