Diagnostic algorithm for relapsing acquired demyelinating syndromes in children

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Running head: relapsing demyelinating syndromes in children

Word Count: 2998 Abstract: 244

Title: 71 characters

References: 40 Figure: 2 Table: 3

Abbreviations: Ab antibody **ADEM** Acute disseminated encephalomyelitis **AP** area postrema **AQP4** aquaporin-4 **CIS** clinically isolated syndrome **CNS** central nervous system **CP** cerebellar peduncle **CSF** cerebrospinal fluid **DIS** disseminating in space **DIT** dissemination in time **EBV** Epstein-Barr virus **EDSS** expanded disability status scale **MDEM** multiphasic disseminated encephalomyelitis **MOG** myelin oligodendrocyte glycoprotein **MS** multiple sclerosis **NMOSD** neuromyelitis optica spectrum disorders **OCB** oligoclonal bands **ON** optic neuritis **RDS** relapsing inflammatory demyelinating syndrome **RON** relapsing optic neuritis **TM** transverse myelitis **TTFR** time to first relapse

Declaration of interests

YH, KM, WKC have no disclosures. FB serves as a scientific consultant to Bayer Schering Pharma, Sanofi-Aventis, Biogen Idec, Teva Pharmaceutical Industries, Merck Serono, Novartis, Roche, Synthon BV, Janssen, Genzyme, and Toshiba Medical systems, and has served on speakers' bureaus for Serono Symposia Foundation and MedScape. AV serves/has served on scientific advisory boards for the Patrick Berthoud Trust, the Brain Research Trust and the Myasthenia Gravis Foundation of America; has received funding for travel and a speaker honorarium from Baxter International Inc and Biogen Inc; serves as an Associate Editor for Brain; receives royalties from the publication of Clinical Neuroimmunology (Blackwell Publishing, 2005) and Inflammatory and Autoimmune Disorders of the Nervous System in Children (Mac Keith Press, 2010); receives/has received research support from the European Union, NIHR Biomedical Research Centre Oxford, Euroimmun AG and the Sir Halley Stewart Trust; and has received Musk antibody royalties and consulting fees from Athena Diagnostics Inc. EW receives research grants from Action Medical Research and MS Society; has received travel grants from UCB, Shire and Biogen Idec, educational grants to organize meetings from Merck Sereno, Novartis, Bayer and Biogen Idec, speaker's fees from Merck Sereno and consultancy fees from Genzyme. ML receives research grants from Action Medical Research, DES society, GOSH charity, NIHR, MS Society, SPARKS charity and; receives research support grants from the London Clinical Research Network and Evelina Appeal; has received consultation fees from CSL Behring; received travel grants from Merck Serono; and awarded educational grants to organize meetings by Novartis, Biogen Idec, Merck Serono and Bayer OC serves as a consultant for GE, Biogen Idec, and Novartis, and all the payments are made to the institution (Queen Square MS Centre, UCL Institute of Neurology, London, UK). CH has received educational and travel grants from Merck Serono and Bayer and Biogen.

Abstract

Objectives: To establish whether children with relapsing acquired demyelinating syndromes (RDS) and myelin oligodendrocyte glycoprotein antibodies (MOG-Ab) show distinctive clinical and radiological features and generate a diagnostic algorithm for the main RDS for clinical use.

Methods: A panel reviewed the clinical characteristics, MOG-Ab and aquaporin-4 (AQP4) Ab, intrathecal oligoclonal bands and Epstein-Barr virus serology results of 110 children with RDS. A neuroradiologist, blinded to the diagnosis, scored the MRI scans. Clinical, radiological, and serological tests results were compared.

Results: 56.4% of children were diagnosed with multiple sclerosis (MS), 25.4% with neuromyelitis optica spectrum disorder (NMOSD), 12.7% with multiphasic disseminated encephalomyelitis (MDEM), and 5.5% with relapsing optic neuritis (RON). Blinded analysis defined baseline MRI as typical of MS in 93.5% of MS children. ADEM presentation was only seen in the non-MS group. 30.7% of NMOSD cases were AQP4-Ab positive. MOG-Ab were found in 83.3% of AQP4-Ab negative NMOSD, 100% MDEM, and 33.3% with RON. Children with MOG-Ab were younger, less likely to present with area postrema syndrome, had lower disability, longer time to relapse, and more cerebellar peduncle lesions than AQP4-Ab NMOSD. A diagnostic algorithm, applicable to any episode of CNS demyelination, leads to four main phenotypes: MS, APQ4-Ab NMOSD, MOG-Ab-associated disease, and antibody-negative RDS.

Conclusion: Children with MS and AQP4-Ab NMOSD showed features typical of adult cases. Since MOG-Ab positive children showed notable and distinctive clinical and MRI features, they were grouped into a unified phenotype (MOG-Ab-associated disease), included in a new diagnostic algorithm.

Introduction

Paediatric relapsing acquired demyelinating syndromes (RDS)[1] of the central nervous system (CNS) define a group of diseases with different phenotypes.

The most common pediatric RDS is multiple sclerosis (MS). The 2010 McDonald criteria enable a diagnosis of MS in children over the age of 11 years, presenting with a clinically isolated syndrome (CIS) and MRI evidence of dissemination in space (DIS) and time (DIT)[2], providing that the clinical presentation does not resemble ADEM[3, 4]. Although a proportion of children present with MS before the age of 12 years, the 2010 McDonald criteria[2] show a low positive predictive value[5] in this patient group.

Another RDS is NMOSD, which is stratified according to the presence/absence of AQP4-Ab[6]. About 30% of AQP4 seronegative NMOSD adult patients have myelin oligodendrocyte glycoprotein antibodies (MOG-Ab)[7]. Comparative studies between MOG-Ab and AQP4-Ab positive NMOSD in adults[8-10] and children[11] have shown that MOG-Ab positive patients are younger, more frequently male, and have a better outcome and more often a monophasic course.

In addition to AQP4-Ab seronegative NMOSD[12], MOG-Ab have been detected in other RDS, such as multiphasic disseminated encephalomyelitis (MDEM)[13], recurrent optic neuritis (RON)[14], and acute disseminated encephalomyelitis, followed by recurrent or monophasic optic neuritis (ADEM-ON)[15].

We evaluated retrospectively a large cohort of children with RDS who underwent clinical assessments, MRI, oligoclonal bands (OCBs) testing in the cerebrospinal fluid, AQP4-Ab, MOG-Ab, and Epstein-Barr virus (EBV) antibody testing in the serum, as part of routine clinical protocols. We aimed to identify the key features of RDS that unify phenotypes, and additionally focused on patients with MOG-Ab, to investigate whether they show distinct clinical and radiological features, independently of their original diagnosis. Our ultimate goal was to develop a diagnostic algorithm that provides advice on how to reach the diagnosis of the newly defined phenotypes, by suggesting sequential diagnostic tests and supporting features.

Methods

Participants

A total of 110 children with RDS were retrospectively studied. Consecutive children attending follow-up visits between September 2014-September 2015 were identified from three UK & Ireland Childhood CNS Inflammatory Demyelination Working Group (UK-CID) centers: Great Ormond Street Hospital, Evelina London Children Hospital, and Birmingham Children Hospital. The diagnosis of RDS was defined as two or more episodes of acquired CNS demyelination lasting > 24 hours involving the optic nerve, brain or spinal cord, associated with T2 lesions on MRI. Patients with monophasic ADEM and CIS (even if meeting McDonald criteria after first event) were not included.

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by Great Ormond Street Hospital Research and Development Department (reference: 16NC10).

Procedure

All clinical records were reviewed by one YH, who summarized patients' demographics, clinical presentations, demyelinating phenotypes at visits, timing and features at relapses, and expanded disability status scale (EDSS) at 2 years from onset. Onset demyelinating phenotype was determined based on the clinical features and neurological examination according to established criteria[3] (without neuroimaging reference) as being optic neuritis (ON), transverse myelitis (TM), neurological deficits associated with encephalopathy (ADEM) or without encephalopathy (a brainstem, cerebellar and hemispheric CIS)

All patients had undergone brain and spinal cord imaging according to local MRI protocols (not routinely including orbits). Gadolinium enhanced imaging was performed in all cases, but not always at the first scan.

Within 1 month of an acute event (either onset or relapse), clinically symptomatic children underwent testing for serum AQP4-Ab and MOG-Ab (not CSF), as part of routine assessments of children with demyelinating diseases, performed at the Clinical Neuroimmunology service at the Oxford Radcliffe Hospital Trust, using live cell-based assays[16, 17](This laboratory receives samples for antibody testing from all over the world including the US where MOG-Ab testing is not available). Qualitative analyses of serum and CSF oligoclonal patterns were performed by isoelectric focusing on agarose gels followed by immunoblotting[18], and serum IgG-Ab directed against Epstein-Barr virus capsid antigens, nuclear antigens (EBNA1), and early antigens were measured using standard ELISA kits, locally.

Clinical review panel

At least two clinicians who were not involved with the direct clinical care of the children and two pediatric neuroradiologists reviewed the case summaries and all neuroimaging, assigned the cases to one of the following diagnostic categories:

1. MS, fulfilling the 2013 International Pediatric Multiple Sclerosis Study Group (IPMSSG) consensus criteria[3] and the 2010 McDonald criteria[2]
2. NMOSD, fulfilling the 2015 Wingerchuk criteria[6]
3. MDEM and ADEM-ON, fulfilling the 2013 IPMSSG consensus criteria[3]
4. Recurrent demyelination in a single CNS area without evidence of clinically-silent disease (e.g., RON)

Blinded radiological analysis

A third neuroradiologist (FB) performed a separate analysis to assess whether imaging characteristics alone can support the diagnosis of a specific syndrome, being blind to the clinical features and the antibody results. The following analysis was repeated separately for baseline MRI and follow-up scans. Lesion morphology, distribution, and location were used to support a diagnosis of MS. MS plaques are ovid and perpendicularly oriented to the ventricular surface, they occur bilaterally, but are typically asymmetrical, and are distributed in both the supra- and infratentorial compartments. Although MS lesions can be located anywhere in the CNS, they frequently seen in the juxtacortical, periventricular, and infratentorial regions[19]. MRI scans were grouped into the following categories; (1) not MS, (2) not typical of MS, (3) some MS features, (4) typical of MS.

For categories 1 and 2, one of the following five main imaging patterns, which included features known to be associated with NMOSD[6, 20] and features recently reported in patients with MOG-Ab[13, 21] was chosen by the neuroradiologist as the predominant pattern: (i) disease localized to brainstem and hypothalamus; (ii) predominantly confluent, hazy/poorly marginated lesions involving both grey matter and white matter; (iii) extensive confluent 'leukodystrophy-like' MRI pattern; (iv) sharply demarcated hemispheric white matter lesions (>3cm); (v) TM and/or ON with normal intracranial appearance or non-specific white matter lesions. Additionally, for the follow-up scans, the two following imaging features were looked for: (i) almost or complete resolution of lesions, (ii) destructive lesions, defined as severe rarefaction of tissue leading to central low signal on FLAIR with associated volumes loss.
Finally, the presence of lesions in the diencephalon, dorsal brainstem, periependymal area surrounding the lateral ventricles, longitudinally extensive TM (LETM), cortical grey matter, thalamus, basal ganglia, juxtacortical and deep white mater involvement more than periventricular, cerebellar peduncles, pons and optic nerves/tracts, considered to be typical of patients with NMOSD with AQP4-Ab (according to Wingerchuk criteria)[6, 20] and MOG-Ab associated demyelination, were recorded.

Statistical analysis

To compare the demographic, clinical, radiological and serological characteristics between the phenotypes, parametric or non-parametric statistical tests (Mann–Whitney U and Kruskal Wallis tests) were used for continuous distributions, as appropriate given normality, and $\chi 2$ or Fisher's exact tests for nominal data. Results associated with a p-value <0.05 were considered significant. Data were analyzed using GraphPad Prism 5.

Results

110 consecutive children with RDS were studied. The median length of follow-up (from first clinical presentation) was 4 years (IQR3-7). During this period, a median of 4 repeated MRI scans were performed (range 3-10). All patients had brain MRI at onset and 95/110 (86.3%) had spinal cord MRI too.

The panel diagnosed 62/110 (56.4%) children with MS, 28/110 (25.4%) with NMOSD, 14/110 (12.7%) with MDEM, and 6/110 (5.5%) with RON (not fulfilling criteria for chronic relapsing inflammatory optic neuropathy[22]).

Patients' demographic, clinical and paraclinical features and clinical disability at 2 year follow-up according to each RDS phenotype, are summarized in **Table 1**.

Patients with MS

At onset, 75.8% of MS patients presented with a brainstem, cerebellar and hemispheric CIS and 98.4% with abnormal MRI, with T2 hyperintense lesions. The majority of MS patients (94.6%) showed OCBs in the CSF and all patients showed EBV IgG. (**Table 1)**. No differences in the clinical, radiological and immunological features between MS children younger (32.3%) and older than 11 years (67.7%) at onset were detected (Supplemental **Table 1**).

Comparison between children with MS and non-MS RDS

48/ 110 (43.6%) children did not have MS; the majority of these patients had NMOSD (28/48, 58.3%), followed by MDEM (14/48, 29.2%), and RON (6/48, 12.5%)(**Table 1, Supplemental Figure 1**). MS patients were older and more likely to present with a brainstem, cerebellar and hemispheric CIS than non-MS RDS; ADEM presentation was only seen in the non-MS group (all p values <0.0001). Brain MRI abnormalities at presentation were more frequently seen in the MS than non-MS RDS (p<0.0001). MOG- and AQP4-Abs were found exclusively in the non-MS group (p<0.0001). All MS patients tested had evidence of remote EBV infection compared to 42.9% of non-MS patients and more frequently showed intrathecal synthesis of OCBs (all p values <0.0001) (**Table 1)**.

The blinded analysis of baseline MRI scans, done in all 110 patients, correctly identified 58/62 (93.6%; 52 category 4; 6 category 3) children with MS and all 48 children with non-MS (100%; 38 category 1; 10 category 2). Follow-up MRI scans analysis only identified an additional 2 MS cases (The MRI scans of

the 4 MS patients with atypical imaging are shown in ***Supplemental Figure 2***). All 45 patients with spinal cord lesions had short segment myelitis by contrast to 12/13 patients in the non-MS group who had LETM (p<0.0001).

Autoantibodies in children with RDS

Thirty-four out of 41 (82.9%) patients with non-MS RDS (who were tested) were positive to either AQP4-Ab or MOG-Ab. In particular, 30.7% (8/26) of NMOSD cases tested were AQP4-Ab-positive. 83.3% (15/18) of AQP4-Ab negative NMOSD cases were MOG-Ab-positive. No patients had antibodies to both antigens. MOG-Ab were found in 100% (9/9) of MDEM tested cases and 33.3% (2/6) of RON tested cases. Seven patients were negative to both antibodies: 4 relapsed with RON and 3 with NMOSD.

Clinical characteristics of patients with AQP4-Ab NMOSD and all patients with MOG-Ab associated disease are detailed in **Table 2**.

Comparison between MOG-Ab-positive and AQP4-Ab-positive children

Children with MOG-Ab grouped together (independently of their original RDS diagnosis) were younger (p=0.048), less likely to present with area postrema syndrome (p=0.0067), more likely to present with ADEM (p=0.034), had lower disability at 2-year follow-up (p=0.03) and a longer time to relapse (p=0.016) than NMOSD with AQP4-Ab (**Table 2** and **Supplemental Figure 3**).

When the MRI patterns and lesion locations between MOG-Ab-and AQP4-Ab-positive patients were compared, patients with AQP4-Ab were more likely to have disease restricted to the brainstem and/or hypothalamus (at onset 38% vs 0%, p=0.0094, and at follow-up 50% vs 0%, p=0.0015). Destructive lesions at follow-up scans were seen in the majority of AQP4-Ab-positive patients (62.5%) and in none of the MOG-positive patients (p=0.002) (**Table 3, Supplemental Figure 4**). Lesions located in dorsal brainstem were more frequently seen in AQP4-Ab-positive patients (87.5% vs 11.5%, p=0.0085), whilst lesions in the cerebellar peduncles were only seen in the MOG-positive patients at onset and follow-up (p=0.03 and p=0.011) (**Table 3**, **Figure 1**). Finally, leukodystrophy-like lesions were only seen in MOG-Ab-positive patients (**Table 3, Figure 1**).

Diagnostic algorithm

We propose a diagnostic algorithm (**Figure 2**), applicable to any episode of CNS demyelination, which leads to four main demyelinating syndromes: MS, APQ4-Ab NMOSD, MOG-Ab associated disease and Ab-negative RDS.

The first recommended diagnostic test is brain and spinal cord MRI. If the clinical features of a CNS attack and MRI findings are considered to be typical/suggestive of MS, then the McDonald diagnostic criteria should be applied.

In children whose MRI is not typical/suggestive of MS, but have clinical and radiological features suggestive of NMOSD, AQP4-Ab testing is recommended, particularly in children having an area postrema syndrome, MRI abnormalities localized to the brainstem and hypothalamus, and destructive lesions. MOG-Ab should be tested in AQP4-Ab negative cases.

In children whose MRI is not typical of MS or NMOSD, but the clinical and radiological presentation resembles ADEM, MOG-Ab testing is recommended. Additionally, MOG-Ab testing is recommended in children who with poorly marginated lesions in the cerebellar peduncle, and in children with a "leukodystrophy-like" MRI pattern.

Consideration of alternative diagnoses (e.g. inflammatory, infectious and neurometabolic) and then monitoring are recommended in the remaining minority of Ab-negative RDS.

Discussion

Based on the observations in this large cohort of 110 children with RDS, a diagnostic algorithm applicable to any episode of CNS demyelination in children was developed to reach the diagnosis of four main phenotypes: MS, AQP4-Ab NMOSD, MOG-Ab associated disease and seronegative RDS children.

Brain and spinal cord MRI is the first diagnostic test to be performed. This helps to reach the diagnosis of MS, which is the most common RDS in our cohort (56.4%), even if the MRI is blindly analysed in isolation. The lack of clinical, radiological and immunological differences between MS children younger and older than 11 years suggests that the 2010 McDonald diagnostic criteria for MS[2] can be applied to children of any age. Current guidance[3] for the diagnosis of MS in children recommends caution for children younger than 12 years, but our study indicates that if clinical and MRI features typical of adult MS are seen in children, there should be confidence about the diagnosis of MS.

None of the children with MS presented with ADEM. Previous studies have shown that 5-29%[23, 24] of children initially diagnosed with ADEM have further demyelinating events that are atypical for ADEM, but leads to a diagnosis of MS. However, MOG-Ab were not tested in these patients. These previous studies have reported that MS in children showed unique MRI features, such as oedema and widespread white-matter involvement, increased frequency of LETM, and lower frequencies of intrathecal oligoclonal band positivity than adults with MS[1, 23], whilst these features were absent in our MS patients, but typical of MOG-Ab associated disease.

AQP4-Ab testing should be performed in patients with clinical and/or radiological features suggestive of NMOSD[6]. We found that two third of non-MS children had NMOSD, of which 30.7% tested were AQP4-Ab-positive, which is lower than previously reported in adult[8, 21, 25] and pediatric cohorts[26]. This is in keeping with previous studies of AQP4-Ab seropositivity exponentially increasing with age, particularly in women[27]. Nevertheless, comparison between different cohorts has to be made with caution if referral pathways are different between centers.

We found that 82.9% of all patients with non-MS RDS were either AQP4-Ab or MOG-Ab positive. We detected MOG-Ab in 83.3% of NMOSD without AQP4-Ab. This was much higher than the previously reported adult cohorts[7], but not surprising since MOG-Ab are known to be common in children[28]. Additionally, increasing recognition of this antibody and the application of the latest diagnostic criteria for NMOSD[6] may also contribute to the observed high number of NMOSD patients[29] and hence MOG-Ab-positive cases. CSF testing for AQP4 and MOG antibodies was not performed as in both these

conditions it has been shown that the antibodies originate from the periphery and serum testing is more sensitive than CSF[30, 31].

As we found that 100% of patients with MDEM are MOG-positive, and previous investigations detected significant association between ADEM and MOG-Ab[13, 32], we feel that performing MOG-Ab testing in all patients presenting with ADEM (both monophasic and relapsing disease) is justified on the bases of expert's opinion consensus. A key question is whether MOG-Ab negative ADEM patients show different clinical and MRI features from MOG-Ab positive ADEM patients. Large ill-defined lesions in the cerebellar peduncle, and a leukodystrophy-like MRI pattern can be a useful clue to this diagnosis.

Despite the low number of children with AQP4-Ab in this cohort we identify differences between children with AQP4-Ab vs children with MOG-Ab grouped together (independently of their original RDS diagnosis). Children with MOG-Ab were younger, less likely to present with area postrema syndrome, more likely to present with ADEM, lower disability at 2-year and a longer time to relapse than AQP4-Ab NMOSD. Therefore, we proposed to group all the MOG-Ab-positive patients into a unified phenotype ("MOG-Ab associated disease"). It is possible that the two antigenic targets (AQP4 and MOG) lead to different diseases, via different pathogenic mechanisms, which cause an autoimmune astrocytopathy in AQP4-Ab-associated disease and autoimmune oligodendrogliopathy in MOG-Ab-associated disease.[33] Recent studies looking at the effects of MOG-Ab in cell cultures and in mouse models showed loss of the microtubule cytoskeleton of oligodendrocytes when incubated with purified IgG from MOG-Ab positive patients,[34] as well as myelin changes and altered expression of axonal proteins when injected directly into mouse brain[35]. By contrast, AQP4-Ab are thought to produce astrocyte damage by complement-dependent cytotoxicity which leads to blood–brain barrier disruption causing leukocyte infiltration, and cytokine release resulting in damage to oligodendrocytes, myelin and neurons which may explain the more destructive lesions seen in this phenotype[36]. Abundance of AQP4 in the area postrema[37] is likely to explain the increased frequency of nausea and vomiting in the AQP4-Ab positive group, together with imaging abnormalities in the dorsal brainstem and hypothalamus, as previously reported.[38]

Overall, the very high percentage (82.9%) of children with non-MS RDS who are positive to one of the two antibodies, suggests that the majority of these children have a known antibody-mediated demyelinating disease. In the rare cases of RDS other than MS, negative to both antibodies, it is important to consider mimics of CNS demyelination.

The blinded MRI analyses successfully distinguished between MS and non-MS cases already at onset. MRI abnormalities in the brainstem and hypothalamus were typical of AQP4-Ab positive patients, as previously reported,[38] whilst ill-defined lesions in the cerebellar peduncle were seen exclusively in the

MOG-Ab positive patients. Future studies will aim to confirm whether this can be used as a marker for MOG-Ab associated disease.

Intrathecal OCBs and the remote EBV infection confirmed previous findings[39, 40], but since we did not calculate the predictive value of these tests and their role independently of MRI, we decided not to include them into the algorithm.

A limitation of our study is its retrospective nature, which led to the inclusion of only relapsing cases. However, this large cohort allowed us to identify notable differences in their clinical, imaging and immunological characteristics that have led us to propose four main distinct phenotypes: MS, NMOSD with AQP4-Ab, MOG-Ab associated disease and seronegative RDS children. The distinction of MOG-Ab-associated disease from the other RDS should be considered in the next revisions of the diagnostic criteria for MS and NMOSD.

Table 1. Demographics, clinical and paraclinical features of children according to their standard RDS diagnosis

		***All non-MS* (n=48)**				
	MS (n=62)	**NMOSD** (n=28)	**MDEM** (n=14)	**RON** (n=6)	***Summary of all non-MS***	***P Value MS vs non-MS***
Age (years) at presentation Median (IQR)	13 (11-14)	8 (5-11)	4.5 (3-5)	10 (9.3-12.5)	7 (5-10)	<0.0001
Sex (M:F)	1:2.1	1:2.5	1:0.75	1:1	1:1.53	0.55
Ethnicity (white: other)	29: 33	13: 15	10: 4	5: 1	28:20	0.25
Demyelinating phenotype at onset						
ADEM	0 (0%)	3 (10.7%)	14 (100%)	0 (0%)	17 (35.4%)	<0.0001
ON	12 (19.4%)	11 (39.3%)	0 (0%)	6 (100%)	17 (35.4%)	0.08
TM	3 (4.8%)	7 (25%)	0 (0%)	0 (0%)	7 (14.6%)	0.51
Brainstem, cerebellar and hemispheric CIS	47 (75.8%)	8 (28.6%)	0 (0%)	0 (0%)	8 (16.7%)	<0.0001
Abnormal brain MRI at onset*	61 (98.4%)	14 (50%)	14 (100%)	0 (0%)	28 (58.3%)	<0.0001
OCB	53/56 (94.6%)	4/25 (16%)	1/10 (10%)	0/6 (0%)	5/41 (12.2%)	<0.0001
EBV IgG	47/47 (100%)	8/12 (66.7%)	2/11 (18%)	2/5 (40%)	12/28 (42.9%)	<0.0001
AQP-Ab	0/56 (0%)	8/26 (30.7%)	0/9 (0%)	0/6 (0%)	8/41 (19.5%)	0.0007
MOG-Ab	0/56 (0%)	15^/26 (57.7%)	9/9 (100%)	2/6 (33.3%)	26/41 (63.4%)	<0.0001
Time to first relapse Median months (IQR)	6 (4-12.5)	6 (3-16)	18.5 (3.8-41.3)	5 (3-27.8)	6.5 (3-21.75)	0.55
EDSS at 2yr Median (IQR)	1 (1-1.6)	1 (0-2)	1.3 (1-3)	1 (0-1)	1 (0-2)	0.6
Follow-up time (years) Median (IQR)	3 (2.5-6)	4 (3-6.75)	9 (4.75-13.25)	3 (2.75-4)	5 (3-8)	0.0143

^All these 15 cases were AQP4-Ab negative, so 83.3% (15/18) of AQP4-Ab negative patients were MOG-Ab positive. * This does not include orbital MRI.

Table 2. Comparison of clinical and paraclinical features in all patients with MOG-Ab-positive and AQP4-Ab-positive children

	AQP4-Ab (n=8)	**MOG-Ab (n=26)**	**P value**
Demographic characteristics			
Age at presentation median (IQR)	10.5 (6.5-12)	6 (4-8)	**0.048**
M: F	1: 7	1: 1.6	0.23
Ethnicity (white: other)	2: 6	17: 9	0.1
Demyelinating phenotype at onset			
ADEM	0 (0%)	11 (42.3%)	**0.034**
ON	1 (12.5%)	11 (42.3%)	0.21
TM	3 (37.5%)	2 (7.7%)	0.072
CIS (other than ON and TM)	4 (50%)	2 (7.7%)	**0.018**
First attack symptoms			
Vision	2 (25%)	12 (46.2%)	0.41
Motor	4 (50%)	7 (26.9%)	0.38
Sensory/parathesia	2 (25%)	3 (11.5%)	0.57
Vomiting/nausea/weight loss (AP syndrome)	4 (50%)	1 (3.8%)	**0.0067**
Cerebellar symptoms	0(0%)	5 (19.2%)	0.31
Cranial neuropathies	0 (0%)	3 (11.5%)	1.0
Seizures	0 (0%)	5 (19.2%)	0.31
Encephalopathy	2 (25%)	11 (42.3%)	0.44
ITU admission	2 (25%)	1 (3.8%)	0.13
Abnormal intracranial MRI at onset	6 (75%)	15 (57.7%)	0.44
OCB	1/8 (12.5%)	2/22 (9.1%)	1.0
EBV IgG	0/1 (0%)	7/17 (41.2%)	1.0
TTFR Median months (IQR)	3 (1.5-4)	9.5 (3.75-24)	**0.016**
Demyelinating phenotype at relapse			
MDEM	0 (0%)	9 (34.6%)	0.077
NMOSD	8 (100%)	15 (57.7%)	**0.034**
RON	0 (0%)	2 (7.7%)	1.0
EDSS at 2-year follow-up Median (IQR)	2 (1.25-3.375)	1 (0-2)	**0.030**

Table 3. Blinded radiological analysis stratified to antibody positivity.

	AQP4-Ab (n=8)	MOG-Ab (n=26)	P value (AQP4 vs MOG)	Both antibodies negative (n=7)	Not tested (n=7)
MRI predominant pattern at onset					
Disease localized to brainstem and hypothalamus	3 (37.5%)	0 (0%)	**0.0094**	1	0
Predominantly confluent, hazy/poorly marginated lesions involving both grey and white matter	2 (25%)	12 (46.2%)	0.422	0	5
Extensive confluent 'leukodystrophy-like' pattern	0	2 (7.7%)	1.0	0	0
LETM and/or ON with normal intracranial appearance or non-specific white matter lesions	3 (37.5%, AP involved in 2)	12 (46.2%)	1.0	6	2
Sharply, demarcated, hemispheric white matter lesions (>3cm)	0	0	1.0	0	0
Lesion location at onset					
Diencephalon	3	4	0.315	1	1
Dorsal brainstem	5	3	**0.0085**	1	1
Periependymal area	0	0	1.0	1	0
LETM	5	4*	0.165	3	1
Cortical grey matter	0	4	0.55	0	2
Thalamus	0	8	0.152	1	2
Basal ganglia	0	2	1.0	1	0
Juxtacortical and deep white mater involvement more than periventricular	0	4	0.55	1	3
Cerebellar peduncles	0	8	**0.030**	0	0
Pons	0	2	1.0	0	0
Optic nerve/tracts	1	9	0.39	3	0
MRI predominant pattern at follow-up					
Disease localized to brainstem and hypothalamus	4	0	**0.0015**	0	1
Predominantly confluent, hazy/poorly marginated lesions involving both grey and white matter	4	11	1.0	1	5
Extensive confluent 'leukodystrophy-like' pattern	0	6	0.30	0	1
LETM and/or ON with normal intracranial appearance or non-specific white matter lesions	0	9	0.077	4	0
Sharply demarcated hemispheric white matter lesions (>3cm)	0	0	1.0	2	0
Significant resolution	2	10	0.68	1	4
Destructive lesions	5	**0**	**0.002**	2	0
Lesion location at follow-up					
Diencephalon	4	2	**0.018**	1	1
Dorsal brainstem	7	3	**0.0002**	1	1
Periependymal area	2	0	0.05	1	0
LETM	7	**6***	**0.033**	3	2
Cortical grey	0	7	0.16	2	4

Thalamus	0	10	0.072	0	2
Basal ganglia	1	2	1.0	1	1
Juxtacortical and deep white mater involvement more than periventricular	0	5	0.31	0	4
Cerebellar peduncles	0	**14**	**0.011**	0	3
Pons	0	3	1.0	0	0
Optic nerve/tracts	1	9	0.38	1	0

*** One child had central cord short transverse myelitis.**

Contributors

YH, OC and CH contributed to the conception and design of the study with suggestions from FB, ML and AV. KM, WKC and FB performed the radiological analysis. YH, EW, ML and CH performed the clinical analysis. YH and OC drafted the manuscript. All authors contributed to editing the final manuscript.

Funding: This research is supported by the NIHR University College London Hospitals Biomedical Research Centre (OC) and the NIHR Great Ormond Street Hospital Biomedical Research Centre (YH, CH)

Acknowledgments

We are very grateful to Prof Brenda Banwell for her thoughtful comments on the manuscript. In addition we would like to thank all the numerous primary physicians and allied health professionals caring for these children during their complex course of disease.

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Legends:

Figure 1: MRI patterns observed in MOG-Ab-positive patients: (A) Deep grey matter disease with linear pattern of external/extreme capsule disease; (B-C) Significant cortical grey matter involvement; (D) poorly marginated lesions with involvement of the dorsal brainstem and spinal cord. (E-F) Cerebellar peduncles & pons: confluent, poorly marginated, mostly reversible lesions; (G-I) Extensive confluent 'leukodystrophy-like' lesions.

Figure 2: Diagnostic algorithm that can be applied to any episode of CNS demyelination in children. The first recommended diagnostic test is brain and spinal cord MRI. If MRI findings are considered to be typical or suggestive of adult MS, then the McDonald diagnostic criteria should be applied. In children whose MRI is not typical or suggestive of MS, but have clinical and radiological features suggestive of NMOSD, AQP4-Ab testing is recommended. In particular, this test is advised in children presenting with an area postrema syndrome, MRI abnormalities localised to the brainstem and hypothalamus, and destructive lesions. If AQP4-Ab are negative, then MOG-Ab should be tested. In children whose MRI is not typical of MS or NMOSD, but the clinical and radiological presentation has features of ADEM, MOG-Ab testing is recommended. Supporting features for MOG-Ab associated disease include lesions in the cerebellar peduncle, and leukodystrophy-like MRI pattern in the very young. Alternative diagnoses should be considered in the remaining antibody negative patients.