A study of the epidemiological, clinical features, and outcomes of patients with neuromyelitis optica spectrum disorder attending a South African tertiary referral hospital.

by

Manqoba Vusumuzi Gule

SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

In fulfilment of the requirements for the degree

Master's in Medicine (Neurology)

Department of Neurology

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

28th December 2022

Supervisors:

Dr E. Lee Pan MBChB, MMed (Stell.)

A. Prof L.M. Tucker MBChB, MSc (Lond.) PhD (Cantab.)

Word Count: 19 881

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ACKNOWLEDGEMENTS

I want to thank my supervisors, Dr E. Lee Pan and Prof LM Tucker, for their guidance and supervision, Dr H. Hussey and the Provincial Government of the Western Cape Data Centre, for their contribution to population platform data and statistical analysis, and my colleagues in the neurology service for referring cases to the audit.

Lastly, I would like to thank the Groote Schuur Hospital Neurology service patients, whom it is my great privilege to serve. In particular, I would like to thank those patients suffering from NMOSD who informed this audit and for whom the results of this study aim to improve standards of care.

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A study of the epidemiological, clinical features, and outcomes of patients with neuromyelitis optica spectrum disorders attending a South African tertiary referral hospital.

Abstract

NMOSD is a severe CNS inflammatory disorder classically characterised by recurrent bouts of optic neuritis and myelitis.

The pathogenic anti-aquaporin-4 antibody is present in most cases and distinguishes it from other forms of inflammatory CNS demyelination. This biomarker has led to recognising a broader clinical spectrum of NMOSD. Another recently discovered antibody, the anti-myelin-oligodendrocyte-glycoprotein-antibody, further broadens the spectrum of pathophysiological mechanisms and clinical presentations of NMOSD.

NMOSD may be associated with infections, autoimmune diseases, and malignancies. Observational data from South Africa suggests an association between NMOSD and tuberculosis.

Ethnicity and geographic locality play an important role in the epidemiology of NMOSD. Worldwide, non-European, particularly Black-African and Asian ethnicity, is associated with the highest incidence, prevalence and severity of NMOSD. Despite this, sub-Saharan African studies are under-represented in the medical literature.

NMOSD is typically associated with aggressive attacks, which may recur, often in temporal clusters. When left untreated, NMOSD may result in severe permanent disability and death.

Immune-based therapies are used to manage acute attacks and prevent recurrences. These include steroids and plasmapheresis, steroid-sparing agents, and large-molecule biological agents. Novel and highly effective disease-modifying treatments are continually being developed and examined in clinical trials. These agents are expensive, restricting their use in low-and middle-income settings.

The prevalence of NMOSD in the study population remains unknown, nor is there local data on the clinical spectrum, or whether an infectious trigger such as TB or HIV plays a role.

This audit evaluated the characteristics of a cohort diagnosed with NMOSD attending a South African tertiary hospital. These included demographic, clinical, serological, radiologic, and therapeutic interventions and patient outcomes.

We highlight serious shortcomings in case recognition and referral pathways. In our setting, NMOSD is under-recognised at district care facilities where 67% (26/39) of early NMOSD attacks presented and were not recognized. A further 38% (15/39) had recurrent admissions with unrecognized attacks. Moreover, 51% of patients with AQP4-Ab-positive serology captured by the PGWC Data-Centre did not attend the referral neurology service.

The demographic profiles of our cohort were similar to others that have been reported. Most of our patients were young women of non-European ancestry: Mixed-race (Coloured) and Black-African ethnicity.

HIV and antecedent or concurrent tuberculosis were the most common comorbidities.

At the neurology service, the AQP4-Ab-positivity rate was lower than compared with international cohorts. This was compounded by 20% of cases being diagnosed with NMOSD despite not meeting diagnostic criteria. This raises the possibility of misdiagnosis and inappropriate management.

Plasmapheresis is an highly effective, albeit an expensive, treatment for acute attacks. Only 30% of patients were treated with plasmapheresis. The most frequently cited reasons were limited access and cost. Although understandable in low-and-middle-income settings, advocating for effective, equitable treatments materially affects patient outcomes. Robust local evidence of the disease burden and overall cost implications of relapses and subsequent disability will support this objective.

Data from this, and other similar audits, will inform the development of evidence-based and cost-effective practices to guide immunotherapy and management strategies for NMOSD in resource-limited settings.

Word Count: 496

List of Abbreviations in alphabetical order

AChR-Ab	acetylcholine receptor antibody
ADEM	acute demyelinating encephomyelitis
ANCA	antineutrophil cytoplasmic antibodies
anti-dsDNA	anti-double stranded DNA
Anti-MOG	anti-myelin oligodendrocyte glycoprotein
aOR	adjusted odds ratio
APLS	antiphospholipid syndrome
AQP4-Ab	anti-aquaporin-4 antibody
ARR	annualised relapse rate
BA	black-african
CBA	cell-based assay
CD4	cluster of differentiation-4
CMV	cytomegalovirus
CNS	central nervous system
CRP	C-Reactive protein
CSA	cyclosporine
CSF	cerebrospinal fluid
CYC	cyclophosphamide
DM	diabetes mellitus
EAE	experimental autoimmune encephalomyelitis
EBV	Epstein-Barr virus
EDSS	extended disability severity scale
ELISA	enzyme-linked immunoassay
ESR	erythrocyte sedimentation rate
FLAIR	fluid attenuated inversion recovery
FTA	fluorescent treponemal antigen
GBS	Guillain-Barré syndrome
GSH	Groote Schuur Hospital
HAART	highly active antiretroviral therapy
HDS	high-dose IV steroids
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen

HREC	Human Ethics Research Committee
HTLV	human T-lymphotropic virus
IFA	Immunofluorescence-tissue assay
IgG	immunoglobulin-G
IPND	International Panel for NMOSD Diagnosis
IV	intravenous
KZN	KwaZulu-Natal
LETM	longitudinally extensive transverse myelitis
MG	myasthenia gravis
MMED	Masters in medicine
MOG	myelin oligodendrocyte glycoproteins
MOGAD	MOG-associated disease
MRA	mixed-race african
MRI	magnetic resonance imaging
MRS	modified rankin scale
MS	multiple sclerosis
NMO	neuromyelitis optica
NMOSD	neuromyelitis optica spectrum disorder
OCB	oligoclonal bands
ON	optic neuritis
PCR	polymerase chain reaction
PLEX	plasma exchange / plasmapheresis
RA	rheumatoid arthritis
RPR	rapid plasma reagin
SLE	systemic lupus erythematosus
SS	Sjogren's syndrome
SSA	steroid sparing agents
TM	transverse myelitis
TPAB	*Treponema pallidum* antibody
VDRL	venereal diseases research laboratory
VZV	varicella zoster virus

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Background and review of the literature:

Search Strategy and Selection Criteria

A search of PubMed for the terms "neuromyelitis optica", "neuromyelitis optica spectrum disorder", "Devic's disease/syndrome", "opticospinal MS", "parainfectious NMOSD", "tuberculosis and NMOSD", "HIV and NMOSD" "NMOSD in Africa" was conducted. We reviewed original articles, unpublished theses, and narrative and systematic reviews that included the above. The bibliographies of relevant sources were also examined to identify further relevant sources where appropriate.

Clinical Features, pathophysiology, radiology, and biomarkers

Neuromyelitis optica spectrum disorder (NMOSD) is a severe CNS inflammatory disorder, which was classically thought to be characterised by optic neuritis and concurrent myelitis. These are typically severe and disabling and may be recurrent. Other characteristic presentations include bouts of intractable hiccups and vomiting due to area postrema involvement. Without intervention, NMOSD often has an aggressive course with multiple relapses associated with the accumulation of severe disabling deficits[1,2].

In the past, NMOSD was widely regarded as an endemic variant of multiple sclerosis (MS) that was particular in some areas of the world, with individuals from the far east being more susceptible. However, it is now clear that NMOSD is a separate clinical entity distinct from MS in terms of clinical presentation and aetiology[3]. NMOSD is typically relapsing but may be self-limiting. Relapses can occur several years and even decades after the initial attack[1,4].

Recently, an underlying autoimmune mechanism has been identified. In most cases, NMOSD is associated with the pathogenic anti-aquaporin-4 antibodies (AQP4-Ab), which target astrocyte epitopes in susceptible individuals[5,6]. This process results in secondary CNS demyelination due to glial cell dysfunction[7], in contrast to the primary demyelination seen in MS[3]. A second specific antibody, the anti-myelin oligodendrocyte glycoprotein (anti-MOG) antibody, has recently been described in association with NMOSD[2].

Other evidence supporting an underlying autoimmune aetiology is that NMOSD is frequently associated with other autoimmune disorders such as hypothyroidism, pernicious anaemia, ulcerative colitis, myasthenia gravis, and idiopathic thrombocytopenic purpura, as well as systemic lupus erythematosus (SLE), antiphospholipid syndrome(APLS), and Sjogren's syndrome (SS)[2,8–10]. NMOSD is occasionally preceded by antecedent infections or associated with malignancy[10–12]. In South Africa, for instance, cases

of NMOSD and subsequent clinical recurrences have been associated with recent or active tuberculosis (TB)[13].

The identification of anti-aquaporin-4 antibody (AQP4-Ab) specific to NMOSD has led to the recognition of a broader spectrum of clinical presentations than initially described. These include the area postrema syndrome, generalised encephalopathy, brainstem, and hypothalamic syndrome. Hypothalamic dysfunction may consist of narcolepsy, excessive daytime sleepiness, obesity, autonomic dysfunction with hypotension, hypothermia, and bradycardia[2].

Several clinical, serological and radiological characteristics support or confirm a diagnosis of NMOSD. Typical clinical features include severe (often bilateral) optic neuritis (ON), para/quadriparesis with longitudinally extensive myelitis (LETM) and intractable hiccups and vomiting due to area postrema syndrome. The AQP4-Ab represents a specific biological marker for NMO, which plays a critical role in the pathogenesis of the disease. This antibody is present in approximately two-thirds of patients with this NMOSD and is readily detectable in blood[5,14].

The radiological hallmark of NMOSD is LETM[2]. LETM is best appreciated on magnetic resonance imaging (MRI) as intrinsic cord T2-hyperintensity extending the length of three or more adjacent spinal vertebral segments[15]. While several CNS infectious, inflammatory, and metabolic diseases may result in similar changes, additional radiological features may help distinguish NMOSD from other disorders[3]. The involvement of the central cord on axial (transverse) sections with a peripheral pattern of gadolinium enhancement and cord expansion, along with area postrema involvement, gadolinium enhancement of optic chiasm and bilateral long segment optic nerves, as well as characteristic patterns of cerebral white matter lesions, all support a radiological diagnosis of NMOSD[15]. While large (greater than 3 cm in diameter) confluent hemispheric (often radial and spindle-shaped) white matter and tumefactive lesions were thought to be most characteristic in NMOSD, it is now recognised that the appearance of cerebral lesions in NMOSD varies greatly, and small non-specific white matter lesion are the most frequently encountered feature[15]. The main utility in describing these characteristic radiographic features is to differentiate NMOSD from other CNS demyelinating disorders, most importantly multiple sclerosis (MS)[16].

Diagnostic Criteria and Nomenclature

Recently, NMOSD has undergone several iterations in its nomenclature and diagnostic criteria (see Figure 1). The first cases of NMOSD were described in 1894 by Eugene Devic. Consequently, the eponym of Devic's disease was adopted, and opticospinal MS was commonly used to describe concurrent optic neuritis and inflammatory myelitis[17]. Previously, this opticospinal syndrome was considered an Asian variant of multiple sclerosis; however, this has been refuted, and characteristic features such as concurrent myelitis (especially LETM) and severe bilateral ON are now used to help distinguish it from MS[3,18,19].

Subsequently, the term neuromyelitis optica (NMO) became the standard naming convention for this condition, which was defined by clinical and radiological criteria[17].

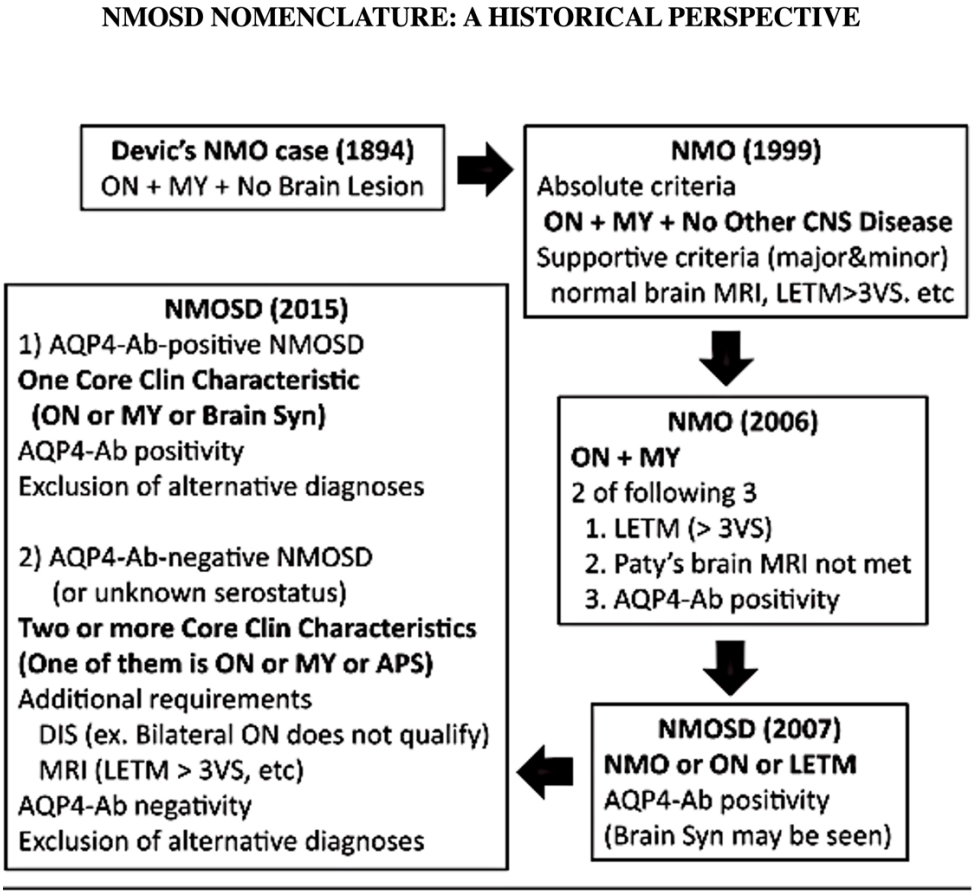


Figure 1: Historical overview of Neuromyelitis Optica nomenclature and diagnostic criteria. Note that while Opticospinal MS has not been formally used in diagnostic criteria, from a historical point of view, cases reported as opticospinal MS in Asian populations are now thought to represent mostly NMOSD.

Fujihara, K. Neuromyelitis optica spectrum disorders: still evolving and broadening. Current Opinion in Neurology. 2019.

In 2004, the discovery of the NMO-specific immunoglobulin-G (IgG) antibody represented the first biomarker for NMOSD[5,20] and allowed for better characterisation of the clinical spectrum of syndromes associated with this antibody. This recognition of other clinical syndromes, such as the area postrema, diencephalic and cerebral syndromes, are now accepted as core syndromes of what is now referred to as neuromyelitis optica spectrum disorder (NMOSD)[2].

In the last decade, a second biomarker associated with NMOSD, the anti-MOG antibody, has been described[2,21]. Distinguishing NMOSD from MS and other causes of inflammatory CNS disease is crucial as it has different therapeutic and prognostic implications[3]. The 2015 International Panel for NMOSD Diagnosis (IPND) criteria are the most permissive to date with regard to clinical features and the inclusion of seronegative cases. These include a definition of NMOSD based on clinical and radiological features alone without serological confirmation (see Figure 2).

The prior 1999 criteria excluded any CNS disease that was not transverse myelitis (TM) or optic neuritis (ON)[1,2] and later the 2006 Wingerchuk criteria recognised that some cases expressed symptoms outside of the classical opticospinal presentation and the utility of the NMO-IgG antibody diagnosis of NMO[22].

The 2015 IPND criteria have been validated in several clinical settings and have substantially increased the number of patients diagnosed with NMOSD[23–25].

2015 INTERNATIONAL DIAGNOSTIC CRITERIA FOR NEUROMYELITIS OPTICA SPECTRUM DISORDERS

Diagnostic criteria for NMOSD with AQP4-IgG

1. At least 1 core clinical characteristic
2. Positive test for AQP4-IgG using best available detection method (cell-based assay strongly recommended)
3. Exclusion of alternative diagnoses[a]

Diagnostic criteria for NMOSD without AQP4-IgG or NMOSD with unknown AQP4-IgG status

1. At least 2 core clinical characteristics occurring as a result of one or more clinical attacks and meeting all of the following requirements:
   a. At least 1 core clinical characteristic must be optic neuritis, acute myelitis with LETM, or area postrema syndrome
   b. Dissemination in space (2 or more different core clinical characteristics)
   c. Fulfillment of additional MRI requirements, as applicable
2. Negative tests for AQP4-IgG using best available detection method, or testing unavailable
3. Exclusion of alternative diagnoses[a]

Core clinical characteristics

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

Additional MRI requirements for NMOSD without AQP4-IgG and NMOSD with unknown AQP4-IgG status

1. Acute optic neuritis: requires brain MRI showing (a) normal findings or only nonspecific white matter lesions, OR (b) optic nerve MRI with T2-hyperintense lesion or T1-weighted gadolinium-enhancing lesion extending over >1/2 optic nerve length or involving optic chiasm (figure 1)
2. Acute myelitis: requires associated intramedullary MRI lesion extending over ≥3 contiguous segments (LETM) OR ≥3 contiguous segments of focal spinal cord atrophy in patients with history compatible with acute myelitis (figure 1)
3. Area postrema syndrome: requires associated dorsal medulla/area postrema lesions (figure 2)
4. Acute brainstem syndrome: requires associated periependymal brainstem lesions (figure 2)

Figure 2: 2015 International consensus diagnostic criteria for neuromyelitis optica spectrum disorders.
1.Diagnostic criteria for AQP4-Ab-positive and seronegative or unknown cases.
2.Recognised Core clinical syndromes with associated radiological correlates in seronegative or AQP4-Ab-unknown cases.

International consensus diagnostic criteria for neuromyelitis optica spectrum disorders
Dean M. Wingerchuk, et al., Neurology Jul 2015, 85 (2) 177-189; DOI: 10.1212/WNL.0000000000001729

There is widespread recognition that seronegative cases of NMOSD exist[2]. The 2015 diagnostic criteria acknowledge that, while the cell-based assay (CBA) for the AQP4-Ab is the gold-standard test for NMOSD, it is not universally sensitive (76%) nor specific (94%), nor is the CBA universally available

in clinical practice[2]. In addition to providing affirmative criteria for the diagnosis of NMOSD, the IPND panel made a note of a set of specific clinical situations in which a diagnosis of seronegative NMOSD should be made, in the absence of AQP4-Ab positivity, albeit with caution[2]. These "red flags" (See Figure 3 below) are important because there are several other common causes of LETM and central nervous system inflammation unrelated to NMOSD.

Although much of the literature on NMOSD focuses on multiple sclerosis as the primary differential diagnosis for NMOSD, many other causes of LETM and NMOSD mimics have been described. These include spinal stroke in the hyperacute setting, other inflammatory conditions such as neurosarcoidosis and neuro-Bechet's disease, CNS infections such as neurosyphilis and tuberculosis, and malignant and paraneoplastic myelopathies[2]. Excluding these diseases is important because they have different therapeutic and prognostic implications[2,26]. Additionally, the IPND criteria advise against indiscriminate testing for the AQP4-Ab in clinical situations that are not recognised as core NMOSD syndromes or screening in asymptomatic individuals, as the implications of a positive test in these clinical settings remain unclear[2].

The recognition of AQP4-Ab seronegative cases fitting the NMO/NMOSD phenotype has contributed to the discovery of other disease-causing antibodies, such as antibodies to myelin oligodendrocyte glycoproteins (MOG)[27]. MOG is expressed on oligodendrocytes[28], and antibodies against epitopes of this protein may cause an NMO-like illness[21,29,30]. However, distinctive features may predict MOD-Ab-associated disease (MOGAD). These include the propensity for MOGAD to affect the anterior optic nerves[21,31], in contrast to the optic chiasm, in AQP4-Ab-associated NMOSD and

MOGAD not demonstrating the same female predilection of AQP4-Ab-associated NMOSD[32]. Importantly, NMOSD associated with anti-MOG antibodies tends to be a monophasic disorder, although the initial presentation is often severe enough to result in significant residual disability[3,29]. MOG antibodies may also be associated with cases of MS, brainstem encephalitis, recurrent optic neuritis and an acute demyelinating encephalomyelitis (ADEM)-like disease[17,29,33].

SERONEGATIVE NMOSD - "RED FLAGS"

1. Clinical features and laboratory findings
Progressive overall clinical course (neurologic deterioration unrelated to attacks; consider MS)
Atypical time to attack nadir: less than 4 hours (consider cord ischemia/infarction); continual worsening for more than 4 weeks from attack onset (consider sarcoidosis or neoplasm)
Partial transverse myelitis, especially when not associated with LETM MRI lesion (consider MS)
Presence of CSF oligoclonal bands (oligoclonal bands occur in <20% of cases of NMO vs >80% of MS)
2. Comorbidities associated with neurologic syndromes that mimic NMOSD
Sarcoidosis, established or suggestive clinical, radiologic, or laboratory findings thereof (e.g., mediastinal adenopathy, fever and night sweats, elevated serum angiotensin converting enzyme or interleukin-2 receptor levels)
Cancer, established or with suggestive clinical, radiologic, or laboratory findings thereof; consider lymphoma or paraneoplastic disease (e.g., collapsin response mediator protein-5 associated optic neuropathy and myelopathy or anti-Ma-associated diencephalic syndrome)
Chronic infection, established or with suggestive clinical, radiologic, or laboratory findings thereof (e.g., HIV, syphilis)

1. Brain
a. Imaging features (T2-weighted MRI) suggestive of MS (MS-typical)
Lesions with orientation perpendicular to a lateral ventricular surface (Dawson fingers)
Lesions adjacent to lateral ventricle in the inferior temporal lobe
Juxtacortical lesions involving subcortical U-fibers
Cortical lesions
b. Imaging characteristics suggestive of diseases other than MS and NMOSD
Lesions with persistent (>3 mo) gadolinium enhancement
2. Spinal cord
Characteristics more suggestive of MS than NMOSD
Lesions <3 complete vertebral segments on sagittal T2-weighted sequences
Lesions located predominantly (>70%) in the peripheral cord on axial T2-weighted sequences
Diffuse, indistinct signal change on T2-weighted sequences (as sometimes seen with longstanding or progressive MS)

Figure 3: "Red Flags" for contexts where diagnosis of seronegative /unknown NMOSD should with caution. These include clinical, laboratory and MRI imaging characteristics which should be viewed with caution when diagnosing seronegative NMOSD

International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Dean M. Wingerchuk, et al., Neurology Jul 2015, 85 (2) 177-189; DOI: 10.1212/WNL.0000000000001729

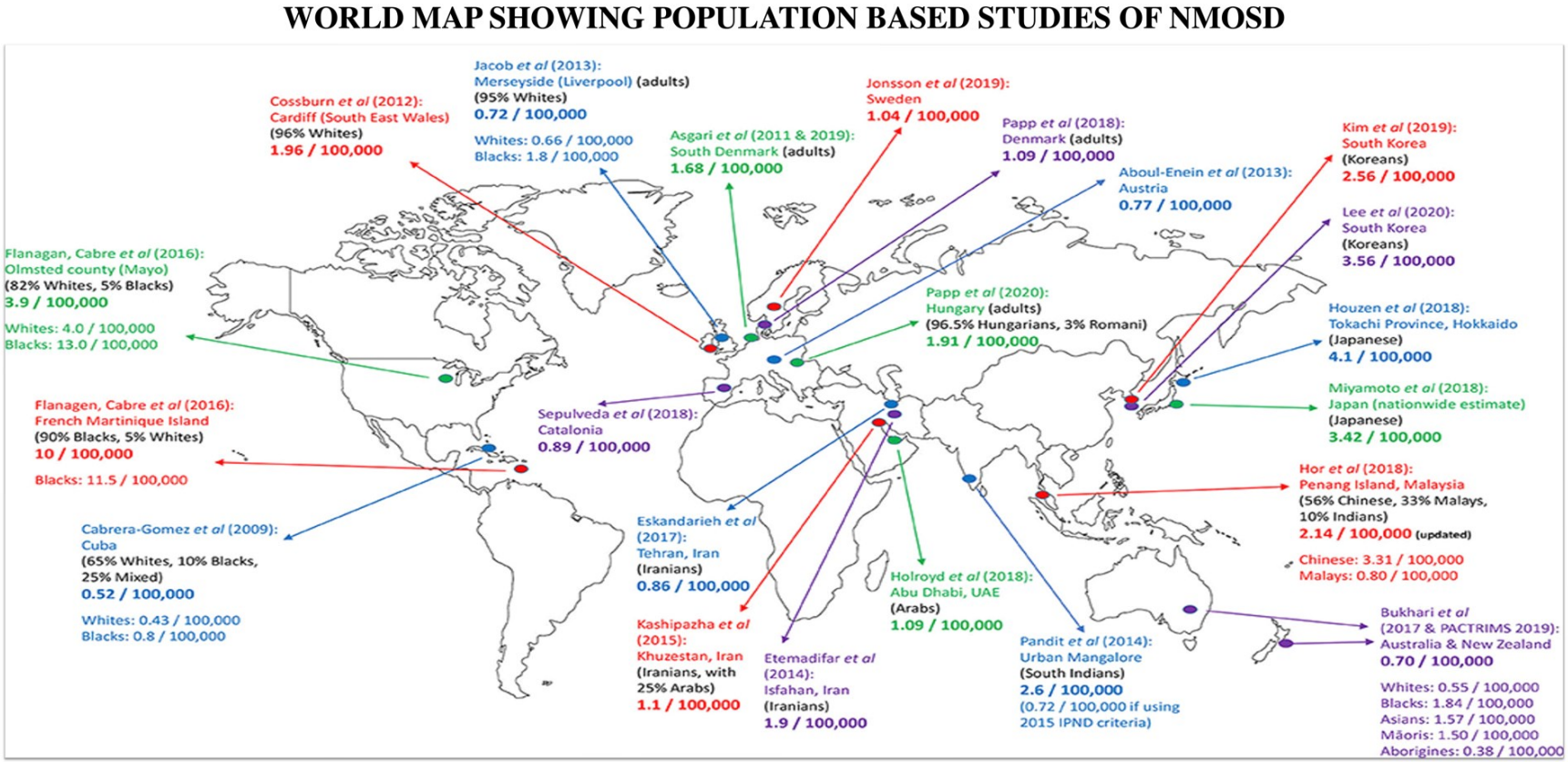


Figure 4: Estimates of the global epidemiologic features of NMOSD cohorts. Where demarcated incidence, prevalence, aquaporin-4 antibody seroprevalence and male-to-female ratio are described. Eight were European studies, ten were Asian, one from Oceania, and two were from the Americas (one Cuban and one joint study from Olmstead County, USA and Martinique Island). The numbers given were prevalence per 100,000 population. In certain studies, the prevalence according to racial groups is provided. Only the adult populations were studied. Note that no African cohorts were included in this analysis.

Hor et. Al., Prevalence of neuromyelitis optica spectrum disorder in the multi-ethnic Penang Island, Malaysia, and a review of worldwide prevalence. Multiple Sclerosis and Related Disorders, Volume 19, 2018, Pages 20-24, ISSN 2211-0348,

Epidemiology and demographic features of NMOSD

Opticospinal MS and NMO were previously thought to be endemic forms of MS and confined mainly to Japan[18] and East Asia; however, NMOSD has now been described in cohorts of patients from across the world[34]. In the past decade, the number and heterogeneity of epidemiologic studies on NMOSD have increased substantially (see world map in Figure 4). In an early systematic review of the epidemiology of NMOSD published in 2013, only five cohorts were reported, reflecting predominantly white/Caucasian populations from Europe and America[34]. In 2020, a systematic review of 22 studies identified more geographic and ethnic heterogeneity in the cohorts reviewed, yet bias towards American and European centres remained[35]. Notably, African and, specifically, sub-Saharan populations remained under-represented[35] (world map, Figure 4). To our knowledge, the epidemiology of NMOSD in South Africa has not been described in an evidence-based manner and remains unknown.

The diagnostic criteria used to define NMOSD have continued to evolve over recent years, mainly influenced by new reports of the incidence, prevalence and clinical spectrum of the condition[35]. Epidemiologic studies have used the 1999, 2006 or 2015 diagnostic criteria and, at times, a combination of these, such as including AQP4-Ab serology along with the 1999 clinical criteria[35]. This primarily reflects the timing of the studies and is likely to have significantly influenced estimations of incidence and prevalence depending on when these studies were performed. In addition, the AQP4-Ab test methodology (Immunofluorescence-tissue assay, immunoprecipitation, enzyme-linked immunoassay/ELISA, and Cell-Based Assay/CBA) has varied in different studies included in the systematic reviews[34,35]. As a result, the prevalence in different populations has varied and may be influenced by the population studied, diagnostic criteria applied, and the AQP4-Ab test methodology used[34,35]. It is also worth noting that the studies included in the most recent (and largest) systematic review of the epidemiology of NMOSD have been mainly retrospective cohort reviews that used older diagnostic criteria[35].

NMOSD is a rare disease with a global incidence ranging from 0.039 to 0.73/100,000 person-years for adults. The international prevalence of NMOSD has been reported as 0.34 to 10/100,000 in adults[35]. Sex, geographic distribution and ethnicity have all been reported to influence prevalence and incidence rates[35].

NMOSD is described in paediatric and adult age groups, with peak incidence noted in the 40 to 59 age group, although 20 to 28% of cases are reported to have an onset over the age of 60 years[35]. Older studies of prevalence suggested that the highest prevalence rates occurred in the 40 to 49 age group[34]

NMOSD affects predominantly young women of non-white and African ancestry[35,36]. This finding is in contrast to earlier studies[34,37,38]. However, these studies involved mainly European and American cohorts and one South American cohort and did not reflect ethnically diverse populations[34]. As newer, more heterogenous cohorts are reported, differences between ethnic groups can be made more verifiably. In white populations, incidence using the 2015 criteria ranged from 0.037 to 0.132/100,000 person-years, and prevalence ranged between 0.7 to 1.91/100,000 using the 2015 IPND criteria[35]. In non-white populations, a higher burden of disease has been consistently reported[35,36,39,40]. Studies involving predominantly Asian populations have reported an incidence of 0.39 to 0.6/100,000 person-years and a prevalence of 1.57 to 4.9/100,000[35]. When performing a breakdown within Asian populations, there appears to be a greater prevalence of ethnic Chinese compared with Malays (3.31/100,000 versus 0.43/100,000)[40].

The highest incidence and prevalence of NMOSD is in populations with African ethnicity at 0.7/100,000 person-years and 10/100,000, respectively[35]. Only a few studies of multi-ethnic populations reported head-head ethnic disparities in the same geographic area[34], and it should be noted that non-white and, specifically, African populations are under-represented in most international reports[34,35]. African and Asian ethnicities not only affect the numerical burden of NMOSD but also, in predictive models, have been shown to affect the number and severity of relapses[41].

A female predominance is consistently reported in studies of NMOSD[35,42]. The extent of this predominance varies between studies and populations under examination. The reported global prevalence of NMOSD was estimated as 2.3 to 7.6 times higher in women than men, but this gender difference is less pronounced in paediatric populations (1.5 to 2.0 times higher)[35].

NMOSD in Africa

The Multiple Sclerosis International Federation Query Data estimated the prevalence of NMOSD in Africa at 5/100,000[43]; this ranks amongst the higher reported prevalence rates globally. Limited data on the epidemiology, clinical characteristics, or disease associations of NMOSD in Africa are availible[36]. Most of the published data is in case reports and small, retrospective cohort reviews, which involve only 10/53 African countries[36]. In the only systematic review of NMOSD epidemiology in Africa by Musubire et al., the largest published cohorts are from North Africa[36,44], and five of the eleven sub-Saharan cohorts described are from

South Africa. The remaining cohorts were from east Africa (Sudan n = 31, Ethiopia n = 4) and west Africa (Niger n = 4, Senegal n = 16 & 5, Nigeria n = 95)[36]

Reports from Africa draw on patients attending urban centre hospitals and thus may not reflect the rural patterns of incidence and prevalence of the disease[36]. Many Africans live in rural settings where gender, economic and geographic barriers often limit healthcare access, so estimates of the NMOSD disease burden may be grossly underestimated[36,45]. Another methodological limitation in reporting the epidemiology of NMOSD in Africa is that the cases described do not originate from cohorts that were not specifically designed to, nor did they aim only to describe instances of NMOSD. In these cohorts, NMOSD cases are often sampled from cohorts of "non-traumatic myelopathy "[36,46]. These cohorts are by their nature heterogeneous and may include conditions that could mimic NMOSD amongst other metabolic, infective and CNS inflammatory conditions[36]. It is well established that access to diagnostic modalities in Africa, such as AQP4-Ab serology, cerebrospinal fluid (CSF) analysis, and MRI, are often unavailable, geographically limited, or restricted due to affordability[45].

Immunosuppressive therapy is the mainstay of management for NMOSD[47]. Relapses are initially treated early with high-dose steroid therapy, and escalation to plasma exchange is often warranted[48,49]. Attacks of NMOSD often occur in temporal clusters[22]. Preventing subsequent attacks is vital as the number and severity of attacks in the first two years often predict long-term outcomes[22]. Prevention of recurrent attacks generally requires the use of long-term immunosuppression[47]. Commonly used steroid-sparing agents include azathioprine (AZA), mycophenolate (MMF), cyclosporine (CSA) and cyclophosphamide (CYC)[50–52]. The use of novel large-molecule biological agents in either the acute management or the prevention of relapses in NMOSD is a topic of ongoing research. In many well-resourced settings, biologic agents are the standard of care[52–55]. However, these agents are frequently too expensive for routine clinical care of patients in Africa, where even more affordable steroid-sparing agents may be unavailable[36,45]. In Africa and other resource-constrained settings, the treatment of NMOSD incurs considerable costs to patients and the health care systems[45].

Parainfectious NMOSD

Several disease associations have been described with NMOSD. These include autoimmune diseases, infections and malignancies[10,11,56]. Parainfectious NMOSD is well recognised, although the exact pathogenic mechanism has yet to be elucidated.

Preceding infections have also been described, with systemic autoimmune disorders such as SLE, Type-1 diabetes mellitus (DM), and rheumatoid arthritis (RA)[12]. In addition, primary CNS inflammatory disorders such as MS, acute disseminated encephalomyelitis, and experimental autoimmune encephalomyelitis (EAE) have all had infectious triggers described[12]. In parainfectious NMOSD, a pathogenic mechanism similar to that described in ADEM is suspected [57]. ADEM is frequently associated with a preceding febrile or viral illness (50–75% of cases)[57].

In NMOSD, parainfectious immune activation via several mechanisms, and more specifically molecular mimicry, bystander activation in response to inflammation in AQP4 antigen-rich tissues, and re-activation or immune amplification of subclinical NMOSD in an infectious inflammatory milieu, have been suggested as possible pathophysiological mechanisms underlying the parainfectious attacks of NMOSD (Figure 5). In addition, an association of parainfectious NMOSD with certain human leukocyte antigen (HLA) genotypes have also been proposed[12,57]. However, there is no definitive model for parainfectious NMOSD, and data regarding the pathophysiology and epidemiology of parainfectious NMOSD are sparse and often contradictory[13,56–60].

PROPOSED MECHANISMS FOR PARAINFECTIOS NMOSD

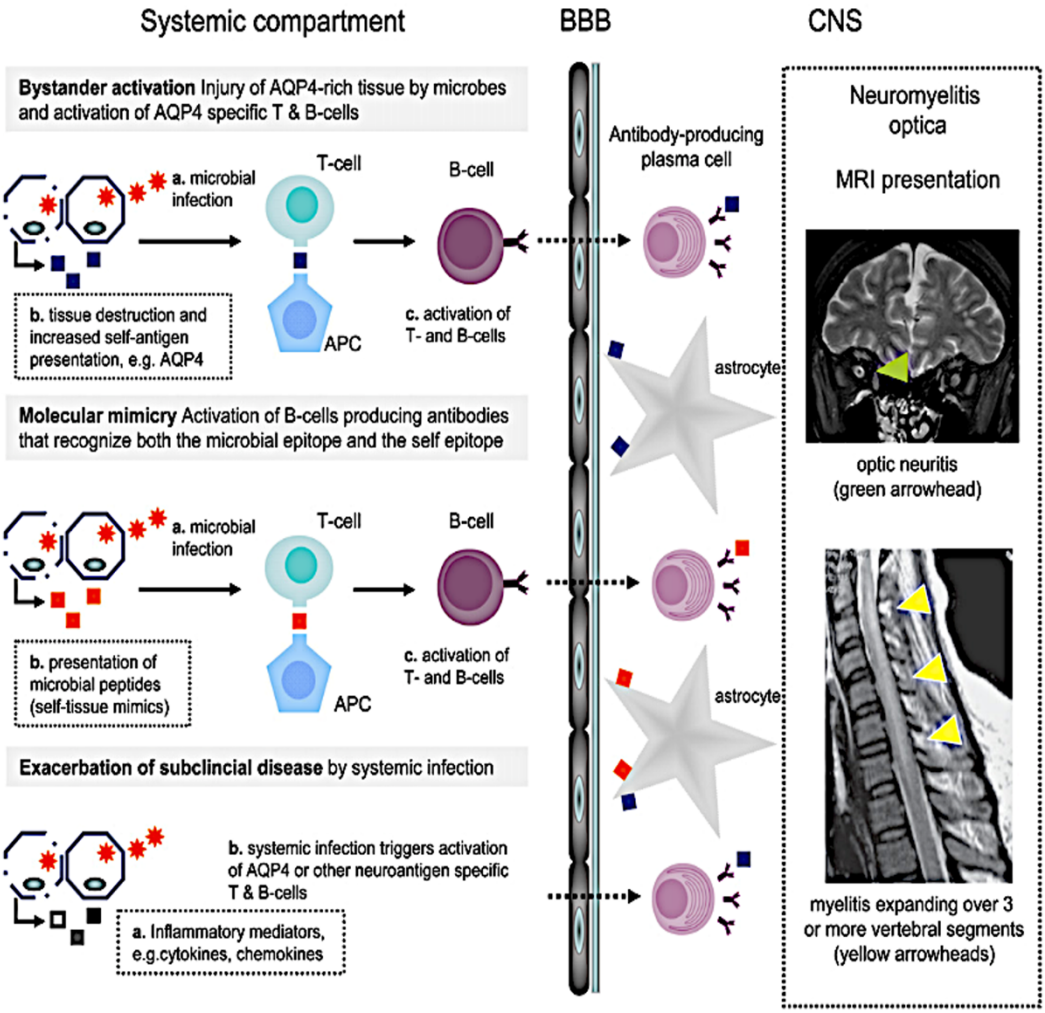


Figure 5: Three proposed mechanisms for para-infectious NMOSD: **1.) Bystander** to inflammation and subsequent cell destruction in AQP4-rich tissues. **2.) Molecular mimicry** of infectious antigen and host AQP4-AB production. **3.)** Infection with general inflammatory milieu **exacerbating underlying subclinical NMOSD**.

Sellner J, Hemmer B, Mühlau M. The clinical spectrum and immunobiology of parainfectious neuromyelitis optica (Devic) syndromes. J Autoimmun. 2010 Jun;34(4):371-9. doi: 10.1016/j.jaut.2009.09.013. Epub 2009 Oct 22. PMID: 19853412.

Organisms associated with parainfectious NMOSD include bacteria such as tuberculosis, *Mycoplasma pneumoniae*, *Treponema pallidum* (syphilis), *Helicobacter pylori*, *Clostridium perfringens* and *Escherichia coli*. Implicated viral infections include the herpes viruses, varicella zoster, cytomegalovirus (CMV), Epstein-Barr virus (EBV), dengue and several others[12,57,58,61].

The rates at which preceding or concurrent infections occur vary across different studies, and much of this information is derived from case reports rather than prospective cohorts[12,57]. Zhong

et al. quoted a figure of 25 to 31% of cases of NMOSD being preceded by non-specific febrile or viral illness[12]. In addition, the role of geographic localisation and endemic organisms may influence the prevalence of; and organisms associated with parainfectious NMOSD[56].

NMOSD, HIV and tuberculosis

In the South African context, the association of NMOSD with TB and HIV is particularly relevant, and there is minimal data on this subject. Cohorts of NMOSD from the South African province of KwaZulu-Natal (KZN)[62] and "non-traumatic myelopathy" from Gauteng[46] recorded a high incidence of concurrent HIV infection (44% and 50%, respectively). Whether the occurrence of HIV and NMOSD reflects a novel underlying pathological mechanism or simply reflects the background high prevalence of HIV is not known. Furthermore, the impact of immunosuppressive therapies and the effect of immune reconstitution in this setting remains uncertain and does not appear to be explored in the current NMOSD literature.

The co-occurrence of TB and NMOSD has been described in small cohorts, and case reports for decades[63,64]. In these reports, the onset of symptoms of NMOSD typically occurs after, or simultaneously with, a diagnosis of TB[13,60,64]. Two South African cohorts have reported a strong association between TB and NMOSD. Silber *et al.* described eight cases of NMO in the setting of preceding TB compared to five patients without TB over the same period[64]. In 2011, Zatjirua *et al*. described the co-occurrence of these two conditions in 79% of their cases[60]. Furthermore, relapses of NMOSD have even been described as coinciding with recurrent bouts of TB[13]. In these cases, evidence of pulmonary or systemic TB was present based on clinical, radiological or microbiological grounds without any evidence of TB in the CNS[13,60,64].

The mechanism of the suspected pathological relationship between TB and NMOSD remains unknown, although molecular mimicry has been postulated[12]. It may be significant that AQP4 channels are expressed in the CNS and many other tissues, including the lungs[65,66]. Moreover, AQP4 channels are highly conserved, as evidenced by the aquaporin-Z channel expressed in the prokaryotic organism *Escherichia coli*, and there is homology between the AQP4 channel in humans and the extracellular epitopes of the aquaporin channels expressed in TB and mycoplasma[12]. It has also been noted that injecting tuberculin antigen can cause demyelination in experimental models[12]. Based on this presumed pathogenic role of TB in the pathogenesis of NMOSD, in 2010, Feng *et al*. described the treatment of 12 cases of steroid-refractory NMO with anti-tuberculous medication to positive effect when compared with controls[67]. These results have not been replicated.

While numerous reports over several decades have described the co-occurrence of TB and NMOSD, some argue against this postulate. The most robust evidence against a relationship between TB and NMOSD comes from the results of a Chinese case-control study where only one case of TB was described in 88 patients with NMOSD[56]. The rate of concurrent TB was no more than that observed in the control populations consisting of patients with myasthenia gravis, polymyositis, dermatomyositis, idiopathic facial palsy and viral meningitis/meningoencephalitis[56]. In addition, it has been noted that anti-tuberculous treatment with ethambutol and isoniazid are neurotoxic[12], and the spectrum of clinical presentations may overlap with NMOSD. Ethambutol commonly causes optic neuropathy, and isoniazid neurotoxicity is well documented and has been reported to cause optic neuropathy and necrotic myelopathy[12].

It should be noted that the geographic and genetic characteristics of cases of parainfectious NMOSD may play an important role and have not been well studied, particularly in South Africa and concerning a preceding or concurrent TB infection. Given that South Africa has a high prevalence of TB and HIV and is also presumed to have an increased incidence of NMOSD, researchers in this county are uniquely positioned to study underlying pathological relationships which may exist between these conditions.

Methodology

Aims of the Audit

The overarching aims of this audit were to describe the clinical characteristics of patients with NMOSD attending a tertiary South African hospital and to identify the challenges in making an early and accurate diagnosis and managing these patients. Conclusions from this audit may inform the development of an evidence-based guide for the management of NMOSD for this study population.

Objectives

The objectives of this cross-sectional, observational audit were as follows:

1. To retrospectively identify and characterise the clinical presentations of cases diagnosed with NMOSD presenting to the Groote Schuur Hospital (GSH) division of Neurology between 1 January 2013 and 31 December 2019.
2. To determine rates of co-infection with HIV and tuberculosis in this cohort
3. Audit acute and long-term disease maintenance therapeutic interventions in this cohort.
4. Retrospectively evaluate outcomes in this cohort using the Modified Rankin scale as a measure of disability.

Audit inclusion criteria

All patients who received a clinical diagnosis of NMOSD, NMO, Devic's syndrome, or opticospinal MS was made, and who attended the GSH neurology service during the period 1 January 2013 to 31 December 2019 were included in this audit.

Audit Exclusion criteria

1. Clinical and MRI findings in keeping with multiple sclerosis:
    a. Dawson's fingers
    b. Juxta-cortical white matter lesions
    c. Lesions confined to the peripheral spinal cord.
2. Patients with an alternative diagnosis in their discharge records (such as an infectious, vascular, or alternative inflammatory autoimmune CNS condition) that better explained their clinical presentations would be cause for exclusion from the analysis.

Data collection and analysis

Case finding

Screening involved a review of all prior laboratory requests for the Aquaporin-4 antibody test performed at GSH or the referring hospitals during the audit period. Opportunistic case collection also occurred by reviewing the folders of patients attending weekly GSH neurology outpatient clinics and requesting GSH clinical staff to volunteer cases they were aware of.

1. Cases were identified retrospectively by using the following strategies:
    1.1. Reviewing all aquaporin-4 (AQP4-Ab) antisera tests performed during the study period, which were requested by GSH and its draining secondary hospitals.
    1.2. Evaluating and correlating AQP4-Ab requests/results with MRI spine (and brain) findings performed at GSH and available on its picture archiving and communication system (PACS) radiology system.
    1.3. We reviewed each of the identified case's clinical records.
    1.4. Additional cases were identified by treating clinicians and referred to the audit.

Data Collection

1. The relevant demographic, clinical, radiological, and serological data were collected and collated into an anonymised data-capturing spreadsheet.
2. All relevant data was captured and anonymised using the allocation of case numbers.
3. We summarised their clinical presentation recorded in their medical notes. AQP4-Ab serology status and radiological features cases were categorised as fulfilling or not fulfilling NMOSD diagnostic criteria. This was based on the 2015 revised IPND criteria.
4. Data collection included all cases with a clinical diagnosis of NMOSD managed at the UCT/GSH neurology service during the period 1st January 2013 to 31 December 2019.
    4.1. Cases were included based on the diagnosis assigned to them by their treating clinician, irrespective of whether or not they fulfilled any NMOSD diagnostic criteria.
5. All participants were categorised according to their predominant presenting clinical phenotypes:
    5.1. Myelitis
    5.2. Optic neuritis
    5.3. Area postrema syndrome
    5.4. Optic neuritis and myelitis
    5.5. Other includes: Brainstem, cerebral syndromes (including focal or generalised encephalopathy) and diencephalic/hypothalamic syndromes

6. All cases were assessed for concurrent infections, but specifically for the presence of:
    6.1. HIV infection
    6.2. Tuberculosis (TB)
        6.2.1. Empiric TB: Cases with clinical and radiological features resulting in a clinical decision to treat for TB without microbiological proof.
        6.2.2. Definitive TB: Microbiologically proven TB diagnosis.
7. Assessment of therapeutic interventions
    7.1. Acute attacks
        7.1.1. High-dose intravenous steroids
        7.1.2. Plasma exchange (PLEX)/plasmapheresis
    7.2. Prevention of relapses
        7.2.1. Oral steroids
        7.2.2. Steroid sparing agents (SSA) and time from first attack to initiation of SSA.
        7.2.3. Biological agents
8. Outcomes
    8.1. Data relating to outcomes was collected. These included death, relapses, and disability using the Modified Rankin Scale (MRS) score, where available, or derived from the findings of neurological examinations and descriptive assessments recorded in the clinical record by attending neurologists or neurology residents at the time of hospital discharge or most recent clinical review.

Data Analysis

Review of clinical documentation

The discharge summaries, clinical notes, and laboratory and radiology records of identified patients were reviewed for appropriateness for inclusion in the study. Clinical information was collected to provide baseline demographic data and medical comorbidities. Clinical characteristics of presentations, inpatient management of acute attacks, maintenance immunotherapy and outcome function were described. These included:

1. All cases with AQP4-Ab positive serology and an appropriate NMOSD clinical syndrome.
2. AQP4-Ab negative cases with clinical characteristics suggestive of probable NMOSD:
    a. bilateral optic neuritis
    b. acute myelitis with longitudinally extensive transverse myelitis (LETM)
    c. area postrema syndrome: intractable hiccups and vomiting

d. MRI features in keeping with NMOSD.
    i. Optic nerve T2 hyper-intense lesions or T1 weighted gadolinium-enhancing lesions involving the optic chiasm or more than half the length of the optic nerve.
    ii. LETM: T2 hyper-intense cord signal abnormality extending three or more contiguous vertebral segments (vertebral body heights) with the exclusion of other causes.
    iii. Dorsal medullary involvement/area postrema lesions
    iv. Associated peri-ependymal brainstem lesions

Statistical Analysis

Logistic regression was performed using Stata version 13.1 to calculate adjusted odds ratios (aOR) for the outcome of severe MRS score. A severe MRS score was defined as an MRS score of 4 or higher (implying loss of functional independence). The regression model included aquaporin 4-positivity, HIV status and concurrent or recent tuberculosis diagnosis (within the preceding six months), age, and sex. Having received a combination of prednisone and SSA (compared to not receiving one or both of these drug classes) was also included as a covariate. Box-plots of MRS scores were prepared and grouped according to AQP4-Ab result, HIV status and TB diagnosis.

Ethical Considerations

This audit was performed at the Groote Schuur Teaching Hospital (GSH), and ethical approval was obtained from both the UCT Human Ethics Research Committee (HREC) of the University of Cape Town (HREC Number 158/2020) and the hospital administration. Ethical approval was subject to annual renewal throughout the study.

This audit represents a retrospective review of clinical information (made available from clinical notes), laboratory results and radiological findings, which are readily available from the clinical records of patients attending the GSH neurology service. Patients were not approached directly to participate in this audit. Data obtained from clinical records were coded and anonymised. In line with principles of patient privacy, at no point was any identifying information captured or made public.

Patient data was anonymised and stored digitally on a password-protected computer. Data capturing and access were limited only to investigators named in the protocol to limit the risk of data breaches.

Planned Outputs and dissemination

This document is intended for an MMED thesis. Data collected from this audit will be disseminated within the Division of Neurology and Department of Medicine at UCT to aid in informing minimums and standards of practice for diagnosing and managing NMOSD in this resource-limited setting.

The results of this study will be made available through dissemination in the form of medical lectures, conference presentations and publication in medical education journals. Preliminary data from this cohort were presented at the Congress of the Neurological Association of South Africa 2020.

Looking forward to the future, we may plan to obtain further ethical approval for the use of this cohort in future prospective projects. These may include data sharing and collaboration with other national and international collaborators and stakeholders invested in NMOSD research and management in similar resource-limited settings.

Results

Case Identification

During the review period, a total of 1703 AQP4-Ab antisera were received at the NHLS from the western cape. Of these, 105 cases tested AQP4-Ab-positive. Thirty-five of these 105 cases came from hospitals or clinics within the GSH referral region. Only 17 of the 35 AQP4 had attended the GSH neurology service.

Screening of records in clinics and routine clinical practice discovered 36 NMOSD cases. All overlapped with cases identified with screening of sera, including all AQP4-Ab-positive cases. Three of the AQP4-positive cases had a clinical diagnosis of NMO made before the availability of the AQP4-Ab assay. These cases had subsequent testing once the assay became available, confirming the diagnosis of NMOSD. One AQP4-Ab-positive patient was diagnosed in Gauteng in 2014, which was corroborated on his presentation to GSH in 2016. Another AQP4-Ab-positive case had been lost to follow-up for two years and resumed follow-up due to her data being captured in this audit.

Screening identified 52 cases of suspected NMOSD during the audit period between 1 January 2013 to 31 December 2019. Three cases were duplicates, and alternate diagnoses were confirmed in five cases. The latter included two cases of multiple sclerosis, one case each of brainstem stroke and inflammatory optic neuropathy with recurrent uveitis, as well as a case of autoimmune encephalitis with a brief psychotic episode that presented with brain MRI, features similar to those of cerebral lesions seen in NMOSD.

After these exclusions, there were 44 cases of possible NMOSD. In all 44 cases, NMO, NMOSD, Oculospinal MS or Devic's disease was documented as the diagnosis in the clinical records. The case acquisition strategy is summarised in Figure 6 below.

UCT NMOSD COHORT CASE FINDING STRATEGY

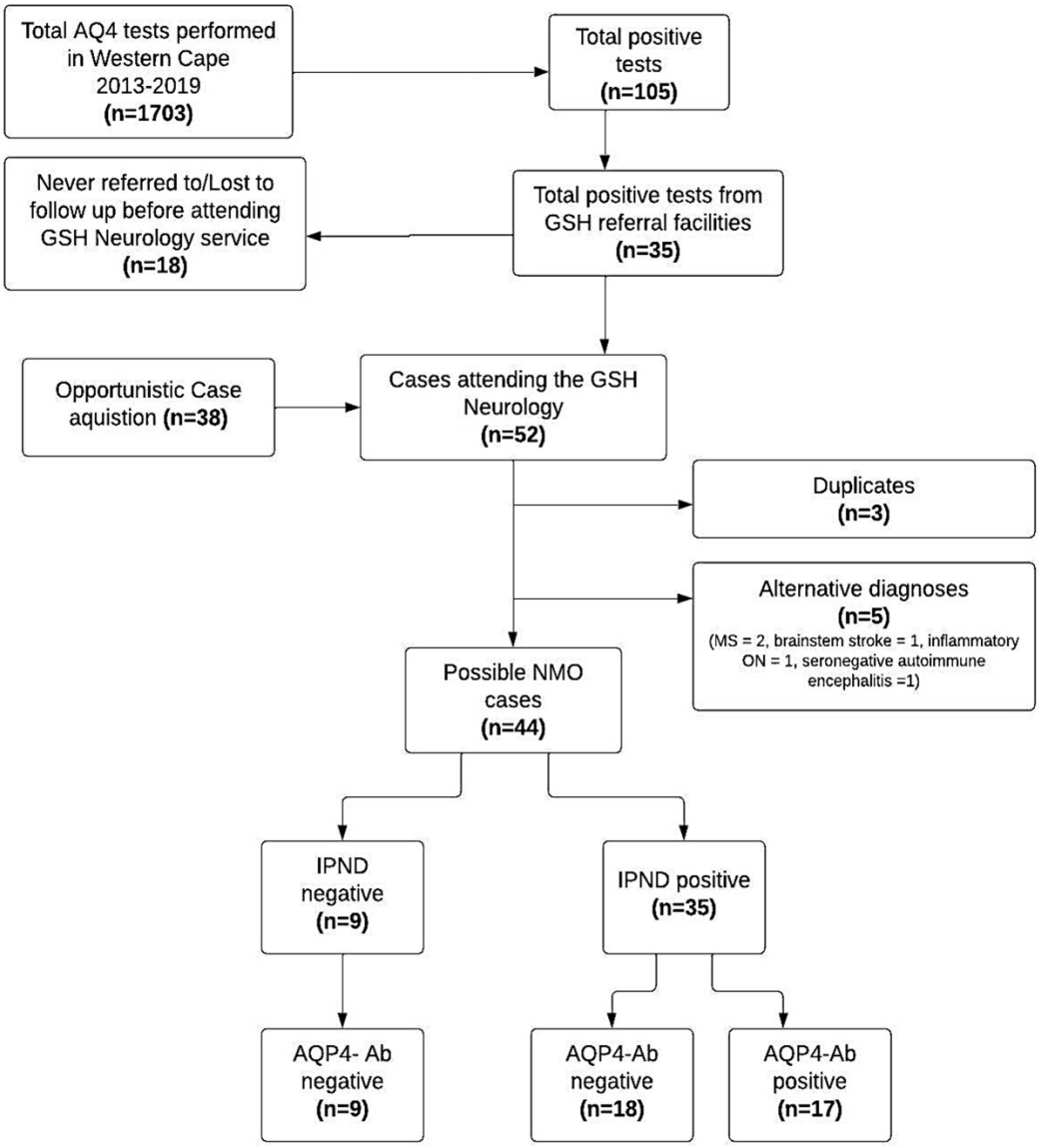
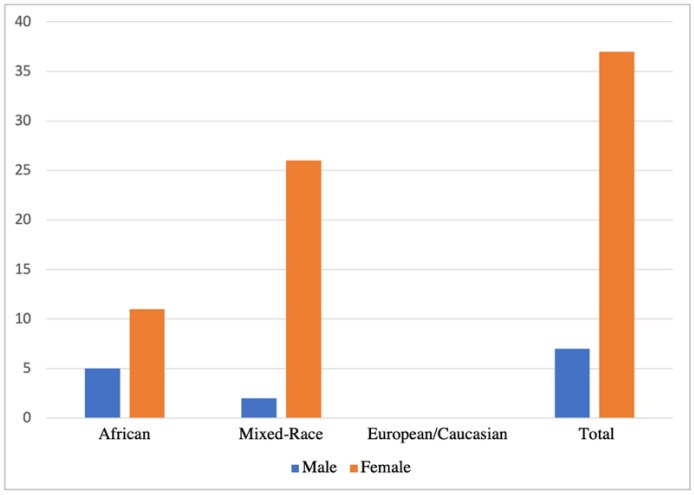


Figure 6: A flow diagram summarising case acquisition strategy in this NMOSD cohort between 1st January 2013 and 31st December 2019. A total of 1703 AQP4-AB tests in performed in the Western Cape, with 105 AQP4-Ab Positive cases. Thirty-five attended facilities within the Groote Schuur hospital drainage area. However, only 17 were recorded as attending the Groote Schuur neurology service — a further 38 cases were identified through opportunistic acquisition at clinics and referrals from colleagues. A total of 52 total cases had a record at GSH Neurology service. Cases excluded were three duplicates and five cases with an alternate diagnosis.

Demographic characteristics

Most cases included in the study (37/44; 84%) were female. This was also true of the AQP4-Ab-positive sub-group, in which 16/17 (94%) were female.

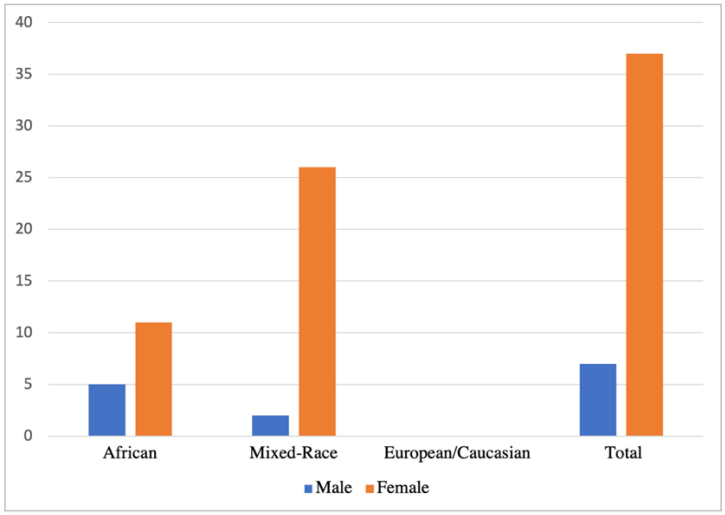


Race	Male	Female	Total
African	5	11	16
Mixed-Race	2	26	28
European/Caucasian	0	0	0
Total	7	37	44

Figure 8: Self-identified race of cases in the NMOSD cohort. Black,-African ancestry in Sixteen cases (five male and eleven female), Mixed-Race decent was noted in twenty-eight cases (two male and twenty-six female)

For the entire cohort, the median age at presentation was 32 years (mean 33.5 years), with a range of 13 to 60 years. In the AQP4-Ab-positive group, the median age was 33 years (mean 34.3 years, range 15 to 58 years), whilst in the AQP4-Ab-negative group, the median age was 33 years (mean 33 years, range of 13 to 60 years).

Race was determined by self-identification in their hospital records. Twenty-eight of the 44 cases (64%) were of mixed-race descent, and 16/44 (36%) were of black-African ancestry. Eleven (65%) of the 17 AQP4-Ab-positive cases were of mixed-race descent, with the remaining six being of black-African ancestry. One of the cases of African ancestry was East African from South Sudan, and one patient of mixed-race origin reported direct ethnic descent from south-east Asia.



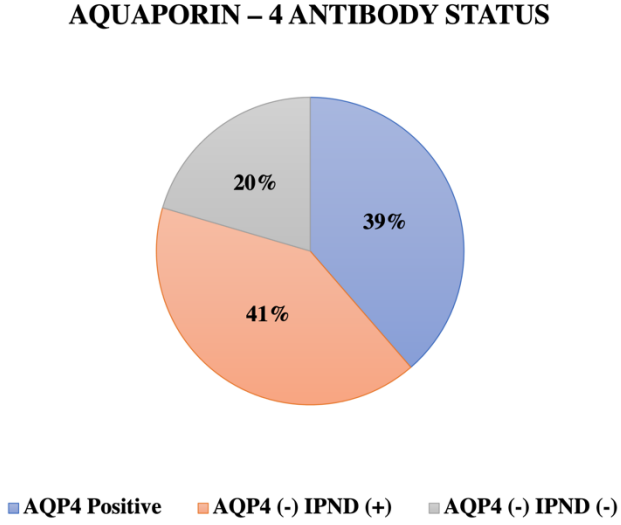
Race	Male	Female	Total
African	5	11	16
Mixed-Race	2	26	28
European/Caucasian	0	0	0
Total	7	37	44

Figure 8: Self-identified race of cases in the NMOSD cohort. Black,-African ancestry in Sixteen cases (five male and eleven female), Mixed-Race decent was noted in twenty-eight cases (two male and twenty-six female)

AQP4-Ab status and 2015 IPND Criteria

Of the 44 cases identified, 35 fulfilled the 2015 IPND criteria for definite NMOSD; 17 were AQP4-Ab-positive and 18 AQP4-Ab-negative.

Nine of the 44 (20%) cases had clinical diagnoses of NMOSD based on their presentation's distinctive clinical and radiological features, despite being AQP4-Ab-negative and not fulfilling the 2015 IPND criteria. Eight presented with idiopathic inflammatory myelitis with long-segment cord lesions on MRI; one had an inflammatory area-postrema syndrome. In addition, all nine were comprehensively investigated for alternative aetiologies, and IPND "red flags" were excluded. This group had the highest morbidity and mortality of the cohort. Five of these nine cases died after their initial attack, four of whom were discharged to the community or rehabilitation facility with an MRS score of 5.



Gender	AQP4 (+)	AQP4 (-) IPND (+)	AQP4 (-) IPND (-)	Total
Male	1	5	1	7
Female	16	13	8	37
Total	17	18	9	44

Figure 9: Aquaporin-4 status. Seventeen cases were AQP4-positive, and twenty-seven were AQP4-negative.

The AQP4-AB negative cases were further characterised into those that fulfilled IPND diagnostic criteria (n = 18) and those that did not, despite having been diagnosed with NMOSD at the time of discharge from hospital (n = 9).

There was no standardised or prespecified protocol for investigations performed in the cohort. Tests typically performed could include full blood and differential counts, serum electrolytes, urea and creatinine liver functions. Metabolic markers such as Vitamin-B12, folate levels and thyroid function, as well as inflammatory markers and serologies such as erythrocyte sedimentation rate (ESR), C-Reactive protein (CRP), antinuclear antibodies (ANA), antineutrophil cytoplasmic antibodies (ANCA), and serum angiotensin-converting enzyme (ACE). Serum and cerebrospinal fluid (CSF) testing for specific infections, including human immunodeficiency virus (HIV) serology and viral load, where appropriate. Syphilis serologies, including *Treponema pallidum* antibody (TPAB), Rapid plasma regain (RPR), and, in some instances, CSF fluorescent treponemal antigen (FTA) and venereal diseases research laboratory (VDRL), were tested. Testing for TB included TB GeneXpert, TB and bacterial culture. Smears for acid-fast bacilli were not routinely performed. All cases had chest radiographs, and abdominal ultrasound was performed in patients with a high suspicion of disseminated TB. It

should be noted that serum testing for human T-lymphotropic virus (HTLV), investigations for schistosomiasis and serum paraneoplastic antibody panels were infrequently performed in our cohort.

COHORT PRIMARY PRESENTING NMOSD CORE SYNDROME

Clinical Presentation	AQP4-AB (+)	AQP4-AB (-) IPND (+)	AQP4-AB (-) IPND (-)	All
ON	2	0	0	2
Myelitis	6	0	8	14
Myelitis & ON	4	15	0	19
Postrema Syndrome	1	0	0	1
Myelitis & Postrema Syndrome	3	1	0	4
Other	1	2	1	4
Total	17	18	9	44

Table 1: Clinical presentation of the cases in the cohort stratified according to their AQP4-Ab sero-status and whether or not they fulfilled 2015 IPND diagnostic criteria for NMOSD.

Clinical Presentations

All recognised core clinical NMOSD syndromes were clinically represented in this cohort except for the diencephalic syndrome. Concurrent optic neuritis (ON) and myelitis were the most common presentation (19/44; 43%), followed by isolated myelitis (14/44; 32%). Myelitis with concurrent area-postrema syndrome occurred in 4/44 (9%), and brainstem syndromes in 2/44, 4.5% (one with associated recurrent ON). In addition, 2/44 (4.5%) presented with cerebral hemisphere lesions, 1/44 (2.3%) with isolated area-postrema syndrome and 2/44 (4.5%) with isolated ON. The clinical data of each case are summarised in Table 2 below.

INDIVIDUAL CASE CLINICAL COURSE & CHARACTERISTICS

AGE	SEX	ETHNICITY	AQP4-AB	CLINICAL SYNDROME	HIV STATUS	TB	PLEX	ATTACKS	MRS
25	F	MRA	Positive	ON, Myelitis	Negative	No	No	5	4
29	F	MRA	Positive	Myelitis, Postrema	Negative	No	No	4	5
62	F	MRA	Negative	ON, Myelitis	Negative	No	No	3	3
25	F	BA	Negative	ON, Myelitis	Negative	No	Day 5	4	3
14	F	MRA	Negative	ON, Myelitis	Negative	Yes	No	3	5
42	F	MRA	Negative	ON, Myelitis	Negative	No	No	4	1
33	F	BA	Negative	ON, Myelitis	Negative	No	Day 5	6	5
18	F	MRA	Negative	ON, Myelitis	Negative	No	No	3	5
30	F	MRA	Negative	ON, Myelitis	Negative	No	No	3	3
53	F	MRA	Negative	Myelitis	Negative	No	No	1	3
24	F	MRA	Negative	Myelitis	Negative	No	No	3	2
23	F	MRA	Positive	Myelitis	Negative	No	Day 21	1	5
26	F	MRA	Negative	Myelitis	Positive	No	Day 7	1	5
24	F	BA	Positive	Myelitis	Positive	Yes	Day 120	4	4
33	M	BA	Positive	ON and Myelitis	Negative	No	Day 15	2	2
58	F	MRA	Positive	Myelitis	Negative	No	Day 10	1	4
31	F	BA	Positive	ON, Myelitis, Postrema	Negative	Yes	No	4	3
14	F	MRA	Positive	Myelitis	Negative	No	No	2	4
37	F	MRA	Positive	Myelitis and cerebral	Negative	Yes	Day10	5	5
31	M	BA	Negative	ON, Myelitis	Positive	No	No	2	2
18	F	MRA	Negative	Myelitis, Postrema	Negative	No	No	2	3
41	F	MRA	Positive	Myelitis, Postrema	Negative	Yes	No	1	5
28	F	MRA	Negative	Myelitis	Negative	Yes	Day 8	2	5
29	F	BA	Positive	Myelitis	Positive	Yes	Day15	2	5
32	F	BA	Negative	ON, Myelitis	Positive	No	Day10	1	2
50	M	MRA	Negative	ON, Myelitis	Positive	No	No	1	2
28	F	BA	Negative	ON, cerebral, Myelitis	Positive	Yes	Day 9	2	2
58	F	BA	Positive	Myelitis	Negative	No	No	5	2
34	F	MRA	Positive	ON, Myelitis	Negative	Yes	Day 9	2	5
31	F	MRA	Positive	Postrema syndrome	Negative	No	No	1	0
54	F	MRA	Positive	ON	Negative	No	No	1	0
38	M	BA	Negative	ON, Myelitis	Negative	No	No	1	3
36	F	MRA	Negative	Myelitis	Positive	No	No	1	5
41	F	MRA	Negative	ON, Myelitis	Negative	Yes	No	3	4
29	F	BA	Negative	Brainstem, Postrema	Negative	No	No	1	0
19	F	BA	Positive	ON	Positive	No	No	3	1
35	M	BA	Negative	ON, Myelitis	Negative	No	No	2	1
37	F	MRA	Negative	ON, Myelitis	Positive	No	No	3	5
40	F	MRA	Positive	ON	Negative	No	No	4	5
49	M	BA	Negative	ON	Negative	No	No	2	1
17	M	MRA	Negative	Myelitis	Negative	No	No	1	3
20	F	MRA	Negative	Myelitis	Negative	No	No	1	5
21	F	BA	Negative	ON, Brainstem syndrome	Negative	Yes	No	3	3
17	F	MRA	Negative	ON, Myelitis	Negative	No	No	1	1

Table 2: Data of individual cases in the cohort. These include demographic, AQPA-Ab status, clinical presentation, number of attacks, concurrent infection (HIV and TB status), intervention with plasma exchange, and Modified Rankin Scale score at the time of discharge..

PLEX = Plasma Exchange (days denote date of initiation from admission)

MRS = Modified Rankin Score BA= Black African MRA= Mixed Race African

Comorbidities

TB and HIV infections were the most common concurrent infectious comorbidities. We also looked specifically for evidence of other concurrent autoimmune conditions described below. None of our patients had concurrent malignancy.

HIV co-infection

HIV was the most common chronic comorbidity occurring in 10/44 cases. The average cluster of differentiation-4 (CD4) count at the time of diagnosis of NMOSD was 482 cells/µl (median CD4 count 484 cells/µl; range 64 to1004 cells/µl), and 7/10 (70%) of these patients were on anti-retroviral therapy. There was no clinically significant difference between the average CD4 counts in the highly active anti-retroviral therapy (HAART)-naïve and the treated groups, with 495 cells/µl in the former and 450 cells/µl in the latter (median 420 cells/µl and 346 cells/µl respectively). The lowest CD4 count was 64 cells/µl in a patient who had started ART three months before presentation.

All the HIV-infected cases on HAART were on first-line therapy at the time of NMOSD diagnosis. Six patients had a viral load available at the time of review, and only one case on HAART had a detectable viral load in her serum (256 copies/ml)

All HAART-naïve patients had CD4 counts above 200 cells/µl; none had an AIDS-defining condition at presentation with NMOSD. AQP4-Ab-seropositivity was confirmed in 3/10 (30%) of the HIV-positive group vs 14/34 (41%) in the HIV-negative group.

CHARACTERISTICS OF HIV INFECTED CASES

AGE	RACE	SEX	AQP4-AB	IPND CRITERIA	CLINICAL PRESENTATION)	FOLLOW-UP (MONTHS)	CD4 COUNT	HAART	TB	TOTAL ATTACKS	MRS
26	MRA	F	Negative	No	Myelitis	0	720	No	No	1	5
24	BA	F	Positive	Yes	Myelitis	74	321	Yes	Yes	4	4
31	BA	M	Negative	Yes	ON and Myelitis	34	259	No	No	2	2
29	BA	F	Positive	Yes	Myelitis	22	371	No	Yes	2	5
32	BA	F	Negative	Yes	ON and Myelitis	13	420	Yes	No	1	2
51	MRA	M	Negative	Yes	ON and Myelitis	13	1004	Yes	No	1	2
28	BA	F	Negative	Yes	ON, Cerebral and Myelitis	9	64	Yes	Yes	2	2
36	MRA	F	Negative	No	Myelitis	0	560	Yes	No	1	5
19	BA	F	Positive	Yes	ON	48	548	Yes	No	3	1
37	MRA	F	Negative	Yes	ON and Myelitis	65	549	Yes	No	3	5

Table 3: Characteristics of the HIV infected subjects: individual demographic data, AQPA-Ab status, clinical presentation, CD4 count at the time of presentation, HAART treatment at the time of presentation, number of attacks, concurrent infection (HIV & TB), intervention with plasma exchange and Modified Rankin Scale score at discharge. **MRS** = Modified Rankin Score HAART = Highly Active Antiretroviral Therapy

Tuberculosis co-infection

In our cohort, 11 (30%) cases had active or concurrent diagnoses of TB. In 6/11 cases, TB diagnoses were made within six months of initial NMOSD presentation (mean 19.83 weeks, median eight weeks), and the other five were diagnosed with TB during their index NMOSD presentation.

Seven cases had TB diagnoses based on definitive microbiological proof. In contrast, four had an empiric diagnosis of TB (i.e. based solely on associated clinical and radiological features. A typical chest radiograph of an NMOSD case with TB is illustrated in Figure 10. Roughly half of the subjects with TB (6/11, 55%) were AQP4-Ab-positive, and the diagnosis and treatment of TB was empiric in 4/6 (67%) of these AQP4-Ab-positive subjects. All AQP4-Ab-negative cases with TB had a definitive microbiological diagnoses of TB outside the CNS. One patient treated for TB empirically did not fulfil IPND criteria for NMOSD.

Three cases of TB were diagnosed in HIV-infected patients (two on HAART), but only one was microbiologically proven. All six HIV-negative patients with TB in this study had positive microbiological confirmation of this diagnosis.

Myelitis was the most common clinical presentation in cases with TB. It occurred in 8/11 (72%) patients (three with myelitis alone, three with myelitis and ON, and two with myelitis and area-postrema syndromes). Two cases had hemispheric lesions, and one presented with a brainstem syndrome subsequently associated with bilateral ON. According to the medical records, none of the 44 patients in this cohort developed TB after their NMOSD diagnosis, regardless of whether or not they received immunosuppression. None of our patients were prescribed isoniazid (INH) as prophylaxis for TB.

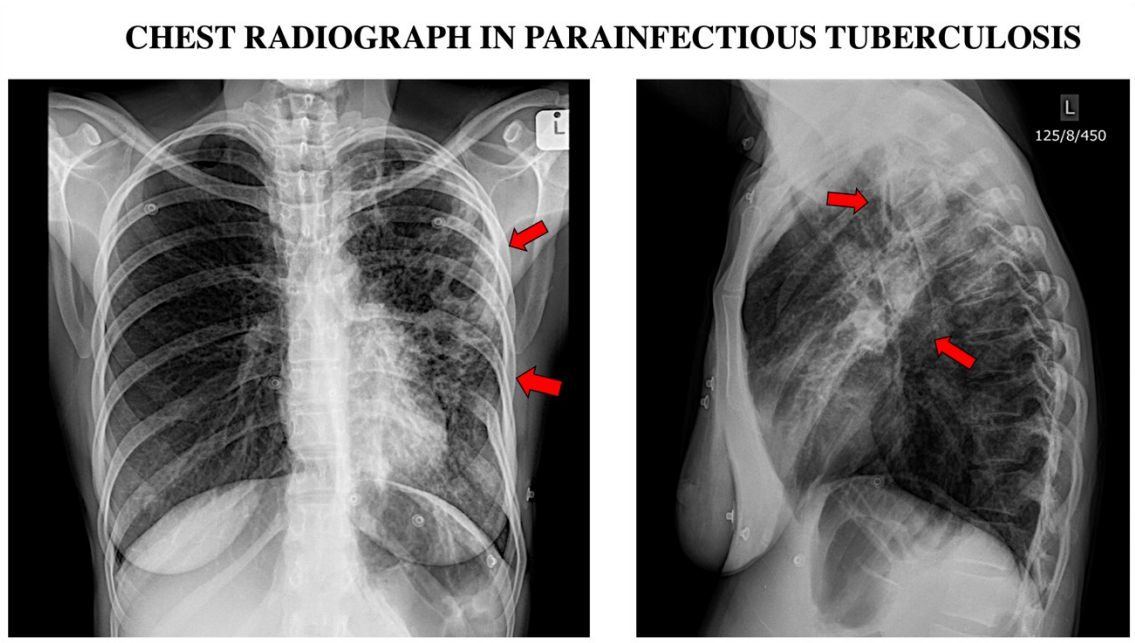


Figure 10: Example of a chest radiograph in case with AQP4-Ab-positive NMOSD and sputum positive TB. Left upper and middle zone cavities, with pulmonary infiltrates typical of reactivation TB.

A TB diagnosis was diagnosed in 11 of the patients in our cohort. 4/11 had a TB diagnosis based on definitive microbiological proof, While 7/11 cases had an empiric diagnosis of TB based on clinical and radiological grounds.

Non-infectious comorbidities

In our cohort, 2/44 cases had concurrent diagnoses of systemic lupus erythematosus (SLE); both of these subjects were female and AQP4-Ab-positive.

The first case with SLE was a 54-year-old female presenting with isolated ON. She was serum positive for ANA and anti-double stranded DNA (anti-dsDNA) antibodies and lymphopenic. APLS screen showed that she was positive for lupus anticoagulant, anticardiolipin and anti-beta-2-Glycoprotein-1 antibodies.

The second case with concomitant SLE was a 29-year-old female who presented with LETM and area-postrema syndrome. She had an ANA titre of 1:320 (speckled) and anti-dsDNA of 32I U/mL, as well as positive Anti-Ro antibodies (240.0 U/ml) and Anti-La antibodies (240 U/ml). She was also lymphopenic with a lymphocyte count of 1.07 $x10^9$/L. Moreover, she had positive serum syphilis serology with a positive RPR (titre 1:16) and positive TPAB. Her CSF FTA was positive. In addition, she had a transient false-positive HIV ELISA, which was negative on confirmatory re-testing. Testing for the antiphospholipid syndrome was negative.

One subject had previously been diagnosed with acetylcholine receptor antibody (AChR-Ab) positive myasthenia gravis (MG). She presented with AQP4-Ab-positive, NMOSD-related myelitis, which was fatal. One subject had concurrent Hashimoto's thyroiditis, and another patient had both type-1 DM and primary APLS.

Concurrent infections, other than TB, were detected in three cases. One AQP4-Ab-positive case had evidence of prior syphilis infection in her blood (TPAB positive and RPR titre = 4) and CSF (FTA positive but VDRL non-reactive).

The remaining two cases were both HIV infected. One was AQP4-Ab-positive, and Epstein-Barr Virus (EBV) was detected in her CSF with 600 viral copies/mL. The second was AQP4-Ab-negative with varicella zoster virus (VZV) polymerase chain reaction (PCR) positivity in his CSF. This patient presented with myelitis and ON. The VZV in his CSF was diagnosed 20 days after his admission after initiating steroid treatment. He was found to be CSF VZV PCR positive with no clinical evidence of VZV zoster reactivation or dissemination.

None of our patients had concurrent malignancy.

Parainfectious NMOSD in our cohort

Concurrent infections, other than TB, were detected in three cases. One AQP4-Ab-positive case had evidence of prior syphilis infection in her blood (TPAB positive and RPR titre = 4) and CSF (FTA positive but VDRL non-reactive).

The remaining two cases were both HIV infected. One was AQP4-Ab-positive, and Epstein-Barr Virus (EBV) was detected in her CSF with 600 viral copies/mL. The second was AQP4-Ab-negative with varicella zoster virus (VZV) polymerase chain reaction (PCR) positivity in his CSF. This patient presented with myelitis and ON. The VZV in his CSF was diagnosed 20 days after his admission after initiating steroid treatment. He was found to be CSF VZV PCR positive with no clinical evidence of VZV zoster reactivation or dissemination.

Cerebrospinal fluid analysis

Cerebrospinal fluid (CSF) was obtained on at least one occasion in 41/44 cases. For this audit, the findings of the first evaluation are noted (unless stated otherwise). As standard, CSF protein, CSF glucose, serum glucose, CSF cell count, CSF bacterial culture, TB culture, TB GeneXpert, cryptococcal and syphilis were tested in most but not all cases. CSF findings and normal values are described in the text and Tables 4 and 5 below.

Across the entire cohort, CSF protein was performed in 39/41, CSF glucose in 38/41 (serum glucose in 21/41), and the IgG index in 31/41 cases. CSF was tested for TB in 39/41 patients (TB GeneXpert PCR or TB culture), and 40/41 had syphilis serology performed. CSF viral panel testing was performed in almost half (21/41) cases. However, the specific viruses tested for varied considerably. In order of frequency: CSF Herpes simplex 1 & 2 PCR, cytomegalovirus viral load, John Cunningham (JC) virus and Epstein-Barr virus PCR. CSF HIV viral load was not performed in this cohort's HIV-infected cases.

Values for CSF chemistry (protein, glucose and IgG index) and cell counts (polymorphonuclear cells and lymphocytes) were calculated and expressed in mean values, interquartile ranges and centiles; with standard deviations. These were compared between AQP4-Ab-positive and AQP4-Ab-negative cases, HIV-positive and HIV-negative cases, as well as those with and without TB. We also compared the CSF indices of the patients who did not fulfil IPND (see Table 4 and charts with box-plots and detailed statistical analysis of CSF results in the supplementary text).

A lymphocytic pleocytosis, with elevated protein and IgG index, was the most common finding, and there was no significant difference between groups when stratified according to AQP4-Ab status, IPND criteria, HIV status or the presence of active TB.

CSF Chemistry and IgG Index

The mean CSF protein for the cohort was 0.5g/L (normal reference range 0.15g/L to 0.45g/L), ranging from 0.14 g/L to 1.46g/L.

In AQP4-Ab-positive cases, the mean CSF protein was 0.60g/L (IQR 0.3g/L to 0.83g/L), and AQP4-Ab-negative cases had a mean protein of 0.58g/L (IQR 0.34g/L to 0.71g/L). HIV-infected patients had a mean CSF protein of 0.72g/L (IQR of 0.54g/L to 0.75g/L), HIV-negative cases had a mean protein of 0.54g/L (IQR 0.33g/L to 0.68g/L). In cases with TB, the mean CSF protein was 0.69g/L (IQR of 0.45 g/L to 0.83 g/L), while HIV negative cases, the mean CSF protein was 0.55g/L (IQR of 0.32g/L to 67g/L).

Serum glucose was available in 21/41 cases. The mean CSF glucose for the cohort was 3.2mmol/L. The reference range quoted by the reference laboratory is 60 - 80% of plasma glucose without expressing an absolute value or time between specimens being obtained.

In AQP4-Ab-positive cases, the mean CSF glucose was 3.4mmol/L (IQR 2.8mmol/L to 3.5mmol/L), and in AQP4-Ab-negative cases, the mean CSF glucose was 3.9mmol/L (IQR 2.95 to 4.6mmol/L). HIV-infected patients had mean glucose of 3.6mmol/L (IQR 2.7mmol/L to 4.7mmol/L), while HIV-negative cases had mean CSF glucose was 3.8mmol/L (IQR 3mmol/L to 4.4mmol/L). In cases with TB, the mean CSF glucose was 3.4mmol/L (IQR 2.7 mmol/L to 3.8 mmol/L), and in the absence of a TB diagnosis, the mean CSF glucose was 3.8mmol/L (IQR 3mmol/L to 4.7mmol/L).

The mean IgG index for all patients in which it was performed was 0.7 (reference range 0.30 to 0.60), with the lowest and highest values of 0.2 to 1.2, respectively. The cohorts' IgG index IQR was 0.4 to 1.0.

In AQP4-Ab-positive cases, the mean IgG index was 0.68 (IQR 0.51 to 0.85). AQP4-Ab-negative patients had a mean IgG index of 0.71 (IQR 0.6 to 0.8). HIV-negative cases had a mean IgG index of 0.68 (IQR0.6 to 0.8), and HIV-infected patients had a mean IgG index of 0.77 (IQR of 0.79 to 1). Cases with TB had a mean IgG index of 0.69 (IQR of 0.51 to 0.85), and those without TB diagnosis had a mean IgG index of 0.7 (IQR 0.6 to 0.8).

CSF Cell Counts

The mean CSF polymorphonuclear cell count was 3/µl (median 0/µl; range 0/µl to 25/µl). Our laboratory provides no reference range for CSF cell counts

In AQP4-Ab-positive cases, the mean polymorphonuclear cell count was 0/µl (IQR 0/µl), with a range from 0/µl to 25/µl. In HIV-infected patients, the mean polymorphonuclear cell count was 2/µl (IQR 0/µl to 1/µl) and in the setting of TB, the mean polymorphonuclear cell count was 1/µl (IQR 0/µl to 1/µl).

The mean CSF lymphocyte count was 25/µl (median 9µl; range 0/µl to 187/µl). In AQP4-Ab-positive cases, the mean lymphocyte count was 17/µl (IQR 4 to 12). In HIV-infected patients, the mean lymphocyte count was 29/µl (IQR 4/µl to 13/µl); and in TB cases, the mean lymphocyte count was 16 (IQR 1/µl to 17/µl).

There were no significant differences in CSF indices of protein, polymorphonuclear and lymphocyte count when stratifying by any of the prespecified clinical categories stated above. CSF IgG-index trended towards higher values in HIV-infected cases.

In addition, the CSF findings of the 9/44 cases that did not fulfil the IPND criteria are tabulated below. As with the rest of the cohort, their CSF showed an inflammatory picture with elevated IgG index and lymphocyte predominance. There were no significant differences in indices of protein, glucose, IgG-index, polymorphonuclear cells and lymphocytosis between this group, the cohort as a whole, the AQP4-Ab-positive patients or other AQP4-negative patients.

CSF FEATURES OF THE NINE IPND NEGATIVE CASES

CSF Prot	CSF IgG-I	CSF Gluc	Serum Gluc	Poly	Lymph	RBC
0.34	0.9	4.6	11.5	24	49	240
0.17	0.6	3.2	_	0	4	0
0.65	0.8	2.9	5.9	0	6	5
0.47	0.80	6.8	15.1	0	17	0
1.26	1.0	2.0	4.4	11	187	0
0.50	0.61	4.6	_	8	76	1000
0.23	0.8	3.2	_	0	6	2

Table: 4: CSF features of the nine IPND negative cases in the UCT/GSH NMOSD cohort. Note that the pattern of a lymphocytic pleocytosis with elevation in IgG index and protein were no different to the rest of the cohorts. Although not documented in table TB bacterial cultures and viral studies were negative in all these CSF samples.

Normal values according to reference laboratory: CSF Protein: 0.15g/L-0.45g/L; **CSF Glucose**: 3.3 to 4.4, range quoted by the reference laboratory is 60-80% of plasma glucose ; **IgG index**: 0.30 to 0.60; **Polymorphonuclear cells**: 0/μl ; **Lymphocytes:** <5 /μl

As noted in the text CSF IgG Index is not routinely measured in HIV infected patients and the clinical or pathophysiological significance of IgG index findings members of this cohort with HIV is not know but not thought to be of any relevance pertaining to NMOSD.

CEREBROSPINAL FLUID CHARACTERISTICS

		Total		HIV negative		HIV positive		No Tuberculosis		Tuberculosis		IPND negative		IPND positive	
		n=44		n=34 (77.27%)		n=10 (22.73%)		n=33 (75%)		n=11 (25%)		n=9 (20.45%)		n=35 (79.55%)	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
Baseline Characteristics	Male	7	15,91	5	14,71	2	20	7	21,21	0	0	1	11,1	6	17,14
	AQ4 positive	17	38,64	14	41,18	3	30	11	33,33	6	54,55	0	0	17	48,57
	HIV positive	10	22,73					7	21,21	3	27,27	2	22,22	8	22,86
	TB	11	25	8	23,53	3	30								
	Presenting age	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
		32,59	12,34	33	13,34	31,2	8,52	33,51	13,4	29,81	8,3	35	12,47	41,11	12,47
CSF	Polymorphs	2,93	6,56	3,25	7,28	1,9	3,54	3,77	7,47	0,63	1,5	6,14	9,1	2,26	5,86
	Lymphocytes	24,95	40,22	23,8	34,18	28,5	57,24	28,2	44,53	16,1	24,57	49,29	66,48	19,94	31,72
	Protein	0,59	0,36	0,54	0,34	0,73	0,37	0,55	0,35	0,69	0,36	0,52	0,37	0,61	0,36
	IgG index	0,7	0,2	0,69	0,19	0,77	0,3	0,71	0,22	0,69	0,2	0,79	0,14	0,68	0,22
	Glucose	3,73	1,38	3,76	1,26	3,64	1,75	3,85	1,44	3,43	1,24	3,9	1,58	3,69	1,36

Table 5: Cerebrospinal fluid characteristics of cohort. Note that this chart is in sections above but included in supplementary text to facilitate easy referencing

1. Baseline characteristics of sex, HIV and TB status; and Age expressed as mean value in each sub-group wåith 95% standard deviation.
2. CSF indices of Polymorphonuclear cells, Lymphocytes, CSF protein, IgG-index and glucose expressed in total cohort and subgroups of HIV and TB status with 95% standard deviation.

Normal values according to reference laboratory

CSF Protein: 0.15g/L-0.45g/L; **CSF Glucose**: 3.3 to 4.4, range quoted by the reference laboratory is 60-80% of plasma glucose ; **IgG index**: 0.30 to 0.60; **Polymorphonuclear cells**: 0/μl ; **Lymphocytes:** <5 /μl (Reference laboratory did not give normal range for cell counts)

As noted in the text CSF IgG Index is not routinely measured in HIV infected patients and the clinical or pathophysiological significance of IgG index findings members of this cohort with HIV is not know but not thought to be of any relevance pertaining to NMOSD.

Radiological Findings

all subjects had at least one magnetic resonance imaging (MRI) performed, but this was only available for review in 40/44 (91%) cases. Radiological characteristics were inferred from the attending radiologists' reports and clinical records of the remaining four patients. Only 31 of the 44 subjects received intravenous (IV) contrast medium during MRI.

Cord lesions

Longitudinally extensive cord lesions were the most common radiological finding in 35/44 cases. Cervical segments were most frequently involved in 54% (28/44) cases. Forty-one per cent (18/44) of subjects had lesions involving both the cervical and thoracic cord. Four (9%) patients had extensive lesions traversing cervical, thoracic and lumbar segments. Lesions isolated to the cervical cord were seen in 14% (6/44) of cases, and (9%) 4/44 patients had lesions confined to the thoracic cord. Two cases had thoracolumbar long-segment lesions. Cord expansion was reported in 26/35 (74%) patients. IV contrast was only administered in cases with cord lesions in 28/35 subjects, 21 (60%) of whom demonstrated enhancement.

Optic nerve lesions

Radiologically-confirmed optic nerve involvement was present on MRI in 8/44 cases. Of these eight cases, only one had dedicated optic nerve sequences performed. Optic nerve lesions were demonstrated in the other patients during the routine brain, Fluid Attenuated Inversion Recovery (FLAIR), T2 or T1 post-gadolinium MRI imaging sequences. Only 4/8 of these patients were administered IV contrast during MRI; two demonstrated optic nerve enhancement. In 5/8 patients, T2- or FLAIR high signal was present in the optic nerves, while atrophy of the affected optic nerves was reported in the remaining 3/8 patients.

Radiological finings according to Aquaporin-4-antibody status and IPND criteria

Aquaporin-4-antibody-positive cases:

Two of the 17 AQP4-Ab-positive cases had isolated ON, while ON was a component of the presentation in 6 of the 17 cases. Cervical (13/17) and thoracic (13/17) cord segments were the most frequently involved. Cord abnormalities occurred over several segments in the majority of cases. Three AQP4-Ab-positive patients had lesions extending across cervical, thoracic and lumbar segments. All three also had cerebral involvement, and two had additional brainstem involvement. Only one patient had isolated cervical LETM, and one had isolated thoracic LETM. None of the AQP4-Ab-positive cases had isolated lumbosacral lesions, and none had conus medullaris lesions.

In the AQP4-Ab-positive group, 5/17 had involvement of both brain and cord: 3/17 had cerebral lesions, 3/17 had brainstem involvement, and 2/17 had area-postrema involvement.

Intravenous contrast was administered in 11/17 AQP4-Ab-positive patients, eight of whom showed contrast enhancement. All but two (6/8) cases with contrast enhancement of the cord also had cord expansion.

Aquaporin-4-antibody-positive cases:

In reviewing the radiological findings of the 27 AQP4-Ab-negative cases, four had no MR imaging to review. As in AQP4-Ab-positive cases, cervical or thoracic lesions segments were most frequently involved (20/23 patients). Cervical involvement was noted in 10/23 and thoracic 15/23 patients. The proportion of AQP4-Ab-negative patients with cervical involvement was lower when compared to the AQP4-Ab-Positive group (43% vs 76%).

In 3/23 AQP4-Ab-negative patients, there was lumbosacral segment involvement; two of these had additional patchy thoracic and cervical cord lesions, with the most contiguous LETM localised to the lumbosacral region with conus lesions—the third having contiguous LETM of the thoracic and lumbosacral cord.

With regard to brain findings in the AQP4-Ab-negative group, seven cases had cerebral involvement, two had ON involvement, one had area-postrema involvement, and four had brainstem involvement.

Three AQP4-Ab-negative patients did not receive contrast, and 16 of the 20, who did, demonstrated enhancement. The MR images of 13 AQP4-Ab-negative cases showed cord expansion.

Cases not fulfilling IPND criteria

Imaging in eight of the nine patients, who did not fulfil IPND criteria, was available for review. Seven had LETM. A radiologist's report was available for the one case whose imaging we could not review, stating that she had cervicothoracic LETM extending from the craniocervical junction to T12. Three had isolated cervical LETM, three had cervicothoracic LETM, and one had thoracic LETM. Of the two IPND-negative cases with brain lesions, one had brainstem lesions, and one had a cerebral lesion (as well as cervicothoracic LETM).

The table below (Table 5) documents which cord segments and brain areas were involved in the entire cohort, and the series of images below (Figures 11 – 15) show examples of characteristics and common radiological lesions encountered in cases from our cohort.

BRAIN AREAS AND CORD SEGMENTS INVOLVED IN THE COHORT

LOCATION	NUMBER	AQP4-AB (+)	AQP4-AB (-)	EXPANSION	ENHANCEMENT	NO CONTRAST
ON	8	6	2	1	2	3
Cerebral	10	3	7	-	5	4
Brainstem	8	4	4	-	1	1
Postrema	3	2	1	-	0	0
Cervical	29	13	16	22	17	6
Thoracic	28	13	15	22	18	6
Lumbar	6	3	3	6	5	1
Any MRI cord Lesion	35	15	20	26	21	7
FREQUENCY OF SEGMENTS INVOLVEMENT ON MRI SCANS						
Cervico-thoracic	17	6	11	13	10	4
Cervico-thoracolumbar	4	3	1	4	4	1
Thoracolumbar	2	0	2	2	2	0

Table 6: MRI features in 40/44 cases described in this table. In the cases there was no the was no images or report to review and one case had not imaging but a detailed result of the MRI findings was available. We reviewed the images and documented involvement of optic nerve, axial brain structures and cord; along with radiographic features of enhancement and expansion (on optic nerve and cord lesions).

Below in figures 11-16 (pages 54-56) we demonstrate a series of images representative MRI's of radiographic features of NMOSD from patients in our cohort:

MRI Series:

RADIOLOGICAL PATTERNS OF MYELITIS

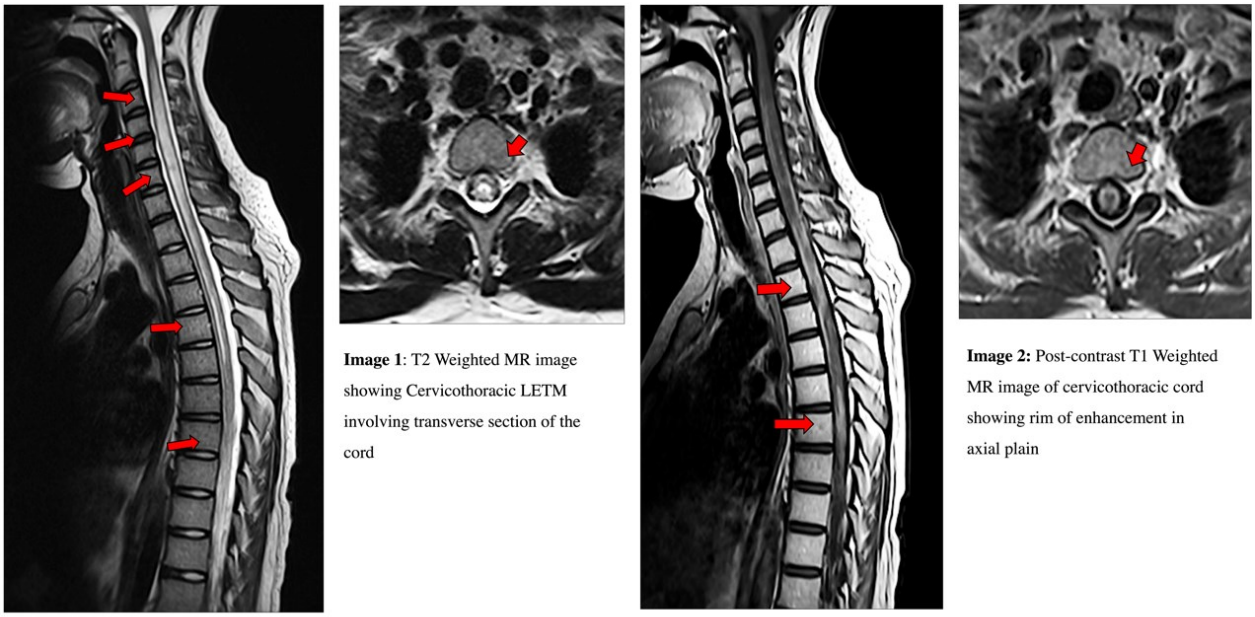


Image 1: T2 Weighted MR image showing Cervicothoracic LETM involving transverse section of the cord

Image 2: Post-contrast T1 Weighted MR image of cervicothoracic cord showing rim of enhancement in axial plain

Figure 11: An example of the cervicothoracic MRI spine imaging characteristics of AQP4-Ab-positive NMOSD case. In **Image 1** note the T2-weighted image showing LETM in the axial plane and central cord hyperintensity in the axial plane. The **Images 2** show the corresponding sagittal and axial T1-weighted Post Gadolinium contrast enhanced MRI sequences. In **Image 2** there is "cigar-like" pattern of enhancement in post-contrast sagittal image and rim of enhancement in the axial plane. See addendum in the supplementary text for pre-contrst T1 images

RADIOLOGICAL IMAGES OF OPTIC NEURITIS

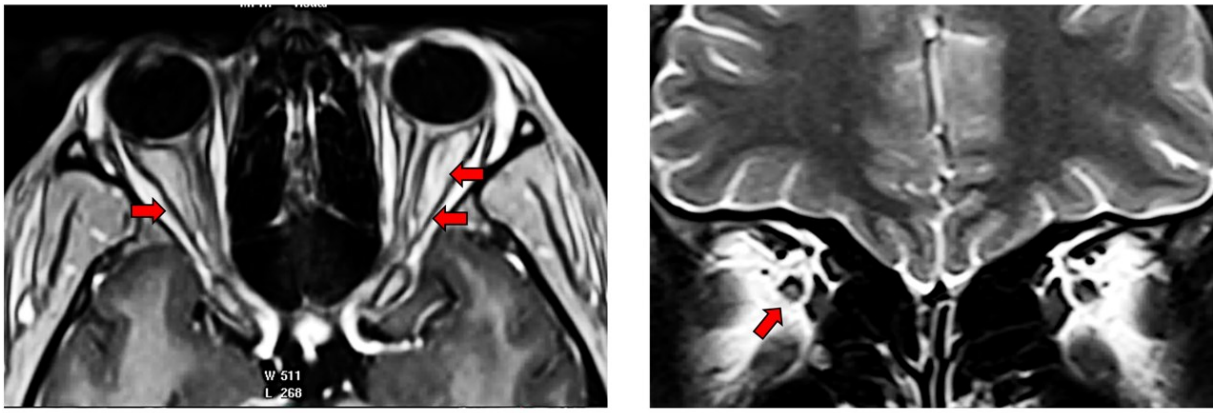


Image 1: Contrast enhanced T1 -weighted MRI with bilateral long segment ON in AQP4-Ab-negative NMOSD case. Note bilateral contrast enhancement along the length of the nerves

Image 2: T2 Weighted MRI; coronal section of the optic nerves showing increased signal in both optic nerves but more avidly on the right

Figure 12: MRI Images of examples of optic neuritis on dedicated Optic Nerve sequences in cases of NMOSD

RADIOLOGICAL PATTERN OF TUMEFACTIVE DEMYELINATING LESION

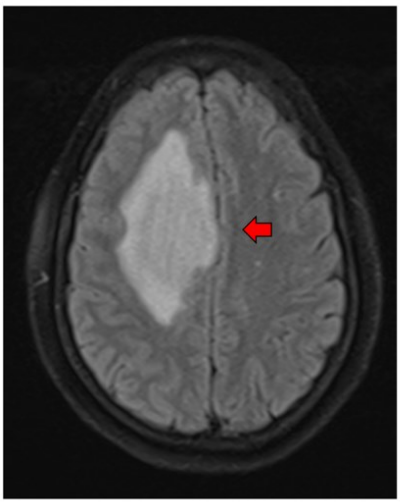


Image 1: FLAIR in axial plane

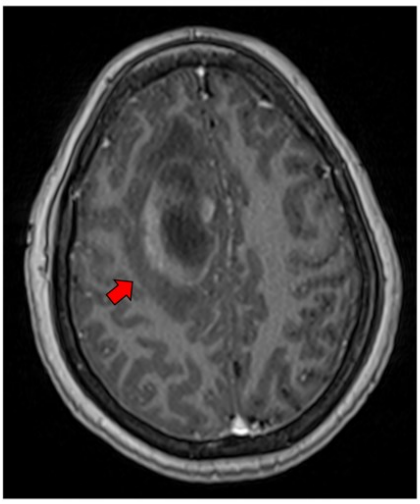


Image 2: T1-wieghted post-contrast in axial plane

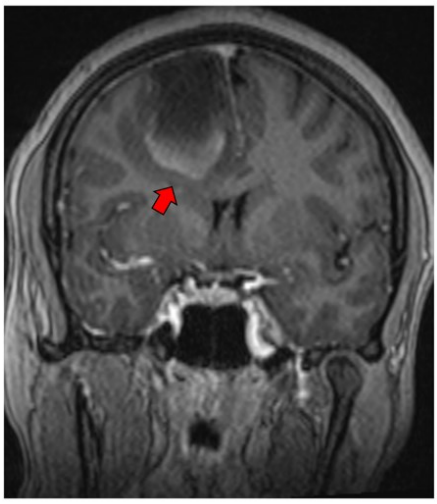


Image 3: T1-wieghted post-contrast in coronal plane

Figure 13: An example of a sequence of images in a AQP4-Ab negative case with tumefactive cerebral lesion. Axial FLAIR showing large right frontal high signal lesion with midline shiftT1-post contrast in axial and coronal plain show incomplete ring of enhancement typical of demyelination.

DIENCEPHALIC CEREBRAL RADIOLOICAL PATTERNS IN NMOSD

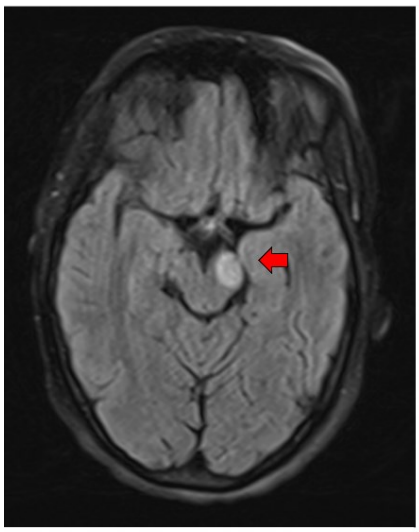


Image 1: Midbrain FLAIR in axial plane

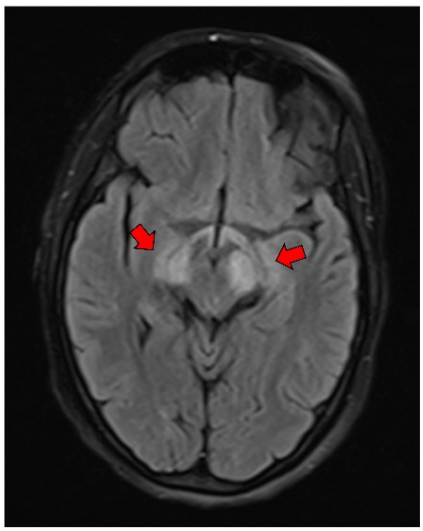


Image 2: Cerebral Peduncle lesions on FLAIR sequence in axial plane

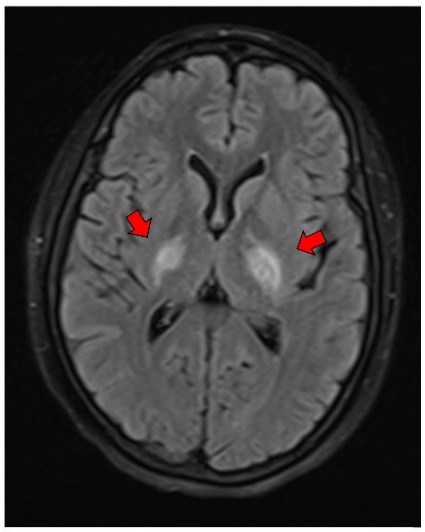


Image 3: Basal ganglia lesions on FLAIR sequence in axial plane

Figure 14: Images 1-3 are of an asymptomatic diencephalic lesion in an AQP4-Ab negative case with multiple relapses. Her original presentation was ON in **Figure 12** then the tumefactive lesions seen in **Figure 13**.

OTHER BRAIN LESIONS IN NMOSD

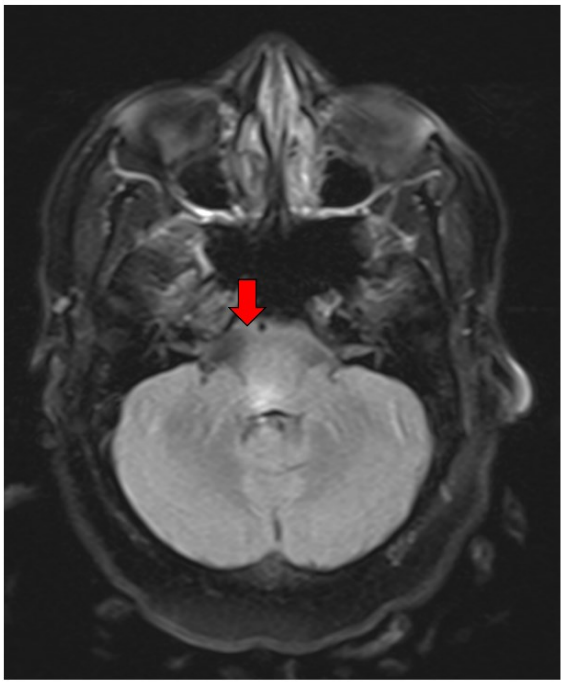


Image 1: Area-Postrma lesion at floor of 4th ventricle on FLAIR sequence in axial plane

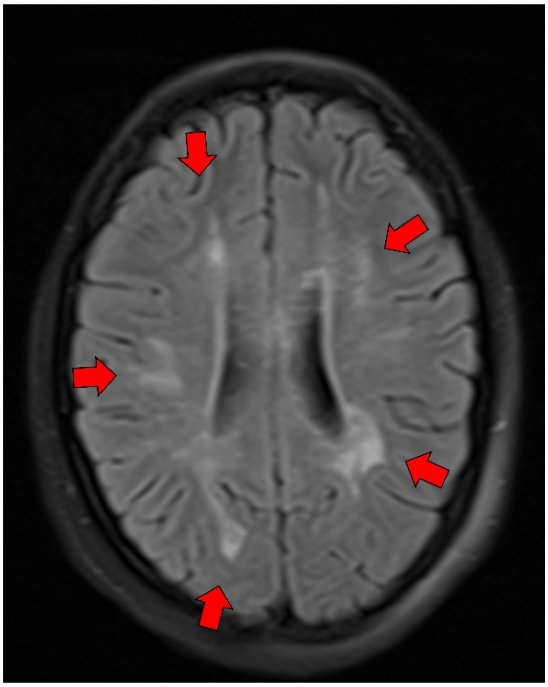


Image 2: non-specific white matter lesions on FLAIR sequence in axial plane

Figure 15: Image 1 Area-Postrema lesions are thought to be highly specific for NMOSD. **Image 2** non-specific white matter lesions in and AQP4-Ab-positive NMOSD with multiple relapses. Note that while not pathognomonic, these are the most common cerebral lesions in NMOSD

Management and Therapies

Acute Attacks

In 42/44 (95%) subjects, attacks were treated with 3–5 days of high-dose IV steroids (HDS). 36/44 had HDS initiated within a week of presentation to the neurology service with an attack. Time to NMOSD diagnosis delayed the initiation of HDS beyond the first week in 6/44 cases, all of which had LETM as part of their presentation, and 3/6 were AQP4-Ab-positive.

Two cases in the NMOSD cohort did not receive any acute treatment. One AQP4-Ab-positive patient had a prior history of a remote attack of isolated unilateral optic neuritis. She was seen as an elective outpatient after the referring service discovered that she had positive serology for NMOSD. The second case to never receive treatment in the context of an acute attack was AQP4-Ab-negative and had LETM. Both of these patients were diagnosed with NMOSD several months after their clinical attack.

Of the 44 patients reviewed, only 30% (13/44) received PLEX in the setting of their acute attacks (Table 7). Of these, 7/13 were AQP4-Ab-positive. The median Modified Rankin (MRS) score in those that received PLEX was 4 (mean value 4). When comparing the seven AQP4-Ab-positive and the six AQP4-Ab-negative cases who received PLEX, the median MRS score was 4 (mean values were 4.3 and 3.6, respectively). The median MRS score in those not receiving PLEX was 3 (mean value 2.87).

ACUTE ATTACKS TREATED WITH PLASMAPHERESIS

PLEX	AQP4-AB (+)	AQP4-AB (-)	Number	(Median) MRS
Yes	7	6	13	4
No	10	21	31	3
Total	17	27	44	3

Table 7: Number of patients treated for acute attacks with PLEX N=13. Median MRS 4. PLEX= Plasma exchange/Plasmapheresis

PLEX was delayed a median of 10 days (mean of six days) from the onset of steroid therapy in 12 of the 13 cases. This excludes a patient in whom PLEX was clinically indicated but delayed after presentation for a full calendar year. The patient was a foreign national, in whom the delay was due to administrative complications relating to her immigration status

and departmental policy. PLEX was instituted a year later following subsequent recurrent attacks

MYELITIS, SEVERE MRS AND INCREASED LIKLIHOOD OF PLASMAPHERESIS

ALL POSSIBLE NMO CASES (N=44)				
		aOR	**95% CI**	
Clinical Presentation	No Myelitis	Ref.		
	Myelitis	263,3	3,06	22684,31
Acute Treatment	No PLEX	Ref.		
	PLEX	7,35	0,61	88,25

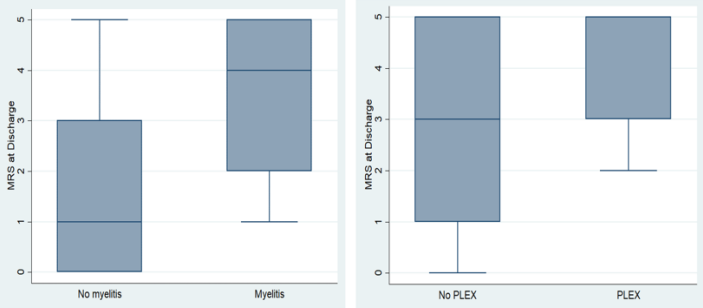


Table 8: with Box Plots shows MRS in cases with myelitis as a component of their presentation. Myelitis is significantly associated with worse at presentation MRS (aOR=263) and while not statistically significant, patents treated with PLEX trended towards more severe MRS at presentation. Note MRS score does not account for visual symptoms which are a prominent component of NMOSD and therefore does not account for all the disability experienced by people suffering from NMOSD.

Maintenance Immunosuppression

Immunosuppressive treatment for the prevention of subsequent attacks was instituted in 41/44 subjects. The most common maintenance therapies prescribed were prednisone and azathioprine in 77% (34/44) of patients. One case (with MG and NMOSD) was treated with azathioprine and methotrexate concurrently. Oral steroid monotherapy was prescribed in 7/44 patients, and one subject was treated with Rituximab. Three seronegative subjects received no maintenance therapy. Two of these had minimal clinical manifestations (one with prior myelitis, MRS score 2, and the other with prior ON and myelitis, MRS score 1) and declined maintenance immunosuppression; and another (MRS score 5) died from complications relating to her myelitis before immunosuppression could be initiated.

Outcomes

This study being a retrospective, cross-sectional audit examining several years of clinical practice around NMOSD at our institution, there was a considerable variation in the duration of follow-up. The intervals between follow-up visits and initial hospital admissions were similarly variable. Consequently, it was not possible to adequately calculate the relationship with the treatment interventions administered at prespecified time intervals.

Measured outcomes included the median Modified Rankin Scale (MRS) score at the time of the last clinical interaction, the number of relapses documented in the clinical record until 31 December 2019, the total number of attacks each case experienced (both before and after NMOSD diagnosis) and death. Poor outcome was defined as an MRS score of 4 or more at their most recent documented clinical interaction. Median rather than mean values have been expressed due to the relatively small sample size and because the dataset was not normally distributed.

Attack(s) before diagnosis with NMOSD

Our review of this cohort assessed patient histories and prior admission details. There was a total of 107 recorded events consistent with NMOSD attacks. Attacks before a formal clinical diagnosis of NMOSD accounted for 39/107 (36%) attacks and there were 26 hospital admissions.

In our cohort, 24/44 (54%) cases had a history consistent with one or more attacks of NMOSD occurring before their formal clinical diagnosis. In 18 of these cases, 26/39 (66%) of the attacks had resulted in a hospital admissions either to their general district/secondary level hospital (15 attacks) or an ophthalmology service (11 attacks).

In six cases of these with hospital admissions 15/26 (57%) were for recurrent attacks before an NMOSD diagnosis being recognised. Four cases were admitted to district hospitals, and two were admitted to ophthalmology services. Two patients had three district hospital admissions with NMOSD attacks before their NMOSD diagnosis.

The highest number of attacks occurring in a single subject was six. Attacks of NMOSD tended to cluster temporally. In the cases with hospital admissions for presumed NMOSD attacks occurring before a formal diagnosis, 11 had their initial attack within one year of their NMOSD diagnosis, and a further three had their first attack within 18 months of their NMOSD diagnosis.

There was a considerable range between the first attack and a diagnosis of NMOSD (0–±288 months). The most prolonged duration was 24 years. This occurred in a case initially diagnosed with multiple sclerosis after presenting with myelopathy and ON in 1994. She had a subsequent attack in February 2002 and was finally diagnosed with AQP4-Ab-positive NMOSD in April 2018 after another attack. Another case with an extended period between her first attack and an NMOSD diagnosis was an AQP4-Ab-positive case with a first attack with cerebral symptoms in November 2000. Her second attack was in February 2002, and she was diagnosed with NMOSD in December 2016 after presenting again with hemiplegia.

Attacks after Diagnosis with NMOSD (relapses)

A total of 68 clinical relapses occurred once the patients were under the care neurology service. Not all documented relapses resulted in hospital admissions. There were 31 relapses in 21 separate cases requiring hospital readmission.

In our cohort 21/44, patients had at least one clinical relapse requiring hospital admission, eight of them in AQP4-Ab-positive cases. The median duration from diagnosis to the first relapse was seven months (0–33 months). Ten patients (four of whom were AQP4-Ab-positive) had two or more admissions for relapses after their NMOSD diagnosis (median 34 months, range 0–151 months).

As stated above, relapses tended to temporally cluster, with 14/21 cases experiencing their first relapse within a calendar year of their NMOSD diagnosis (18/21 within two years of diagnosis). 20/21 patients with relapses were discharged after their initial presentation on maintenance immunosuppression (20 were on steroids and 19 on at least one steroid-sparing agent).

Relapse rates

A total of 107 attacks were recorded in the cases of our cohort, with 39 (26 requiring hospital admission) of these occurring before a formal diagnosis of NMOSD was made. Six patients (accounting for seven attacks) were not admitted to the hospital with their first attack(s) with NMOSD symptoms (three myelitis, two ON and two area-postrema attacks).

Sixty-eight relapses occurred after a diagnosis of NMOSD had been made. Thirty-one of these resulted in hospital admission. It is of concern that, in 36 events, the relapses were identified retrospectively in the clinical record as relapse events where the patient did not present to, or the attack was not recognised by, health care services. In all cases, 2136 patient-months (178 patient-years) after a diagnosis of NMOSD were recorded, with a crude

annualised relapse rate of 0.38 relapses per patient-year. The median number of attacks was two per subject (mean 2.4), ranging from one to six attacks.

Treatment of Relapses

Only 13 cases in our cohort were treated with PLEX, and 15/21 patients with relapses (five of whom were AQP4-Ab-positive) had never been treated with PLEX, either for their first attack or subsequent relapses. Of the six cases treated with PLEX for relapses, three were AQP4-Ab-positive. Steroid responsiveness was why PLEX was not administered in 13 patients and was unavailable for two more patients.

Modified Rankin score with covariates of Myelitis, AQP4-Ab-Positivity, HIV and TB

A clinical presentation with myelitis was generally associated with a worse outcome. The aOR of severe MRS score when considering the presence of myelitis versus its absence was 263,30 (95% CI 3.06–22684,31). Cases with myelitis had greater odds of being treated with PLEX with an aOR of 7.35 (95% CI 0.61–88,25). Note that these wide confidence intervals are a function of the low number of patients who presented without myelitis (see Table 8 below and box-plots in the supplementary text).

The mean MRS score at discharge was 3.29 (median 3). Of the 7/44 (15.9%) subjects, who were male, none had an MRS score of 4 or higher. In the members of the cohort that fulfilled IPND criteria for NMOSD, the first logistic regression model, which included the covariate of sex and AQP4-Ab-positivity, was significantly associated with a severe MRS score with an aOR of 8.65 (95% CI 1.14–65.43).

Being HIV positive and having recent tuberculosis were also associated with a higher MRS score, but these findings were not statistically significant, with aORs of 1.32 (95% CI 0.16–10.86) and 7.52 (95% CI 0.91–-62.22), respectively. Sex was included in this model, but there were no males with severe MRS scores in this cohort, and thus the male observations were dropped from the model.

In the second logistic regression model, sex was removed as a covariate, allowing all 44 possible NMOSD patients' findings to be included in the analysis. The aORs in this model were similar to the previous model, but the slightly larger population size resulted in some narrowing of the confidence intervals. AQP4-Ab-positivity was now associated with an aOR of 6.78 (95% CI 1.23–37.37), HIV infection was associated with an aOR of 1.80 (95% CI 0.30–10.92) and TB infection with an aOR of 7.70 (95% CI 1.12–53.13).

ADJUSTED ODDS RATIO FOR SEVERE OUTCOME (MRS > 3)							
		Criteria Positive NMO cases (n=37)			All possible NMO cases (n=44)		
		aOR	95% CI		aOR	95% CI	
AQP4	Negative	Ref			Ref		
	Positive	8,65	1,14	65,43	6,78	1,23	37,37
Sex	Female	(omitted)*	-	-	-	-	-
	Male	(omitted)*	-	-	-	-	-
Age	Age < 30	Ref			Ref		
	Age 30-39	0,22	0,02	2,01	0,28	0,04	2,26
	Age 40-49	7,09	0,33	151,29	1,79	0,19	16,94
	Age 50 +	0,44	0,04	4,93	0,43	0,05	3,72
HIV	Negative	Ref			Ref		
	Positive	1,32	0,16	10,86	1,80	0,30	10,92
TB	No	Ref			Ref		
	Yes	7,52	0,91	62,22	7,70	1,12	53,13

Table 9: Table of the logistic regression model of the adjusted odds ratios of poor outcome. Poor out come determined as MRS of greater than 3 and loss of functional independence. Males not included in second model as no males with poor outcomes. AQP4-Ab-positivity, HIV infection, TB and age between 40-49 associated with severe outcome. Note wide confidence intervals and lack of statistical significance due to small sample size.

* No males with severe MRS, therefore perfect protection and dropped from regression model (therefore male sex dropped as covariate in second model, allowing all observations to be included)

Mortality

Seven patients died from complications of their NMOSD during the study period. All deaths occurred in cases with myelitis. 5/7 of the deaths were from the "AQP4-Ab-negative, IPND negative" group. These patients died as a consequence of their initial attack of myelitis. Only three of these fatalities had received PLEX during their admission (delayed for 7, 8 and 9 days, respectively, after onset of symptoms/presentation).

Discussion

This study is a retrospective audit of the demography, presenting clinical features, serological profile, infective comorbidities, acute and maintenance therapies, and functional outcomes in a cohort of patients diagnosed with NMOSD at Groote Schuur Hospital (GSH). This is a large university teaching hospital in South Africa's public healthcare sector with a specialist neurology referral service. The audit covers the period from 1st January 2013 to 31st December 2019. A consultant neurologist managed all cases described in this cohort.

To our knowledge, this is one of the largest cohorts of NMOSD from sub-Saharan Africa, consisting of 44 patients, of whom 35 fulfilled the 2015 IPND diagnostic criteria.

The key findings of this audit are that, while the demographic and several clinical features were consistent with international cohorts, a significant proportion of our cases had TB and HIV as comorbidities. This finding contrasts the findings of the most comprehensive study that specifically interrogated the relationship between TB and NMOSD and did not find any association[56]. That said, several reports of NMOSD with parainfectious TB have been described from South Africa[13,60,64,68].

In addition, at an operational level, we have noted systemic problems in recognition and referral pathways for NMOSD patients from peripheral centres. In contrast, at the neurology service, patients with unexplained CNS inflammatory disorders were labelled as having NMOSD without fulfilling consensus criteria and had the worst outcomes in the cohort.

All cases diagnosed with NMOSD underwent various investigations to find alternative aetiologies for their presentation. The specific investigations performed were at the discretion of their respective treating neurologists. These investigations commonly included blood and CSF analysis, CNS magnetic resonance imaging (MRI), and electroencephalography (EEG). In this way, other causes of myelopathy and ON, such as inflammatory conditions like MS, SLE and sarcoidosis, and infectious diseases such as TB and syphilis, were excluded.

In describing this cohort, we acknowledge the ongoing evolution in NMOSD diagnostic criteria, the increasingly recognised clinical spectrum of NMOSD, and the advances made in NMOSD management during the study period. For instance, during this time, the 2006 Wingerchuk criteria were replaced by the 2015 IPND criteria. Before 2013, the diagnosis of NMOSD was relatively rare in our clinical setting as AQP4-Ab testing only became readily available in South Africa's public sector hospitals in 2013, and anti-MOG antibody testing was not available for any of the patients during the period of this audit.

Demographic features of our NMOSD cohort

Characteristic demographic features have consistently been reported in cohorts of NMOSD, with female sex and African and Asian ethnicity widely regarded as risk factors[35,36]. Our cohort was overwhelmingly female (84%), and this predominance applied irrespective of AQP4-Ab antibody status (females constituted 94% of AQP4-Ab positive and 78% of AQP4-Ab negative subjects). This female predominance is typical of NMOSD described by other groups from Africa and around the world[35,36,43].

Our cohort's average age at the initial presentation was 32 years, ranging from 14–62 years. This age demographic is similar to other international and African cohorts[34–36]. Previous South African cohorts and cases have similarly described patients who typically present in their second and third decades of life but with a broad range. The youngest recorded South African case was eight years old, and the oldest documented NMOSD case was 42 years old[13,46,60,62,69]. Our cohorts' age range is broader than that described in other cases and cohorts from South Africa but is in keeping with international trends.

Historical descriptions of NMOSD proposed an ethnic and geographic distribution, with many initial reports describing the condition in people of Asian ethnic origin[3,18,19]. Before the development of AQP4-Ab in NMOSD, reports from Asia, especially Japan, had long described a syndrome referred to as oculospinal MS[18,19], which is clinically indistinguishable from NMOSD[4]. Authors recognised that this disorder was distinct from "western MS" with respect to its clinical features, aggressive course, findings on CNS imaging, and resistance to traditional therapies for MS[18,19]. Subsequently, many of these cases were diagnosed with NMOSD[19]. Cases of NMOSD have now been reported worldwide[34,35]. It seems clear from cohorts from multi-ethnic urban settings in North and South America and Asia that NMOSD tends to affect non-white individuals disproportionately and more severely than white populations[35,41,42,70,71]. This is also reflected in our cohort.

An early systematic review did not identify any association between NMOSD and ethnicity. However, earlier studies of NMOSD were limited in number and over-represented populations with homogenous and predominantly European populations[34,37]. Later cohorts and systematic reviews performed internationally and on the African continent have confirmed non-white ethnicity as a risk factor[35,42,45,71,72]. In their study of the epidemiology of NMOSD in the Americas, Eoin *et al.* compared the characteristics of the population of predominantly European ancestry in Olmsted County, Minnesota, USA, with the Afro-

Caribbean population of Martinique. They found that NMOSD was more common and had a higher AQP4 seroprevalence in the Afro-Caribbean group[72].

Similarly, studies in multi-ethnic settings in South America[39,73,74] have found NMOSD more prevalent in non-white participants of African and Afro-Caribbean descent. Researchers studying large longitudinal cohorts in North America have found that NMOSD is over-represented in non-white (Black-African, Asian and Latin/Hispanic) populations[42,70]. In multi-ethnic Asian populations (where the opticospinal presentation was first described in detail), these ethnic predispositions have been confirmed[40]. In describing the epidemiology of NMOSD, Malaysia Hor *et al.* found that NMOSD was less prevalent in people of south-east Asian descent compared to people of Han-Chinese ancestry. In addition, in their description of the global prevalence of NMOSD and ethnic correlates, they found that in Asian populations, Chinese ethnicity had the highest incidence, followed by those with south-east Asian ancestry and then by Japanese ancestry (see Figure 4)[40]. In a review of NMOSD in Toronto, Canada, Stratos *et al.* found that NMOSD in Asians was associated with worse EDSS compared to Caucasian cases in the same healthcare system, despite Asians having fewer relapses[71]. They also found that black-African ancestry cases were less likely to have "typical" NMOSD presentations and less likely to conform to the older 2006 diagnostic criteria with the "classical" opticospinal presentation[71]. Stratos *et al.* also noted that long-term visual outcomes were worse, and there was more brain atrophy in studies of African patients[71].

NMOSD has been under-reported in sub-Saharan Africa[36]. In our study, 29/44 (65%) of the subjects self-identified as mixed-race and 15/44 (34%) as black African. None of the cases in our study identified as being primarily of white/European, Asian or Indian ethnicity. One AQP4-Ab case identified as "coloured"/mixed-race ethnicity noted that she had Malaysian ancestry. The ethnic characteristics of our cohort may, in part, reflect the demography of patients attending our state-funded healthcare centre.

Similarly, all other South African reports of NMOSD were from large urban university teaching hospitals. Modi *et al.* in 2001 reported a series of eight cases with a "demyelinating disorder of the central nervous system in black South Africans" between 1996–2000. In their description, Modi *et al.* noted disseminated cerebral white matter lesions in 6/8 of their cases, which excluded NMO based on the diagnostic criteria at the time. In retrospect (based on the information provided in the publication), all eight of these cases fulfilled the 2015 IPND diagnostic criteria for NMOSD [75]. Since then, a further three South African case series of NMOSD have been described. These were from Cape Town (2011, n = 14)[60], KZN (2017, n = 27)[62], and Gauteng (2017, n = 15)[69]. Furthermore, in 2011, two cases of NMOSD were

identified in HIV-infected patients in a cohort of 100 cases of non-traumatic myelopathy attending Chris Hani Baragwanath Hospital in Gauteng[46].

In South African cohorts, patients' ethnicity has been overwhelmingly non-white (only two cases from the 2017 Gauteng cohort of 15 patients were white)[60,62,69,75,62]. In further support of this pattern of non-European/non-white predominance, cases reported from a cohort described in Kwa-Zulu Natal (KZN) province, all patients were non-white. Their cohort was 76% African, 17% Indian and 7% mixed race[62]. Our cohort had no subjects that identified themselves as primarily Indian ethnicity. Again this racial breakdown may just represent the geographic and ethnic distribution of the people of South Africa. South Africa has one of the largest populations of Indian diaspora in the world74, and this population is concentrated in KZN[77]. The most recent South African census data noted that Indian ethnicity accounted for 7.4% of the people of KZN while only making up 1% of the population of the Western Cape[77].

In the context of our study population, "mixed race" likely includes a combination of Black African !San, Indian, south-east Asian/Malay and European ancestry. In other studies of NMOSD patients from the Western Cape, ethnicity was not reported[60,64]. While ethnic disparities are widely reported in NMOSD, when describing the clinical course of NMOSD in cases with similar ethnicities but in different geographic contexts, Soares-dos-Reis *et al.* found that geographic context affected outcomes in Afro-Caribbean patients but not in Asian patients. This may indicate that, in addition to the risks conferred by genetic factors, disparities in modifiable environmental factors may also be relevant[78].

AQP4-antibody status

Only 38% (17/44) of patients in our cohort were AQP4-Ab positive. The AQP4-Ab-positivity increased to 49% (17/35) in patients who fulfilled the IPND diagnostic criteria for NMOSD. These figures for AQP4-Ab-positivity are relatively low compared with those reported in other cohorts of mixed and African ancestry. In their systematic review of 19 cohorts (totalling 410 cases) of NMOSD in African populations, Musubire *et al.* noted an average AQP4-Ab-positivity rate of 53% and a positivity rate range of 29–95%[36]. They stated that ELISA and cell-based assays were variably used in different geographical clinical settings[36]. Other studies from South Africa have reported AQP4-Ab-positivity rates that are generally higher than in our cohort. For example, AQP4-Ab-positivity rates were 53% (8/15)[69] and 65% (15/23)[62] in series from Gauteng and KZN, respectively. In our case-finding strategy, we identified 18 AQP4-Ab-positive cases from within our hospitals' referral catchment area, who were not referred, or did not attend our neurology service. This accounts for 51% of all AQP4-Ab positive cases between January 2013 to December 2019. When including these cases, our positivity rate increases to 66% in patients fulfilling IPND criteria and 56% in the cohort as a whole. Presuming that these tests were performed in the correct clinical context and are not duplicates, this finding would represent a serious systemic inequity in access to the appropriate referral services.

Cases not fulfilling IPND criteria

A significant proportion (9/44, 20%) of our cases did not fulfil the 2015 IPND diagnostic criteria for NMOSD. Including these cases reduced the overall AQP4-Ab-positivity rate in our cohort. Nevertheless, these cases had a clinical diagnosis of NMO, Devic's disease, or NMOSD. These cases are an intriguing clinical dilemma that deserves discussion.

Myelopathy was the primary diagnosis in 8/9 cases. Myelopathy, NMOSD, was diagnosed because of their typical MRI findings of cervicothoracic LETM with cord expansion and a peripheral pattern of cord enhancement in 5/8 patients (see examples in figures below). Contrasted MRI sequences were not performed in 2/8 cases.

These cases underwent extensive investigation to exclude alternative causes for their presentation. These included investigations for CNS TB, sarcoidosis, neurosyphilis, SLE including antinuclear antigens (ANA), extractable nuclear antigens including Sjogren's syndrome antibodies (anti-Ro and anti-La), ANCA-associated vasculitis and vitamin B12 deficiency.

In all nine cases, CSF results were available (CSF panels are shown below in Table 9). They showed mild inflammatory changes, with lymphocytic pleocytosis and elevated CSF IgG

index being the most common findings. While nonspecific, these findings do not differ significantly from other cases described in our cohort or the NMOSD literature[79].

With regard to comorbidities, one patient had a history of fully treated sputum-confirmed tuberculosis six months before her presentation with acute myelitis and had never had any evidence of microbiologically proven CNS TB. Two cases had comorbid HIV infection, one of whom was HAART naïve. Both HIV-infected patients had high CD4 counts (720 and 560), and neither had evidence of opportunistic infections. One case had evidence of autoimmunity with autoimmune thyroid disease.

Treating clinicians initiated high-dose steroid therapy in 8/9 cases; two had PLEX. Five of the eight non-criteria fulfilling cases did not have AQP4-Ab serology performed before starting immunosuppressive treatment, and their AQP4-Ab results were only available after their hospital discharge.

Several explanations may account for these nine cases being diagnosed with NMOSD despite failing to fulfil NMOSD criteria. Firstly, the AQP4-Ab detection assay is known to play a significant role in the NMOSD diagnostic algorithm. The ELISA assay, with its' mean sensitivity of 63%–64%, has a lower sensitivity than the gold standard CBA, which has a mean sensitivity 76.7%[2]. Some of these cases may have tested as false negatives by the best available test methodology at the time of their presentation. The AQP4-Ab detection modality used currently at our laboratory is the Euroimmun™ CBA. This assay has been validated in several clinical and research settings and performed similarly (if not better) to previously cited CBA sensitivities[14,80]. While our laboratory is currently using a highly accurate CBA testing methodology, they could not confirm that a CBA was used in all our seronegative cases as the test was previously referred to a private laboratory in the earlier period of this study (i.e. those diagnosed before 2013, but still being followed up in our service). In addition, some patients in our study may be anti-MOG antibody positive, and, as previously stated, this test was unavailable during our audit period.

VALIDATION OF COMMERCIAL CELL-BASED AQP4-AB ASSAY

Group tested	*N*	T-IIF	ELISA	EI-M1/M23	EI-CBA	Ox-CBA	MOG
CASE SENSITIVITY—n +ve/*N* (%)							
NMOSD [95% CI for sensitivity]	80	62/78 (78) [69–87]	25/42 (60) [45–73]	38/42 (90) [78–96]	34/36 (94) [82–99]	33/36 (92) [78–97]	0/48 (0) [0–7]
Suspected NMOSD	101						8/79 (10)
CONTROL SPECIFICITY—n –ve/*N* (%)							
Suspected NMOSD	101	99/99 (100)	62/64 (97)	61/64 (95)	42/43 (98)	49/49 (100)	
Multiple sclerosis	101	98/98 (100)	48/48 (100)	48/48 (100)	20/20 (100)	21/21 (100)	52/52 (100)
Inflammatory disease	49	49/49 (100)	43/49 (88)	49/49 (100)	49/49 (100)	49/49 (100)	48/49 (98)
Blood donors	103	99/100 (99)	102/103 (99)	103/103 (100)	103/103 (100)	82/82 (100)	89/90 (99)
Overall [95% CI for specificity]	354	346/346 (99.7) [98–100]	255/264 (97) [94–98]	242/245 (99) [97–100]	214/215 (99.5) [97–100]	201/201 (100) [98–100]	189/191 (99) [96–100]

T-IIF, tissue-based indirect immunofluorescence; ELISA, enzyme linked immunosorbent assay; EI M1/M23, Euroummun® M1/M23 biochip slide; EI-CBA, Euroimmun® AQP4 fixed cell-based assay; Ox-CBA, Oxford AQP4 live cell-based assay; MOG, myelin oligodendrocyte glycoprotein antibody assay; NMOSD, neuromyelitis optica spectrum disorders.

Figure 16: Comparison of sensitivity and specificity of Euroummun AQP4 cell based assay compared against other available testing modalities including Indirect immunoflourecence, ELISA, Academic centre cell based assay and anti MOG antibody test. AQP4-Ab-positive cases and cases with suspected NMOSD with controls included cases of multiple sclerosis, other inflammatory disorders and asymptomatic blood donors.

Zamvil, S. S. et al. AQP4 Antibody Assay Sensitivity Comparison in the Era of the 2015 Diagnostic Criteria for NMOSD. Front. Neurol. 10, 1–7 (2019)

We should acknowledge that when including the cases that tested AQP4-Ab positive and were never assessed by the neurology service (n = 18), the total cohort then constitutes 62 cases with a 56% AQP-Ab-positivity rate. When counting only AQP4-Ab positive and seronegative cases fulfilling IPND criteria, our AQP4-Ab-positivity rate increases further to 66%. Accounting for these cases would place our AQP4-Ab-positivity rate more in line with internationally reported figures[2,35,36]. However, this statement remains conjecture without knowing the clinical context of these 18 AQP4-Ab positive cases.

Secondly, several of our AQP4-Ab negative cases were administered high-dose steroid therapy for their acute presentation before serum was sent for AQP4-Ab testing. This being the case, AQP4-Ab testing may have been performed late in their admission or after discharge, when they were already established on immunosuppressant therapy and affected the AQP4-Ab titres. In their studies of AQP4-Ab assays, their titres and their relationship to immunosuppressive therapies, Jarius *et al.* in 2008 and Takahashi *et al.* in 2007 demonstrated that AQP4-Ab titres showed a strong inverse correlation with immunosuppressive therapy but never fell below a detectable threshold in the majority of patients[20,81]. Valentino *et al.* showed that immunosuppression demonstrated a titration effect on the AQP4-Ab titre in patients treated with Rituximab. Although the AQP4-Ab titre diminished with Rituximab therapy, it only became negative in a minority {58/316; 18%) of previously positive samples[82]. Valentino *et al.* also found that AQP4-Ab titres only decreased in cases with clinical a response to Rituximab therapy[82]. These findings suggest

that while immunosuppression may have influenced the AQP4-Ab titres but this would not likely result in titres below detection thresholds, and the role of immunosuppression alone would be an unlikely explanation for the low seroprevalence in our clinical setting.

This group of nine cases who did not fulfil the 2015 IPND diagnostic criteria was also the group with the highest mortality, with 5/9 points dying from complications of fulminant myelitis. Some of these cases may have been actual seronegative cases of NMOSD who, due to the severity (and outcome) of their initial attack, never had the opportunity to present with a second attack or another core NMOSD syndrome, which would allow them to fulfil criteria for seronegative NMOSD. However, 10 of the 18 criteria fulfilling seronegative NMOSD cases had clinical or historical evidence of attacks (median = 1; range = 1–3 attacks) before presenting to our neurology service. The poor clinical outcomes and the disproportionate number of deaths in this group may imply that seronegative NMOSD was the incorrect diagnosis in these patients. Misdiagnosis may explain their poor response to our conventional NMOSD treatment regimens.

Whether these cases were misdiagnoses, false-negative AQP4-Ab NMOSD or MOGAD remains unknown. Our small sample size and the retrospective nature of our data collection preclude us from drawing any definitive conclusions. These findings argue for a prospective audit of cases with stricter adherence to IPND criteria for case definitions. This would assist in a more representative characterisation of NMOSD in our clinical context.

Another explanation for the low AQP4-Ab-positivity rate in our cohort is the under-recognition and under-referral of cases of NMOSD. This is exemplified by the 18 clinically unaccounted-for AQP4-Ab positive cases discussed above. The under-recognition of NMOSD is further supported by the fact that in several instances within our cohort, the patients were only referred to the neurology service after having had prior attacks consistent with NMOSD (range 1–3). In our cohort, 24/44 (55%) cases reported previous clinical events compatible with NMOSD and numbering 39 attacks in total. These mainly were episodes of visual loss, sensory myelopathic symptoms or area post-trema symptoms. In six cases, the patients did not seek healthcare, and in 18 cases, their presentations were not recognised as a manifestation of NMOSD at their primary healthcare facility. There were 26 hospital admissions for attacks consistent with NMOSD before the diagnosis. Eleven admissions were for ON (in two, the ON was recurrent), 14 had myelitis, and one episode was of area postrema syndrome in a poorly controlled type-1 diabetic without diabetic ketoacidosis. These cases subsequently presented with myelitis or another core syndrome leading to an eventual diagnosis of NMOSD.

The 2015 IPND criteria for NMOSD have increased diagnostic yield in many clinical settings[23–25,83]. Yet, others have experienced the same clinical quandary with a group of cases of idiopathic CNS demyelination. In evaluating the impact of the 2015 IPND criteria, Hamid *et al.* showed an increased diagnostic yield of NMOSD in their cohort of 176 cases of CNS inflammatory disorders. Using the 2006 criteria, 63 of 176 (36%) of their subjects had a diagnosis of NMOSD, while with the 2015 IPND criteria, 111 of 176 (63%) of their patients had an affirmative diagnosis of NMOSD. However, they still had a worrying group of 65 cases (36%) who did not fulfil IPND criteria (see Figure 17 below), despite having features that could be compatible with NMOSD[23]. Hamid *et al.* advocated for caution and reappraisal when cases did not meet the 2015 diagnostic criteria[23], and Wingerchuck and Weinschenker echoed this sentiment in their editorial response[26]. As in our study, anti-MOG antibody testing was unavailable in the Hamid *et al.* cohort. They suggested broad differential diagnoses for LETM and mimics NMOSD should be considered[23,26]. These included atypical presentations of MS, systemic and CNS Inflammatory conditions, paraneoplastic syndromes, and rare genetic disorders[23,26]. Some of these diseases can be tested for in routine clinical practice, while others are more difficult to exclude. Ultimately, Hamid *et al.* concluded that clarity regarding aetiology in these cases could only be obtained through long-term, prospective follow-up so that clinical phenotype and course of these cases of idiopathic CNS demyelination can be compared to seropositive and seronegative NMOSD. Lastly, our findings may represent the true seroprevalence in our setting. If this is the case, it would be of particular interest and worthy of further investigation.

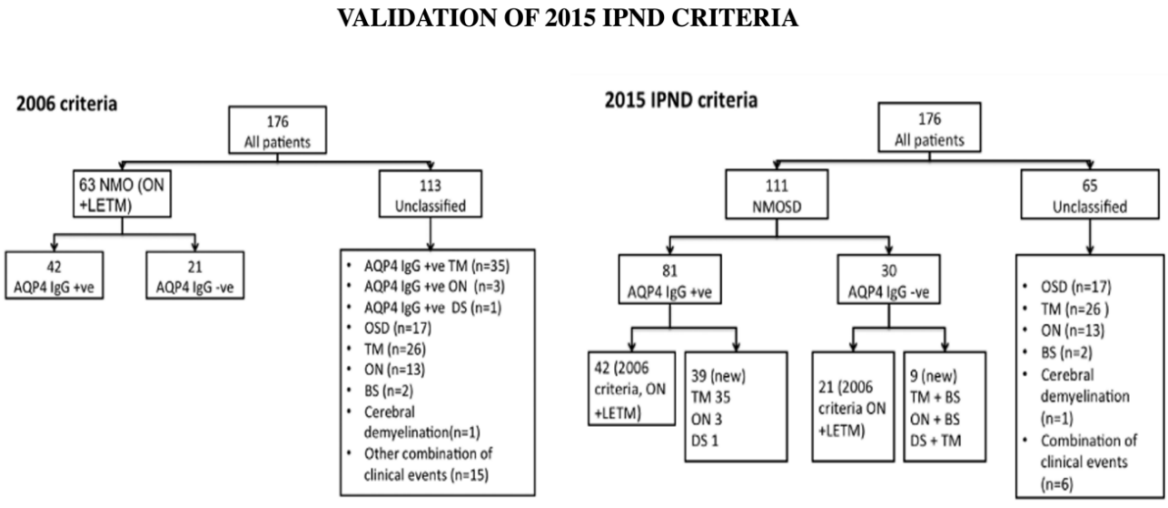


Figure 17: Real world validation and description of diagnostic impact of the of 2015 IPND diagnostic criteria for NMOSD. Note that while significantly reducing the number of "unclassified" cases 65 of total cohort of 176 still remain unclassified.

Hamid SH, Elsone L, Mutch K, Solomon T, Jacob A. The impact of 2015 neuromyelitis optica spectrum disorders criteria on diagnostic rates. Mult Scler. 2017 Feb;23(2):228-233. doi: 10.1177/1352458516663853. Epub 2016 Sep 28. PMID: 27553618.

Clinical Features

Our cohort's most common clinical presentation was concurrent ON and LETM, which occurred in 20/44 (46%). This was followed by isolated myelitis (32%) and concomitant area postrema syndrome and isolated myelitis (9%). Only two cases of isolated optic neuritis and one isolated area postrema syndrome were identified.

NMOSD case series indicate that ON is one of the most common presentations[84,85]. However, isolated ON is under-represented in our cohort and was recorded in only 5% of cases. This may be due to both under-recognition of NMOSD as a cause of isolated ON and referral bias. During the period of this audit, patients with isolated ON were routinely managed by the ophthalmology service without referral to the neurology service. The Groote Schuur ophthalmology service reported a cohort of 117 cases of optic neuritis between 2002 and 2012[86]. They described optic neuritis as having a secondary cause in 51% (60/117; with the secondary cause attributed to HIV infection in 50%)[86]. Importantly in their idiopathic group (n=57), they noted that a large proportion (n=43/57) of their cases demonstrated features of an atypical optic neuritis[86] (with typical features such as subacute onset with unilateral, painful ON with variable degree of visual loss predicting a high likelihood of progressing to MS) and worst visual outcomes[86]. These cases were predominantly of African ethnic background[86]. The AQP4-Ab had not yet been described during this study's early years, and AQP4-Ab testing was unavailable in their routine clinical practice in the latter years. In addition, MRI imaging was performed in only 20/117 cases[86]. This data is in keeping with our findings that, during the early years of this audit, AQP4-Ab antibody testing was rarely performed when evaluating isolated ON. Many of our patients did not have dedicated MRI imaging performed on their optic nerves despite ON being a component of their clinical presentation. Limited access to protocolised MRI sequences for optic nerves and restricted use of contrast media might have negatively affected the quality of optic nerve and chiasmal MR imaging. Consequently, relevant abnormalities such as chiasmal, long-segment optic nerve involvement and optic nerve enhancement may have been missed in our subjects.

A South African case series of NMOSD from KZN reported isolated ON in a third of cases[62]. Modi *et al.* published a series of relapsing idiopathic CNS demyelination from Gauteng in 2001 (all patients would fulfil 2015 IPND criteria for NMOSD). Isolated ON was the first presentation in three of their eight cases, and in five of the eight cases, ON at relapse was described. Later cohorts from Gauteng (Modi *et al.* 2011, Moola *et al.* 2017) and the Western Cape focused primarily on myelitis cases in NMOSD and reported ON only in combination with concurrent myelitis[60,69]. This under-reporting of ON in South African

NMOSD cohorts may reflect regional differences in patterns of case referral, clinical practice and reporting, as described at our referral centre.

In 9/44 (20%) of the subjects in our cohort, a clinical diagnosis of NMOSD was made, despite their not fulfilling current diagnostic criteria for this condition. Extensive investigations were performed to exclude other explanations for their presentation in all nine cases. Of these, 8/9 presented with idiopathic LETM. Four did not have AQP4-Ab performed at their original presentation and were only tested later when immunosuppressive therapy had already been instituted. Immunosuppression reduces AQP4-Ab titres, and low serum AQP4-Ab titres are associated with periods of clinical remission[20,81]. However, the finer points of this argument and why it may not be sufficient justification to diagnose NMOSD in these nine cases have been discussed above.

One case presenting with AQP4-Ab negative LETM had intractable nausea and vomiting, ascribed to side effects of azathioprine. However, azathioprine toxicity was never conclusively proven through blood metabolite levels. Similarly, MRI imaging was not repeated in this subject to determine if there was radiological evidence of area postrema involvement.

Only one case did not have myelitis in the subgroup of nine subjects who did not fulfil IPND criteria. She presented with an inflammatory brainstem syndrome and area postrema symptoms with supportive MRI features. In her case, AQP4-Ab serology was only performed after she had already started immunosuppressive therapy and was negative.

Five of the eight cases of IPND-negative myelitis died ostensibly from complications relating to their myelopathy. These deaths accounted for 5/7 of the total deaths in our cohort.

Imaging Findings

MRI is one of the most critical tools used to assess and differentiate, respectively, CNS demyelination conditions and inflammatory diseases. Although MRI was performed in all cases, no standardised MRI protocol regarding anatomical regions scanned (brain, optic nerve and spinal imaging), sequences acquired, or IV contrast media administration (31/44 cases). This was despite CNS inflammation or opticospinal clinical syndromes being the indication for imaging in most patients. These shortcomings affected the entire cohort, especially those who were seronegative, because the diagnostic criteria explicitly note the need for the imaging to be consistent with NMOSD in seronegative cases[2]. Thus the diagnosis of seronegative NMOSD relies on the premise that the relevant regions are imaged and that contrast is applied to support the diagnosis[2]. Standardised imaging protocols for CNS demyelinating disease are essential in diagnosing and monitoring MS[87] and differentiating NMOSD from MS[31,88]. Literature on NMOSD continues to shed light on the role and specificity of imaging characteristics in our understanding of NMOSD[89,90], not only in differentiating NMOSD from MS but also in distinguishing AQP4-Ab-positive NMOSD from MOGAD[31].

We did not have access to MOGAD testing. However, we could compare and contrast the imaging findings between AQP4-Ab-positive and negative cases. The significant differences were the higher proportion of AQP4-Ab-positive cases with cervical cord LETM, the absence of isolated lumbosacral LETM or conus lesions in the AQP4-Ab-positive group, and the higher proportion of patients with cerebral involvement in the AQP4-Ab-negative group.

In the AQP4-Ab-negative group, cervical involvement occurred in 43% compared to 76% of the AQP4-Ab-positive group. In addition, seven (30%) AQP4-Ab-negative cases had concurrent cerebral involvement. This was almost double the proportion seen in the AQP4-Ab-positive group.

By definition, an isolated core clinical syndrome should exclude a diagnosis of seronegative NMOSD; however, the IPND criteria do not explicitly state this about the radiological findings[2]. Six AQP4-Ab-negative cases had radiologically isolated LETM, and four did not clinically fulfil IPND criteria.

While we do not have a definitive explanation for the differences between AQP4-Ab positive and negative cases, the findings of lumbosacral and conus lesions restricted to AQP4-Ab-negative cases might be because these cases had MOGAD, where sacral lesions are well recognised as typical[91,92]. Similarly, AQP4-Ab-positive NMOSD is known to affect the cervicothoracic cord segments predominantly[15]. The differences in the cerebral lesions (with

a greater proportion of AQP4-Ab-negative cases having cerebral lesions) are less easy to explain, and while misdiagnosis is a possible explanation, only one case with cerebral lesions did not fulfil IPND criteria. It is important to acknowledge that while there was a large difference in the proportion of patients with cerebral lesions, the absolute numbers were small (3/17 vs 7/23).

CSF Findings

In our cohort, CSF investigations were available in the majority of cases. The predominant finding was a lymphocytic pleocytosis with elevated CSF IgG index (23/31 patients). In addition, CSF protein was elevated in 22/31 cases. These findings helped confirm an inflammatory cause. Negative CSF cultures, serologies and PCR results helped exclude CNS infections, notably syphilis and TB, in our most of our cases. The finding of either of these two infections in the CSF would likely have been cause for re-evaluation regarding the NMOSD. This would be in keeping with the recommendations of the IPND consensus document[2]. However, one AQP4-Ab-positive case in our cohort did test positive for syphilis. The nuances of this case and other parainfectious cases of NMOSD are discussed in detail in the section on parainfection in NMOSD.

Regarding CSF chemistry, concentrations of the CSF protein in cases with HIV and TB trended towards higher values, but this was not statistically significant. Cases of HIV had higher IgG index values than cases that were not HIV infected, but again, this was not statistically significant. In our practice, the CSF IgG index is not routinely performed in HIV-infected patients, as abnormalities are not specific, and the cause of abnormal CSF-IgG index in HIV cannot be distinguished from findings resulting from immune dysregulation observed in the CSF of patients with HIV[93–95]. CSF glucose values were not significantly different in cases when stratifying by AQP4-Ab status, HIV or TB infection.

Concerning the CSF cell counts, lymphocytic pleocytosis was the predominant picture. Interestingly, values of pleocytosis seemed to be highest in cases that did not have TB. None of these findings were statistically significant. Similarly, the elevated mean cell counts in cases without TB were skewed towards appearing higher by outlier values in a minority of cases. Our findings corroborated the finding of the CSF profiles in cases described by Bhigjee *et al.* in HIV-infected patients of NMOSD[62]. CSF analysis for oligoclonal bands (OCB) was not performed in our cases. This was in contrast to the cases reported in the KZN cohort of HIV-infected and may again reflect regional differences in clinical practice[62].

Jarius *et al.* evaluated the characteristics of CSF in 211 lumbar punctures performed in cases of NMOSD.[79] They noted a few critical features, also reflected in our cohort. Firstly, the most common picture was CSF lymphocytic pleocytosis. This was observed in about half of the cases (98/194, with a median of 19 cells/µl). Marked lymphocytosis (>100 cells/µl) was atypical and only seen in 6% of cases. Inflammatory changes were characteristically only seen during relapses and less common in attacks of isolated ON than in patients with myelitis[79]. Jarius *et al.* also observed that oligoclonal bands may be positive in a minority (16%) of cases of NMOSD.

In cases of MS, CSF OCB remain positive without any evidence of a concurrent clinical relapse; however, elevated intrathecal immunoglobulin production in NMOSD was limited to clinical attacks[79]. The impact of HIV or TB co-infection on the CSF profiles in cases of NMOSD attacks has not been systematically studied and remains unknown.

Parainfectious NMOSD and our cohort

All patients with myelopathy had an extensive evaluation to exclude primary or concurrent infection causes for myelopathy. Although CSF infectious and viral panel testing was performed in the majority of our cases, testing did not lead to a diagnosis in most patients. None of our patients were tested for HTLV, and only four were tested for Schistosomiasis. These are both common causes of myelopathy in Africa; however, neither infections are thought to be common or endemic in Cape Town (unlike KZN)[96–98], and the clinical characteristics of HTLV tend to be chronic and progressive[99], which is inconsistent with the acute myelopathies typically seen in NMOSD[100].

Syphilis serology was recorded as negative in all subjects included in this study except one with evidence of a concomitant syphilis infection. This patient was AQP4-Ab positive (titre 1:100) and presented with myelitis and area postrema syndrome. Her serum *Treponema pallidum* antibodies (TPAB) were positive, as was her serum RPR with a titre of 1:16. CSF FTA was positive, and VDRL testing of her CSF was negative. Her CSF protein was elevated at 1.26 g/l with a CSF glucose of 2.3 mmol/L (serum 5.5 mmol/l), a polymorphonuclear count of 4/µl, lymphocyte count of 3/µl, and a CSF IgG index of 1.18. This subject was also positive for SLE on serological testing but negative for antiphospholipid syndrome.

Two cases had positive viral studies in the CSF, and both were HIV positive. The first was a 50-year-old male with a history of HIV infection, a CD4 count of 1004 cells/µl and an HIV viral load suppressed in his blood for several years. He was AQP4-Ab negative and had an opticospinal presentation with a mildly inflammatory CSF at his initial presentation. His initial CSF protein was 0.56 g/L, had one polymorphonuclear cell and five lymphocytes. On his 20th day of admission, he had a third CSF sample performed (after already completing a course of high-dose IV steroids). The CSF VZV PCR was positive; however, the sample was acellular and had no other inflammatory changes. His two prior CSF specimens had tested negative for VZV. The clinical time course did not correlate with VZV being the cause of his myelitis, nor did it fit with VZV being a viral trigger of his NMOSD. It was thought that the VZV PCR result most likely represented an asymptomatic viral reactivation, which was not contributing to his clinical presentation. Nevertheless, he was treated with IV Acyclovir for fourteen days.

The second case was a 19-year-old female who was AQP4-Ab positive, presenting with bilateral ON. She had HIV with a CD4 count at presentation of 548 cells/ul and HIV viral suppression in her serum. Her CSF showed a total protein of 0.25 g/l, CSF glucose of 4.7 mmol/l, no polymorphonuclear cells and five lymphocytes. She had a positive EBV viral

load of 600 copies/ml. At the time, this was deemed clinically irrelevant and not investigated further. In retrospect, the EBV infection may have been an immunological trigger of her AQP4-Ab-positive NMOSD attack.

Viral precipitants have been noted in NMOSD. EBV is well known to be associated with CNS inflammatory demyelinating disease. EBV is associated with MS, and there is ongoing debate regarding the veracity of its role in the pathogeneses of MS[101]. In the *in vitro* setting, EBV has been used as a viral trigger in models of EAE[102]. Several cases of EBV and herpes viruses (HSV 1 & 2 and CMV) have been reported in NMOSD[58,103,104].

NMOSD has been recognised to occur with concurrent infections[12]. Indeed, evaluating the relationship between NMOSD and TB in our cohort is one of the primary goals of this audit and has repeatedly been reported in South African cases of NMOSD[13,60,64].
Pre/parainfectious NMOSD has been associated with several common bacterial and viral organisms[12]. In our and other South African cohorts, concurrent TB has been documented in up to 73% of cases[60].

It is important to note that several of the organisms implicated in parainfectious NMOSD can, in their own right, cause CNS inflammation, myelitis and even LETM[2,57,97,100]. It should also be noted that in our cohort and many other clinical series of pre/parainfectious NMOSD, there was no evidence of active bacterial infection in the CNS (especially no evidence of CNS TB). One of our cases had a positive CSF FTA, while her CSF VDRL was negative. Her serum AQP4-Ab was positive, making NMOSD likely the cause of her presentation (specificity 99%). CSF syphilis has been described in cases of NMOSD[105,57,106,107]; however, the positive FTA may be explained by a remote infection or previously treated syphilis. What role, if any, this plays in the pathogenesis of attacks of NMOSD remains uncertain. Nevertheless, vigilance and clinical judgement must be applied in NMOSD and concurrent infection.

Other common bacterial infections associated with NMOSD are *E. coli*, clostridial infections and *Helicobacter pylori*[12]. *H. pylori* infection is endemic to Africa, with a prevalence of up to 92% of people in North Africa and up to 66% in a South African cohort[108]. Yet, none of our NMOSD cases had documented co-infection. *H. pylori* infection is often asymptomatic and can be associated with gastrointestinal illnesses, including gastric cancers[108]. Cancer-associated NMOSD has been reported with gastric cancer11, but, again, this was not the case not in our cohort. *H. pylori* infection can be detected by breathalyser, blood, stool and gastric biopsy samples[108]. However, *H. pylori* testing is invasive in our setting, requiring gastroscopy and gastric biopsy. None of our cases were tested for *H. pylori.* Without a

clinical indication, direct clinical benefit or guidelines dictating the testing of otherwise asymptomatic patients with NMOSD for *H.pylori*, this was not part of our routine management of cases of NMOSD. Similarly, none of our patients were routinely tested for *E. coli* or clostridial infection. Based on the supposition above and our institutional experience, extensive investigations for an associated infection may not be prudent, especially when the infection is asymptomatic. However, TB, HIV and neurosyphilis are obvious exceptions in our setting.

Local population genetics, prevalence and patterns of infections may be critical in the pathogenesis of parainfectious NMOSD[109]. This may explain discrepancies in pre/parainfectious TB findings between NMOSD studies.

HIV and NMOSD in this cohort

HIV was the most common chronic comorbidity identified in our cohort affecting 10/44 cases; 7/10 of these patients were on HAART at presentation. There was a considerable variation in CD4 counts amongst HIV-infected subjects (64 – 1004 cells/µl) but no significant difference in CD4 counts between HAART-naïve subjects and those receiving HAART. 3/10 of our HIV-infected cases had concurrent tuberculosis. In two of these, the diagnosis was empiric and based on clinical evidence (both were AQP4-Ab positive). The only AQP4-Ab negative subject on TB treatment had sputum-positive TB. During her third month of anti-TB therapy, she presented with ON, myelitis, and a large hemisphere lesion. She had no evidence of CNS TB on CSF analysis and culture or brain biopsy (which showed acute demyelination and chronic inflammation with gliosis). None of the HIV-positive subjects had any AIDS-defining illnesses or history of AIDS-defining opportunistic infections before their presentation with NMOSD.

In January 2017, Bhigjee *et al*. published a retrospective series of 29 cases of NMOSD presenting to tertiary referral hospitals in KZN over ten years; 12/29 of these cases were HIV positive. While they did not describe the clinical presentations of all their patients, they noted through illustrative cases that their patients presented with opticospinal presentations along with other core NMOSD syndromes and concomitant HIV infection. They stated that while isolated LETM or ON in HIV-positive subjects may be attributable to opportunistic infections, drugs, concurrent metabolic illness or HIV itself; NMOSD was the most likely aetiology in subjects with concurrent or relapsing opticospinal presentations[62]. Of the AQP4-Ab negative members of our HIV-infected cases, 5/7 had an opticospinal presentation, fulfilling 2015 IPND criteria, and two AQP4-Ab negative, HIV-infected patients did not fulfil criteria for NMOSD. Both latter cases presented with fulminant LETM and died following their initial presentation.

In the series described from KZN, AQP4-Ab testing was only performed in 10/12 of their cases. In four of their HIV-positive group, AQP4-Ab was positive vs. 6/11 of their HIV-negative group[62]. In contrast, all our HIV-positive cases had AQP4-Ab antibodies performed, but only 2/9 were AQP4-Ab positive, while 15/36 of our HIV-negative patients were AQP4-Ab positive. As noted above, this seropositivity rate is lower than observed internationally or in the KZN cohort. Bhigjee *et al.* noted that while three of their cases had AQP4-Ab testing at a private laboratory, the rest used the same Euroimmun™ testing methodology used in our cases. Therefore, the testing methodology did not account for the differences in AQP4-Ab seroprevalence between our cohorts. Similarly, ethnicity did not differ significantly between our HIV-infected cases (12/12 in KZN and 8/10 in Cape Town were of black-African descent). Clinical presentations of all subjects in the KZN cohort were not described in detail, and it would be of interest to find out if their cohort differed clinically from our own and, if so, how.

The role that HIV plays in the development of NMOSD, if any, remains a matter of debate. It has been postulated that HIV or other concomitant infections, including TB or certain viremias, may represent autoimmune triggers[12,62,110]. The literature on HIV and NMOSD remains sparse and, apart from the series described above, is limited mainly to case reports. In a 2018 review dealing with the co-occurrence of NMOSD and HIV, Matthew *et al.* recorded only six cases in the medical literature, all from case reports. These authors did not include cohorts of NMOSD and HIV co-occurrence from South Africa (most notably 12 patients reported by Bhigjee *et al. in 2017,* two cases reported by Modi *et al.* in *2011*, one from Zatjirua *et al.* in 2011 and four cases from a 2017 unpublished cohort from Gauteng by Moola). These reports amount to 25 published cases of NMOSD and HIV in the medical literature[46,62,69,75]. Our study contributes a further nine cases to the medical literature.

There remains a dearth of literature dealing with the implications of HIV infection on the pathogenesis, relapse rate and management of NMOSD. More information is required with particular relevance to the South African setting, as demonstrated by our cohort.

TB and NMOSD in this cohort

Our cohort describes 11/44 cases of TB associated with NMOSD. In 6/11 cases, the diagnosis of TB preceded the symptoms of NMOSD, while 5/11 were diagnosed at the time of their NMOSD diagnosis. In 7/11, a positive microbiological diagnosis of TB (from sputum in six and lymph node biopsy in one) was made, whilst in the remainder (4/11), the diagnosis was empiric and based on either clinical or radiological grounds. None of our cases

had evidence of TB in the CSF. The most prolonged period between TB diagnosis and presentation with NMOSD was 54 weeks. 3/11 subjects diagnosed with TB had a comorbid HIV infection.

In 1990, Silber *et al.* described a series of eight patients presenting with a fulminant opticospinal syndrome in Cape Town in the setting of tuberculosis. While these cases were clinically diagnosed with NMO, questions remained about whether the opticospinal presentation resulted from TB or neurotoxicity from TB medication. They found that, in five patients, treatment was only started after the onset of neurological symptoms and that their cases of TB-associated NMO were clinically indistinguishable from *de novo* cases of NMO. In addition, one patient had an autopsy that showed extensive demyelination of the spinal cord and optic nerves [64]. Given these findings, Silber *et al.* postulated a possible pathophysiological association between TB and NMO/Devic's disease.

Another study from the Western Cape published by Zatjirua *et al.* in 2011 reported TB in 11/14 cases of NMOSD, with an odds ratio of 4.6 of having TB compared with their control cohort of patients with Guillain-Barré syndrome (GBS)[60]. They concluded that there was a pathogenic and possible causal link between concurrent TB and NMOSD presentations in cases from the Western Cape. In contrast, a large Chinese study of 88 cases of NMOSD compared with a control group of various inflammatory and infectious neurological conditions identified only one case of TB in their NMOSD group, which did not differ significantly from their control group[56]. The authors noted their findings were not generalisable because their study population was drawn from a single region.[56]. This limitation also applies to our audit.

The World Health Organization has categorised TB in South Africa as a "severe endemic", with an estimated incidence of > 500/100 000 new or relapsed cases per year. The South African Medical Research Council performed a large-scale, nationwide, multiple-cluster assessment of the South African TB prevalence in adults above 15. In this study, they found the national prevalence of TB to be 852 cases per 100 000 population. This further increased to 1734 per 100 000 HIV-infected people, in whom the highest prevalence was noted[111,112]. The finding of co-occurrence of TB in a quarter of our NMOSD cases, while not as high as the 78% reported by Zatjirua *et al.,* is nevertheless notable. It is higher than the background community prevalence rate for populations in South Africa [111,112]

South Africa has a very high prevalence of TB, especially in patients with underlying immunosuppression such as HIV[111,113]. In addition, TB often goes unrecognised or is minimally symptomatic in the community[111]. The relationships between TB, HIV and

NMOSD remain controversial, and South Africa's high prevalence of TB and HIV means that it is an ideal location to study these relationships prospectively.

Therapeutic Interventions

Acute Management of Attacks

High-dose IV steroid administration is widely regarded as the first-line therapeutic intervention in NMOSD[3]. The efficacy of plasmapheresis (PLEX) in acute attacks of NMOSD is well established as the gold standard of treatment in cases that do not respond to high-dose steroids[48,49,114–116], with some even suggesting that PLEX should be used as a first-line treatment[115–117].

In our cohort, 68/107 attacks occurred while our neurology service managed them. All cases, bar two, were initially treated with high-dose steroids, and only 13 of our 44 patients received PLEX. In the majority of these cases, the reason documented for PLEX not being administered was restricted access rather than the lack of a clinical indication. In our setting, access to PLEX was limited mainly due to cost and administrative hurdles such as limited availability, scheduling, and staffing. We could not interpret meaningful outcome measures due to PLEX administration, maintenance therapies or the timing thereof because of the retrospective nature of our audit and the lack of standardised/protocolised time points for clinical re-evaluations. Our cohort's crude annualised relapse rate (ARR) estimation was 0.38 per patient-year. This is similar to relapse rates of cases on first-line immunosuppressive therapy in a large systematic review performed by Giovannelli *et al.* on behalf of the NEMOS (Neuromyelitis Optica Study Group in Germany), NOMADMUS (Neuromyelitis Optica Study Group in France), and OFSEP (Observatoire Francßais de la sclerose en Plaques) groups. These authors also noted that tracking ARR, rather than time to first relapse or change in ARR, was not as clinically applicable because of the severe morbidity that can be associated with even a single relapse[118]. Non-European and specifically African participants were under-represented by Giovannelli *et al.* in a retrospective review of first-line therapy with azathioprine in a multi-ethnic setting in Brazil (where 11/19 cases were of mixed African descent), Gomes *et al*. reported an ARR of 0.1 (range = 0–0.35)[119], which was similar to ours.

Our cohort used a crude ARR measured by patients' historical recollections of clinical and subclinical events in the hospital folders and admission records of relapses. Calculating changes in ARR or time to first relapse was not possible, nor would it have been reliable in our setting, as many relapses went unrecognised at the time of the attack. This applied to attacks that occurred both before and after a diagnosis of NMOSD was made. Whether our ARR of 0.38 is due to therapeutic interventions such as PLEX and long-term preventive therapies or under-recognition of clinical relapses is unclear. A prospective follow-up could answer several of these questions.

In our cohort, patients had a disability at discharge in those who received PLEX and those who did not (MRS score of 4 and 2.64, respectively). Disability was worse in those that received PLEX, which may have led to greater motivation for this treatment. Nevertheless, it is generally accepted that PLEX is highly effective in the acute management of NMOSD and CNS demyelinating disease[48,120], which applies to both antibody-positive and negative cases[121]. Therefore, patients must receive the best possible treatment early in their disease course to mitigate the accumulation of disabling deficits[1].

Maintenance Immunosuppression Therapy

43/44 patients received immunosuppression during the study period. 7/44 patients were prescribed oral steroid monotherapy, while 34/44 were treated with oral steroids and a steroid-sparing agent (SSA). The most common SSA prescribed was azathioprine. Methotrexate was used in only one case. Of the subjects who were not prescribed an SSA, only 3/7 were treated with PLEX, and 4/7 died due to their initial attack of myelitis. Three cases received no maintenance therapy after their initial presentation. As noted before, one of these patients died following their first attack. At the same time, the other two (both AQP4-Ab negative) had minimal discernible deficits at follow-up and were subsequently lost to follow-up. Only one case in our study was treated with a biologic agent (Rituximab). Rituximab and other biologic agents have been restricted in our setting due to their cost. Because steroids and SSA, such as azathioprine, are more affordable and also effective in preventing relapses of NMOSD, their use is a more pragmatic compromise in our setting[45,47,122].

Given this study's retrospective, cross-sectional nature, long-term outcomes of therapeutic interventions could not be reliably measured. This is a limitation of our study. A prospective study would be required to evaluate the effect of various treatment modalities on long-term outcomes.

Outcomes

In our cohort, the durations and intervals between clinical follow-ups varied considerably. For example, the duration of follow-up ranged from 0–151 months after a patient's diagnosis of NMOSD. This is in large part due to the retrospective nature of the study, and thus we could not determine outcomes at predetermined times. As such, this audit could not accurately compare differences in outcomes related to treatment modalities and relapse rates at prespecified time intervals. It should be noted that the disability documented in some cases may reflect the severity of the acute attack and failure of acute rescue therapies (HDS and PLEX) rather than the effects of relapse prevention therapies.

Several disability outcome scales have been used in the context of NMOSD trials[123,124]. However, the MRS score, rather than Extended Disability Severity Scale (EDSS), ~~w~~as used to measure disability in this cohort as it is readily calculated and was frequently documented in clinical records. This way, we could assess disability at the presentation and the last clinical interaction with the hospital.

As expected, patients presenting with myelitis, either alone (median MRS score of 3; mean MRS score 3.78) or with ON (median 3; mean MRS score 3.3) and area postrema syndrome (median 3, mean MRS score 4), were associated with poor MRS score. The median MRS score for cases without myelitis was also 3. However, due to the small number of cases (seven) and non-normative distribution of outcome results, a single patient with an MRS score of 5 due to hemiplegia skewed the average results for cases without myelitis. The mean MRS score for patients without myelitis was 1.6, and excluding this case with an MRS score of 5, the median MRS score in cases without myelitis was 0 (mean 1).

The aOR of severe MRS score when considering the presence of myelitis vs its absence was 263,30 (95% CI 3.06-22684,31); however, there is a very wide confidence interval due to the low number of controls without myelitis. Similarly, cases with myelitis had greater odds of receiving PLEX (aOR 7.35 95% CI 0,61–88.25). This access to PLEX may have been a function of the perception of worse disease or deficits in cases with myelitis. Note that the MRS score focuses primarily on motor functions and functional independence[123]. This focus may underestimate neurologic deficits such as visual loss and minimise the severity of the underlying disease process. However, the MRS score has been used as a composite of outcome measures in NMOSD drug trials[123,124]. Furthermore, all deaths in our cohort occurred in the clinical setting of myelitis. Due to their low occurrence or absence in this cohort, it is difficult to draw any conclusions regarding syndromes such as the area postrema brainstem and diencephalic syndromes.

Across the whole cohort, characteristics that were associated with worse disability were AQP4-Ab-positivity (aOR 6,78), HIV infection (aOR1,80) and recent or active TB (aOR 7.70) and age between 40-49 (aOR 17.9).

In our cohort, there was a total of 107 attacks experienced by our patients, 39 of which occurred before the clinical diagnosis of NMOSD had been made. Many preceding episodes were treated at peripheral hospitals and not recognised as NMOSD. More concerning, however, was that 18 cases, which could not be incorporated into our audit, were AQP4-Ab positive but were never assessed by the neurology service. This means these cases were denied the benefit of early and appropriate management (both for their acute attacks and the prevention of future attacks).

In our cohort, female sex, AQP4-Ab-positivity, recent TB before presentation, and HIV infection appeared to be associated with worse functional outcomes as reported in the discharge record or most recently available clinical records at the time of the audit. However, these findings were not statistically significant due to the small sample size. Prospective and preferably multi-centre studies involving larger cohorts would be helpful to validate these associations.

7/44 cases died, and all deaths occurred following discharge from health care services. All of these cases had myelopathy, with 4/5 having an MRS score of 5, implying severe disability at hospital discharge. These clinical characteristics were similar regardless of AQP4-Ab status. These deaths may represent shortfalls in general myelopathy care in the community clinical setting. However, nine other cases in our cohort were discharged with disability of similar severity (MRS score of 5) who did not die. Another explanation for the deaths may be that a fulminant attack occurred while in the community setting. Under-recognition of attacks (especially area postrema, diencephalic and brainstem syndromes) and limited access to timeous intervention precipitated their deaths. Again, the fact that five of these deaths occurred in cases that did not fulfil IPND diagnostic criteria means that an alternative and undescribed diagnosis may underlie their lack of response to usual NMOSD therapies.

Limitations of this Audit

1. This descriptive audit was retrospective, cross-sectional and based on medical records from routine clinical care. The study did not include a control cohort. Furthermore, the follow-up duration varied markedly between cases and as a result, no definitive conclusions could be drawn regarding annualised relapse rates or long-term functional outcomes and therapeutic interventions.

2. This study, involving a rare condition, was performed at a single centre, tertiary level, public-sector referral hospital in an urban setting. This resulted in geographic and socioeconomic referral bias. Consequently, caution should be exercised when applying the findings to a broader national or international context.

3. Definitions of NMOSD evolved during the period, with the criteria becoming more inclusive in terms of the clinical spectrum of syndromes recognised. Consequently, we may have underestimated the actual burden of disease. However, nine cases (20%) in this cohort did not meet any diagnostic criteria for NMOSD. These cases also had worse clinical outcomes regarding MRS score and death. Including them in this cohort study may have skewed our data set towards worse clinical outcomes. It should be borne in mind that these cases were included because they met the inclusion criteria for this audit, namely, having a clinical diagnosis of NMO/NMOSD recorded in their clinical record and no red flags for an alternate diagnosis. The decision to include them in the cohort also reflects the "real world" practice in our setting. The treating clinicians' decision to label them as NMO/NMOSD despite their lack of diagnostic criteria informed their treatment and management, and this may have affected their overall outcomes.

4. During this audit, no standardised protocols were in place to guide the investigation of CNS inflammatory and demyelinating diseases for serological tests, other blood tests, testing for co-infection, CSF analysis, and MR imaging. Similarly, no standardised protocols were in place to guide management. This was most pronounced in applying protocols for administering PLEX for acute attacks, adopting standardised MRI protocols, and administering contrast media when investigating suspected CNS inflammatory disorders.

This audit of our clinical service is of value in several ways

1. In our clinical setting, we have comprehensively described the demographic, clinical, laboratory and radiological features of NMOSD and idiopathic inflammatory CNS demyelination with LETM.
2. We have described the clinical burden of NMOSD, and, in doing so, this has enabled us to plan, apportion and advocate for recourses for our patients with NMOSD, supported by a data-driven model of a described clinical cohort. This study serves to:
    a. Increase the recognition of NMOSD, its' clinical presentations and relapses, enabling early referral for evaluation.
    b. Ascertain the actual burden of NMOSD and associated relapse rates to allow us to calculate the cost of NMOSD to the health care services at GSH and extrapolate to future service provision. This will enable us to advocate for using PLEX, SSA and biological disease-modifying therapies in our routine clinical care and research settings (i.e., in clinical trials).
    c. Empower healthcare professionals to advocate for the care of patients with NMOSD within their communities because all the deaths occurred following hospital discharge:
        i. This may take the form of advocating for more education and better myelopathy care at community health centres and referral hospitals, as well as for more recourses such as home-based and clinic-based care for patients with severe disability.
        ii. Acknowledging the impact of new visual deficits/blindness and creating relationships with organisations such as Friends of the Blind may help patients adjust to life and reintegrate into society and their work environments.

3. It has allowed us to document shortcomings and has created the opportunity to remediate discrepancies in the diagnostic and therapeutic practices for NMOSD, which may disadvantage patients.
    a. Most AQP4-Ab-positive cases from within our referral area were never clinically assessed by the neurology service. This deprived patients of the required expertise and therapies to manage their condition and placed them at risk for future relapses.
    b. Our series acknowledges that we may have over-diagnosed cases of idiopathic CNS inflammatory disorders as seronegative NMOSD. In doing so, we may have subjected patients to prolonged immunosuppression and

the inherent burden and complications without being informed by empirical evidence.

c. In evaluating our cases, we noted the lack of the appropriate MRI protocols for suspected CNS demyelinating diseases.
    i. Specifically, regarding the approach to MRI imaging in cases of CNS inflammatory/demyelinating disorders, we recommend adopting an MRI demyelination protocol which would include sequences of the brain, spinal cord, and optic nerves, along with the routine administration of contrast media.

4. Our audit has allowed us to describe the clinical features and comorbid diseases that have not been reported extensively in the literature. We have drawn attention to questions that continue to require further clinical research to understand the underlying relationships, namely:
    a. TB was diagnosed concurrently with NMOSD in a greater proportion of our cases than expected from the background community prevalence. However, whether this is geographically specific and confined to areas with a high prevalence of TB, such as Cape Town and the rest of South Africa, is yet to be addressed.

    b. The high incidence of comorbid HIV raises questions about the interactions between HIV and NMOSD. This includes the effects of HAART, the possibility of immune reconstitution syndromes and the complications of immunosuppressive treatments in people with NMOSD and concurrent HIV.

5. We have highlighted the under-representation of African cohorts in the academic discourse involving NMOSD. Inequity in NMOSD research disadvantages patients from novel treatments and researchers in better describing the disease's pathophysiology and characteristics.
    a. The exclusion of a region presumed to have one of the world's highest prevalences of NMOSD means that important clinical and basic science features, which may contribute to the understanding of NMOSD, are being overlooked.

This cohort and other cohorts from South Africa and sub-Saharan Africa need to be recorded and followed prospectively to understand and determine the true national incidence, prevalence, relapse and complication rates. NMOSD is a rare disease, and local collaboration

between institutions may help better characterise the condition in our context. Raising awareness of the disease allows for increased recognition. Local clinical and academic partnerships and advocacy also enable the establishment of uniform, equitable standards of care that do not disadvantage patients while being cost-effective and evidence-based.

The burden of NMOSD on patients, their families, the greater community, and health care systems in general is considerable. Advocating for patients from low- and middle-income countries to access resources and actively participating in the international NMOSD research agenda can only be beneficial.

Concluding Statement and Relevance of this Audit

In conclusion, we note that NMOSD is understudied in South Africa and sub-Saharan Africa, despite the high prevalence of the disease in this region. Clinical associations of NMOSD with HIV and TB have been reported in the medical literature but require further clarification. As a region with some of the world's highest prevalence of HIV and TB; and purportedly one of the world's highest prevalence of NMOSD, South African centres are ideally placed to study these diseases and their relationships.

As stated above, the key findings of our study were the systemic shortcomings in the referral pathways of NMOSD cases and the under-recognition and under-referral of AQP4-Ab-positive cases. This can be mitigated by active surveillance and reporting AQP4-Ab assay seropositivity directly between the laboratory and an NMOSD working group. As local researchers and clinicians interested in NMOSD, we must identify cases of NMOSD systematically. Patients identified at the peripheral hospitals must be referred to centres of expertise for early and appropriate management. Another important finding is that our neurology service diagnosed NMOSD in patients who did not fulfil the IPND (or any other) criteria for NMOSD. It is crucial to adhere to international consensus criteria and, in this way, avoid over-diagnosis NMOSD in cases which should more correctly be described as idiopathic (demyelinating) CNS inflammatory disorders. Adhering to IPND criteria is critical and will allow us to enrol our patients into studies of novel agents. More importantly, as demonstrated in our cohort, adhering to diagnostic criteria will avoid the morbidity and mortality associated with misdiagnosis.

International investigations into newer, typically more expensive treatment modalities continue while our patients, who have the most to benefit from advances in understanding NMOSD, are neglected and poorly represented.

In Africa, healthcare professionals interested in NMOSD and people affected by the condition must form local and international ties to improve awareness of NMOSD in sub-Saharan Africa. Improving this situation requires intervention at multiple levels. It requires interacting with patients and their communities to assess and increase their knowledge of and how they interact with the disease. It also requires researchers and medical professionals advocating for their patients to raise awareness of NMOSD and to support trials assessing new treatments. Lastly, it requires organisation through further collaborative multi-centre studies to formally describe the burden of disease of NMOSD in the South African health care context. Through rigorous evidence-based knowledge of the characteristics of NMOSD

in South Africa, we can participate with the international community of stakeholders in NMOSD and improve the lives of people living with NMOSD in our context.

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Supplementary Data

Documentation and Poster

1. UCT Health Sciences Human Ethic committee (HREC) Approval Letter

2. Neurological Association of south Africa poster presentation March 2020

Tables and Charts

1. Supplementary Charts and Tables of CSF indices
    a. Box-plots of CSF indices commonly measured in the cohort
    b. Box-plots of CSF IgG index stratified by AQP4-ab status; HIV and TB infection as referred to results discussion
        i. Tables of statistics of IgG-index data (stratified by AQP4-ab status; HIV, TB infection and IPND criteria)
    c. Box-plots of CSF protein stratified by AQP4-ab status; HIV and TB infection
        i. Tables of statistics of CSF protein data (stratified for variables AQP4-ab status; HIV, TB infection and IPND criteria)
    d. Box-plots of CSF glucose stratified by AQP4-ab status; HIV and TB infection
        i. Tables of statistics of CSF Glucose data (stratified for variables AQP4-ab status; HIV, TB infection and IPND criteria)
    e. Box-plots of CSF Lymphocyte counts stratified by AQP4-ab status; HIV and TB infection
        i. Tables of statistics of CSF lymphocyte counts (stratified for variables AQP4-ab status; HIV, TB infection and IPND criteria)

2. Radiology: Spinal imaging MRI of LETM with Pre and Post contrast T1 weighted sequences.

Human Ethics Committee Approval Letter

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room G50 - G Floor
Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 650 1236
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

16 March 2020

HREC REF: 158/2020

Dr E Lee Pan
Neurology
E8, New Groote Schuur Hospital

Dear Dr E Lee Pan

PROJECT TITLE: AUDIT OF THE CLINICAL, RADIOGRAPHICAL, AND IMMUNOLOGICAL FEATURES OF PATIENTS DIAGNOSED, AND MANAGED WITH NEUROMYELITIS OPTICA (NMO) BETWEEN 2013 AND 2018. SUBSEQUENT CORRELATION WITH THERAPEUTIC INTERVENTIONS AND CLINICAL OUTCOME MEASURES. MMED CANDIDATE DR MV GULE

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 March 2021.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

The HREC acknowledge the student: Dr MV Gule will also be involved in this study.

Please also note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

[1] The original ethical approval was for the audit was from 2013-2018; this was amended to extend the review period until the end of 2019. Given our results, subsequent amendments have been made to survey AQP4-Ab and MOG-Ab serology results prospectively for future audits.

Neurological Association of South Africa Poster

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES

Review of clinical and serological phenotypes of Neuromyelitis Optica Spectrum Disorders presenting to Groote Schuur Hospital

M.V. Gule and E. Lee Pan

Background

Neuromyelitis Optica Spectrum Disorders (NMOSD) is a severe CNS inflammatory disorder characterized classically by episodes of optic neuritis and/or myelitis and is associated with positive aquaporin-4 (AQP-4) antibodies. Without intervention, NMOSD is characterized by multiple relapses, an aggressive progression with the accumulation of disability.

While description of NMOSD and its disease associations continues to grow, knowledge regarding the clinical phenotypes, serological and imaging features in the South African context remains limited and there is no consensus on the association between NMOSD HIV infection and TB co-infection.

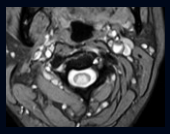
Questions of interest are what proportion of our patients are Aquaporin-4 antibody (AQP-4) positive, and what are the clinical and outcome characteristics of the AQP-4 seropositive and negative patients?

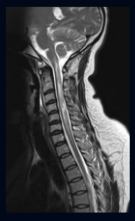
Methodology

We performed a retrospective audit of cases of NMOSD at Groote Schuur Hospital (GSH) between 2013 and 2019. Strategies to identify cases included a retrospective search for of AQP-4 sera performed at our reference laboratories and corresponding this with hospital records for clinical information. Prior to the period of review, the AQP-4 antibody test was performed infrequently at a private laboratory. In 2013 became available to state, hospital Laboratories. Lastly, a convenience sampling of cases of NMOSD attending the Neurology service clinic was conducted as an adjunct to source further cases.

Clinical information was collated to provide baseline demographic and medical comorbidities. Clinical characteristics of presentations, inpatient management of Acute attacks, maintenance immunotherapy and outcome function was assessed.

Cases were primarily separated into 3 groups: 1. AQP-4 positive 2. AQP-4 negative - IPND, criteria positive NMOSD, and 3. strongly clinically suspected to be NMOSD despite AQP4 negativity and unable to fulfil IPND criteria and without clinical “red-flags”. NMOSD cases regardless of AQP4 status were further separated according to their HIV seropositivity and concurrent or prior of tuberculosis at their presentation.





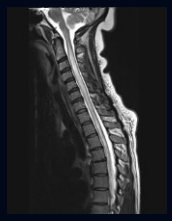


Figure 1: An example of Transverse section of myelitis (above) and LETM in an AQP4 Positive (right) and AQP4 negative patients

Results

It is important to acknowledge that the NMOSD diagnostic criteria changed during the period of this study.

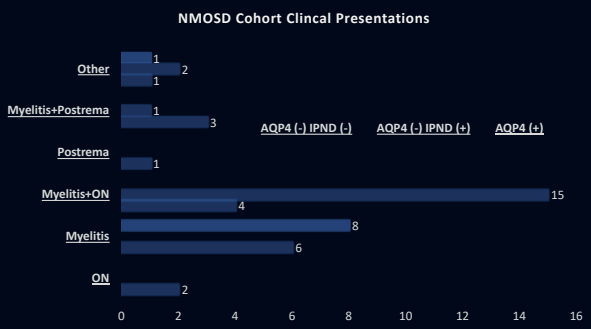
52 cases of suspected NMOSD being managed by the GSH Neurology service were identified. Duplicates, incompatible cases and cases with incomplete data were excluded. In total 44 cases were included in the final analysis. The AQP4 positive group consisted of 17 patients. 18 cases were AQP4 negative NMOSD and 9 cases were thought to represent possible NMOSD despite not fulfilling criteria.

Most of our patients were female (80%) and had an average age of 38. One seropositive patient presented with optic neuritis (ON) and transverse myelitis (TM) in the setting of a recent pregnancy.

Although not self-reported ,the ethnicities were captured from the records. Cases were predominantly of mixed-African and African ancestry. One patient mixed reported Malay/Philipino ancestry. None of our group had reported White/Caucasian ancestry.

AQP-4 positive cases presented predominantly with inflammatory TM and ON however, cases were described across all prescribed phenotypes. AQP-4 negative patients fulfilling IPND criteria all bar one had concurrent ON and Myelitis as their core clinical syndromes. The group of suspected NMOSD not fulfilling NMOSD criteria comprised of cases with unexplained long segment idiopathic inflammatory myelitis, and a case of brainstem syndrome.

One HIV negative patient presenting with Seropositive NMOSD was concomitantly diagnosed with SLE and Antiphospholipid syndrome.

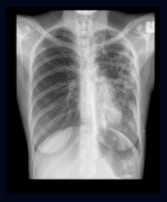


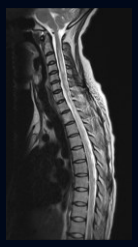
Disease associations: HIV/TB and NMOSD

HIV was the most common comorbidity accounting for 8 of 44 cases. The average CD4 count at the time of diagnosis of NMOSD was 464 and 5/8 patients were on antiretroviral therapy. The lowest CD4 count in the group was 64, in a patient who started ART 3 months prior. All HAART naïve patients had CD4 counts above 200 and none had AIDS defining conditions. The AQP-4 seropositivity was 25% (2/8) amongst the HIV positive and 38% (13/34) in HIV negative patients.

A diagnosis of TB occurred 9 (21%) of the NMOSD cohort. Three cases of TB were diagnosed in the HIV infected patients, however only one case was microbiologically proven. 4/6 HIV negative patients with TB had a positive microbiological diagnosis.

All TB diagnoses were made within six months of NMO presentation (average 12.7 weeks). Four patients were diagnosed with TB at the time of their NMOSD presentation, 2/4 were empiric TB diagnoses. None of the our NMOSD patients have subsequently been diagnosed with TB, regardless of treatment with immunosuppression.





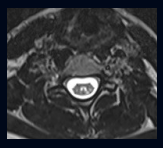


Figure 3: An HIV infected case presented with bilateral symmetrical long segment ON a subsequent relapse encephalitic a month later, even after a response to Plasma Exchange and immunosuppressive therapies. This, in the context of an antecedent TB infection and CD4 count of 64.

Outcomes

Measures outcome included Modified Rankin Score after initial attack or relapse, total number of attacks, escalation of immunotherapy with plasmapheresis (PLEX) and maintenance steroid sparing or biological agents. A total number of 42 relapses were recorded. The average number of attacks was 1.3/case over the review period (range 0-4). The average MRS at follow up was 3.28 (Range 0-5), six patients had died at the time of data collection, usually from complications of their NMOSD attacks.

Of the 40 patients analyzed, only six received PLEX. Resource access constraints rather than clinical decisions were the main reason for not receiving PLEX. PLEX was delayed an average of six days from onset of steroid therapy (this average excluded one case in whom PLEX was delayed a full calendar year and was only instituted after recurrent applications, and finally accessing the intervention through private means).

Conclusions

This limited retrospective folder review represents the experience of a single center. It demonstrates that NMOSD creates several clinical challenges in its diagnosis and management. This is all in the setting of an aggressive disease where timely intervention is required to limit potentially severe and lasting disability, therapeutic options are limited and costly.

From our cohort there does not appear to be a consistent association between cases of NMO and HIV infection or tuberculosis. This warrants further exploration. Our experience with HIV infected patients shows that their management is particularly complex, with diagnostic uncertainties, multiple concurrent diagnoses, and the risks of initiating immunosuppressant therapy in these patients with pre-existing immunocompromise

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Cerebrospinal Fluid Indices, Charts and Statistics

CEREBROSPINAL FLUID INDICES IN COHORT

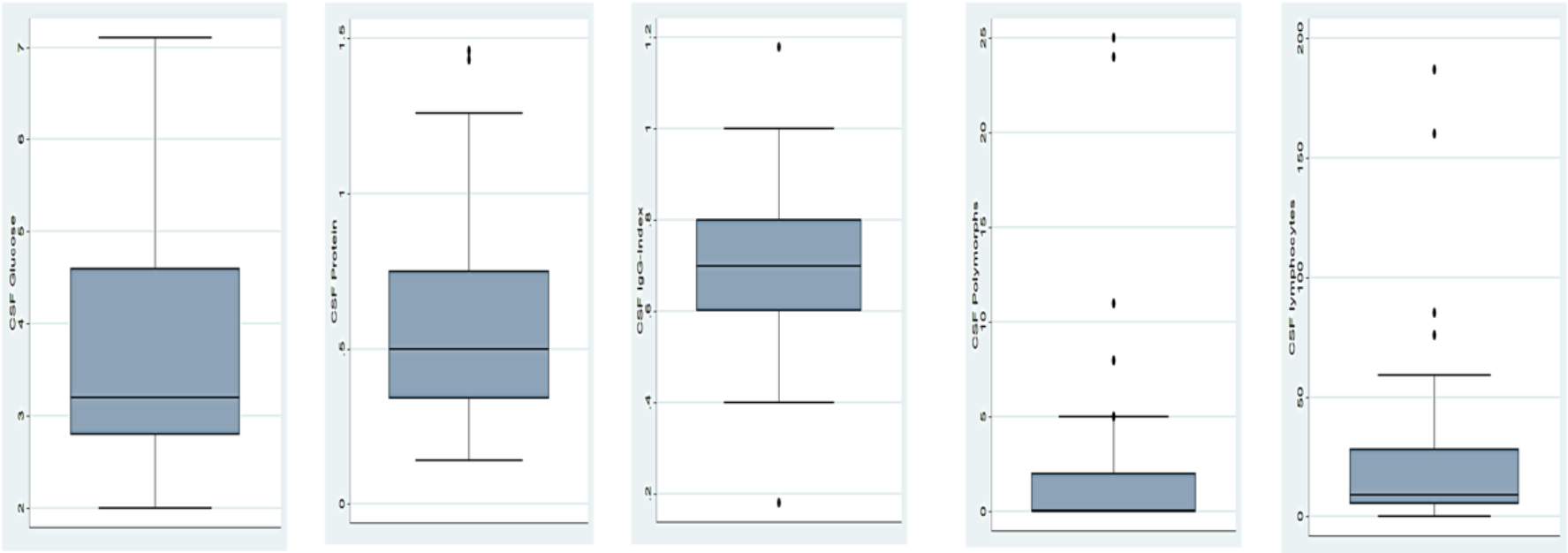
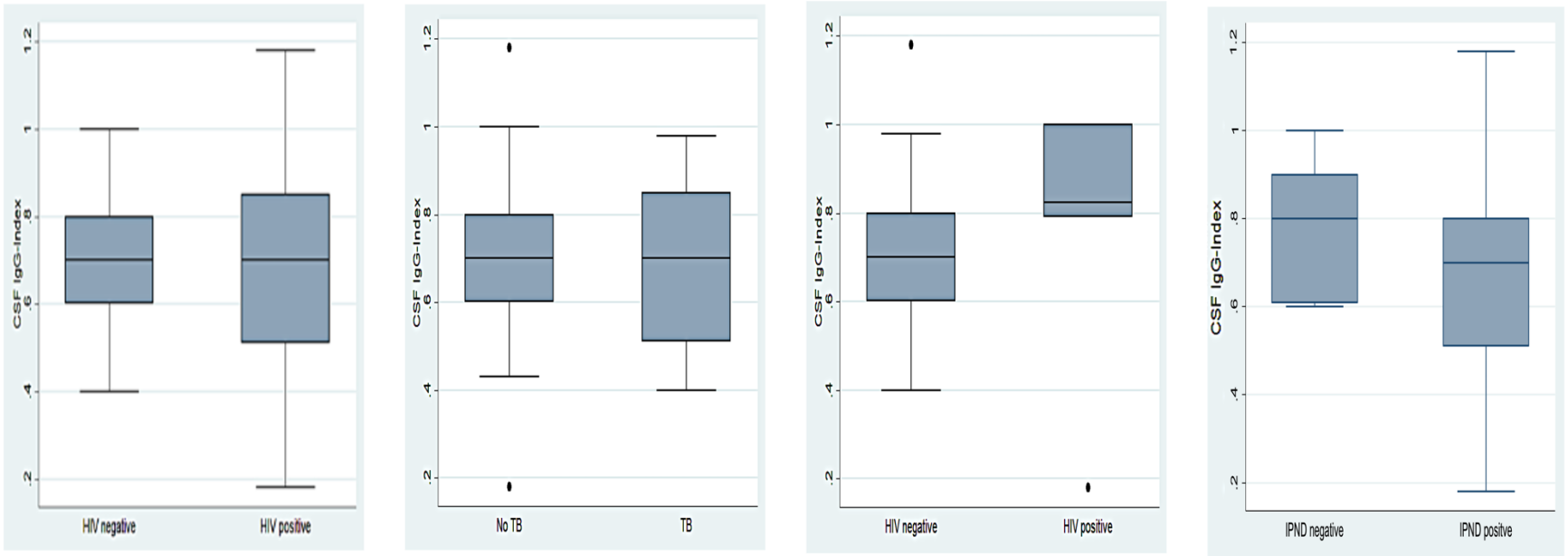


Figure 20 - Supplementary Charts

Box plots of CSF indices commonly measured in the cohort. Indices include CSF Protein, Glucose, IgG index, CSF Polymorphonuclear cells, CSF Lymphocytes as referred to in text of results, discussion and Table-4. Note the pattern of the mild lymphocytic pleocytosis with mildly elevated protein.

Values stratified by clinical characteristics of HIV status, AQP4-Ab status, IPND criteria and TB infection are shown in the tables and charts below with box plots and values for medians, and interquartile ranges of each index.

CSF IgG-INDEX STRATIFIED AQP4-AB STATUS, HIV & TB INFECTION, & IPND CRITERIA



Supplementary Charts – Figure 21
Box plots of CSF IgG index stratified by AQP4-ab status; HIV and TB infection as as referred to results discussion and in Table-4 where the text delineates numerical values of results in tested in each index. See tables below actual with data numerical values values 50% is the mean value, with 25 and 75% being the interquartile range

As noted in the text CSF IgG Index is not routinely measured in HIV infected patients and the clinical or pathophysiological significance of IgG index findings members of this cohort with HIV is not know but not thought to be of any relevance pertaining to NMOSD.

CSF IgG INDEX STRATIFIED BY AQP4-AB STATUS

-> aquaporin = 0

CSF IgG-I

Percentiles		Smallest		
1%	.4	.4		
5%	.43	.43		
10%	.51	.51	Obs	21
25%	.6	.6	Sum of Wgt.	21
50%	.7		Mean	.7147619
		Largest	Std. Dev.	.1673804
75%	.8	.9		
90%	.9	.9	Variance	.0280162
95%	1	1	Skewness	-.0788499
99%	1	1	Kurtosis	2.328303

-> aquaporin = 1

CSF IgG-I

Percentiles		Smallest		
1%	.18	.18		
5%	.18	.43		
10%	.305	.51	Obs	10
25%	.51	.51	Sum of Wgt.	10
50%	.7		Mean	.684
		Largest	Std. Dev.	.28949
75%	.85	.8		
90%	1.08	.85	Variance	.0838044
95%	1.18	.98	Skewness	-.0040819
99%	1.18	1.18	Kurtosis	2.45502

Supplementary Charts - Figure 21 (a.
Tables demonstrating numerical values values CSF IgG index Stratified AQP4-ab status; 50% is the median value, with 25 and 75% being the interquartile range. Cases with AQP4-Ab-positivity had the highest 99% value but also the largest variance in IgG index, when HIV positive cases were excluded.

CSF IgG INDEX STRATIFIED BY HIV STATUS

-> HIV = 0

CSF IgG-I

Percentiles		Smallest		
1%	.4	.4		
5%	.43	.43		
10%	.43	.43	Obs	25
25%	.6	.51	Sum of Wgt.	25
50%	.7		Mean	.6892
		Largest	Std. Dev.	.1857175
75%	.8	.9		
90%	.9	.9	Variance	.034491
95%	.98	.98	Skewness	.5903997
99%	1.18	1.18	Kurtosis	3.256774

-> HIV = 1

CSF IgG-I

Percentiles		Smallest		
1%	.18	.18		
5%	.18	.79		
10%	.18	.8	Obs	6
25%	.79	.85	Sum of Wgt.	6
50%	.825		Mean	.77
		Largest	Std. Dev.	.3038421
75%	1	.8		
90%	1	.85	Variance	.09232
95%	1	1	Skewness	-1.409773
99%	1	1	Kurtosis	3.570929

Supplementary Charts – Figure 21 (b.

Tables demonstrating numerical values values CSF IgG index Stratified HIV infection. HIV infected cases had the overall highest median IgG index and the heist absolute value but as noted in the text CSF IgG Index is not routinely measured in HIV infected patients and the clinical or pathophysiological significance of IgG index findings members of this cohort with HIV is not know but not thought to be of any relevance pertaining to NMOSD.

CSF IgG INDEX STRATIFIED BY AQP4-AB STATUS

-> tb = 0

CSF IgG-I

	Percentiles	Smallest		
1%	.18	.18		
5%	.43	.43		
10%	.43	.43	Obs	24
25%	.6	.51	Sum of Wgt.	24
50%	.7		Mean	.70875
		Largest	Std. Dev.	.2159773
75%	.8	.9		
90%	1	1	Variance	.0466462
95%	1	1	Skewness	-.1722471
99%	1.18	1.18	Kurtosis	3.348839

-> tb = 1

CSF IgG-I

	Percentiles	Smallest		
1%	.4	.4		
5%	.4	.51		
10%	.4	.6	Obs	7
25%	.51	.7	Sum of Wgt.	7
50%	.7		Mean	.6914286
		Largest	Std. Dev.	.2026844
75%	.85	.7		
90%	.98	.8	Variance	.041081
95%	.98	.85	Skewness	-.0473352
99%	.98	.98	Kurtosis	1.851789

Supplementary Charts - Figure 21 (c.

Tables demonstrating numerical values values CSF IgG index stratified TB infection 50% is the median value, with 25 and 75% being the interquartile range. The was no noticeable difference in the values of those with and those without TB.

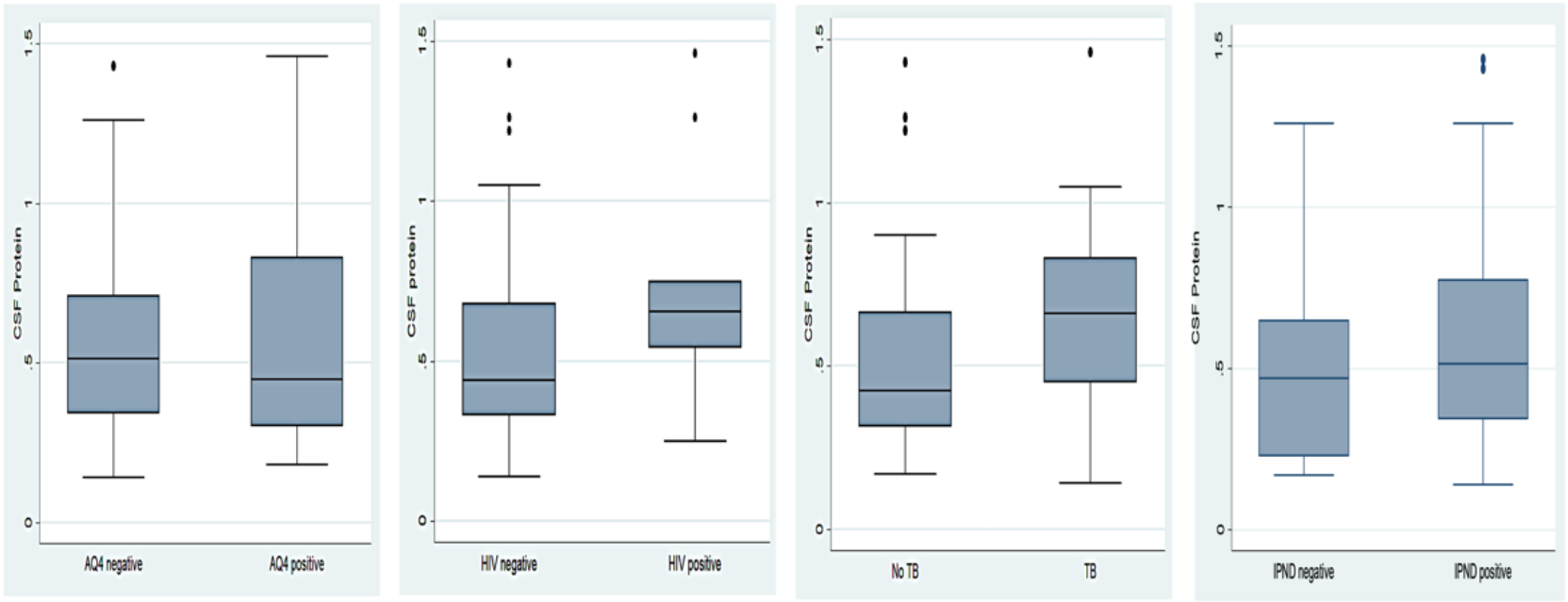
CSF PROTEIN STRATIFIED BY IPND STATUS

```
. bysort ipnd_corr: summ CSFProt , det

-> ipnd_corr = 0

                          CSF Prot
-------------------------------------------------------------
      Percentiles      Smallest
 1%           .17           .17
 5%           .17           .23
10%           .17           .34       Obs                   7
25%           .23           .47       Sum of Wgt.           7

50%           .47                     Mean           .5171429
                        Largest       Std. Dev.      .3665931
75%           .65           .47
90%          1.26            .5       Variance       .1343905
95%          1.26           .65       Skewness       1.246395
99%          1.26          1.26       Kurtosis       3.522082

-------------------------------------------------------------
-> ipnd_corr = 1

                          CSF Prot
-------------------------------------------------------------
      Percentiles      Smallest
 1%           .14           .14
 5%           .18           .18
10%           .27           .25       Obs                  32
25%          .345           .27       Sum of Wgt.          32

50%          .515                     Mean           .6078125
                        Largest       Std. Dev.      .3567934
75%          .775          1.22
90%          1.22          1.26       Variance       .1273015
95%          1.43          1.43       Skewness       .9934596
99%          1.46          1.46       Kurtosis       3.109192
```

Supplementary Charts – Figure 22 (d.

Tables demonstrating numerical values values CSF Protein Stratified by IPND criteria 50% is the median value, with 25 and 75% being the interquartile ranges

CSF PROTEIN STRATIFIED BY AQP4-AB STATUS, HIV& TB INFECTION, & IPND CRITERIA



Supplementary Charts – Figure 22

Box plots of CSF protein stratified by AQP4-ab status; HIV and TB infection. Note that Table-4 in text above delineates numerical values and numbers tested in each index. See tables below actual with data numerical values values 50% is the mean value, with 25 and 75% being the interquartile ranges.

CSF PROTEIN STRATIFIED AQP4-AB STATUS

-> aquaporin = 0

CSF Prot

Percentiles		Smallest		
1%	.14	.14		
5%	.17	.17		
10%	.23	.23	Obs	24
25%	.34	.27	Sum of Wgt.	24
50%	.515		Mean	.5841667
		Largest	Std. Dev.	.3477245
75%	.71	1.05		
90%	1.22	1.22	Variance	.1209123
95%	1.26	1.26	Skewness	1.030726
99%	1.43	1.43	Kurtosis	3.235607

-> aquaporin = 1

CSF Prot

Percentiles		Smallest		
1%	.18	.18		
5%	.18	.25		
10%	.25	.29	Obs	15
25%	.3	.3	Sum of Wgt.	15
50%	.45		Mean	.6033333
		Largest	Std. Dev.	.3794294
75%	.83	.83		
90%	1.26	.9	Variance	.1439667
95%	1.46	1.26	Skewness	1.004683
99%	1.46	1.46	Kurtosis	2.991616

Supplementary Charts - Figure 22 (a.

Tables demonstrating numerical values CSF protein stratified AQP4-ab status; 50% is the median value, with 25 and 75% being the interquartile range. AQP$-Ab positive cases had the highest variance in CSF protein, however this was not statistically. Nor did we deem this clinically significant.

CSF PROTEIN STRATIFIED HIV INFECTION STATUS

```
bysort HIV: summ CSFProt , det
```

-> HIV = 0

CSF Prot

Percentiles		Smallest		
1%	.14	.14		
5%	.17	.17		
10%	.18	.18	Obs	29
25%	.33	.23	Sum of Wgt.	29
50%	.44		Mean	.5441379
		Largest	Std. Dev.	.3442644
75%	.68	1.05		
90%	1.22	1.22	Variance	.118518
95%	1.26	1.26	Skewness	1.148718
99%	1.43	1.43	Kurtosis	3.361181

-> HIV = 1

CSF Prot

Percentiles		Smallest		
1%	.25	.25		
5%	.25	.39		
10%	.32	.54	Obs	10
25%	.54	.59	Sum of Wgt.	10
50%	.655		Mean	.729
		Largest	Std. Dev.	.3692771
75%	.75	.74		
90%	1.36	.75	Variance	.1363656
95%	1.46	1.26	Skewness	.8866807
99%	1.46	1.46	Kurtosis	2.872083

Supplementary Charts – Figure 22 (b.
Tables demonstrating numerical values values CSF IgG index Stratified AQP4-ab status; HIV and TB infection 50% is the median value, with 25 and 75% being the interquartile ranges

CSF PROTEIN STRATIFIED TB INFECTION STATUS

-> tb = 0

CSF Prot

	Percentiles	Smallest		
1%	.17	.17		
5%	.18	.18		
10%	.23	.23	Obs	28
25%	.315	.25	Sum of Wgt.	28
50%	.425		Mean	.5517857
		Largest	Std. Dev.	.3536168
75%	.665	1.22		
90%	1.26	1.26	Variance	.1250448
95%	1.26	1.26	Skewness	1.24539
99%	1.43	1.43	Kurtosis	3.435961

-> tb = 1

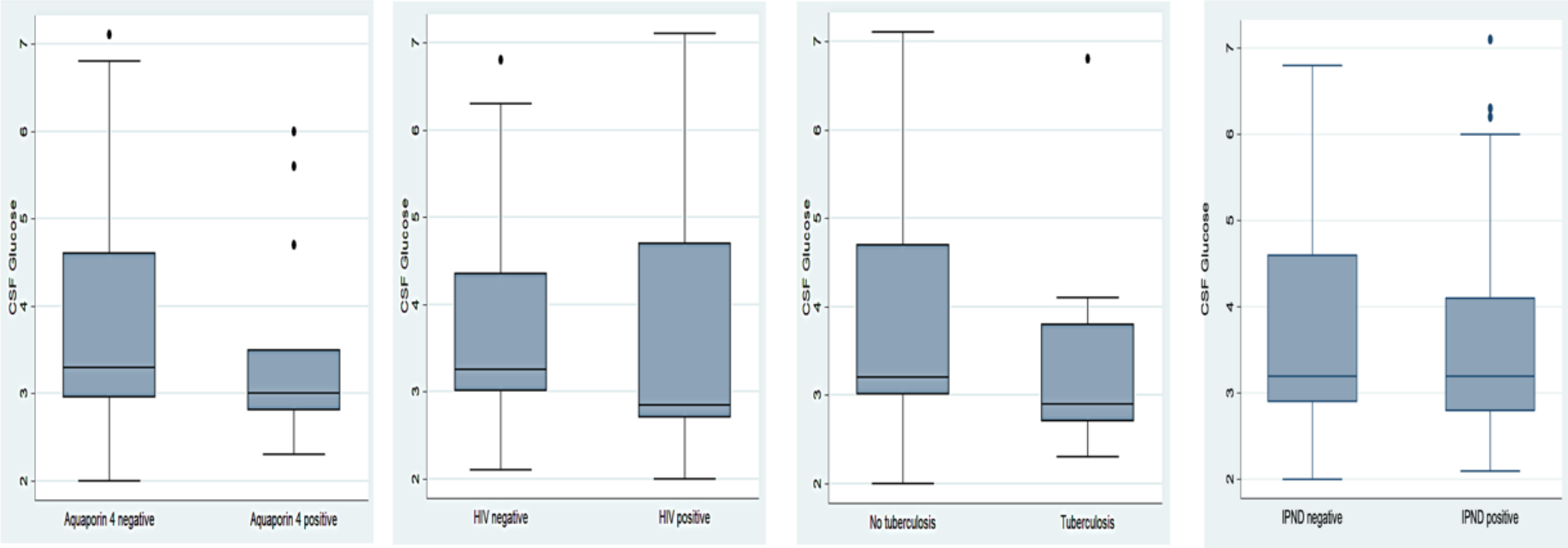
CSF Prot

	Percentiles	Smallest		
1%	.14	.14		
5%	.14	.38		
10%	.38	.45	Obs	11
25%	.45	.47	Sum of Wgt.	11
50%	.66		Mean	.6927273
		Largest	Std. Dev.	.3559239
75%	.83	.8		
90%	1.05	.83	Variance	.1266818
95%	1.46	1.05	Skewness	.6425425
99%	1.46	1.46	Kurtosis	3.236715

Supplementary Charts – Figure 22 (c.

Tables demonstrating numerical values for CSF protein stratified by TB infection. 50% is the median value, with 25 and 75% being the interquartile ranges. Note how cases with TB marginally had the highest median and IQR for CSF protein. This is in the absence of any evidence of CNS TB. This may indicate TB playing a role in provoking an inflammatory milieu that may bring about an attack of NMOSD in susceptible individuals.

CSF GLUCOSE STRATIFIED ACCORDING TO AQP4-AB STATUS, HIV & TB INFECTION, & IPND CRITERIA



Supplementary Charts – Figure 23

Box plots of CSF glucose stratified by AQP4-ab status; HIV and TB infection. Note that Table-4 in text above delineates numerical values of the numbers tested in each index. See tables below actual with data numerical values values 50% is the median value, with 25 and 75% being the interquartile ranges. Note that cases with TB and AQP4-Ab-positivity had the lowest glucose values. Corresponding serums glucose values were infrequently available.

CSF PROTEIN STRATIFIED BY IPND STATUS

```
. bysort ipnd_corr: summ CSFProt , det

-------------------------------------------------------------------------------
-> ipnd_corr = 0

                          CSF Prot
-------------------------------------------------------------------------------
      Percentiles      Smallest
 1%           .17           .17
 5%           .17           .23
10%           .17           .34       Obs                   7
25%           .23           .47       Sum of Wgt.           7

50%           .47                     Mean           .5171429
                      Largest         Std. Dev.      .3665931
75%           .65           .47
90%          1.26            .5       Variance       .1343905
95%          1.26           .65       Skewness       1.246395
99%          1.26          1.26       Kurtosis       3.522082

-------------------------------------------------------------------------------
-> ipnd_corr = 1

                          CSF Prot
-------------------------------------------------------------------------------
      Percentiles      Smallest
 1%           .14           .14
 5%           .18           .18
10%           .27           .25       Obs                  32
25%          .345           .27       Sum of Wgt.          32

50%          .515                     Mean           .6078125
                      Largest         Std. Dev.      .3567934
75%          .775          1.22
90%          1.22          1.26       Variance       .1273015
95%          1.43          1.43       Skewness       .9934596
99%          1.46          1.46       Kurtosis       3.109192
```

Supplementary Charts – Figure 22 (d.

Tables demonstrating numerical values values CSF Protein Stratified by IPND criteria 50% is the median value, with 25 and 75% being the interquartile ranges

CSF GLUCOSE STRATIFIED BY AQP4-AB STATUS

-> aquaporin = 0

CSF Gluc

Percentiles		Smallest		
1%	2	2		
5%	2.1	2.1		
10%	2.3	2.3	Obs	24
25%	2.95	2.5	Sum of Wgt.	24
50%	3.3		Mean	3.891667
		Largest	Std. Dev.	1.503306
75%	4.6	6.2		
90%	6.3	6.3	Variance	2.259928
95%	6.8	6.8	Skewness	.8771066
99%	7.1	7.1	Kurtosis	2.586943

-> aquaporin = 1

CSF Gluc

Percentiles		Smallest		
1%	2.3	2.3		
5%	2.3	2.7		
10%	2.7	2.7	Obs	14
25%	2.8	2.8	Sum of Wgt.	14
50%	3		Mean	3.442857
		Largest	Std. Dev.	1.142005
75%	3.5	3.5		
90%	5.6	4.7	Variance	1.304176
95%	6	5.6	Skewness	1.364972
99%	6	6	Kurtosis	3.398168

Supplementary Charts – Figure 23 (a.
Tables demonstrating numerical values CSF glucose values stratified AQP4-ab status; HIV and TB infection 50% is the median value, with 25 and 75% being the interquartile range

CSF GLUCOSE STRATIFIED BY TO TB INFECTION

-> tb = 0

CSF Gluc

Percentiles		Smallest		
1%	2	2		
5%	2.1	2.1		
10%	2.3	2.3	Obs	27
25%	3	2.5	Sum of Wgt.	27
50%	3.2		Mean	3.848148
		Largest	Std. Dev.	1.440451
75%	4.7	6		
90%	6.2	6.2	Variance	2.0749
95%	6.3	6.3	Skewness	.8152223
99%	7.1	7.1	Kurtosis	2.417545

-> tb = 1

CSF Gluc

Percentiles		Smallest		
1%	2.3	2.3		
5%	2.3	2.7		
10%	2.7	2.7	Obs	11
25%	2.7	2.8	Sum of Wgt.	11
50%	2.9		Mean	3.427273
		Largest	Std. Dev.	1.240235
75%	3.8	3.5		
90%	4.1	3.8	Variance	1.538182
95%	6.8	4.1	Skewness	1.972497
99%	6.8	6.8	Kurtosis	6.145433

Supplementary Charts - – Figure 23 (b.
Tables demonstrating numerical values CSF glucose values stratified AQP4-ab status; HIV and TB infection 50% is the median value, with 25 and 75% being the interquartile range

CSF GLUCOSE STRATIFIED BY HIV INFECTION STATUS

-> HIV = 0

CSF Gluc

Percentiles		Smallest		
1%	2.1	2.1		
5%	2.3	2.3		
10%	2.5	2.5	Obs	28
25%	3	2.8	Sum of Wgt.	28
50%	3.25		Mean	3.757143
		Largest	Std. Dev.	1.262692
75%	4.35	5.8		
90%	6	6	Variance	1.594392
95%	6.3	6.3	Skewness	1.072117
99%	6.8	6.8	Kurtosis	3.042854

-> HIV = 1

CSF Gluc

Percentiles		Smallest		
1%	2	2		
5%	2	2.3		
10%	2.15	2.7	Obs	10
25%	2.7	2.7	Sum of Wgt.	10
50%	2.85		Mean	3.64
		Largest	Std. Dev.	1.750048
75%	4.7	3		
90%	6.65	4.7	Variance	3.062667
95%	7.1	6.2	Skewness	1.084977
99%	7.1	7.1	Kurtosis	2.639159

Supplementary Charts - – Figure 23 (c.

Tables demonstrating numerical values CSF glucose values stratified AQP4-ab status; HIV and TB infection 50% is the median value with 25 and 75% being the interquartile ranges.

CSF GLUCOSE STRATIFIED BY IPND STATUS

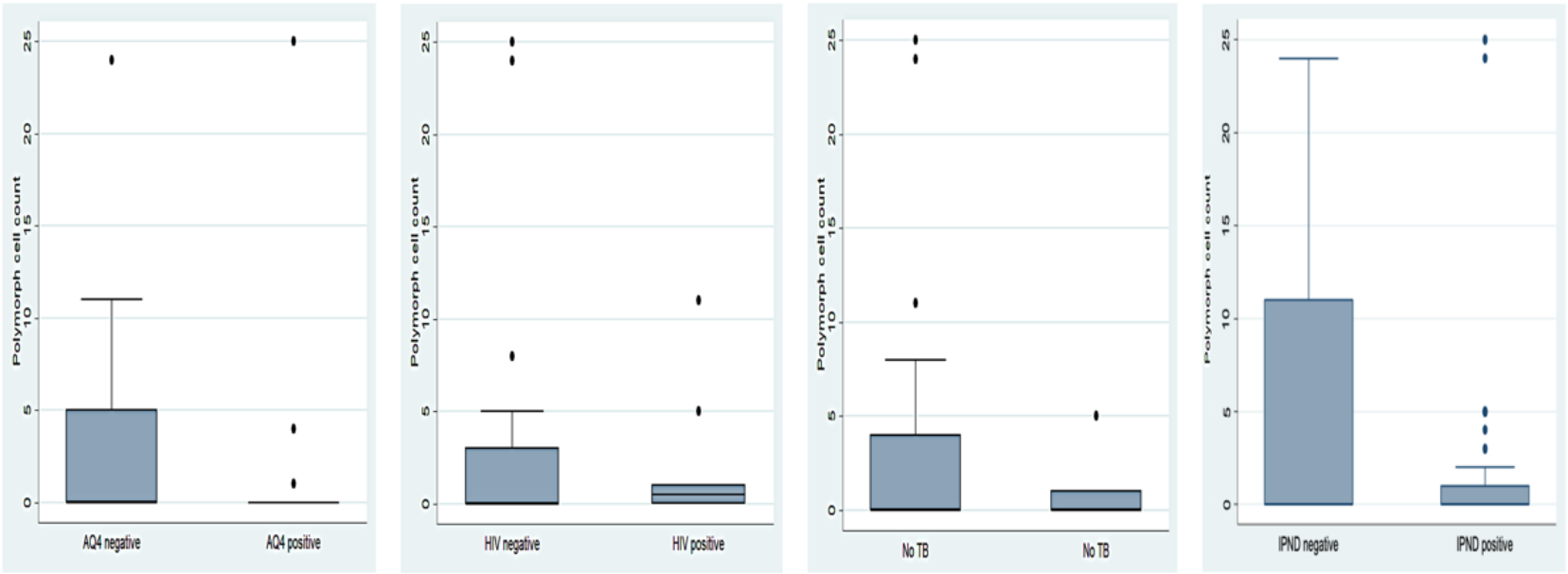
```
. bysort ipnd_corr: summ CSFGluc , det
```

```
-> ipnd_corr = 0

                          CSF Gluc
-------------------------------------------------------------
      Percentiles      Smallest
 1%             2             2
 5%             2           2.9
10%             2           3.2       Obs                   7
25%           2.9           3.2       Sum of Wgt.           7

50%           3.2                     Mean                3.9
                        Largest       Std. Dev.       1.580084
75%           4.6           3.2
90%           6.8           4.6       Variance        2.496667
95%           6.8           4.6       Skewness        .7543174
99%           6.8           6.8       Kurtosis        2.673989

-------------------------------------------------------------
-> ipnd_corr = 1

                          CSF Gluc
-------------------------------------------------------------
      Percentiles      Smallest
 1%           2.1           2.1
 5%           2.3           2.3
10%           2.5           2.3       Obs                  31
25%           2.8           2.5       Sum of Wgt.          31

50%           3.2                     Mean            3.687097
                        Largest       Std. Dev.       1.359839
75%           4.1             6
90%             6           6.2       Variance        1.849161
95%           6.3           6.3       Skewness        1.157166
99%           7.1           7.1       Kurtosis        3.085807
```

Supplementary Charts - – Figure 23 (d.

Tables demonstrating numerical values CSF glucose values stratified IPND criteria; HIV and TB infection 50% is the median value with 25 and 75% being the interquartile ranges.

CSF POLYMORPHONUCLEAR CELLS STRATIFIED BY AQP4-AB STATUS, HIV &TB INFECTION, & IPND CRITERIA



Supplementary Charts - 24

Box plots of polymorphonuclear cell counts stratified by AQP4-ab status; HIV and TB infection. Note that Table #4 in text above delineates numerical values of . Numbers tested in each index. See tables below actual with data numerical values values 50% is the median value, with 25 and 75% being the interquartile range

CSF POLYMORPHONUCLEAR CELLS COUNTS BY AQP4-AB STATUS

-> aquaporin = 0

Poly

Percentiles		Smallest		
1%	0	0		
5%	0	0		
10%	0	0	Obs	25
25%	0	0	Sum of Wgt.	25
50%	0		Mean	3.6
		Largest	Std. Dev.	6.78233
75%	5	8		
90%	11	11	Variance	46
95%	24	24	Skewness	2.291911
99%	24	24	Kurtosis	7.221979

-> aquaporin = 1

Poly

Percentiles		Smallest		
1%	0	0		
5%	0	0		
10%	0	0	Obs	16
25%	0	0	Sum of Wgt.	16
50%	0		Mean	1.875
		Largest	Std. Dev.	6.249
75%	0	0		
90%	4	1	Variance	39.05
95%	25	4	Skewness	3.467627
99%	25	25	Kurtosis	13.34437

Supplementary Charts – Figure 24 (a.

Tables demonstrating numerical values CSF polymorphonuclear cells stratified AQP4-ab status; HIV and TB infection 50% is the median value, with 25 and 75% being the interquartile range

CSF POLYMORPHONUCLEAR CELLS COUNTS BY AQP4-AB STATUS

-> aquaporin = 0

Poly

Percentiles		Smallest		
1%	0	0		
5%	0	0		
10%	0	0	Obs	25
25%	0	0	Sum of Wgt.	25
50%	0		Mean	3.6
		Largest	Std. Dev.	6.78233
75%	5	8		
90%	11	11	Variance	46
95%	24	24	Skewness	2.291911
99%	24	24	Kurtosis	7.221979

-> aquaporin = 1

Poly

Percentiles		Smallest		
1%	0	0		
5%	0	0		
10%	0	0	Obs	16
25%	0	0	Sum of Wgt.	16
50%	0		Mean	1.875
		Largest	Std. Dev.	6.249
75%	0	0		
90%	4	1	Variance	39.05
95%	25	4	Skewness	3.467627
99%	25	25	Kurtosis	13.34437

Supplementary Charts – Figure 24 (a.

Tables demonstrating numerical values CSF polymorphonuclear cells stratified AQP4-ab status; HIV and TB infection 50% is the median value, with 25 and 75% being the interquartile range

CSF POLYMORPHONUCLEAR CELLS STRATIFIED TB STATUS

-> tb = 0

Poly

Percentiles		Smallest		
1%	0	0		
5%	0	0		
10%	0	0	Obs	30
25%	0	0	Sum of Wgt.	30
50%	0		Mean	3.766667
		Largest	Std. Dev.	7.468016
75%	4	11		
90%	17.5	24	Variance	55.77126
95%	24	24	Skewness	2.154648
99%	25	25	Kurtosis	6.253513

-> tb = 1

Poly

Percentiles		Smallest		
1%	0	0		
5%	0	0		
10%	0	0	Obs	11
25%	0	0	Sum of Wgt.	11
50%	0		Mean	.6363636
		Largest	Std. Dev.	1.501514
75%	1	0		
90%	1	1	Variance	2.254545
95%	5	1	Skewness	2.513373
99%	5	5	Kurtosis	7.87552

Supplementary Charts – Figure 24 (c.

Tables demonstrating numerical values CSF polymorphonuclear cells stratified AQP4-ab status; HIV and TB infection 50% is the median value, with 25 and 75% being the interquartile range.

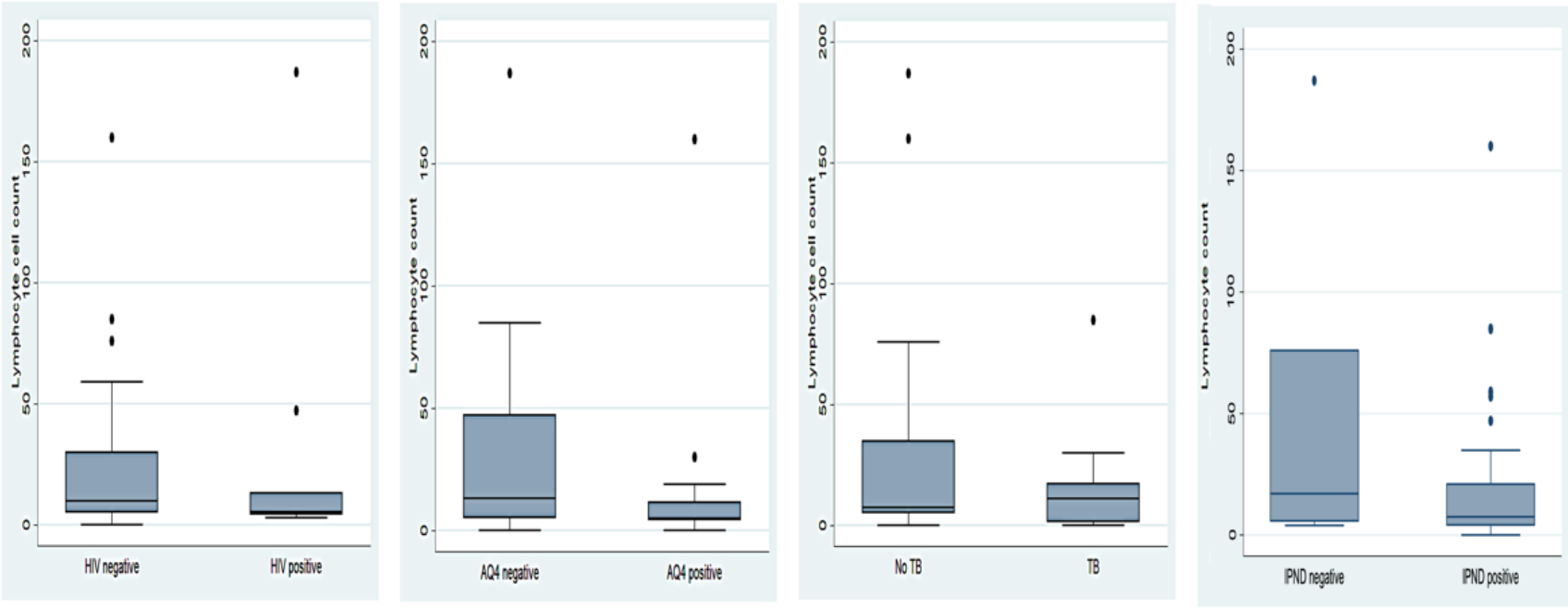
CSF POLYMORPHONUCLEAR CELLS COUNTS BY IPND STATUS

```
. bysort ipnd_corr: summ Poly , det

-------------------------------------------------------------------------------
-> ipnd_corr = 0

                            Poly
-------------------------------------------------------------------------------
      Percentiles      Smallest
 1%             0             0
 5%             0             0
10%             0             0       Obs                   7
25%             0             0       Sum of Wgt.           7

50%             0                     Mean           6.142857
                        Largest       Std. Dev.      9.099974
75%            11             0
90%            24             8       Variance       82.80952
95%            24            11       Skewness       1.167714
99%            24            24       Kurtosis       3.060874

-------------------------------------------------------------------------------
-> ipnd_corr = 1

                            Poly
-------------------------------------------------------------------------------
      Percentiles      Smallest
 1%             0             0
 5%             0             0
10%             0             0       Obs                  34
25%             0             0       Sum of Wgt.          34

50%             0                     Mean           2.264706
                        Largest       Std. Dev.      5.863647
75%             1             5
90%             5             5       Variance       34.38235
95%            24            24       Skewness       3.329558
99%            25            25       Kurtosis       12.97105
```

Supplementary Charts – Figure 24 (d.

Tables demonstrating numerical values CSF polymorphonuclear cells stratified IPND criteria; HIV and TB infection 50% is the median value, with 25 and 75% being the interquartile range.

CSF LYMPHOCYTE CELLS STRATIFIED AQP4-AB STATUS, HIV & TB INFECTION, & IPND CRITERIA



Supplementary Charts – Figure 25
Box plots of CSF Lymphocyte counts stratified by AQP4-ab status; HIV and TB infection. Note that Table-4 in text above delineates numerical values of and numbers tested in each index. See tables below actual with data numerical values values 50% is the median value, with 25 and 75% being the interquartile ranges

CSF LYMPHOCYTES STRATIFIED BY AQP4-AB STATUS

-> aquaporin = 0

Lymph

Percentiles		Smallest		
1%	0	0		
5%	0	0		
10%	1	1	Obs	25
25%	5	3	Sum of Wgt.	25
50%	13		Mean	29.76
		Largest	Std. Dev.	41.17572
75%	47	59		
90%	76	76	Variance	1695.44
95%	85	85	Skewness	2.436894
99%	187	187	Kurtosis	9.545189

-> aquaporin = 1

Lymph

Percentiles		Smallest		
1%	0	0		
5%	0	0		
10%	0	3	Obs	16
25%	4	3	Sum of Wgt.	16
50%	5		Mean	17.4375
		Largest	Std. Dev.	38.76419
75%	11.5	12		
90%	30	19	Variance	1502.662
95%	160	30	Skewness	3.393459
99%	160	160	Kurtosis	13.02259

Supplementary Charts – Figure 25 (a.

Tables demonstrating numerical values CSF lymphocyte counts stratified AQP4-ab status; 50% is the median value, with 25 and 75% being the interquartile range. AQP4-Ab positivity was associated with the highest CSF polymorphonuclear cell count.

CSF LYMPHOCYTES STRATIFIED BY HIV STATUS

-> HIV = 0

Lymph

Percentiles		Smallest		
1%	0	0		
5%	0	0		
10%	0	0	Obs	31
25%	5	0	Sum of Wgt.	31
50%	10		Mean	23.80645
		Largest	Std. Dev.	34.18423
75%	30	59		
90%	59	76	Variance	1168.561
95%	85	85	Skewness	2.423861
99%	160	160	Kurtosis	9.371476

-> HIV = 1

Lymph

Percentiles		Smallest		
1%	3	3		
5%	3	3		
10%	3	4	Obs	10
25%	4	5	Sum of Wgt.	10
50%	5.5		Mean	28.5
		Largest	Std. Dev.	57.24072
75%	13	12		
90%	117	13	Variance	3276.5
95%	187	47	Skewness	2.432224
99%	187	187	Kurtosis	7.284605

Supplementary Charts – Figure 25 (b.
Table demonstrating numerical values of CSF lymphocyte cell counts stratified by HIV status where 50% is the median value, with 25 and 75% being the interquartile range

CSF LYMPHOCYTES STRATIFIED BY TB STATUS

-> tb = 0

Lymph

Percentiles		Smallest		
1%	0	0		
5%	0	0		
10%	3	3	Obs	30
25%	5	3	Sum of Wgt.	30
50%	7.5		Mean	28.2
		Largest	Std. Dev.	44.52694
75%	35	59		
90%	67.5	76	Variance	1982.648
95%	160	160	Skewness	2.478454
99%	187	187	Kurtosis	8.624453

-> tb = 1

Lymph

Percentiles		Smallest		
1%	0	0		
5%	0	0		
10%	0	1	Obs	11
25%	1	3	Sum of Wgt.	11
50%	11		Mean	16.09091
		Largest	Std. Dev.	24.56605
75%	17	13		
90%	30	17	Variance	603.4909
95%	85	30	Skewness	2.223467
99%	85	85	Kurtosis	6.891379

Supplementary Charts – Figure 25 (c.

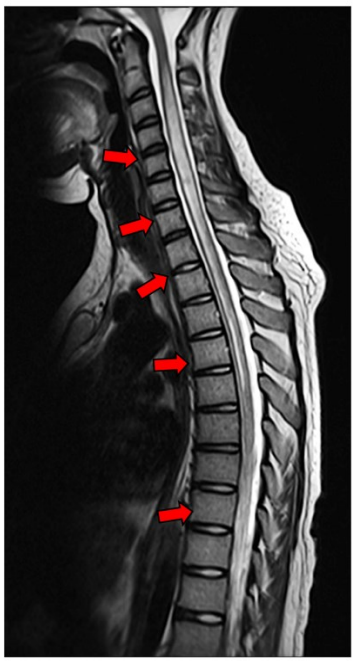
Tables demonstrating numerical values CSF Lymphocyte cell counts stratified by TB status. 50% is the median value, with 25 and 75% being the interquartile rang

CSF LYMPHOCYTES STRATIFIED BY IPND STATUS

```
. bysort ipnd_corr: summ Lymph , det

-------------------------------------------------------------------------------
-> ipnd_corr = 0

                            Lymph
-------------------------------------------------------------
      Percentiles      Smallest
 1%             4             4
 5%             4             6
10%             4             6       Obs                   7
25%             6            17       Sum of Wgt.           7

50%            17                     Mean           49.28571
                        Largest       Std. Dev.      66.48236
75%            76            17
90%           187            49       Variance       4419.905
95%           187            76       Skewness       1.434866
99%           187           187       Kurtosis       3.707651

-------------------------------------------------------------------------------
-> ipnd_corr = 1

                            Lymph
-------------------------------------------------------------
      Percentiles      Smallest
 1%             0             0
 5%             0             0
10%             0             0       Obs                  34
25%             4             0       Sum of Wgt.          34

50%           7.5                     Mean           19.94118
                        Largest       Std. Dev.      31.71554
75%            21            57
90%            57            59       Variance       1005.875
95%            85            85       Skewness       2.960389
99%           160           160       Kurtosis       12.62194
```

Supplementary Charts – Figure 25 (d.
Tables demonstrating numerical values CSF Lymphocyte cell counts stratified by IPND criteria status 50% is the median value, with 25 and 75% being the interquartile rang

MRI: Longitudinally Extensive Transverse Myelitis Images in NMOSD



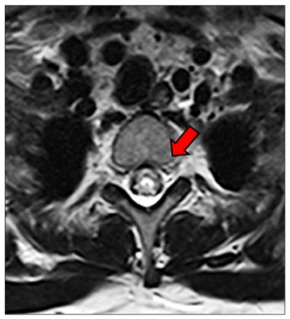
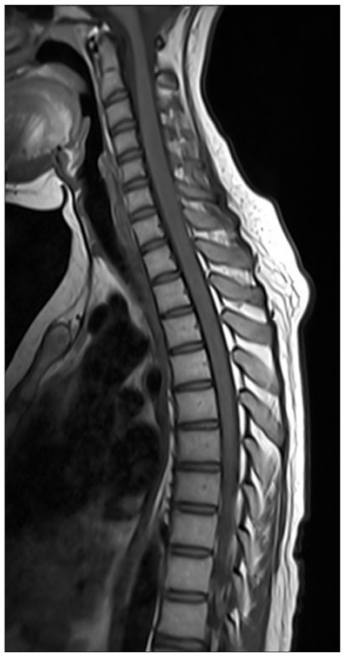


Image 1: T2 Weighted MR image showing Cervicothoracic LETM involving transverse section of the cord



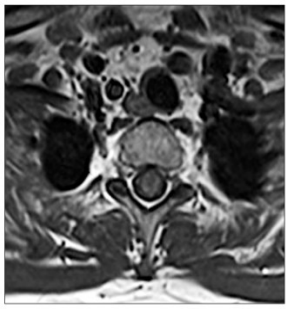
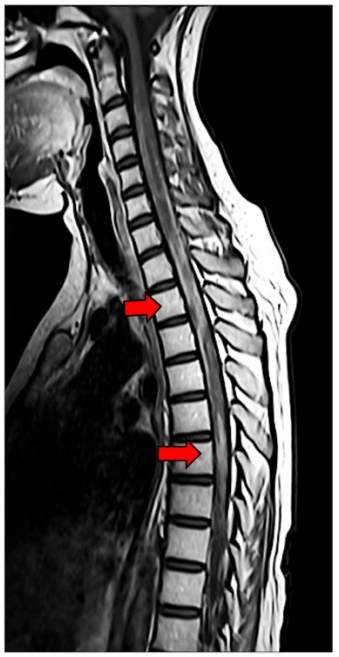


Image 2: Pre-contrast T1 Weighted MR image of cervicothoracic cord



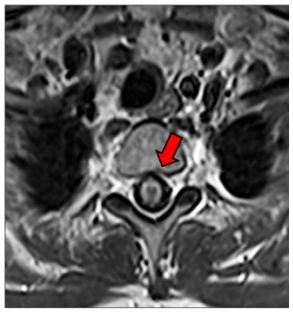


Image 3: Post-contrast T1 Weighted MR image of cervicothoracic cord showing rim of enhancement in axial plain

Figure 11: An example of the cervicothoracic MRI spine imaging characteristics of AQP4-Ab-positive NMOSD case. In **Image 1** note the T2-weighted image showing LETM in the axial plane and central cord hyperintensity in the axial plane. The **Images 2 & 3** show the corresponding sagittal and axial T1-weighted Pre and Post Gadolinium contrast enhanced MRI sequences. In **Image 3** there is "cigar-like" pattern of enhancement in post-contrast sagittal image and rim of enhancement in the axial plane.

Dedication

As a final indulgence, I would like to thank my friends, family and my partner, Jessica, for their enduring support and understanding during my many years of training and their continued support in the exciting and challenging times to come.

End.