NEUROMYELITIS OPTICA SPECTRUM DISORDERS THAT HAVE NO ANTIBODIES TO AQUAPORIN-4

Shahd HM Hamid

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Abstract

Introduction and objectives: neuromyelitis optica (NMO) is an autoimmune inflammatory disease that affects the central nervous system. It was thought to be a subclass of multiple sclerosis (MS); however, keen clinical observation, advances in imaging and diagnostic immunology have proved it to be a separate disorder. The discovery of antibodies to aquaporin-4 antigen (AQP4-IgG) has further advanced the field. The spectrum of disorders that are categorised under this name has widened and a new criterion has evolved. While we now have a fair understanding of aquaporin-4 NMO spectrum disorders (NMOSD), there is now an increased need to understand NMOSD without antibodies to aquaporin-4, including the disease associated with the new antibody to myelin oligodendrocytes glycoprotein (MOG-IgG).

Hypothesis: neuromyelitis optica without AQP4-IgG antibodies is a different disease from that observed in neuromyelitis optica with AQP4-IgG antibodies.

Aims: To study the widening spectrum of NMOSD, and to describe the role of other antibodies in seronegative NMOSD, namely antibodies to MOG. Specifically, I have studied three questions:

1. What is the effect of the 2015 criteria for diagnosis of neuromyelitis optica spectrum disorders on diagnostic rates?
2. What proportion of AQP4-IgG-negative NMO spectrum-disorder patients are MOG-IgG positive?
3. How common is the occurrence of seizures and encephalitis in myelin oligodendrocyte glycoprotein IgG disease compared with figures for aquaporin 4 IgG disease?

Methods: I conducted the study in the NMO and non-MS central nervous system (CNS) demyelination clinic, part of the UK NMO service at the Walton Centre NHS Foundation Trust. After an initial detailed review, prospective follow-up was performed with imaging and additional investigations that included new antibody tests as indicated, based on new information from the rapidly evolving field. All patients were tested for AQP4-IgG and MOG-IgG antibodies at the Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital,

Oxford, through use of the best available cell-based assay. Clinical and paraclinical information was gathered between 2013 and 2016 and analysed according to the study questions. Statistical analysis included demographic data, percentages, mean, median, interquartile range and Fisher test. This study formed part of the UK NMO study (MREC 02/8/082, Northwest Medical Research Ethics Committee).

Results:

1. Application of the new NMOSD criteria has led to a significant increase in the numbers of diagnoses of NMOSD by 76% and has widened the spectrum of the disease.

2. MOG-IgG is present in 42% of patients who satisfy the clinical criteria for NMOSD but lack AQP4-IgG. However, some cases with MOG-IgG (20%) do not satisfy NMOSD criteria.

3. Seizures occur in about 14% of MOG-IgG-positive NMOSD patients compared with 1% in AQP4-IgG positive.

Conclusion: The ongoing research, which includes this work, has changed our understanding of NMOSD significantly in the last few years. The criteria that were introduced by the 2015 International Panel for NMO Diagnosis (IPND) bring a significant change in the approach to the diagnosis and classification of demyelinating syndromes that are not typical MS. The study of AQP4-IgG-negative NMOSD reveals that a significant group of patients have a different antibody (MOG-IgG) and exhibit unique clinical features including seizures. MOG-IgG disease now is considered a different disease entity. It is expected that these observations will lead to a revision of the IPND criteria. These revised criteria could influence future clinical trials.

Acknowledgments

Four years ago I stepped into a clinical research fellowship at Liverpool's renowned Walton Centre to pursue a longstanding ambition for a career in neuroimmunology.

I was fortunate to spend these years working as part of the NMO-UK service, a dream team of highly professional and dedicated individuals looking after a special group of patients with neuromyelitis optica.

I am forever grateful to my inspiring supervisor Dr Anu Jacob, who taught me a lot about neurology, neuroimmunology and life! He has been a constant source of encouragement and support.

I was fortunate to work with a talented multidisciplinary team comprised of nurses (particularly Kerry Mutch), therapists and coordinators. I would like to thank them for their wonderful support.

I also want to thank Dr Martin Wilson (Consultant Neurologist and MS specialist) for his mentoring and for giving me the opportunity to become involved in key clinical trials, and Dr Maneesh Bhojak (Consultant Neuroradiologist) for guiding me through the assessment and interpretation of neuroradiological imaging.

I also had the wonderful opportunity to visit our twin NMO-UK centre in Oxford, where all antibody assays were tested, and I have to thank Patrick Waters and Dr Mark Woodhall for welcoming me and for the opportunity to further my learning in clinical neuroimmunology and cell-based assays. It was an absolute pleasure to be acquainted with Prof Angela Vincent and take some inspiring lifetime career advice.

I am grateful to my academic supervisor Professor Tom Solomon, who has provided guidance and wisdom when needed. I would also like to thank Dr Benedict Michael, a colleague, friend and an advisor.

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Finally, I want to wholeheartedly thank my husband and partner in this world, Mohamed, for his patience, understanding, compassion, and encouragement and for keeping Ghazi (our son) entertained whilst Mummy worked on her research. My gratitude also extends to

my parents for helping me keep everything in perspective.
And last but not least, I want to thank all my patients and their families. I hope this work will be beneficial to them and their future management. It is only by their contributions and goodwill that advances in this field can happen.

Declaration

I declare that all the work described in this thesis is my own, with the exceptions mentioned in the acknowledgments.

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List of Abbreviations:

AQP4-IgG	Aquaporin-4 immunoglobulin G
CSF	Cerebrospinal fluid
IPND	International panel for NMO diagnosis
LETM	Longitudinally extensive transverse myelitis
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MOG-IgG1	Myelin oligodendrocytes antibodies (immunoglobulin subclass G1)
MOG-IgG Encephalomyelitis	MOG-EM
MOGAD	MOG-IgG antibody disease
NMO	Neuromyelitis optica
NMO-IgG	Neuromyelitis optica immunoglobulin G
NMOSD	Neuromyelitis optica spectrum disorder
OCB	Oligoclonal bands
ON	Optic neuritis
TM	Transverse myelitis

Original peer reviewed publications arising from this work:

1: Hamid SHM, Whittam D, Saviour M, Alorainy A, Mutch K, Linaker S, Solomon T, Bhojak M, Woodhall M, Waters P, Appleton R, Duddy M, Jacob A. Seizures and Encephalitis in Myelin Oligodendrocyte Glycoprotein IgG Disease vs Aquaporin 4 IgG Disease. JAMA Neurol. 2018 Jan 1;75(1):65-71.

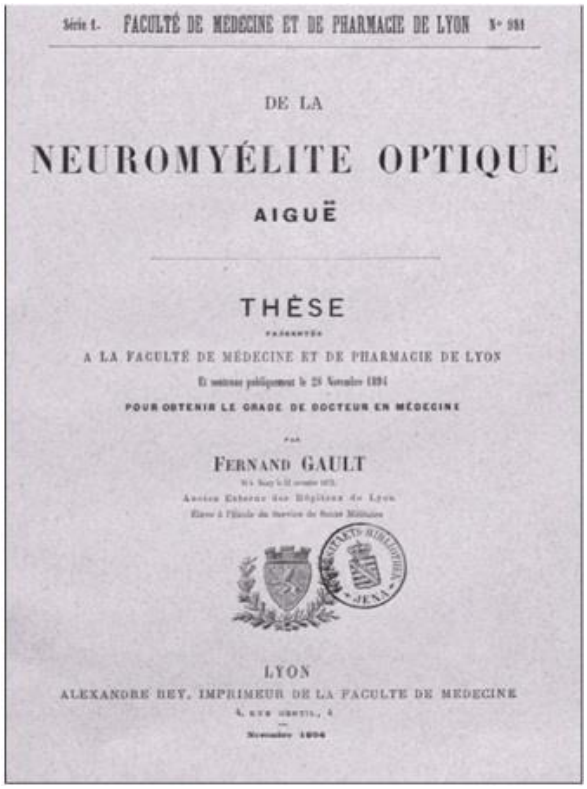
2: Hamid SHM, Whittam D, Mutch K, Linaker S, Solomon T, Das K, Bhojak M, Jacob A. What proportion of AQP4-IgG-negative NMO spectrum disorder patients are MOG-IgG positive? A cross sectional study of 132 patients. J Neurol. 2017 Oct;264(10):2088-2094.

3: 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.

Chapter 1 Introduction

1.1 Neuromyelitis optica

Neuromyelitis optica (NMO) is an autoimmune disease that affects the central nervous system (CNS). It acquired this name when it was first described in 1894 by French physicians who were based in Lyon, Fernand Gault and Eugene Devic. Fernand Gault (1873-1936) was a student who published his doctoral thesis on neuromyelitis optica: "De La Neuromylitie Optique Aigue" (Figure 1). Eugene Devic (1858-1930) was his supervisor. Their work was based on a case of acute bilateral optic neuritis and tetraparesis and a review of earlier similar case reports. The eponym "Devic's disease" (1) has been used for nearly a century, yet the first report in the literature of this disease is from 1870, when Sir Clifford Allbutt


Série t. FACULTÉ DE MEDECINE ET DE PHARMACIE DE LYON N° 981

DE LA

NEUROMYÉLITE OPTIQUE

AIGUË

THÈSE

PRÉSENTÉE

A LA FACULTÉ DE MÉDECINE ET DE PHARMACIE DE LYON

Et soutenue publiquement le 28 Novembre 1894

POUR OBTENIR LE GRADE DE DOCTEUR EN MÉDECINE

PAR

FERNAND GAULT

Né à Sury-le-Comtal (Loire)

Ancien Externe des Hôpitaux de Lyon

Elève à l'École du Service de Santé Militaire

LYON

ALEXANDRE REY, IMPRIMEUR DE LA FACULTE DE MEDECINE

4, RUE GENTIL, 4

Novembre 1894

FIGURE 1. THE TITLE PAGE OF DR GAULT'S DOCTORAL THESIS (DE LA NEUROMYELITE OPTIQUE AIGUE 1894)

published in the Lancet "On the ophthalmoscopic signs of spinal disease" (2).

Initial enthusiasm in diagnosis of this new condition was confined to the optic nerves and spinal cord, but this gave way to a more variable spectrum of symptoms that were used in diagnosis: unilateral optic neuritis, relapsing rather than monophasic in course, mild attacks and a range of time intervals between the occurrence of optic neuritis and myelitis.

Meanwhile, an understanding of multiple sclerosis (MS) patterns and diagnoses that was based on dissemination of space and time was evolving. As a consequence, NMO gradually became considered to be a severe subcategory of MS for more than a century, although the lack of typical MS brain lesions puzzled physicians (3).

Renewed interest in NMO as a separate disease came when the NMO antibody (NMO-IgG) was discovered in 2004 in the Mayo Clinic by Professor Vanda Lennon and her colleagues. They identified NMO-IgG in the serum of patients with NMO and Asian opticospinal multiple sclerosis with sensitivity of 73% and 91% and specificity of 58% and 100% respectively. The terminology Asian opticospinal MS was used for patients who were thought to have MS but showed a normal or atypical brain in magnetic resonance imaging (MRI), and predominant involvement of optic nerve and spinal cord. The NMO-IgG outlined the CNS microvessels, pia, subpia and Virchow space, and was bound to protein at or near the blood-brain barrier (BBB). It could be used to clearly distinguish between NMO and MS (4).

In subsequent work, the Lennon group (2005) concluded that NMO-IgG bound selectively to the aquaporin-4 water channel that was expressed abundantly on the surface of the astrocytic foot processes at the BBB (5). Outside North America, the antibody was increasingly referred to as anti aquaporin-4 IgG. At the time, this antibody had not been proven to be pathogenic, but several findings suggested that it might be, including the similarity of the immunohistochemical pattern of NMO-IgG binding to mouse CNS tissues with the pattern of immune complex deposition in autopsied patients' spinal cord tissue (6).

Several case reports from other international centres confirmed the presence of NMO-IgG in their NMO patients. A large case series from Japan tested for the antibody in what the researchers had classified as opticospinal MS, clinically typical MS, other neurological disorders and healthy controls. The results showed that NMO-IgG was present in (13/48, 27%), (3/54, 5.6%), (0/52) and (0/35) of the patients that were studied in these four groups, respectively. These findings supported the hypothesis that opticospinal MS was more likely to be NMO than MS (7). It was not until 2007 that another two independent labs, in Oxford and Berlin, confirmed and replicated the Mayo Clinic findings (8).

Using the antibody as a biomarker for the disease has helped us to learn much more about NMO. Up to 80% of patients with typical NMO (bilateral optic neuritis and long transverse myelitis) have AQP4-IgG antibodies (4).

As interest increased and research continued, more challenges became apparent. We came to understand that there remained a group of patients who did not have the antibody but who exhibited similar clinical features. We also learned that some patients with AQP4-IgG antibodies may have signs and symptoms other than the typical optic neuritis or myelitis. In fact, some of these patients would only develop optic neuritis or myelitis years later, after the appearance of other neurological symptoms.

Over the previous two centuries, many diagnostic criteria had been proposed by neurologists worldwide, almost all of them focused on the involvement of the optic nerve and spinal cord and paucity of brain lesions (9). The most recent criteria that had been drawn up at the time that this research was started were the 2006 criteria. They adhered to the classical picture of involvement of both optic neuritis and myelitis along with two of: normal brain as seen in MRI, long myelitis and positive AQP4-IgG (10). Other clinical and immunological associations with the disease were also apparent: substantial female dominance (up to 10:1); presentation in extremes of age as young as three years old and as old as the ninth decade (11, 12); association with other immunological diseases; and the presence of either organ-specific or non-specific autoantibodies or both as an immunological epiphenomenon (13). The latter was observed among AQP4-IgG-positive cases more than seronegative cases.

The discovery of the AQP4-IgG antibody revolutionised the concept of NMO. As neurologists and physicians became more familiar with its significance, it became common to test patients once they developed any one of the classic clinical presentations but had not necessarily developed the full house. This led to the emergence of the concept of an NMO spectrum (2007) that contained limited forms of NMO, i.e. optic neuritis or transverse myelitis in the presence of AQP4-IgG, which considered the antibody as a pathognomonic biomarker (14). However, many cases do not meet the current criteria for diagnosis of NMO or of MS. Some of these patients may test positive for other antibodies (e.g. antibodies to myelin oligodendrocyte glycoproteins (MOG-IgG) (15)) or may have no known antibodies. Although there are many studies on the epidemiology, immunology, therapy and outcome

of AQP4-IgG-positive NMO, the characteristics of NMO disease without AQP4-IgG remain largely unknown. The patients who fall into this category form the focus of this project.

1.2 Epidemiology and genetics

Neuromyelitis optica spectrum disorders are rare, but a few studies provide population-based incidence and prevalence estimates. In the UK, according to available epidemiological studies, the prevalence varies from 7.2/million (95% CI 3.1-14.2) to 19.6/million (95% CI 12.2-29.7) (16, 17). In the last few years, the prevalence has risen with early diagnosis and evolving criteria. Worldwide, the figures now range between 40/million and 100/ million (18-20). Its incidence rate peaks at approximately age 40, but it can occur at any age .Virtually all reports of NMO worldwide describe female predominance, with female: male ratios as high as 10:1. This striking predominance is not found in seronegative cases, which comprise about 30% of NMOSD cases (12).

The disease is not clearly familial, nor has a specific genetic susceptibility been identified. However, genetic studies that have involved large sample sizes have shown that human leukocyte antigen (HLA) alleles and other non-HLA genetic loci may have variable associations with NMO/NMOSD. Some alleles seem to increase susceptibility to the disease, while others appear to be protective. For example, a Japanese study has suggested that HLA-DRB1*1602 and DPB1*0501 alleles are associated with anti-AQP4-IgG-positive NMO/NMOSD, but not with anti-AQP4-IgG-negative NMO/NMOSD (21). Another recent study from Japan that has used next-generation sequencing has also reported significant association of HLA-DQA1*05:03 with NMOSD (22). Interestingly, there are approximately 20 families in which two family members are reported to be affected (23).

1.3 Neuroimaging

The hallmark changes in NMO that are observed in MRI are: optic-nerve inflammation; longitudinally extensive transverse myelitis (LETM) with central cord lesion that extends ≥ three vertebral levels and/or to the brain stem; and a brain MRI that is not diagnostic of MS. Very long lesions that extend over six or more segments and involvement of >50% of cord area are observed more frequently in seropositive than negative cases. In recent years, brain involvement has been reported in many patients with NMOSD (24). Certain types of brain lesions are now regarded as characteristic of NMO: e.g. those in periependymal areas,

the corticospinal tract and the hemispheric white matter. It is increasingly recognised that MS and NMO brain images can sometimes have similar appearances, but there are some differences. NMO is indicated by the presence of: areas with higher expression of AQP4; hypothalamic, periaqueductal grey and area postrema lesions; and extensive signal changes in optic nerves that extend posteriorly beyond chiasm, along with extensive involvement of corpus callosum.MS is suggested by the presence of: cortical and juxta cortical, U-fibre, linear Dawson's finger collosal and short spinal cord lesions (mainly posterior) (25). The diagnostic challenge occurs when these patients are seronegative to AQP4 antibodies.

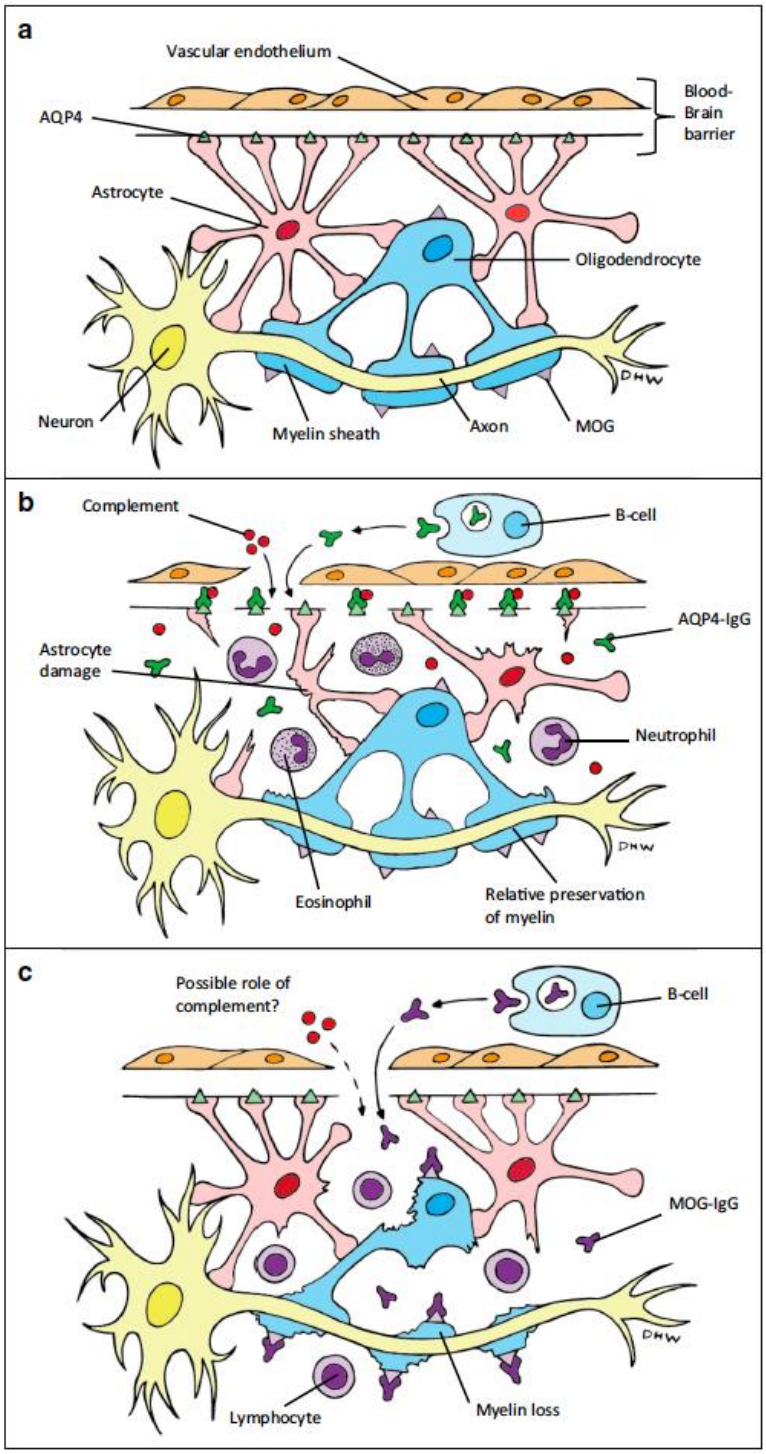
The imaging characteristics of AQP4-IgG seronegative NMO are poorly understood. Recent research on MOG-IgG-positive NMO has revealed that it shares some MRI features with AQP4IgG antibody-positive cases, such as LETM, hemispheric brain lesion and brain stem lesions; however; the extension of the signal change in the optic nerves seems to be more anterior than posterior. Short spinal-cord lesions do exist in MOG-IgG-positive patients, but they tend to affect >50% of axial cord thickness instead of favouring posterior tracts, as is found in MS. Conus lesions are found more often in MOG-IgG1 antibody-positive patients and MS patients than in AQP4-IgG antibody-positive patients (25-27.

These observations lead to the question of whether NMOSD with positive MOG-IgG1 antibodies are the same diseases as AQP4-IgG antibody-positive diseases. The more intriguing question revolves around those who are double seronegative (28).

1.4 Pathology and pathogenesis

NMO is primarily an astrocyte disease rather than a demyelinating disease like MS. The astrocytes are glial cells of the CNS, which have multiple branches and end-feet processes. They function as a scaffold for the CNS, form glial scars in response to injury and play a major role in the maintenance of homeostasis as they are part of the BBB system. AQP4 in the adult brain mainly plays a role in homeostasis and water permeability. It is densely expressed in the astrocytes foot process at the BBB, but it is also found in circumventricular organs that lack the BBB (29, 30)

Outside the CNS, AQP4 is expressed in the collecting ducts of the kidney, and the parietal cells of the stomach, airways, secretory glands, and skeletal muscle (31). However, it is believed that these organs are not involved or damaged in AQP4 autoimmunity, due to local complement inhibition, which is lacking in the brain (32).



a)AQP4 IS EXPRESSED AT THE BLOOD–BRAIN BARRIER, ON THE ''FOOT-LIKE'' ENDS OF ASTROCYTES, WHEREAS MOG IS EXPRESSED BY OLIGODENDROCYTES AND ON THE OUTERMOST SURFACES OF MYELIN SHEATHS. b) AQP4-IGG IS SYNTHESISED IN THE BLOODSTREAM BY MATURE B-CELLS. ON CROSSING THE BLOOD–BRAIN BARRIER, IT ACTIVATES COMPLEMENT-MEDIATED ASTROCYTE DAMAGE WITH RELATIVE PRESERVATION OF MYELIN INITIALLY. THE INFLAMMATORY RESPONSE INVOLVES ACCRUAL OF NEUTROPHILS AND EOSINOPHILS. c) MOG-IGG IS ALSO PRODUCED OUTSIDE THE CNS. IT CAUSES DEMYELINATION, BUT THE MECHANISM IS NOT WELL UNDERSTOOD. REPRINTED WITH PERMISSION FROM WHITTAM D, WILSON M, HAMID S ET AL. WHAT'S NEW IN NEUROMYELITIS OPTICA? A SHORT REVIEW FOR THE CLINICAL NEUROLOGIST. J NEUROL 2017;264:2330–44

FIGURE 2. DIAGRAM ILLUSTRATES THE SITE OF EXPRESSION OF AQUAPORINE 4 (AQP4) AND MYELIN OLIGODENDROCYTE GLYCOPROTEIN (MOG) WITHIN THE CNS:

In NMO, AQP4-IgG antibodies are produced by B cells (plasma cells) in the peripheral lymphoid tissue and the antibodies circulate in the systemic circulation. It is unclear how they cross the BBB. However, it is proposed that they help to increase the BBB permeability. The inflammatory process involves the AQP4-IgG antibodies and induces complement activation, interleukin6 (IL6) production and signalling, immunocomplex precipitation in the astrocytic foot processes and subsequent destruction of AQP4 water channels. This results in water influx, necrosis and axonal loss. Other inflammatory cells, including eosinophils and macrophages, are involved due to chemotaxis, and this causes further BBB damage and enhanced entry of AQP4-IgG antibody. Natural killer cells are also activated and produce antibody-dependent cytotoxicity. In the process, oligodendrocytes can be affected and this results in secondary demyelination (33, 34) (see Figure 2).

Neuropathological findings in autopsied and biopsied specimens from patients with NMO have shown differences compared with specimens obtained from those with MS. They are similar to the specimens that are produced in experimental animal models of NMO diseases. Demyelination and necrosis have been reported in both grey and white matter; blood vessels are thickened and they show a pink, glassy appearance (hyalinisation). Leukocytic infiltration is prominent in active lesions, along with complement precipitation and loss of AQP4. There is usually oedema and deposition of immune complexes around the blood vessels (35).

Chronic lesions are characterised by gliosis, cystic degeneration, cavitation and atrophy. Glial fibrillary acidic protein (GFAP) is an intracellular protein that is found in astrocytes, where it maintains their structural integrity. It has been found to be present in high levels in the cerebrospinal fluid (CSF) of NMO patients during acute events, which indicates that it could be a marker for disease (36)OBJ

However, it is unclear whether the pathological changes that occur in seronegative NMO are similar to those reported for seropositive cases. Until recently, very little information was available for this group. One paper has reported changes in patients with AQP4-IgG-negative

NMOSD who presented with Baló concentric brain lesion (BCL); the researchers report extensive loss of APQ4 in the Baló concentric lesions of four Baló concentric sclerosis patients. The loss occurred in both demyelinated and myelinated layers of the BCLs (37). In patients who are AQP4-IgG negative but have other antibodies to MOG, studies suggest that primary demyelination occurs, in which case myelin basic protein (MBP) rather than GFAP would be expected to be present in the CSF. Very recently (May 2020), a collaborative study between researchers in Japan and Austria was published. The authors reported histopathological findings in a group of MOG-IgG1 antibody-positive cases (n=11). These authors found brain lesions that were similar to those found in acute disseminated encephalomyelitis (ADEM); these lesions involved perivenous demyelination (no astrocytopathy as in AQP4-IgG antibody-positive cases) in disseminated lesions with loss of MOG, but no typical MS-like lesions. At a cellular immunity level, there was infiltration of CD4+ inflammatory cells. This infiltration is similar to the AQP4-IgG antibody immune reaction rather than the actions of CD8+ T-cells as in MS (38). Although previous reports highlighted deposition of activated complements and observation of MS pathology pattern II (39), this was not a prominent feature that was observed in this cohort.

1.5 .Immunology

Serum tests for AQP4-IgG form the standard method that is used to confirm a diagnosis of NMO spectrum disorder. AQP4 antibody-seronegative NMO poses a diagnostic challenge in clinical practice and represents a source of uncertainty in NMO clinical research. The antibody was first discovered as NMO-IgG by the Mayo Clinic group in 2004 (4) using indirect immunofluorescence (IIF) on sections of CNS tissue. This was the gold standard technique for many years. The identification of AQP4 as the target for NMO-IgG in 2005 led to the development of various techniques that were based on recombinant AQP4 to identify and quantify NMO-IgG (5). These techniques include tissue-based IIF assay to detect NMO-IgG, the enzyme-linked immunosorbent assay (ELISA), GFP-AQP4 fluorescence immunoprecipitation assay (FIPA), visual fluorescence-observation cell-based assay (CBA) and a quantitative flow cytometry (fluorescence-activated cell sorting [FACS]) assay.

The CBA and FACS methods have been found to have the highest sensitivity (77%, 73%) and specificity (both 100%) but their successful application requires good technical skills (40). However, a commercially available form of CBA that involves visual fluorescence-

observation and that incorporates fixed HEK293 cells that are transfected singly with either human AQP4-M1 or M23 isoform (available from EUROIMMUN), has been found to be reasonably sensitive (68%) and specific (100%) and its use requires fewer technical skills (41).

It is believed that AQP4-IgG is synthesised peripherally and then reaches the AQP4 in the astrocytic foot processes after disruption of the BBB. It can be identified in CSF, but proportionally, its titres in serum are very high (42); hence, for diagnostic purposes its measurement is superior in serum.

About half of AQP4-IgG-positive NMO patients harbour one or more serum auto-antibodies, such as antinuclear antibody and extractable nuclear antigen, and about one-third have one or more systemic autoimmune diseases, such as thyroid disease or lupus. Those who are seropositive for AQP4-IgG are likely to have coexisting autoimmune diseases rather than lupus myelitis or a Sjogren's-related myelopathy or myelitis (13).

Other reported antibodies that have been associated with NMO are antibody to MOG and, far less significantly, to aquaporin 1 (AQP1). Recently, anti-glycine antibodies were isolated in a Turkish patient who was NMO AQP4-IgG-negative (43). The significance of this is unclear. It is known that glycine receptors are abundantly expressed in the spinal cord and optic nerves, as glycine is one of the major inhibitory neurotransmitters in the CNS. Studies have shown that anti-glycine antibodies can be present in some patients with MS, transverse myelitis or isolated optic neuritis. Perhaps other pathological rules apply in other CNS autoimmune disorders such as stiff person syndrome and progressive encephalomyelitis with rigidity and myoclonus (44). The rare presence of anti-glycine antibodies in NMO patients is thought to be a form of bystander effect (45).

Anti-AQP1 auto-antibodies (AQP1-ab) have been described by some centres in a subgroup of patients who have chronic demyelination in the CNS with similarities to anti-AQP4 seronegative NMO. Moreover, these auto-antibodies have been detected in patients who are positive for AQP4-IgG, and some patients with MS. These findings suggest that these auto-antibodies may offer a potential biomarker for CNS demyelination disorders (46). However Long et al. evaluated the diagnostic value of AQP1-ab and concluded that the specificity was low and significantly lower than that for AQP4-IgG (47).

Myelin oligodendrocyte glycoproteins (MOGs) are CNS-specific antigens that are normally expressed on the surface of myelin sheaths. The MOG-IgG was initially described in MS and ADEM cases, particularly in paediatrics (48). Recently it has been reported in patients who show clinical features of NMO and do not have AQP4-IgG (15). This has led to further work on MOG-IgG assays and further research, and the recent development of live-cell-based assays that have high sensitivity and specificity. MOG-IgG is believed to be specific to demyelination syndromes that are distinct from MS (49). Evidence for the pathogenicity of MOG-IgG comes from in-vitro studies that have demonstrated complement-mediated cytotoxicity (50), and from the development of NMOSD-like syndromes in animal models (51). However, these studies in rodents have found that MOG-IgG causes reversible alterations to myelin without complement activation or inflammatory cell infiltration (52).

Intrathecal synthesis of the oligoclonal band (OCB) is uncommon in NMOSD in either AQP4-IgG positive or seronegative patients, but it has been identified in about 10-20% of cases (53).

1.6 Treatment

As of early 2019, there were no licensed treatments for NMO or non-MS CNS demyelination. Many of the treatments that are available are based on retrospective studies, case reports and expert reviews. No prospective controlled trials in NMO were conducted until late 2014. Most study designs that involved placebo treatment were considered ethically challenging; the 90% certainty of relapse in the presence of AQP4-IgG, which can be severely disabling, meant that allocation of people to the placebo arm with no immunosuppressant cover was considered unacceptable.

Current treatment for an acute attack involves a high dose of steroids; intravenous methylprednisolone is favoured over oral therapy (54). In the case of poor clinical response or a refractory attack, plasma exchange is used as a stepping-up rescue therapy (55). In patients in whom neither steroids nor plasma exchange improve symptoms, treatment with intravenous immunoglobulins has been found to hasten recovery, mainly in AQP4-IgG positive patients (56). Intravenous cyclophosphamide is also reported to be effective in the improvement of neurological symptoms in the acute phase if the initial measures fail (57).

More than 90% of patients with NMO and AQP4-IgG ultimately relapse, although this figure is lower in seronegative patients (54). A high morbidity is associated with NMO exacerbations as is a stepwise accumulation of disability (>50% would be functionally blind or would lose independent ambulation within five years if left untreated) (58). Therefore immunosuppressive therapy is typically instituted after the first attack in AQP4-IgG positive patients.

1.6.1 Most commonly used immunosuppressive therapies

1- **Azathioprine**, which is a thiopurine that antagonises endogenous DNA and RNA purines. It is widely available, cheap and usually well tolerated. At a dose of 2.5-3mg/kg, it has a modest effect in the reduction of relapse rates (59).

2- **Mycophenolate mofetil** inhibits lymphocyte proliferation and antibody synthesis. Its use in rheumatological disease and other immune-mediated neurological disorders (e.g. myasthenia gravis) prompted its use for NMO. At a median dose of 2,000mg/day, it is effective in the reduction of relapse rates and development of disabilities (60). It is likely to be superior to azathioprine in reducing relapse rates (61).

3- **Rituximab** is a monoclonal antibody that selectively targets CD20 B cells. It is well-tolerated, and several studies have shown that it has high efficacy in relapse and disability reduction and causes significant recovery in neurological function (62). It is expensive compared with the oral therapies. However, its high efficacy and suitability for individualised treatment, in which CD27 and/or CD19 (memory B-cell markers) are monitored and frequency of dose is subsequently adjusted, make it cost-effective (63, 64).

1.6.2 Other therapies

Since the completion of my study, three drugs have been found to be effective after Randomised controlled trials (RCTS): eculizumab, satralizumab (anti IL-6) and inebilizumab (anti-CD-19) (65-67).

Other less frequently used treatments include methotrexate (68), cyclosporine (69), cyclophosphamide, tacrolimus and mitoxantrone (70). Regular plasma exchange has also been used as maintenance therapy (71).

Table 1. Comparison of main clinical features of NMO AQP4-IgG positive, NMO AQP4-IgG negative and MS

	NMO AQP4-IgG seropositive	**NMO AQP4-IgG seronegative**	**Multiple sclerosis**
Median age at onset	40	38	32
Clinical picture	Optic neuritis and/or myelitis	Optic neuritis and long myelitis	Symptoms and signs of brain demyelination, optic neuritis, myelitis (usually short)
Relapsing course	92%	76%	100% in relapse remittance course Occasional/rare relapses in secondary progressive or primary progressive
Co-existing autoimmunity	23.8%	5.7%	
Treatment	Immunosuppressant initiated at diagnosis	Immunosuppressant in case of relapse	Disease-modifying therapies in RRMS and early PPMS
MRI changes	MRI brain :Normal/atypical to MS MRI spine: long myelitis (> 3 vertebral segments, occasional short myelitis	MRI brain: Normal/atypical to MS and long myelitis	MR Brain: dissemination in space if two of: Three or more periventricular lesions Infratentorial lesion/s Spinal cord lesion/s (short) Optic nerve lesion Cortical/juxtacortical lesions Dissemination in time is demonstrated radiologically by presence of enhancing and none enhancing lesions
Antibodies	AQP4-IgG	MOG-IgG in some cases	None
Pathology	Astrocytopathy and secondary demyelination	Unknown, Demyelination?	Periventricular and subcortical plaques of inflammation (T lymphocytes infiltrates) and demyelination, with relative sparing of axons in early disease; subsequent irreversible axonal loss in progressive late stages.
CSF	Pleocytosis, OCB in 20%	Pleocytosis, OCB in 20%	WBCs usually less than 20, +ve OCB in up to 80%
M:F	1:9	1:2	1:3

1.7 Study rationale

A previous study of NMO by my supervisor (Anu Jacob, 2003-2009) involved 49 patients. Since then, the national NMO UK Centre in Liverpool, a twin to the centre in Oxford, has been established. With increasing awareness of NMO, the number of diagnoses has significantly increased. The centre also sees patients who pose a diagnostic challenge for instance with atypical demyelination, non-NMO opticospinal demyelination, recurrent optic neuritis or transverse myelitis. These patients do not satisfy the criteria for diagnosis of NMO or NMOSD. It has become important to assess how the new NMOSD criteria that were introduced in 2015 by the International Panel for NMO Diagnosis (IPND) have changed patient numbers and how often the newly described MOG-IgG is present in seronegative NMOSD. We have also observed cases in which patients experienced seizures with MOG-IgG. This occurrence was not seen previously in NMO and we felt it was important to describe this new feature.

Chapter 2. General methods

2.1 Studies undertaken and clinical settings:

I – What has been the impact of the 2015 criteria for diagnosis of neuromyelitis optica spectrum disorders on diagnostic rates?

II - What proportion of AQP4-IgG negative NMO spectrum-disorder patients are MOG-IgG positive? A cross-sectional study of 132 patients.

III – Do seizures and encephalitis indicate MOG-IgG disease rather than AQP4-IgG disease?

All studies were performed in the NMO and non-MS CNS demyelination clinic, which is part of the UK NMO service at the Walton Centre for Neurology and Neurosurgery National Health Service Foundation Trust, Liverpool, United Kingdom. This specially-funded clinic is a referral centre for cases that are suspected to be NMO or its variants. It is one of two national multidisciplinary specialist clinics for patients with NMOSD and non-MS

FIGURE 3. CATCHMENT AREA FOR NMO UK CLINIC IN LIVERPOOL

demyelination disorders that form part of the UK NMOSD service; the other is in Oxford. The clinic takes an average of 45 new referrals and 211 follow-ups each year. The clinic in Liverpool covers Merseyside and the northern part of England, North Wales, Scotland and Northern Ireland (Figure 3).

2.2 Methods

An initial detailed review was conducted by the NMO team, which included: myself; Dr Anu Jacob, the NMO lead; Kerry Mutch, the advanced nurse practitioner; the orthoptist and the therapist team of orthoptists. Prospective follow-up was then performed with imaging and additional investigations that included new antibody tests based on new information from the rapidly evolving field. The methods specific to each study are outlined in the results chapters. For ease of presentation and continuity, I have described detailed methods in each chapter.

I collected the data prospectively on a master EXCEL sheet that contained all patients' details, for example:

- Demographics:
    - Age
    - Gender
    - Region
    - Ethnicity
- Consent and date of consents
- Clinical details:
    - Dates of disease onset
    - Nature of events and relapses
    - Dates and clinical details of events/relapses, specific symptoms, examination findings, expanded disability status scale (EDSS), investigation findings
    - Treatment (steroid, additional immunosuppressant, starting dates, dates of any switch and reason, last follow up)
- Co-morbidities
- Family history etc.
- Investigations
    - Antibody testing: dates, titres, other antibodies
    - CSF (cells, protein, sugar, OCB, other tests)
    - MRI details (dates, brain, orbits, spinal cord, description of lesion: location, length, numbers, contrast enhancement)
    - Visual evoked potentials
    - Other tests

- Annualised relapse rate
- Dates for follow-up and EDSS in last follow-up
- Surveys for employment, bladder and bowel symptoms were completed during clinic attendance or over the phone (this was conducted by Kerry Mutch, advanced nurse practitioner)

2.3 Laboratory tests

All patients were tested for the presence of AQP4-IgG antibodies and MOG-IgG antibodies at the Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital, Oxford, through application of the best available cell-based assays for AQP4-IgG and MOG-IgG antibodies.

AQP4-IgG antibody testing. The test used was a cell-based assay (CBA) that employed transfected human embryonic kidney (HEK) cells. The cells that were transfected with AQP4 M1 and M23 were then washed and incubated with fluorescence-conjugated goat anti-human IgG. Positive sera were identified by fluorescence at the cell surface. This CBA had the highest sensitivity (76.7%) and specificity (99.8%) compared with other available testing methods such as enzyme linked immunosorbent assay (ELISA).

MOG-IgG1 antibody testing. The CBA was used with HEK cells that were transfected with full-length (FL) human MOG, which has been found to be more sensitive than the shorter length (SL). The test has a sensitivity of 25% and specificity near to 100%.

2.4 Ethics

This study formed part of the UK NMO study (MREC 02/8/082, Northwest Medical Research Ethics Committee) and was approved by the Research Ethics Service, NRES Committee, London (Ref No 15/LO/1433).

2.5 Statistical analysis

I performed simple statistical analysis for each study, and I used the following statistical tests:

- Median, for:
  - Age at disease onset among patients
  - Disease duration
  - Age at study analysis

        - Inter-attack intervals
    - Interquartile range (IQR) for median age at time of analysis
    - Percentages to illustrate proportions of patients regarding:
        - Group of disease
        - Antibodies status
        - Females and males
        - Patients with relapsing and monophasic disease
        - Change in diagnosis
        - Ethnicities
        - Patients who experience seizures
    - Fisher test to compare occurrence of seizures between two patient groups

RESULTS

Chapter 3. Overall description of the cohort

3.1 General description of the cohort

I assessed a total of 261 patients who had non-MS/atypical CNS inflammatory conditions in this study (data collected to cover the period 2013-2017; I collected and analysed data prospectively over the period 2014-2017, and retrospectively for 2013 and 2014). The majority of patients were of Caucasian ethnicity (79.6%), while 14.3% were Asian, 4.1% African/ Afro-Caribbean and 2% were of mixed or other ethnicity. Most were female (75%) and median age at onset was 38 yrs (range 12-82 years).

For the 2016-2017 year: there were 215 patients who were classed as active and 46 new referrals. Figure 4 shows the diagnoses.

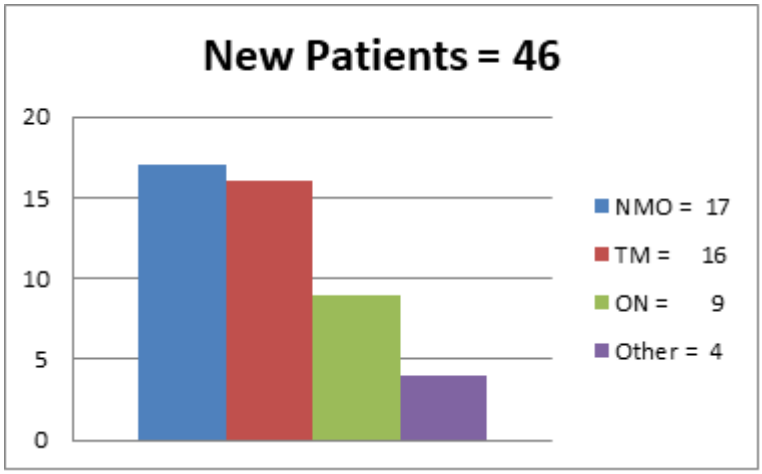
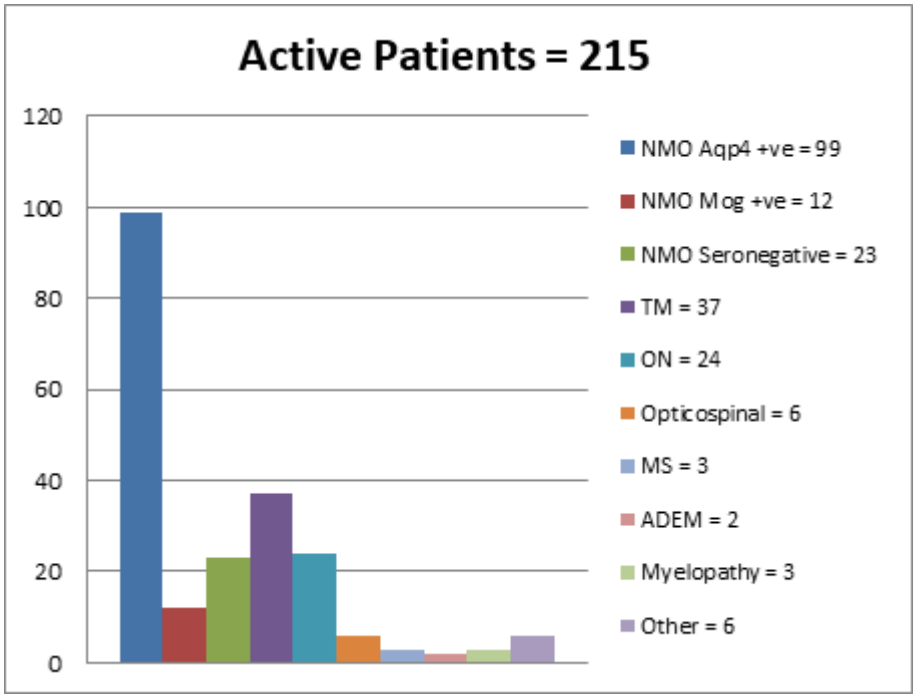


FIGURE 4 DIAGNOSES OF NEW REFERRALS TO THE CENTRE IN 2017

FIGURE 5 DIAGNOSES OF PATIENTS FOLLOWED UP IN CLINIC 2016-2017



3.1.1 Clinic attendance by postal region

The map below shows attendances by postal area of the patient's home address at the date of the last appointmentin 2017. The highest numbers of referrals come from Northwest and North Wales and Scotland.

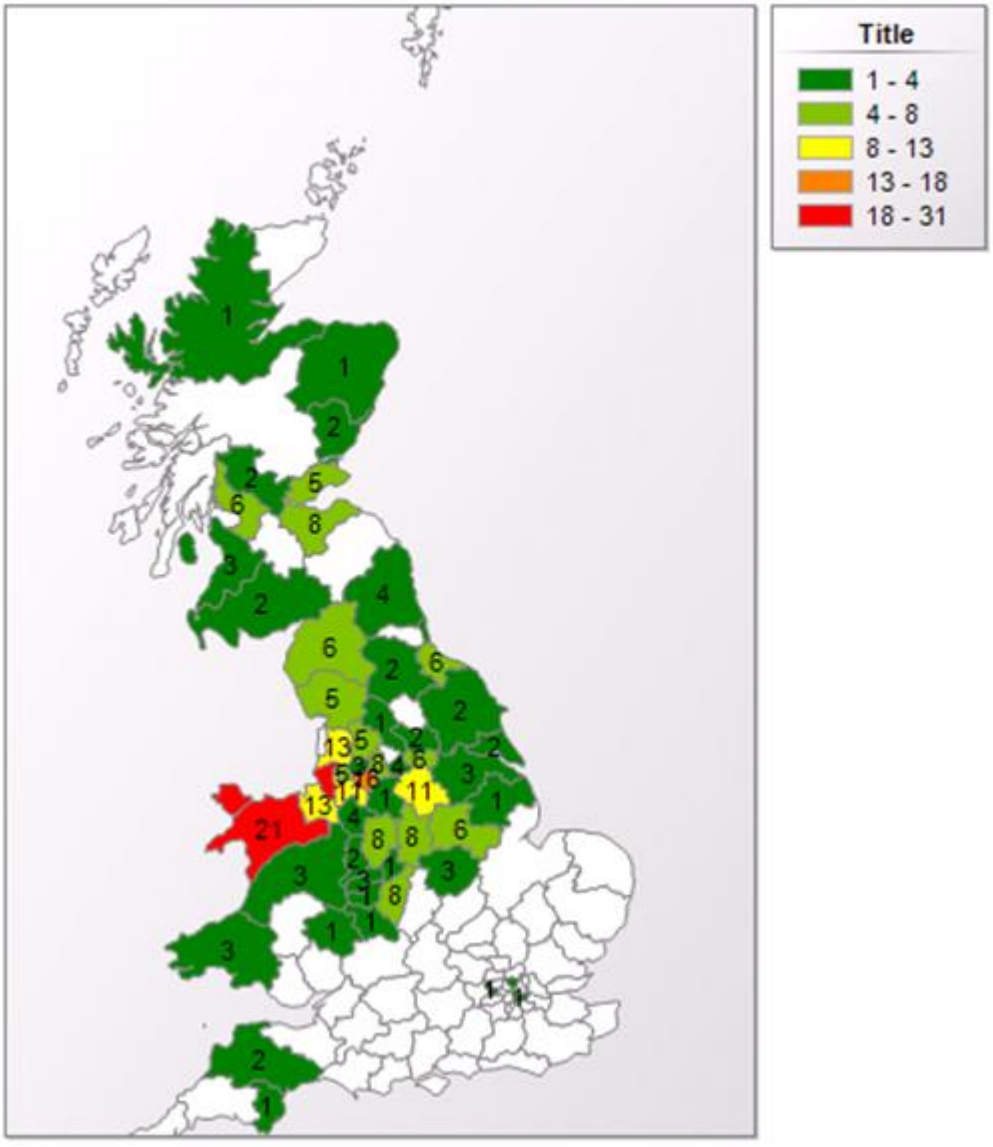


FIGURE 6 NUMBER OF REFERRALS BY REGION

3.2 Relapses

3.2.1 Annualised relapse rates for NMOSD, 2016-2017:

Table 2. Mean and median of annual relapse rate (ARR) pre- and post-enrolment in NMO service

	MEDIAN ARR PRE-SERVICE	MEAN ARR PRE-SERVICE	ARR RANGE PRE-SERVICE	MEDIAN DURATION OF ILLNESS PRE-SERVICE (YEARS)	MEDIAN ARR POST-SERVICE	MEAN ARR POST-SERVICE	ARR RANGE POST-SERVICE	MEDIAN DURATION IN NMO SERVICE (YEARS)
NEW Patients (n = 17)	1.39	2.33	0.23 – 7.30	0.93	0	0	0 – 0	0.62
FOLLOW-UP Patients (n = 117)	0.94	1.58	0.10 – 18.25	3.93	0	0.19	0 – 1.22	3.97
COMBINED (n = 134)	0.97	1.67	0.10 – 18.25	3.68	0	0.16	0 – 1.22	3.45

The relapse rates are seen to decline after the patients are enrolled with the service and have entered into joint follow-up with the NMO service and local specialist MS/neuroinflammation service. Those services ensure that appropriate immunosuppressants are prescribed for all AQP4-IgG antibody-positive cases and for relapsed seronegative cases, and that close follow-up and easy accessibility are arranged for all patients.

3.3 Disability

3.3.1 EDSS

Median EDSS for the whole cohort was 6 with a range of 1-8., The most disabling attack was usually found to be the index event. Relapse-independent progression is not commonly observed in NMOSD.

3.3.2 Visual acuity

Visual acuity (VA) ranged from 6/5 to no perception of light (blindness). A total of 17% of the patients had severe visual loss in both eyes (VA <6/18) and another 42% exhibited severe visual loss in one eye.

3.3.3 Sphincter dysfunction

We performed a cross-sectional study in 2015 that was led by our NMO nurse Kerry Mutch of 60 patients with LETM NMOSD. This study showed that 47/60 (78%) of NMOSD patients who had LETM exhibited both bladder and bowel symptoms; 87% had either bladder or bowel dysfunction. It was found that 65% had developed these symptoms after their first episode of myelitis. Unsurprisingly, 70-83% experienced severely restricted lifestyles due to this function impairment (72).

3.3.4 Effect of neuromyelitis optica spectrum disorder on employment

A cross-sectional study on employment was performed by our team, led by NMO specialist nurse Kerry Mutch, among 103 patients with NMOSD. All these patients fulfilled the 2015 criteria for diagnosis as they tested positive for anti-aquaporin 4. Patients were contacted and asked about their employment status at the time of their first attack and at the time of questioning.

Overall, 85% of these NMOSD patients had stopped or reduced their work soon after diagnosis; 41% stopped work following the first attack. This was mainly due to visual/motor disability. It is known that loss of income and financial hardship have major impacts upon financial, family and social life besides the already known physical effects. These findings highlight the need for early aggressive treatments in NMOSD. This result was presented by Kerry Mutch in the Association of British Neurologists meeting in 2017. I contributed by collecting clinical data.

3.4 Opticospinal demyelination

In unpublished work, I followed up Dr Jacob's research in which the aim was to identify the natural history of non-MS opticospinal demyelination. This was a prospective longitidunal study that had been conducted in 2011 for patients (n=67) who had been recruited in 2003-2005. By 2015 I managed to obtain the outcomes of 64 of these 67 original participants; 58% of the 64 who could be contacted had a diagnosis of NMOSD, 17% were diagnosed as having MS, and 21% remained without specific diagnosis.

When I started my MD studies, research in AQP4-IgG antibody-positive NMO was advancing rapidly. I started to investigate the cases of NMO patients who lacked AQP4-IgG antibodies and those whose diseases behaved like NMO but whose conditions did not fulfil the criteria that were in use for diagnosis at that time.

In the next chapters I present results that answer some specific questions that led to the chosen subject of this work.

Chapter 4. Result 1: The impact of 2015 neuromyelitis optica spectrum disorders criteria on diagnostic rates

4.1 Introduction

The discovery of antibodies to aquaporin 4 (AQP4-IgG) in 70%–90% cases of NMO has changed the diagnostic and treatment approach to disorders presenting with presumed demyelinating aetiology (5). This was reflected in the previous diagnostic criteria (Table 2) (10). However, further advances in the field and the segregation of several clinical syndromes, symptoms, signs or imaging features(26), hitherto not associated with the classical phenotype of NMO (i.e. optic neuritis with longitudinally extensive transverse myelitis), have been reported in patients who have AQP4-IgG antibody although no formal criteria were proposed(14, 73). There also remains a substantial group of patients with otherwise typical clinical or imaging features of NMO that do not have AQP4-IgG antibodies. Some may have other serum markers, for example, myelin oligodendrocyte glycoprotein (MOG) IgG(74). The recent diagnostic criteria developed by an International Panel attempts to encompass and incorporate these developments (75) (Table 3). The term 'neuromyelitis optica spectrum disorders' (NMOSD) was felt to better represent the disease and has replaced the term NMO. It is as yet uncertain how much these new criteria will increase the number of cases diagnosed. Therefore, we systematically applied both the new and old criteria to a large cohort of cases.

Table 3. 2006 NMO diagnostic criteria (10)

2006 NMO diagnostic criteria
Optic neuritis and Transverse myelitis + 2 of the following: Normal MRI brain Transverse myelitis with MRI changes> 3 vertebral segment AQP4-IgG positive

Table 4. the 2015 IPND* criteria for Neuromyelitis optica spectrum disorders (55)

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
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. Fulfilment 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
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 6. Symptomatic cerebral syndrome with NMOSD-typical brain lesions
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 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 (3. Area postrema syndrome: requires associated dorsal medulla/area postrema lesions 4. Acute brainstem syndrome: requires associated periependymal brainstem lesions

4.2 Patients and methods

The aim of this study was to estimate the change in number of patients diagnosed with the new criteria. I conducted this study was in the NMO and non-multiple sclerosis (MS) central nervous system (CNS) demyelination clinic, part of the UK NMO service at the Walton Centre for Neurology and Neurosurgery NHS foundation Trust. This specially funded clinic in a tertiary hospital in the north of England is a referral centre for cases suspected to be NMO or its variants. After an initial detailed review, prospective follow up is done with imaging and additional investigations including new antibody tests as indicated based on new information from the rapidly evolving field. All patients were tested for AQP4-IgG antibodies performed at Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital and Oxford using the best available cell-based assay(41). This study formed part of the UK NMO study (MREC 02/8/082, Northwest medical research ethics committee, United Kingdom). We identified all patients seen in the clinic between January 2013 and May 2015 (n = 198). We excluded patients where an alternative diagnosis like MS (n = 15) or other well-described CNS inflammatory disorder (n = 7), for example, Behcet's disease or sarcoidosis, was made in the clinic on reinvestigation. We systematically applied 2006 and 2015 criteria to the remaining 176 patients.

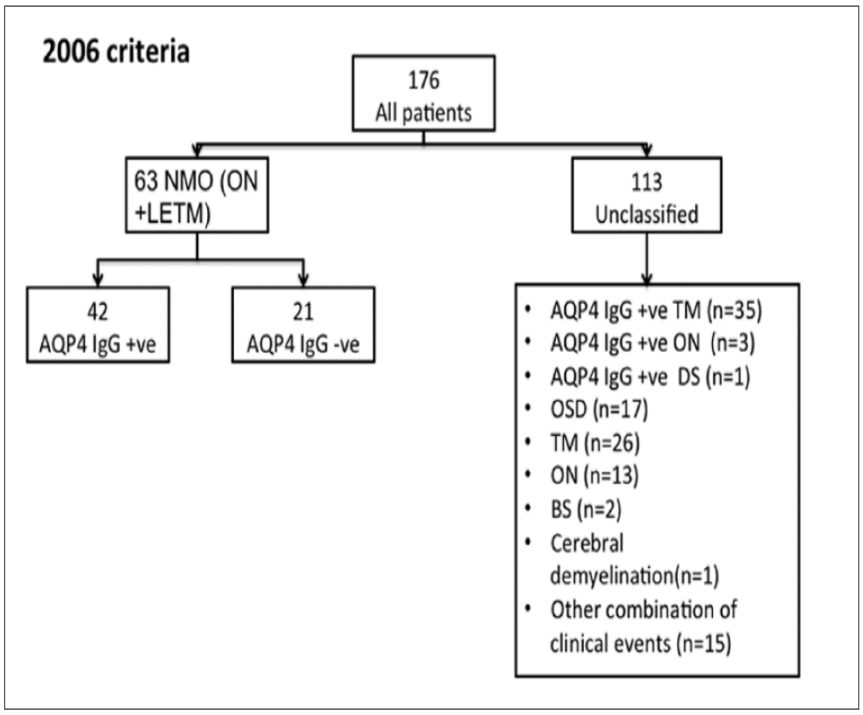
4.3 Results

The 2006 criteria classified 63 of the 176 patients as NMO (42 AQP4-IgG positive and 21 AQP4-IgG negative, 46 females and 17 males, ratio 2.7:1). All these patients had both optic neuritis and longitudinally extensive myelitis, either simultaneously (n = 16) or in subsequent attacks (n = 47). Five had single events (8%) and 58 had a relapsing course (92%). The remaining patients (n = 113) did not satisfy 2006 criteria for NMO (Figure 7). We then applied the 2015 criteria to the same cohort of 176 patients. A total of 111 patients fulfilled the new criteria (82 females, 29 males, ratio 2.8:1, ratio in relapsing cases 3.3:1 and in AQP4-IgG +ve patients is 5:1). In all, 81 were AQP4-IgG positive and 30 AQP4-IgG negative, an increase of 48 patients (76% rise). All the 2006 cases of NMO (n = 63) remained as NMOSD. The AQP4-IgG +ve NMOSD group included the 42 patients with both optic neuritis (ON) and transverse myelitis (TM) (as in 2006) and a further 39 newly included cases (Figure 8). The AQP4-IgG negative group included all the seronegative NMO in 2006 (n = 21) and nine more newly included cases. The remaining 65 patients with a variety of clinical

presentations (all are AQP4-IgG −ve) did not satisfy the new criteria (table 4). All patients who were AQP4 negative were tested for MOG antibodies, 15 of AQP4-IgG –ve NMOSD were positive for MOG-IgG (50%).

FIGURE 7. FLOWCHART CLASSIFYING 176 PATIENTS AS PER 2006 CRITERIA.

OSD: optic neuritis with short segment demyelination and normal MRI brain; NMO: neuromyelitis optica; AQP4: aquaporin 4; IgG: immunoglobulin G, ON: optic neuritis; LETM: longitudinally extensive transverse myelitis; TM: transverse myelitis; BS: brainstem demyelination. Combination of clinical events: combination of events other than long myelitis and optic neuritis, for example, optic neuritis and cerebral/brainstem syndrome, LETM and cerebral/brainstem syndrome, DS: diencephalic syndrome.



4.4 Discussion

The application of the 2015 International Panel for NMO Diagnosis (IPND) criteria in a large cohort of non-MS demyelination demonstrates a rise in diagnosis of NMOSD by 76%. The AQP4-IgG +ve group contributed 62%, and AQP4-IgG −ve group contributed 14%. All patients diagnosed as NMO by the previous criteria in 2006 are still diagnosable as NMOSD. All cases who met the new criteria and did not meet 2006 criteria were previously classified and followed up in the clinic as 'atypical non-MS demyelination-probable NMO'. This

apparent rise may not be evident in specialist clinical settings, where neurologists have been treating all patients with AQP4-IgG as NMO for the past few years based on emerging evidences despite the absence of formal criteria till now.

However, in settings where NMO is still diagnosed with the 2006 criteria, there could be a substantial rise in diagnostic rates. There are many patients who still remain unclassifiable (Figure 8) as they do not satisfy even the present criteria, but are in the authors' practice being treated as NMOSD. These are all AQP4-IgG −ve patients, with more than one clinical event with dissemination in space but don't appear typical for MS. Some patients also do not meet the criteria despite typical clinical events for one of the core criteria (optic neuritis and brainstem symptoms) as they did not have magnetic resonance imaging (MRI) abnormalities specified in the new criteria. Some have clinical syndromes other than that prescribed in the criteria but with typical MRI changes, for example, patients who had typical periependymal changes in area postrema without history of nausea, vomiting or hiccups.

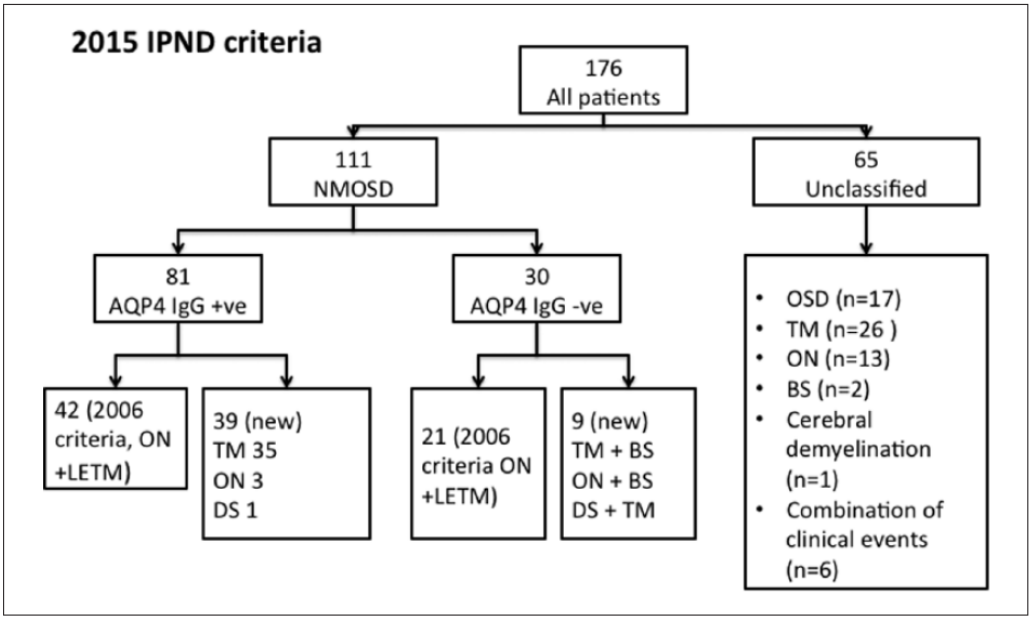


FIGURE 8. FLOWCHART CLASSIFYING 176 PATIENTS AS PER 2015 CRITERIA

AQP4 IgG: aquaporin 4 immunoglobulin G; NMOSD: neuromyelitis optica spectrum disorder; ON: optic neuritis; LETM: longitudinally extensive transverse myelitis; OSD: optic neuritis with short segment demyelination and normal MRI brain; TM: transverse myelitis; BS: brainstem demyelination; DS: diencephalic syndrome. Combination of clinical events: combination of events other than long myelitis and optic neuritis, for example, optic neuritis and cerebral/brainstem syndrome, LETM and cerebral/brainstem syndrome.

A further category is patients who may have recurrent long myelitis without history/signs of ON, but abnormal visual evoked responses. Relapsing forms of site-restricted demyelination like ON, TM and brainstem syndromes too remain without a specific nosological category. These and similar syndromes need to be studied prospectively to see if they will evolve into typical NMOSD and may merit inclusion in future revisions of the criteria. Recent collaborative work showed how the diagnosis of this group can be controversial even among specialists(76). The emergence of MOG antibodies associated with AQP4-IgG −ve cases has added another layer of complexity to the NMOSD field. As in this series, MOG-IgG can be positive in a proportion of AQP4-IgG−ve NMOSD patients. But not all MOG-IgG +ve cases fulfil the NMOSD criteria(77, 78). MOG-IgG positive patients may not have the same severity or clinical prognosis of AQP4-IgG cases. Indeed, many are monophasic. The test is not yet available widely. The non-inclusion of MOG in the present IPND criteria seems appropriate until we learn more about MOG-associated disease. As most cases of NMOSD are relapsing, an accurate early diagnosis and initiation of immunosuppressive treatment should lead to reduction in relapse rates,disability and better long-term outcomes. An earlier diagnosis also alerts the physician to treat a relapse aggressively with longer duration of steroids and or plasma exchange(79). So if the underlying premise of the 2015 IPND criteria is correct, then this increase in the number of patients diagnosed as NMOSD will lead to a larger number of patients being treated earlier(80) and better and would avoid wrong treatments that could be deleterious, for example, some MS drugs can worsen NMO(81). The rise in numbers may also create interest from the pharmaceutical industry and facilitate recruitment into clinical trials. We are aware of only one other study assessing the impact of the new criteria(80). Hyun et al. from South Korea applied the 2006 and 2015 criteria to 594 patients with CNS inflammation (including MS) and compared diagnostic rates. NMOSD was diagnosed in 136 patients with the 2006 criteria (23%) and 252 (42%) with the 2015 criteria, an increase of 85%, quite similar to our study. They estimated that the time to diagnosis reduces to 11 months by 2015 criteria from 53 months by 2006 criteria.

There are limitations to this study. The application of new criteria to data (history, MRI) collected in the past poses some problems. Older MRIs may not be available for review. The nature, duration and severity of symptoms that were thought of as nonspecific and poorly documented or remembered (e.g. nausea, vomiting or excessive sleepiness/narcolepsy) may

now acquire significance causing recall bias. This is perhaps reflected in the paucity of cases with confirmed area postrema syndrome in our cohort. We also acknowledge that our specialised clinical setting may not reflect the typical settings (MS or general neurology clinics) where such patients are followed up globally. We also have ready access to a sensitive and specific AQP4-IgG assay, which may not be the case elsewhere. Notwithstanding these limitations, we believe that our findings should stimulate clinicians to reassess their patients with non- MS or atypical MS to see if they fit the 2015 IPND NMOSD criteria.

The next revision of the criteria should address the above issues. It is anticipated that both validated cell based AQP4 and MOG-IgG tests will become available globally by that time facilitating our understanding of the differences between these groups. Importantly, the IPND should suggest unifying terminology to the as yet unclassifiable cohort of patients to further research.

4.5 Conclusion

The 2015 IPND criteria is a significant change in the approach to the diagnosis and classification of demyelinating syndromes that are not typical MS. It increases the diagnosis of NMOSD by 76%. It also makes NMOSD a differential diagnosis for many previously unclassifiable CNS disorders. It may allow earlier diagnosis and management, which has significant implications to clinical practice, and research including clinical trials and health care costs. Prospective validation of the criteria in a large multiethnic, multinational cohort through international collaboration is essential.

Chapter 5. Results 2: What proportion of AQP4-IgG negative NMO spectrum disorder patients are MOG-IgG positive? A cross- sectional study of 132 patients

5.1 Introduction

73%-90% of Neuromyelitis optica spectrum disorder (NMOSD) patients diagnosed according to the 2015 International panel on NMO diagnosis have aquaporin-4 antibodies (AQP4-IgG) (80, 82). It is presumed that at least a proportion of the remaining 10-27% of patients, classified as seronegative NMOSD have another disease specific antibody. Antibodies to myelin oligodendrocyte glycoprotein (MOG-IgG) have been increasingly reported in a variety of CNS neuroinflammatory conditions including patients with phenotypes typical for NMOSD(83). We aimed to determine the prevalence of MOG-IgG in AQP4-IgG negative NMOSD.

5.2 Methods

The Walton Centre Neurosciences NHS Trust in Liverpool, United Kingdom, is a tertiary neurology hospital that hosts one of the two national multidisciplinary specialist clinics for patients with NMOSD and non-MS demyelinating disorders as part of the UK NMOSD service. We systematically reviewed all patients seen in this clinic over the last 4 years (after the availability of MOG-IgG testing), including clinical information, MRI and antibody tests. Both AQP4-IgG and MOG-IgG were tested using a validated live cell-based assay with high specificity (John Radcliffe Hospital, Oxford, UK) (41, 49). This study was approved by Research Ethics Service, NRES Committee London – Hampstead, Ref No 15/LO/1433

5.3 Results

261 unique patients with non-MS/atypical CNS inflammatory conditions attended the clinic and were assessed for NMOSD. All patients were tested for AQP4-IgG. 132 cases satisfied the 2015 NMOSD diagnostic criteria. Of these 96 [73%] were AQP4-IgG positive and 36 [27%] AQP4-IgG negative. These 36 patients, were tested for MOG-IgG and 15/36 (42%) tested positive. This would account for 11% (15/132) of the total cohort of NMOSD patients (Figure 9 and Table 4). All MOG-IgG–ve patients were Caucasians with a median age of onset of 18 years (range 8-44) and median disease duration of 4.7 (2-16 Years). The predominant clinical phenotype of the demyelinating event was ON (60%), TM (21%), brain (12%) and brainstem (4%).

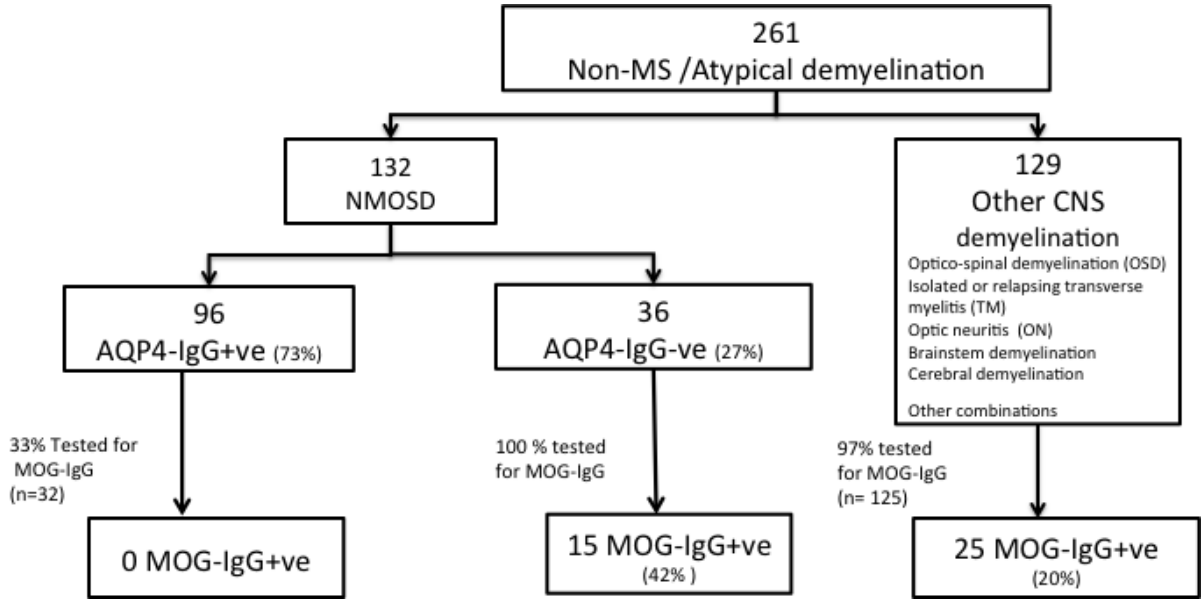


FIGURE 9. CLASSIFICATION OF NON-MS / ATYPICAL DEMYELINATION BASED ON 2015 NMOSD CRITERIA

AQP4-IgG and MOG-IgG testing. NMOSD: neuromyelitis optica spectrum disorder AQP4 IgG: aquaporin 4 immunoglobulin G. MOG-IgG: antibody to myelin oligodendrocyte glycoprotein. OSD -optico-spinal demyelination with normal brain MRI

While we tested all AQP4-IgG negative patients for MOG-IgG (n=36), only a proportion (33%) of AQP4-IgG positive patients (n=32) were tested (as double positives are exceptionally rare) (Figure 9). None were definitely positive. However one patient was 'low positive/possibly negative. This patient with one episode of long myelitis also had antinuclear antibodies (1/80 titre with homogenous pattern (nuclear antigens all negative) and was 'low positive' for anti-glycine antibodies too. The significance of the MOG-IgG in the context of these additional antibodies is uncertain and may reflect a heightened humoral autoimmune response rather than truly pathogenic dual positivity. This patient has not been included in the MOG cohort in this paper.

We also tested the majority of patients with a demyelinating syndrome referred to the service who did not fulfil the NMOSD criteria (125/129, 97%). Twenty five (20%) were positive for MOG-IgG. Details of these cases will be the subject of an upcoming separate research paper and are not discussed further here.

We also assessed how many of the MOG-IgG patients with NMOSD phenotype had a relapsing course. Thirteen patients (86%) had a relapsing course. However a relapsing course was the reason for referral to the clinic in the first place (n=13/13). The median duration of illness for the relapsing patients was 4.7 years (2-16 Years). The median inter

attack interval was 1 year (0.16-17) and median EDSS in the relapsing MOG group at last follow up was 3 (0-9, Table 4). All relapsing patient are on immunosuppressants (Table 4).

We also assessed the proportion of patients with optic neuritis and long myelitis who fulfil Wingerchuk 2006 criteria(10) that are MOG-IgG positive, as this is a clinical question often posed. Of the whole cohort of 261 patients, 75 patients had long myelitis and optic neuritis. Of these 49 were AQP4-IgG positive (66%) and 10 were MOG-IgG positive (13%, or 38% of AQP4-IgG negative patients) and 16 remained seronegative (21%). Serial testing where done in 14/15 patients (13 relapsing); MOG-IgG was detected in all. Treatment with steroid or immunosuppression does not seem to have an effect on MOG-IgG serostatus in this cohort of predominantly relapsing patients (Table 5).

5.4 Discussion

In a cohort of well characterised NMOSD patients (n=132), 73% were AQP4-IgG and 11% were MOG-IgG seropositive and 16% remain seronegative. MOG-IgG disease accounts for 42% of the AQP4-IgG negative seronegative cohort. MOG-IgG was present in 38% of patients with long myelitis and optic neuritis who do not have AQP4-IgG.

86% (13/15) of our patients who satisfy criteria for NMOSD who are MOG-IgG positive patients have relapsing disease, similar to a recent study (84) who reported that 80% of their MOG-IgG positive cohort (n=50) followed a relapsing course. However a relapsing course was the reason for referral to the clinic in the first place (n=13/13) making this a biased sample. Long term follow ups of a cohort of MOG-IgG positive patients after the very first event is required to obtain the true risk of relapse.

Importantly 20% of patients with non-MS/atypical demyelination who do not satisfy criteria for NMOSD tested positive for MOG-IgG (fig5). Double positive cases (both AQP4-IgG and MOG-IgG) are rare (28, 85, 86) with none of the tested patients were definite positives. Since we have tested only 52% (68 /132) of the total NMOSD cohort for MOG-IgG this requires further clarification in future studies.

5.5 Conclusion

Our study provided the best possible answers at the current time on several questions on the frequency of MOG-IgG patients : NMOSD who are AQP4-IgG negative and MOG-IgG positive (42%), NMO (as per Wingerchuk 2006) with optic neuritis and long myelitis who are AQP4-IgG negative but MOG-IgG (13%). We also found that MOG-IgG is found in 20% of non-NMOSD /non-MS demyelination. It is also estimated that at least 11% of all NMOSD (as per 2015 criteria) is MOG-IgG positive.

Our study has important practical implications. Firstly the definite diagnosis of MOG-IgG associated disease offers patients and physicians a better diagnostic label than seronegative NMOSD. Secondly as nearly one in every two of seronegative NMOSD, and 1/5 of atypical non MS demyelination is MOG-Ig positive, testing for these cohorts will be high yield and worthwhile, compared to testing every demyelination (which in most Caucasian predominant populations is likely to be MS) with attendant costs and risk of false positive results. Thirdly, it is likely that the long term disease course and therefore treatment strategies of AQP4-IgG and MOG-IgG is different. If this is the case, MOG-IgG status, should be part of inclusion/exclusion criteria or a variable for stratification in clinical trials. The latter issue may have importance for currently recruiting trials that include seronegative NMOSD.

Table 5. Demographic, clinical and radiological characteristics of the 15 NMOSD patients with MOG-IgG

Patient no	Age	Sex	Age at onset	Disease duration (years)	Course	Total No of events	Clinical phenotype (No of attacks)	First inter-attack interval	Spinal MRI	Baseline brain MRI	CSF oligoclonal bands	EDSS	Current treatment
1	31	F	18	13.4	R	13	ON (13) TM (1)	3 years	LETM	Normal	Negative	4	Subcutaneous IGs (Immunoglobulins) and oral prednisolone
2	55	M	44	11	R	7	ON (2) TM (1) brainstem (1) brain syndrome (5)	7 years	Short mid thoracic lesion	Brain stem , cortical and subcortical extensive demy	Positive	3.5	Steroid & mycophenolate
3	31	F	15	16.4	R	2	ON (1) TM (1)	4 years	LETM	Normal	Negative	9	Azathioprine and oral prednisolone
4	21	M	18	2.5	R	5	Brain stem (1) Brain syndrome (1) TM (1) ON (5)	2 months	Multiple short lesions on thoracic cord	Large area of high T2 signal in the posterior brainstem both sides of mid brain	Negative	1.5	Azathioprine switched to Rituximab
5	22	M	17	4.7	R	>7	ON (>7) and TM (2)	2 months	LETM	Normal	Unknown	3	Tocilizumab, IVIG 6-weekly and oral prednisolone
6	30	F	28	2	R	2	ON (1) TM (1)	1 year	LETM	Cerebral ring enhancing lesion supracollosal subcortical	Negative	0	Mycophenolate

7	23	F	8	14.4	R	3	ON (2) TM (2) Brain syndrome (1)	3 years	LETM	Multiple non-specific white matter lesions	Negative	6	Azathioprine and oral prednisolone
8	24	F	17	6.9	R	2	ON (1) TM (1) Brain syndrome (1)	3 months	LETM	Brainstem, left cerebral peduncle and few non-specific white matter lesions	Negative	1	Azathioprine and oral prednisolone
9	14	F	10	4	R	3	Brain syndrome (1) ON (3) TM (1)	3 month	LETM	Bilateral hemispheric white matter changes	Negative	2.5	Rituximab and mycophenolate
10	28	M	19	8.2	R	4	ON (3) TM (1)	6 years	LETM	Normal	Unknown	4	Mycophenolate
11	44	M	13	31	R	5	ON (3) TM (2)	17 years	LETM	Normal	Negative	3.5	Azathioprine
12	39	F	36	3.1	R	2	Brain stem (1) ON (2)	2.2 years	Normal	Lesion on Pons	Negative (161)	3	Mycophenolate and oral prednisolone
13	42	M	38	3.6	R	2	TM (1) Brain stem (1)	2 months	LETM	Peri ependymal pons lesion	Unknown	6	Azathioprine and oral prednisolone
14	28	M	26	2	Single event	1	ON+LETM	Simultaneously	LETM	Normal	Positive	1.5	Mycophenolate
15	45	M	40	5	Single event	1	ON+LETM	Simultaneously	LETM	Normal	Negative	2	None

F female, M male, R relapsing, ON optic neuritis, TM transverse myelitis, LETM longitudinally extensive transverse myelitis, and IVIG intravenous immunoglobulins

Table 6. MOG-IgG testing in relation to disease course and immunosuppressive treatment

Patient no	Date of onset	Date of 1st relapse	Last relapse	Date of start on steroid	Date of start on maintenance immunosuppressive treatment	First positive MOG-IgG test	Subsequent MOG test Year	Titre	Comments
1	Jan-02	May-05	Jul-05	Jan-08	2009	2011	2013, 2014 Both positive	NA	Data not clear if was on steroid in first or last relapse, but was on immunosuppressant when tested positive for MOG-IgG
2	2004	2011	2015	2014	2014	2014	2015, 2016, 2017 All positive	300	Patient was not on steroid in 1st or last relapses, but was on immunosuppressant when tested positive for MOG-IgG after diagnosis and remained positive
3	Jan-99	Apr-03	May-03	Unknown	2003	Apr-14	Jul-14 positive	NA	Data not clear if was on steroid in first or last relapse, but was on immunosuppressant when tested positive for MOG-IgG subsequently
4	Sep-14	Nov-14	May-17	Nov-14	Dec-14	2014	2015 positive 2016 positive	300 400	Patient was not on steroid in 1st relapse, but was on steroid and immunosuppressant in last relapse and when MOG-IgG tested and remained positive
5	Sep-10	Oct-10	Jul-13	At onset	2011	2012	2014, 2015, 2016 all positive	NA	Patient was on reducing dose of steroid in 1st relapse, and on immunosuppressant and steroid in last

									relapse and when MOG-IgG was tested and remained positive
6	Aug-13	Sep-14	Sep-14	Sep-14	May-15	Sep-14	2016, 2017 Both positive	NA	Patient was not on steroid in 1st relapse, was on steroid when tested for MOG-IgG initially and in 2016 but off steroid in 2017 and remained positive
7	2001	2004	2010	At onset	2010	2013	2014, 2016 both positive	NA	Patient was not on steroid in 1st or last relapse, she was on immunosuppressant when tested for MOG-IgG subsequently.
8	Jul-08	Nov-08	Nov-08	At onset	Nov-08	Apr-11	May-11 positive	NA	Data unavailable if patient was on steroid in 1st relapse, she was on immunosuppressant when tested positive for MOG-IgG
9	Apr-12	July-12	Aug-15	At onset	2012	2012	2015, 2016 positive	NA	Patient was on steroid in first relapse and when tested positive for MOG-IgG. She was also positive when was on steroid and immunosuppressant in subsequent relapses.
10	Mar-07	Jul-13	Dec-15	At onset	Jul-14	Apr-14	2016 positive	NA	Patient was not on steroid in first relapse, or first MOG-IgG test. He was on immunosuppressant in last relapse and when remained positive in subsequent testing
11	1984	2001	Mar-13	At onset	2013	2015	No further tests	NA	No available data whether patient was on steroid in first or last relapse, but he was on immunosuppressant when tested positive for MOG-IgG.
12	May-12	Aug-14	Aug-14	At onset	May-15	May-15	2016 positive	NA	Patient was not on steroid in 1st relapse, but was on steroid when tested positive for MOG-IgG and was on immunosuppressant on subsequent positive test

13	Oct-12	Jan-13	Jan-13	At onset	Aug-13	Jul-13	2014 –ve 2015 +ve	NA	Patient was on steroid in 1st relapse, however immunosuppressant was initiated after MOG-IgG returned positive in 2013, later test one year apart was negative in 2014, and subsequent test in 2015 was positive while still on immunosuppressant.
14	Mar-14			At onset	Apr-14	Apr-14	2015, 2016, 2017 All positive	NA	Only one event but patient chose to go on treatment
15	Jun-12			At onset	Not on immunosuppressant	Jun-12	2015 positive	NA	Not on immunosuppression

NA: not available

Chapter 6. Results 3:

Seizures and encephalitis in myelin oligodendrocyte glycoprotein IgG disease versus aquaporin-4 IgG disease

6.1 Introduction

Antibody-associated central nervous system inflammation is increasingly recognized to cause a wide spectrum of relapsing neurologic diseases. Myelin oligodendrocyte glycoprotein (MOG), a membrane protein expressed on oligodendrocyte cell surfaces and on the outermost surface of myelin sheaths(83), is the target for one such antibody, MOG-IgG. Initially, MOG-IgG was linked to childhood-onset multiple sclerosis(87), but more recently it has been found in a proportion of patients who meet the clinical criteria for neuromyelitis optica spectrum disorder (NMOSD) but who lack antibodies against aquaporin 4 IgG (AQP4-IgG)(15, 50). Although the NMOSD phenotype appears common to these 2 antibodies, the pathogenesis is distinct, with AQP4-IgG triggering complement-mediated astrocyte death rather than targeting myelin and oligodendrocytes(77). Differences in phenotype are also emerging: patients with MOG-IgG are more likely to have a milder or less disabling clinical course compared with patients with AQP4-IgG and less likely to be female, and association with other autoimmune disorders is less common(88). In addition, and in spite of the broadening spectrum of NMOSD outlined in the 2015 International Panel for NMO Diagnosis criteria(75, 82), some patients with MOG-IgG have limited or different phenotypes to typical AQP4-IgG NMOSD(77).Whether MOG-IgG–associated demyelination is part of an evolving NMOSD or a distinctive disease is hotly debated and highlights the potential importance of any clinical feature that appears unique to one or the other antibody. In our cohort of patients with NMOSD and similar disorders, we noticed that some with MOG-IgG antibodies had presented with seizures or an encephalitis-like illness that we had not observed in patients with AQP4-IgG–positive NMOSD. Review of the literature found isolated reports of patients with MOG-IgG–associated disease having seizures or encephalopathy (Table 6)(28, 89-95). Consequently, we considered it important to study this association further.

6.2 Methods

All patients in this case series were under the care of the NMO UK Service, a specialist multidisciplinary clinic for patients with NMOSD and similar non–multiple-sclerosis–related

demyelination based at The Walton Centre NHS Foundation Trust, Liverpool, England, between January 2013 and December 2016. I reviewed the clinical and T2-weighted magnetic resonance imaging (MRI) data of all patients with MOG-IgG antibodies (n = 34) (all of Caucasian) seen at the centre and the 100 most recent AQP4-IgG–positive patients (74% white, 16% Asian, 7% African or Afro-Caribbean, and 3% mixed or other race/ethnicity). Both AQP4-IgG and MOG-IgG were detected in patients' serum using a validated live cell–based assay with high specificity (developed at John Radcliffe Hospital, Oxford, England)(41, 49). For titration purposes, we used antihuman MOG-IgG1 (heavy and light chain) secondary assay. Neuromyelitis optica spectrum disorder was diagnosed in all 100 AQP4-IgG–positive patients and in 17 (50%) of MOG-IgG–positive patients according to the 2015 International Panel for NMO Diagnosis criteria(75). Data analysis was completed January 4, 2017. This study was approved by the Research Ethics Service, NRES Committee London. All patients provided written informed consent.

6.3 Results

Thirty-four patients with MOG-IgG disease (20 female) with a median age at analysis of 30.5 years (interquartile range [IQR], 15-69 years) and 100 AQP4-IgG–positive patients (86 female) with a median age at analysis of 54 years (IQR, 12-91 years) were studied. Most patients were of Caucasian race. One of the 100 AQP4-IgG–positive patients (1%) experienced seizures. This patient experienced her first focal seizure 5 years before her presentation with NMOSD. The patient experienced additional focal seizures and was diagnosed as having focal epilepsy. Magnetic resonance images of the brain were normal. Her AQP4-IgG titer was 1:1600. Five of the 34 MOG-IgG–positive patients (14.7%) presented with seizures at the time of a major episode of central nervous system inflammation, based on both clinical and radiological findings. The difference between our AQP4-IgG–positive and MOG-IgG–positive patients in terms of seizure occurrence was statistically significant (1 of 100 AQP4-IgG–positive patients vs 5 of 34 MOG-IgG–positive patients; 2-sided $P < .008$, Fisher exact test). All 35 MOG-IgG–positive patients were AQP4-IgG negative. Four of these 5 patients had clinical encephalopathy during these particular events. Demographic, clinical, and immunologic profiles for the 5 patients are summarized in Table 7 and described below.

6.3.1 Case 1

A preteen girl presented with generalized tonic-clonic seizures (GTCSs) preceded by fatigue, headache, photophobia, confusion, and vomiting. Magnetic resonance imaging demonstrated bilateral hemispheric abnormalities (Figure10 A and B). She concurrently had right optic neuritis and transverse myelitis. Eight weeks after initial symptoms, MOG-IgG was reported to be positive. She was treated with methylprednisolone administered intravenously and plasmapheresis. Shortly thereafter, she developed optic neuritis affecting the left eye, which was treated with mycophenolate mofetil, 1.25 g/d. She was relapse free for 2 years but then developed optic neuritis in the right eye. Mycophenolate was withdrawn and treatment with rituximab was commenced. At last follow-up, she was in remission for 17 months and no longer receiving antiepileptic treatment. Her MOG-IgG results were persistently positive during the 5 years since her initial presentation. No MOG-IgG titers are available for this patient because samples are not available at present.

6.3.2 Case 2

A Caucasian man in his 50s presented with 8 GTCSs, each lasting 3 to 4 minutes following 5 episodes of demyelination across 10 years (2 brainstem events, 1 transverse myelitis, 1 cerebral event, and optic neuritis, after which he was discovered to be MOG-IgG positive, with a titer of 1:300). He was taking prednisolone, 10 mg/d, which had been tapered from a 60-mg/d dosage commenced during his last relapse, 5 months previously. He had started treatment with oral azathioprine 1 month before this episode. An MRI of the brain showed residual inflammatory lesions in the left frontal, temporal, and occipital lobes (Figure10 C and D). Six weeks later, he had a GTCS in association with severe optic neuritis. No new lesions were present on MRI. His immunosuppressive therapy was switched to mycophenolate, antiepileptic drugs were optimized, and, at last follow-up, he had remained stable for the past 12 months. However, he showed significant residual cognitive damage, including severe expressive aphasia. He remained MOG-IgG positive at subsequent testing across 3 years.

6.3.3 Case 3

A man in his early 20s experienced his first neurological event with optic neuritis and brainstem demyelination and was treated with methylprednisolone and immunoglobulin, both intravenously. Six weeks later, he presented with a cluster of GTCSs, the first during

sleep and the second 4 days later. Magnetic resonance imaging showed a new inflammatory lesion (Figure 11); this time, he was found to be MOG-IgG positive, with a titer of 1:300. He received methylprednisolone intravenously, followed by oral prednisolone, 60 mg/d, and levetiracetam, 1 g/d, for a year, after which it was withdrawn with no recurrence of seizures. His MOG-IgG test remained positive, with a titer of 1:300 twelve months after initial testing.

6.3.4 Case 4

A boy in his early teens presented with fluctuating level of consciousness, extensor posturing of his limbs, and a 4-minute focal seizure affecting predominantly his head, the right side of his face, and the right arm. Magnetic resonance imaging showed marked signal abnormality in the left temporal lobe, particularly within the gray matter (Figure 12). He made a good initial recovery after receiving methylprednisolone intravenously. However, he was readmitted 2 weeks later with severe left-sided retro-orbital and forehead pain; within 24 hours, he experienced 2 focal seizures affecting the left side of his face that progressed to a GTCS. An MRI scan performed at this time showed progression of the previous lesion. His symptoms resolved completely following methylprednisolone delivered intravenously and a tapering course of oral prednisolone, and he continued on a regimen of levetiracetam 2 g/d.

Three years later, seizures recurred. A severe headache associated with pallor and profuse vomiting developed, followed by rhythmic twitching of his head and eyes to the right, without loss of consciousness. No new changes were found on MRI. He was treated by increasing his levetiracetam dosage to 3 g/d. Despite this treatment, he presented with additional clusters of focal seizures with secondary generalization, occurring almost every 4 weeks. The MRI was repeated and showed a new parietal lobe lesion. Following consultation at our center (4 years from initial presentation), MOG-IgG was checked and was positive. His titer was 1:800, and follow-up samples were positive 1 year later. The patient opted not to take long-term immunosuppressant therapy. At last follow-up, he continued to take a combination of levetiracetam and carbamazepine and had been seizure free for 8 months.

6.3.5 Case 5

A Caucasian woman in her early 40s experienced a seizure associated with her first demyelinating event. Her symptoms started with lethargy, confusion, and altered sense of smell and taste. She was noted to behave oddly and had left-sided weakness. She experienced a focal seizure that was recorded during an electroencephalogram. An MRI showed widespread white matter changes, particularly confluent over the right hemisphere and involving both basal ganglia (Figure 12 B and C), and her MOG-IgG test results returned positive 4 weeks after presentation. She received methylprednisolone intravenously and by the third day was able to walk. Her cognition improved gradually and, while taking levetiracetam, she experienced no further seizures for approximately 2 years. A second episode of optic neuritis occurred during a gradual withdrawal of prednisolone treatment, which she had been taking orally. Mycophenolate therapy was introduced, and no further neurological events occurred over the ensuing 20 months. She continued to test positive for MOG-IgG, and her titer was 1:400 when retested 22 months after the second episode.

None of the 5 patients described here had any other identified cause, including infective or drug related, for their focal or generalized seizures. There was no known family history of epilepsy. None of these patients tested positive for other antineuronal antibodies, including anti-N-methyl-d-aspartate receptor and anti–voltage-gated potassium channel antibodies; leucine-rich, glioma inactivated 1 (LGI1); contactin-associated protein 2 (CASPR2); glutamic acid decarboxylase (GAD); and paraneoplastic antibodies (antibodies against Hu, Yo, Ri, Tr, CV2, and amphiphysin).

6.4 Discussion

We describe 5 patients with MOG-IgG–associated inflammatory central nervous system disease and seizures. All had brain cortex involvement, and 4 of the 5 had encephalopathy. Viral or autoimmune encephalitis was the initial diagnosis in these 4 patients. Four of the 5 patients meet the 2015 diagnostic criteria for NMOSD without AQP4-IgG(75). The main clinical spectrum for MOG-IgG–positive disorders is optic neuritis, transverse myelitis, acute disseminated encephalomyelitis, clinically isolated syndrome, paediatric multiple sclerosis, and NMO(77, 96). Acute disseminated encephalomyelitis-like disorders, including encephalopathy, associated with MOG-IgG have previously been reported(77, 92, 93). A recent report(93) identified 4 cases of MOG-IgG–associated encephalitis from a cohort of 24

cases of steroid-responsive encephalitis. These patients were all men with unilateral and "benign" lesions with full resolution. Our case reports suggest that this is not always true. Two of the 5 patients were women. The encephalitis can be severe with lasting damage in some (case 2). Unprovoked seizure recurrence (epilepsy) occurred in 2 of these patients, indicating possible underlying gliosis. Follow-up imaging showed gliosis atrophy or persistent T2-weighted lesions in 3 patients.

In contrast, only 4 studies in the English-language literature reported seizures among AQP4-IgG–positive patients with NMOSD. One is a Japanese study(97) describing 3 of 31 (9.6%) AQP4-IgG–positive patients with NMO who had seizures. One of them had evident seizures associated with an inflammatory event, and it is unclear whether antibody testing was conducted by enzyme-linked immunosorbent assay or cell-based assay. The other 3 are paediatric studies: McKeon et al(98) studied 88 AQP4-IgG–positive, mainly non-Caucasian children with NMO and reported that 2 (2%) had focal seizures. Lotze et al(99) reported 2 Latin American children who had NMO and NMO antibody and presented with seizures. A third study also reports seizures but is unclear about the serostatus of the patients described(100). In our cohort, it was striking that none of the 100 AQP4-IgG–positive patients experienced seizures as part of an inflammatory event. The difference between our AQP4-IgG–positive and MOG-IgG–positive patients in terms of seizure occurrence was statistically significant (1 of 100 AQP4-IgG–positive patients vs 5 of 34 MOG-IgG–positive patients; 2-sided $P < .008$, Fisher exact test).

The encephalopathic disorders and seizures that occurred in these patients were both likely triggered by an episode of demyelination caused by MOG-IgG. There was no evidence that infective encephalitis or other associated autoimmune antibodies (eg, anti-N-methyl-d-aspartate receptor or anti–voltage-gated potassium channel antibodies) were responsible for the seizures. A study of N-methyl-d-aspartate receptor antibodies in 215 patients with a range of inflammatory demyelinating diseases,11 including 22 MOG-IgG–positive patients with cognitive problems, seizures, or both, concluded that double seropositivity is rare(92). Jarius et al(28) described an MOG-IgG–positive patient who experienced seizures complicated with cerebral venous thrombosis and localized brain edema following intravenous treatment with a high dose of methylprednisolone(28). In our cohort, there was no evidence of treatment-induced seizures. It is worth mentioning that epilepsy prevalence

in normal population in UK is about 0.97% (https://www.epilepsyscotland.org.uk/wp-content/uploads/2019/05/Joint_Epilepsy_Council_Prevalence_and_Incidence_September_11_3.pdf)

6.5 Limitations

There are several limitations to this study. This was a single-center, cross-sectional study with retrospective collection of data. The clinic is highly specialized and serves as a national referral centre. It is possible that we have encountered only the severe forms of MOG-IgG disease and that, perhaps in a larger cohort including milder cases, seizures may be rarer. There could have been recall bias about details of seizures, although we relied on hospital notes whenever possible. Larger studies on MOG-IgG disease that specifically ask for the presence of seizures as a clinical feature will be needed to validate this observation further.

6.6 Conclusion

In this cohort, patients with MOG-IgG–associated disease were more likely to present with an encephalopathic disorder and seizures compared with AQP4-IgG–positive cases. The spectrum of MOG-IgG–associated disease continues to expand and includes atypical cerebral inflammatory lesions, which may have been previously characterized as relapsing steroid-responsive autoimmune encephalitis, acute disseminated encephalomyelitis, atypical multiple sclerosis, or central nervous system vasculitis. Myelin oligodendrocyte glycoprotein IgG–associated demyelinating disease is not always benign and can have a relapsing course and cause significant residual damage. Our study further supports the view that AQP4-IgG– and MOG-IgG–associated central nervous system inflammation are 2 different diseases with some overlapping phenotypes, particularly opticospinal inflammation. We recommend that testing for MOG-IgG be considered in patients with atypical inflammatory brain lesions, particularly those with an encephalitis-like presentation.

Table 7. Cases of MOG-IgG-associated seizures identified on literature review

Study	Number of patients	Age /Sex	Ethnicity	Other antibodies	MOG assay	Type of seizure	Clinical syndrome	Cognitive changes	Disease course	MRI brain changes
Hino-Fukuyo et al(89)	3	12M 14M 5M	Japanese	None	CBA	NA	ADEM	NA	Monophasic Relapsing Monophasic	1-Multiple WM, lt.Basal ganglia and bilateral .thalami (one month after onset) 2-Multiple WM, bilateral . Basal ganglia (at third attack) 3-Unknown
Tsuburaya et al(90)	1	7M	Japanese	None	CBA	Partial (eye deviation & L arm clonic seizures)	ADEM ON	No	Relapsing	T2-hyperintense plaque involving subcortical white matter in the right frontal lobe
Ramberger et al(91)	22?	NA	NA	None	CBA	NA	ADEM	NA	NA	NA
Titulaer et al(92)	1	4F	Hispanic	NMDAR	CBA	NA	Seizures, hemiparesis; later: mutism, chorea, orofacial dyskinesias	Yes	Relapsing	Multifocal areas of T2/FLAIR increased signal: periventricular, basal ganglia, cerebellum, and pons
Ogawa et al(93)	4	39M 36M 23M 38M	Japanese?	None	CBA	GTC GTC GTC+ focal GTC	Encephalopathy, ON Seizure, ON Encephalopathy Seizure, aphasia and right hemiparesis	Yes During seizure Yes During seizure	Monophasic Monophasic Monophasic Monophasic	R frontoparietal cortex R frontoparietal Cortex R parietal cortex L hemisphere cortex
Fujimori et al(94)	1	46 M	Japanese	None	CBA	Focal progressed to secondary	Encephalopathy, paraplegia	Yes	Relapsing	Bilateral frontal cortex, corpus collosum

						generalized				

Abbreviations: ADEM, acute disseminated encephalomyelitis; BG, basal ganglia; CBA, cell-based assay; F, female; FLAIR, fluid-attenuated inversion recovery; GTC, generalized tonic-clonic; L, left; M, male; MOG, myelin oligodendrocyte glycoprotein; MRI, magnetic resonance imaging; NA, not available; NMDAR, N-methyl-D-aspartate receptor; R, right; ON, optic neuritis; WM, white matter.a All studies used a cell-based assay for MOG antibody testing.

Table 8. Demographic and clinical features for the five cases of MOG-IgG1-associated seizure

Patient	1	2	3	4	5
Sex	F	M	M	M	F
Age at onset	10	44	18	12	39
Disease duration (years)	5	12	2	4	2
Disease course/ total no. of attacks	R/3	R/7	R/4	R/2	R/2
Clinical phenotype (no of attacks)	Brain syndrome (1) ON (3) LETM (1)	ON (2) TM (1) brainstem (1) brain syndrome (5)	ON (2) TM (1) brainstem (1) brain syndrome (5)	Brain syndrome (2)	brain syndrome (seizure+ hemiparesis+ cognitive changes) (1) ON (1)
Seizure type	GTC	GTC	GTC	PS and GTC	CPS
Altered sensorium/encephalopathy along with seizures	Yes	yes	No	Yes	Yes
Interval between index event and second demyelinating event	3 months	7 years	2 months	4 years	7 months
Recurrence of unprovoked seizures (epilepsy)	No	Yes	No	Yes including one episode of status epilepticus	No
Residual neurological impairment at last follow up	Unilateral pale optic disc	Aphasia right hemiparesis, cognitive impairment , right eye impaired vision	Impaired colour vision	Impaired memory and mild cognitive dysfunction	Nil
Last EDSS	1	5	1.5	1 .5	0
CSF: • OCB • WBCS	 Negative 50	 Positive Unavailable	 Negative 154	 Negative 550	 Positive High
MOG-IgG titres	NA	300	300, 400	800	400
Brain MRI	Bilateral cortical and hemispheric WM changeS	Brain stem , cortical and subcortical	Cortical lesion extending	Temporo-pareital cortical and	Wide spread WM changes in right

		extensive demyelination	to deep WM along long tracts and large area of signal changes in the brainstem and both sides of mid brain	subcortical lesion	hemisphere and involves basal ganglia and cortex
Residual Brain MRI changes	Nil	Atrophy occipital gliosis	Nil	Yes temporal gliosis	Yes white matter lesions
Current treatment	Rituximab and MMF	MMF and steroid, Levetiracetam	MMF	Steroid, Levetiracetam, carbamazepine	MMF

Abbreviations: CPS, complex partial seizures; CSF, cerebrospinal fluid; EDSS, Expanded Disability Status Scale; F, female; GTC, generalized tonic-clonic; L, left; LETM, longitudinal extensive transverse myelitis; M, male; MMF, mycophenolate mofetil; MOG, myelin oligodendrocyte glycoprotein; MRI, magnetic resonance imaging; NA, not available; OCB, oligoclonal bands; ON, optic neuritis; PS, partial seizures; R, relapsing; TM, transverse myelitis; WBCs, white blood cells; WM, white matter. A Cerebral syndrome is defined as seizure or hemiparesis or cognitive changes.

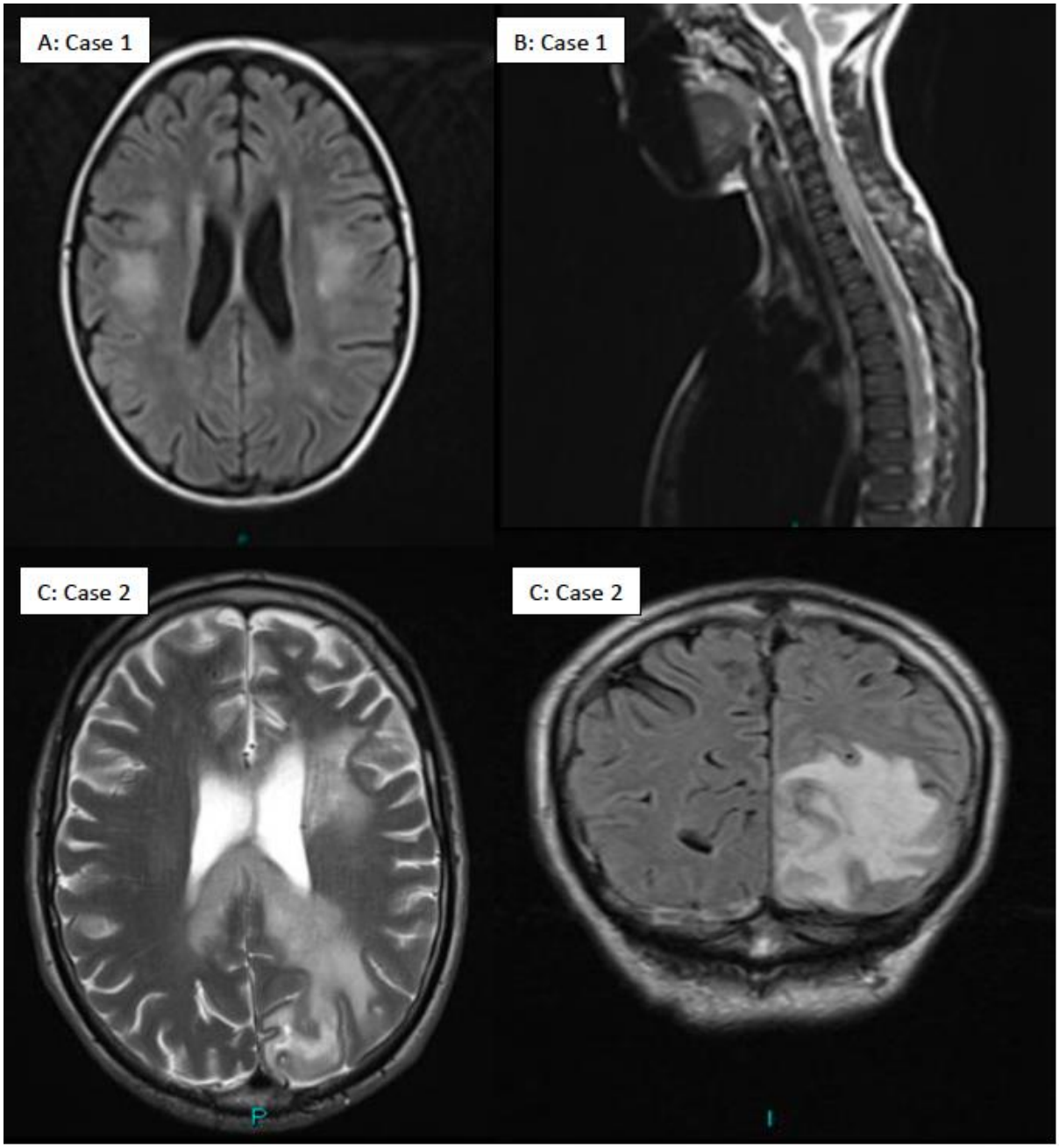
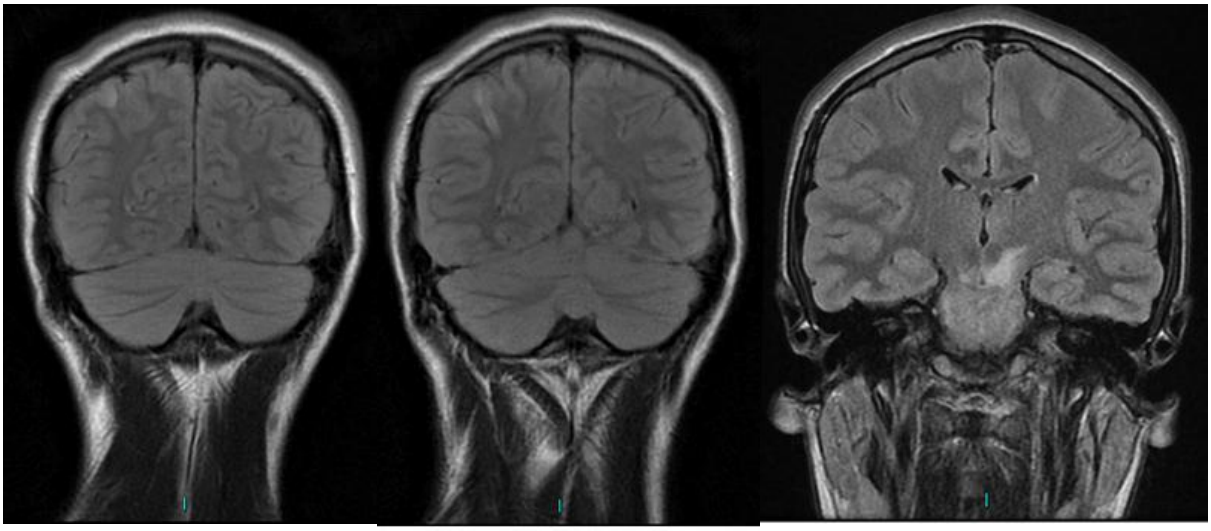


FIGURE 10. MAGNETIC RESONANCE IMAGING (MRI) OF THE BRAIN AND SPINAL CORD, CASES 1 AND 2

A, Axial T2-weighted fluid-attenuated inversion recovery image of the brain shows bilateral hemispheric lesions. B, Sagittal T2-weighted MRI of the spinal cord shows long myelitis. C, Axial T2-weighted MRI shows involvement of the frontal and occipital cortex and transcallosal lesion. D, An extensive occipital lesion can be seen.



3A 3B 3C

FIGURE 11. MAGNETIC RESONANCE IMAGING OF THE BRAIN, CASE 3

A and B, A lesion extends from the cortex to deep white matter. C, A lesion can be seen in the brainstem.

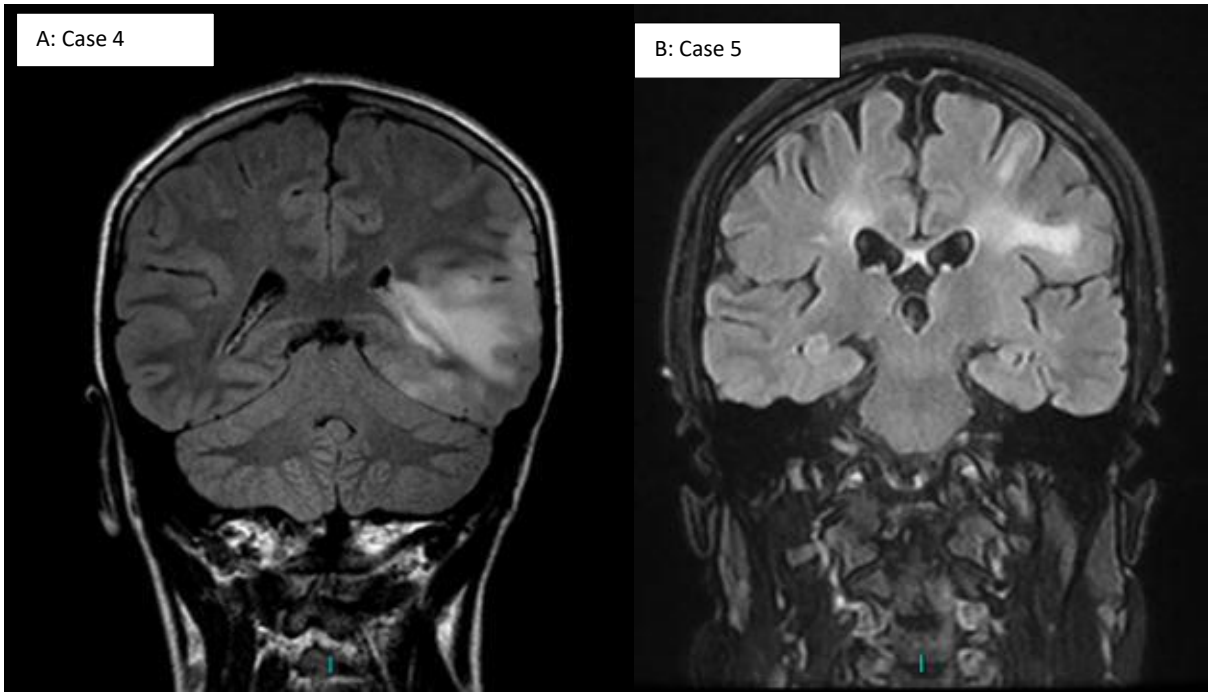


FIGURE 12. MAGNETIC RESONANCE IMAGING OF THE BRAIN, CASES 4 AND 5

A: Coronal T2-weighted fluid-attenuated inversion recovery (FLAIR) image shows an extensive lesion affecting both the left temporal and parietal lobes. B, Coronal T2-weighted FLAIR image shows bilateral hemispheric white matter changes

Chapter 7. General discussion of the results

I have discussed the main results in the relevant chapters. In this chapter I aim to provide an overview and further developments that have been made after my work.

7.1 Implications of the new criteria

In Chapter 4, I presented the results of one of the first studies to describe the implications of the new criteria. The application of the 2015 IPND criteria to a large cohort of patients who exhibited non-MS demyelination demonstrated an increase of 76% in the diagnosis of NMOSD. Of this increase, the AQP4-IgG positive group comprised 62% and the AQP4-IgG negative group 14%. Conditions that did not fit the old criteria were referred to as 'atypical non-MS demyelination – probable NMO'. This was the situation in a tertiary centre where there is access to the specialist clinical settings of the national NMO service, and where neurologists have treated all patients who test positive for the presence of AQP4-IgG as having NMO for the past few years based on emerging evidence.

However, in most settings where NMO is still diagnosed using the 2006 criteria, there could be a substantial rise in diagnostic rates by application of the new criteria. There are many patients who remain unclassifiable (Figure 8) as they do not satisfy even the present criteria but are treated as having NMOSD. These are all AQP4-IgG-negative patients who have experienced more than one clinical event with dissemination in space but whose symptoms do not appear typical for MS. Some patients also do not meet the criteria despite the occurrence of typical clinical events that meet one of the core criteria (optic neuritis and brainstem symptoms) as they have not shown the MRI abnormalities that are specified in the new criteria. Some have clinical syndromes other than those described in the criteria, but they show typical MRI changes; for example, some patients have had typical periependymal changes in the area postrema but no history of nausea, vomiting or hiccups. A further category is that of patients who may have recurrent long myelitis without history or signs of ON, but with abnormal visual evoked responses. Relapsing forms of site-restricted demyelination such as ON, TM and brainstem syndromes also remain without a specific nosological category. These and similar syndromes should be studied prospectively to see whether they will evolve into typical NMOSD and may merit inclusion in future revisions of the criteria. Recent collaborative work has shown that the diagnosis of this

group can be controversial, even among specialists (76). Dr Dean Wingerchuk (the first author of the new IPND criteria paper) commented on these findings in the accompanying editorial when the work was published:

> *"Hamid and colleagues' work highlights not only the utility of a more liberal and unified diagnostic criteria but also the work that remains to characterize AQP4-IgG-seronegative patients and assess the complete diagnostic properties of the 2015 IPND criteria" (101).*

The emergence of MOG antibodies that are associated with AQP4-IgG-negative cases has added another layer of complexity to the NMOSD field. MOG-IgG can be positive in a proportion of AQP4-IgG-negative NMOSD patients, but not all MOG-IgG-positive cases fulfil the NMOSD criteria (77, 78). Moreover, the test for MOG-IgG is not yet widely available. The exclusion of MOG from the present IPND criteria seems appropriate until we learn more about MOG-associated disease. As most cases of NMOSD are discovered during relapse, accurate early diagnosis and initiation of immunosuppressive treatment should lead to a reduction in relapse rates and reduced development of disability, which should lead therefore to improved long-term outcomes. Early diagnosis also prompts the physician to treat a relapse aggressively with longer duration of steroids or plasma exchange (79). If the underlying premise of the 2015 IPND criteria is correct, then this increase in the number of patients who are diagnosed as experiencing NMOSD will lead to a larger number of patients being treated earlier (80) and better, and should avoid the application of inappropriate treatments that can be deleterious; for example, some MS drugs can worsen NMO (81). The rise in numbers may also create interest from the pharmaceutical industry and facilitate recruitment into clinical trials. At the time of publication of this work, there was only one other study that had assessed the impact of the new criteria (80). Hyun et al. from South Korea applied the 2006 and 2015 criteria to 594 patients with CNS inflammation (including MS) and compared the diagnostic rates. NMOSD was diagnosed in 136 patients (23%) when the 2006 criteria were applied and 252 (42%) when the 2015 criteria were applied. These findings showed an increase of 85% with application of the 2015 criteria, which was quite similar to the results found in our study. They estimated that the time to diagnosis could be

reduced to 11 months by use of the 2015 criteria from 53 months under application of the 2006 criteria.

Since then, other centres have tried to validate the criteria and test their implications on their patient cohorts. A nationwide study in Denmark showed an 86% increase in diagnoses of NMOSD through application of the 2015 criteria (102); similarly, diagnoses increased by 62% in Latin America according to a multicentre study that involved Argentina, Brazil and Venezuela (103). Sepulveda et al. in Spain found that rates of prevalence and incidence of NMOSD were 1.5-fold higher than those that had been reported under the 2006 criteria (104).

It will be interesting to see results that seek to validate the criteria in developing countries such as my country of origin (Sudan), where AQP4-IgG antibody testing is not available and neurologists there send serum and CSF samples abroad for testing. The availability of this system is limited, since most patients have low socioeconomic status and such tests incur a fee. Also, the test method that is used is usually ELISA rather than the superior CBA (105).

There are limitations to my study. The application of the new criteria to data (history, MRI) that have been collected in the past poses problems. Old MRIs may not be available for review. The nature, duration and severity of symptoms that were considered at the time of data collection to be nonspecific and were therefore poorly documented or remembered (e.g. nausea, vomiting or excessive sleepiness or narcolepsy) may have acquired significance, and this causes recall bias. This is perhaps reflected in the paucity of cases that had confirmed area postrema syndrome in this cohort. Worldwide, the application of these criteria along with clinical observation may address the need either to modify the criteria again, or to introduce new criteria for cases that lie in-between the current diagnosis conditions. Moghadasi recently reported the case of a seronegative patient with ON whose MRI showed asymptomatic LETM (106), and highlighted this issue (107). A similar case was reported for an AQP4-IgG-positive patient (108).

It is to be hoped that the next revision of the criteria will address these issues. It is anticipated that both validated cell-based AQP4-IgG and MOG-IgG tests will be available globally by then, and this test availability will enhance our understanding of the differences

between these groups. Importantly, the IPND should suggest unifying terminology to the as-yet unclassifiable cohort of patients for further research.

7.2 MOG-IgG in NMOSD

Once international consensus had been achieved regarding NMOSD criteria and the MOG-IgG1 assay had been developed to its best possible sensitivity and specificity, I considered the percentage of tests that showed MOG-IgG1 antibody positivity in NMOSD patients and the percentage that showed MOG-IgG positivity in other non-MS-non-NMO cases. I kept in mind that the latter group had experienced a shift in diagnosis towards NMOSD after application of the new criteria.

In a cohort of well-characterised NMOSD patients (n=132), 73% were found to be AQP4-IgG positive and 11% were MOG-IgG seropositive, while 16% remained seronegative. MOG-IgG disease accounts for 42% of the AQP4-IgG negative seronegative cohort. MOG-IgG antibodies were present in 38% of patients who had long myelitis and ON but who did not test positive for AQP4-IgG.

About 86% (13/15) of our patients who satisfy the criteria for NMOSD and who are MOG-IgG positive have relapsing disease. These figures are similar to those reported in a recent study (84), which showed that 80% of a MOG-IgG positive cohort (n=50) followed a course of relapses. However, relapse was the reason for referral to the clinic in the first place (n=13/13); hence this was a biased sample. Long-term follow-up of a cohort of MOG-IgG positive patients after the very first event is required to determine the true risk of relapse.

About 20% of patients with non-MS/atypical demyelination in our study, who did not satisfy the criteria for diagnosis of NMOSD, tested positive for MOG-IgG (Figure 9). Double positive cases (both AQP4-IgG and MOG-IgG) are rare (28, 85, 86) (n=8-10) and none of the patients who tested positive could be defined as definite positives. Since we have tested only 52% (68/132) of the total NMOSD cohort for MOG-IgG, this requires further clarification in future studies.

This study provided the best possible answers that could be obtained at the current time to several questions on the frequency of the occurrence of MOG-IgG in patients. It found that

42% of NMOSD patients were AQP4-IgG negative and MOG-IgG positive, and that the percentage who had NMO (as per Wingerchuk, 2006) with ON and long myelitis and who were AQP4-IgG negative but MOG-IgG positive was 13%. I also found that MOG-IgG was found in 20% of patients who exhibited non-NMOSD/non-MS demyelination. It was also estimated that at least 11% of all NMOSD patients (as per 2015 criteria) were MOG-IgG positive.

This study has important practical implications. Firstly, the definite diagnosis of MOG-IgG-associated disease offers patients and physicians a better diagnostic label at this time than the label of seronegative NMOSD. Secondly, as nearly one in two cases of seronegative NMOSD and one in five cases of atypical non-MS demyelination is MOG-IgG1 positive, the performance of tests for these cohorts will be high yield and worthwhile compared with testing every case of demyelination (which in most Caucasian-predominant populations is likely to be MS) with the attendant costs and risk of false-positive results. Thirdly, it is likely that the long-term course of disease and therefore treatment strategies in the cases of discovered AQP4-IgG and MOG-IgG will be different. If this is the case, a patient's MOG-IgG status should be part of the inclusion/exclusion criteria or a variable for stratification in clinical trials. This issue may have importance for trials that are currently recruiting participants with seronegative NMOSD.

A UK study that involved the largest cohort of patients with antibodies to MOG-IgG1 to date (n=252) studied demographics, disease presentation, disease course and clinical outcomes of MOG-IgG1 antibody-associated disease. Our cohort was part of this study (109). The results of this study were similar to those of my work. It showed that, in MOG-IgG disease, the first presentation of symptoms was likely to be in the form of ON, either unilateral or bilateral. The disease showed less female predominance compared with AQP4-IgG disease; 57% of patients in the cohort were female, and 44-56% had a relapsing course. The relapses were found to be likely to occur within the first six months or during the first year, hence there was a general consensus that steroid therapy should be continued for that period. Persistent seropositivity was also a predictor of future relapses. Some 59% of patients had disabilities and 25% were rated as moderate to severe sufferers of the disease. One example of a patient who experienced this non-benign course was one of our patients; he had about

six relapses over 12 years and was left with significant cognitive changes along with poor vision and motor weakness (110).

The UK MOG study formed a basis for subsequent potential national practical guidelines regarding the management of MOG-IgG disease (111).

MOG-IgG1 antibody testing is not readily available in all neurology centres internationally. However, MOG-IgG antibody disease (MOGAD) is now a hot topic for debate regarding its classification; the debate centres on whether it represents a unique subgroup of CNS demyelination or is part of a widening spectrum of NMOSD.

An increasing number of neuroscientists is now studying MOGAD and referring to it as a possible different disease. A recent paper by Reindl and Waters discussed different phenotypes of MOG-IgG-associated neurological disorders. It summarised the ways in which the currently available literature pointed to different presentations according to age group, with ADEM-like presentation mainly in children and opticospinal presentation in adults (112).

To regulate what can be referred to as MOG-related neurological disease, international collaboration among neurological experts in this field has proposed recommendations for testing and diagnosis for what they called MOG encephalomyelitis (MOGEM) (Table 9) (113). Another group has proposed the term MOG-IgG-associated optic neuritis, encephalitis, and myelitis (MONEM) (114). All these potential terms imply that MOGAD exhibits a wider spectrum than NMOSD and is likely to be a distinct disorder.

Histopathological findings could offer another strong indicator that it might be a distinct disease. Unfortunately, there are very limited data on pathological lesions in the CNS that are caused by MOG-IgG1 antibody.. Di Puli and Berger have summarised all the literature (nine cases to date), and the results of these were variable. Most cases showed lesions akin to Type II MS brain lesions and others showed nonspecific inflammatory infiltrates. However, none of these cases showed the changes that are usually seen in AQP4-IgG-positive lesions, such as astrocyte loss, necrosis, complement activation, focal perivascular or confluent extensive demyelination, and eosinophilic and neutrophilic cell infiltration (115).

However, there is evidence that some treatments display different efficacies in NMOSD AQP4-IgG antibody-positive cases compared with MOGEM/MOGAD cases. In an international retrospective study that involved multiple centres across the globe, Whittam et al. found that treatment with rituximab reduced relapse in 37% of patients who had MOGAD, (116) while in AQP4-IgG antibody-positive patients the corresponding figure varied from 55% (117) to 100% (118) of patients, although the latter study observed efficacy for only 72 weeks.

All these findings have made clearer the answer to part of my research question; MOG-IgG1 antibody-positive patients have a disease that is different from AQP4-IgG antibody-positive patients. But what about those patients who have NMOSD and are classified as double seronegative? Also can NMOSD be an umbrella term for heterogeneous diseases that share a clinical phenotype but have different immunotypes? Or should these disorders be classified according to similar pathology in homogeneous groups?

I believe the answers to these questions depend on what we look for. For practical reasons, neurologists will continue to deal with these groups of disease that behave similarly (NMOSD AQP4-IgG antibody positive, NMOSD MOG-IgG1 antibody positive and NMOSD seronegative) with a unified treatment approach. Long follow up and outcome measures will determine whether this is the correct approach.

Table 9. Recommended indications that the presence of MOG-IgG1 antibody should be tested in patients who present with acute CNS demyelination of putative autoimmune aetiology (96)

1. Monophasic or relapsing acute ON, myelitis, brainstem encephalitis, encephalitis, or any combination thereof,
and
2. radiological or, only in patients with a history of ON, electrophysiological (VEP) findings compatible with CNS demyelination,
and
3. at least one of the following findings:
MRI
a. Longitudinally extensive spinal cord lesion (≥3 VS, contiguous) (so-called LETM)
b. Longitudinally extensive spinal cord atrophy (≥3 VS, contiguous) in patients with a history compatible with acute myelitis
c. Conus medullaris lesions, especially if present at onset
d. Longitudinally extensive optic nerve lesion (e.g., >1/2 of the length of the pre-chiasmal optic nerve, T2 or T1/Gd)
e. Perioptic Gd enhancement during acute ON
f. Normal supratentorial MRI in patients with acute ON, myelitis and/or brainstem encephalitis
g. Brain MRI abnormal but no lesion adjacent to a lateral ventricle that is ovoid/round or associated with an inferior temporal lobe lesion and no Dawson's finger-type of juxtacortical U-fibre lesion (Matthews Jurynczyk criteria)
h. Large, confluent T2 brain lesions suggestive of ADEM
Fundoscopy
i. Prominent papilloedema/papillitis/optic disc swelling during acute ON
CSF
j. Neutrophilic CSF pleocytosis or CSF WCC > 50/µl
k. No CSF-restricted OCB as detected by iso-electric focusing at first or in any follow-up examination (applies to continental European patients only)
Histopathology
l. Primary demyelination with intralesional complement and IgG deposits
m. Previous diagnosis of "pattern II MS"
Clinical findings
n. Simultaneous bilateral acute ON
o. Unusually high ON frequency or disease mainly characterised by recurrent ON
p. Particularly severe visual deficit/blindness in one or both eyes during or after acute ON
q. Particularly severe or frequent episodes of acute myelitis or brainstem encephalitis
r. Permanent sphincter and/or erectile disorder after myelitis
s. Patients diagnosed with "ADEM", "recurrent ADEM", "multiphasic ADEM" or "ADEM-ON"

t. Acute respiratory insufficiency, disturbance of consciousness, behavioural changes, or epileptic seizures (radiological signs of demyelination required)
u. Disease started within 4 days to ~ 4 weeks after vaccination
v. Otherwise unexplained intractable nausea and vomiting or intractable hiccups (compatible with area postrema syndrome)
w. Co-existing teratoma or anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis (low evidence)
Treatment response
x. Frequent flare-ups after intravenous methylprednisolone (IVMP) therapy, or steroid-dependent symptoms (including chronic relapsing inflammatory optic neuropathy (CRION))
y. Clear increase in relapse rate following treatment with interferon-beta or natalizumab in patients diagnosed with MS (low evidence)

7.3 Seizures and encephalopathy in MOG compared with AQP4

The third objective of my study was to try to describe the unique features of AQP4-IgG-negative NMOSD, compared with seronegative NMOSD. I reported the results in Chapter 5.

One of the major findings was that 42% of this group of patients were positive for MOG-IgG1. There have been reports regarding ways in which MOG-IgG1 antibody-positive patients are different from AQP4-IgG-positive patients. These were largely focused on disease demographics, severity and progression. However, most of these findings were based on short follow-up studies, and in later, larger studies that involved longer follow-ups, including studies of our cohort, these findings were altered slightly (109).

In Chapter 5, I described findings from clinical observation of unique presentations of MOG-IgG1-positive patients with encephalitis and seizures. These symptoms caught my attention because I did not see this presentation in AQP4-IgG-positive patients.

I described five patients with MOG-IgG1-associated inflammatory CNS disease who suffered seizures. All had brain cortex involvement, and four of the five had an encephalopathy. Viral or autoimmune encephalitis was the initial diagnosis in these four and they met the 2015 diagnostic criteria for NMOSD without AQP4-IgG (75).

The main clinical spectrum for MOG-IgG1 antibody-positive disorders is ON, TM, ADEM, clinically isolated syndrome (CIS), paediatric multiple sclerosis and NMO (77, 96). ADEM-like disorders that include encephalopathy and are associated with MOG-IgG1 antibody have previously been reported (77, 92). A recent report identified four cases of MOG-IgG1

antibody-associated encephalitis in a cohort of 24 patients with steroid-responsive encephalitis (93). These four patients were all men with unilateral and benign lesions with full resolution. In contrast, two of our five patients were women. The encephalitis can be severe with lasting damage (case 2, Chapter 5). Unprovoked seizure recurrence (epilepsy) occurred in two of our patients, which possibly indicated underlying gliosis. Follow-up imaging showed gliosis atrophy or persistent T2 lesions in three patients.

There have been only four studies that have reported seizures among NMOSD AQP4-IgG-positive patients. One was a Japanese study that described 3/31 (9.6%) NMO AQP4-IgG-positive patients who had seizures. Only one had evident seizures that were associated with an inflammatory event and it is unclear whether antibody testing was conducted by ELISA or CBA (97). The other three studies were of children. McKeon et al. (98) studied 88 AQP4-IgG positive, mainly non-Caucasian children with NMO and reported that two of them (2%) had focal seizures. Lotze et al. (99) reported the cases of two Latin American children who had NMO and NMO antibodies and who presented with seizures. A third study also reported seizures but was unclear about the serostatus of the patients. In our cohort, it was striking that none of the 100 AQP4-IgG-positive patients had experienced seizures as part of an inflammatory event. The difference between our AQP4-IgG antibody-positive and MOG-IgG antibody-positive patients in terms of seizure occurrence was statistically significant (1 of 100 AQP4-IgG antibody-positive patients vs. 5 of 34 MOG-IgG antibody-positive patients; 2-sided $p<0.008$, Fisher exact test).

The encephalopathic disorders and seizures that occurred in these patients were probably triggered by an episode of demyelination caused by MOG-IgG1 antibody. There was no evidence that infective encephalitis or other associated autoimmune antibodies (e.g., NMDAR or voltage-gated potassium channel antibodies) were responsible for the seizures. A study of N-methyl-d-aspartate receptor antibodies in 215 patients with a range of inflammatory demyelinating diseases, including 22 MOG-IgG1 antibody-positive patients with cognitive problems, seizures, or both, concluded that double seropositivity was rare (92). Jarius et al. (28) described a MOG-IgG1 antibody-positive patient who experienced seizures that were complicated by cerebral venous thrombosis and localised brain oedema. These seizures followed intravenous treatment with a high dose of methylprednisolone (28). In our cohort, there was no evidence of treatment-induced seizures.

There are several limitations to this study. This was a single-centre, cross-sectional study with retrospective collection of data. The clinic is highly specialised and serves as a national referral centre. It is possible that we have encountered only severe forms of MOG-IgG1 antibody disease and that, in a larger cohort that includes milder cases, seizures may be more rare. There could also have been recall bias regarding details of seizures, although we relied on hospital notes whenever possible. Studies that are larger than ours on MOG-IgG1 antibody disease that specifically request that participants have undergone seizures as a clinical feature will be needed to validate this observation further.

Following this work, there have been increases in the number of reports of seizures and encephalitis among MOG-IgG1 antibody-positive patients (119-121). Some case reports had associations with NMDAR antibodies (122, 123).

A recent large Chinese study retrospectively investigated a database for all NMOSD and MOGEM cases and found seizure present in 21% of MOGEM patients (13/61) and in 0.4% of NMOSD patients (2/565); 11 of the MOGEM patients had suffered seizures at the same time as the onset of symptoms, and only 15% of the 13 went on to develop epilepsy (124).

In all these studies and in my cohort, most patients exhibited cortical or subcortical brain changes. This was in keeping with findings in the comparative study of brain lesion in AQP4, MOG and MS (125).

In the cohort that was studied for this project, patients with MOG-IgG1 antibody-associated disease were more likely to present with an encephalopathic disorder and seizures compared with the AQP4-IgG positive cases. The spectrum of MOG-IgG1 antibody-associated disease continues to expand and includes those conditions that involve atypical cerebral inflammatory lesions, which may have been previously characterised as relapsing steroid-responsive autoimmune encephalitis, acute disseminated encephalomyelitis, atypical MS or CNS vasculitis. Myelin oligodendrocyte glycoprotein IgG-associated demyelinating disease is not always benign; it can have a relapsing course and cause significant residual damage. My study supports the view that central nervous system inflammation that is associated with AQP4-IgG antibodies is a different disease to CNS inflammation that is associated with MOG-IgG1 antibodies, although they have some overlapping phenotypes, particularly opticospinal inflammation. I recommend that testing

for MOG-IgG1 antibody should be considered in patients with atypical inflammatory brain lesions, particularly those with an encephalitis-like presentation.

The lack of a gold standard for diagnosis of MOG-IgG1 antibody-positive disease means that it is difficult to characterise the sensitivity of assays, and hence we do not know how many cases are overlooked.

A reconsideration of the literature indicates that there are some other unique clinical presentations/associations that have been described with MOG-IgG1 antibody positive cases that are uncommonly observed in AQP4-IgG antibody-positive cases. An example is specific cranial nerve involvement; trigeminal, vestibulocochlear and oculomotor nerves have been described that show nerve lesions at the root level (126). Aseptic meningitis has also been reported as an initial manifestation of MOG-IgG1 antibody-positive NMOSD (127) .These findings support the idea that there is a wider spectrum for MOGEM/MOGAD than is commonly thought.

Work by Orlandi et al. posed the question regarding the inclusivity of this new recommendation for diagnosis of MOGEM/MOGAD. In a retrospective study, the researchers estimated that the sensitivity of the proposed MOGEM criteria was 28.3% and specificity was 86.7%. If instead we keep it simple and test for all CNS demyelinating events in patients who do not have other evidence to suggest MS diagnosis, fewer MOGEM/MOGAD cases will be missed (128).

7.4 Conclusion

There is a lot to explore in the field of non-MS CNS demyelination. The on-going research, including this work, has changed our understanding of NMOSD significantly over the last few years, yet it raises more questions. The 2015 IPND criteria represent a significant change in approach to the diagnosis and classification of demyelinating syndromes that are not typical MS. It has widened the spectrum of NMOSD and increased the diagnosis of this disorder by 76%, mainly because it has been agreed that AQP4-IgG antibody is pathognomonic for NMOSD. However, is the 14% increase among the AQP4-IgG antibody-negative group a real increase or does this group have different disease? The study of AQP4-IgG-negative NMOSD reveals that a significant group of patients who present with this clinical picture has a different antibody (MOG-IgG1) and their symptoms show unique clinical features such as seizures. MOG-IgG1 antibody disease is now considered a different disease entity, with its own wide spectrum, yet many patients have the NMOSD phenotype. This may have implications in future therapeutic clinical trials. I expect that these findings, among others in the field, may lead to a consideration to revise the IPND criteria, and debate regarding whether to include or exclude MOG-IgG1 disease and the other seronegative cases.

7.5 Next Steps:

This work may have helped to reveal some new understanding of NMOSD and MOGEM/MOGAD. There have been significant leaps in this field since completion of this project, yet there are many questions that remain to be answered.

For instance, we can question now whether Devic's disease is actually AQP4-IgG antibody-positive NMOSD or MOG-IgG1 antibody-positive, or double seronegative. Similarly, is Asian opticospinal MS likely to be MOGAD/MOGEM or AQP4-IgG antibody-positive NMOSD? Is there variability in prevalence and clinical presentations of MOGEM/MOGAD and AQP4-IgG antibody-positive NMOSD among different ethnicities? There may be genetic or specific HLA-typing associations. Are there patients in MS services who are undiagnosed MOGEM/MOGAD sufferers? We have some evidence that therapies that are prescribed to modify MS disease can worsen the condition of patients with NMOSD who are AQP4-IgG antibody-positive, but we do not know the effects of these drugs on MOGEM/MOGAD or

their safety in these cases. Is it sensible to look back at all those patients with MS diagnoses who have mild or atypical changes in the MRI brain and test them for MOG-IgG1 antibody?

The period 2019-2020 has been the time for therapeutic breakthroughs for NMOSD. Three randomised controlled trials have provided evidence of treatment efficacy in NMOSD. However, in spite of the strict inclusion criteria for AQP4-IgG antibody-negative cases, one would wonder what the results might be in a more homogeneous group with a known, similar pathology.

Although I tried to look into AQP4-IgG antibody-negative NMOSD, my main results were centred on MOGEM/MOGAD. Hence the remainder of the NMOSD seronegative group of diseases, along with the seronegative non-MS CNS demyelination conditions, remain as uncharted areas with limited information available. My successor (the current NMO fellow at Liverpool) is currently working on this latter group. Just as the discovery of the AQP4-IgG antibody revolutionised our understanding of NMOSD more than a decade ago, there may be another antibody or breakthrough discovery in the pipeline that will change everything! However, to have more powerful and research and meaningful findings, the best way forward is international collaboration, in this case of rare neurological disorders.

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Appendix1: Expanded Disability Status Scale (EDSS)

Score	Description
1.0	No disability, minimal signs in one FS
1.5	No disability, minimal signs in more than one FS
2.0	Minimal disability in one FS
2.5	Mild disability in one FS or minimal disability in two FS
3.0	Moderate disability in one FS, or mild disability in three or four FS. No impairment to walking
3.5	Moderate disability in one FS and more than minimal disability in several others. No impairment to walking
4.0	Significant disability but self-sufficient and up and about some 12 hours a day. Able to walk without aid or rest for 500m
4.5	Significant disability but up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance. Able to walk without aid or rest for 300m
5.0	Disability severe enough to impair full daily activities and ability to work a full day without special provisions. Able to walk without aid or rest for 200m
5.5	Disability severe enough to preclude full daily activities. Able to walk without aid or rest for 100m
6.0	Requires a walking aid - cane, crutch, etc. - to walk about 100m with or without resting
6.5	Requires two walking aids - pair of canes, crutches, etc. - to walk about 20m without resting

Score	Description
7.0	Unable to walk beyond approximately 5m even with aid. Essentially restricted to wheelchair; though wheels self in standard wheelchair and transfers alone. Up and about in wheelchair some 12 hours a day
7.5	Unable to take more than a few steps. Restricted to wheelchair and may need aid in transferring. Can wheel self but cannot carry on in standard wheelchair for a full day and may require a motorised wheelchair
8.0	Essentially restricted to bed or chair or pushed in a wheelchair. May be out of bed itself much of the day. Retains many self-care functions. Generally has effective use of arms
8.5	Essentially restricted to bed much of day. Has some effective use of arms retains some self-care functions
9.0	Confined to bed. Can still communicate and eat
9.5	Confined to bed and totally dependent. Unable to communicate effectively or eat/swallow
10.0	Death due to MS

Appendix 2: Publications originating from this work