

1 **Diagnostic algorithm for relapsing acquired demyelinating syndromes in children**

2 Yael Hacoen MRCPCH DPhil^{1,2,3}, Kshitij Mankad FRCP⁴, WK 'Kling' Chong FRCP⁴, Frederik Barkhof
3 MD PhD^{5,6}, Angela Vincent FRS⁷, Ming Lim MRCP PhD⁸, Evangeline Wassmer FRCPCH⁹, Olga
4 Ciccarelli FRCP PhD^{2*}, Cheryl Hemingway FRCPCH PhD^{1*}

5 *These authors are joint senior authors

- 6 1. Department of Paediatric Neurology, Great Ormond Street Hospital for Children, London, UK.
- 7 2. Department of Neuroinflammation, Queen Square MS Centre, UCL Institute of Neurology,
8 London, UK
- 9 3. Department of clinical neuroscience, UCL institute of child health, London, UK
- 10 4. Paediatric Neuroradiology, Great Ormond street Hospital, London, UK
- 11 5. Institutes of Neurology and Biomedical Engineering, UCL, London, UK
- 12 6. Department of Radiology and Nuclear Medicine, VU University Medical Centre, Amsterdam,
13 NL
- 14 7. Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital, University of Oxford,
15 Oxford.
- 16 8. Children's Neurosciences, Evelina London Children's Hospital @ Guy's and St Thomas' NHS
17 Foundation Trust, King's Health Partners Academic Health Science Centre, London, UK
- 18 9. Department of Paediatric Neurology, Birmingham Children's Hospital, Birmingham, UK
- 19

20 Address for correspondence:

21 Dr Yael Hacoen, Department of Neurosciences. Great Ormond Street Hospital for Children, Great
22 Ormond Street, London, WC1N 3 JH, UK

23 Email: yael.hacoen@gosh.nhs.uk

24 Running head: relapsing demyelinating syndromes in children

25 Word Count: 2998 Abstract: 244

26 Title: 71 characters

27 References: 40 Figure: 2 Table: 3

28

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

36

1 Declaration of interests

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

1 **Abstract**

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

5

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

10

11 **Results:** 56.4% of children were diagnosed with multiple sclerosis (MS), 25.4% with neuromyelitis
12 optica spectrum disorder (NMOSD), 12.7% with multiphasic disseminated encephalomyelitis (MDEM),
13 and 5.5% with relapsing optic neuritis (RON). Blinded analysis defined baseline MRI as typical of MS in
14 93.5% of MS children. ADEM presentation was only seen in the non-MS group. 30.7% of NMOSD
15 cases were AQP4-Ab positive. MOG-Ab were found in 83.3% of AQP4-Ab negative NMOSD, 100%
16 MDEM, and 33.3% with RON. Children with MOG-Ab were younger, less likely to present with area
17 postrema syndrome, had lower disability, longer time to relapse, and more cerebellar peduncle lesions
18 than AQP4-Ab NMOSD. A diagnostic algorithm, applicable to any episode of CNS demyelination, leads
19 to four main phenotypes: MS, AQP4-Ab NMOSD, MOG-Ab-associated disease, and antibody-negative
20 RDS.

21

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

25

1 **Introduction**

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

4

5 The most common pediatric RDS is multiple sclerosis (MS). The 2010 McDonald criteria enable a
6 diagnosis of MS in children over the age of 11 years, presenting with a clinically isolated syndrome
7 (CIS) and MRI evidence of dissemination in space (DIS) and time (DIT)², providing that the clinical
8 presentation does not resemble ADEM^{3,4}. Although a proportion of children present with MS before the
9 age of 12 years, the 2010 McDonald criteria² show a low positive predictive value⁵ in this patient group.

10

11 Another RDS is NMOSD, which is stratified according to the presence/absence of AQP4-Ab⁶. About
12 30% of AQP4 seronegative NMOSD adult patients have myelin oligodendrocyte glycoprotein antibodies
13 (MOG-Ab)⁷. Comparative studies between MOG-Ab and AQP4-Ab positive NMOSD in adults⁸⁻¹⁰ and
14 children¹¹ have shown that MOG-Ab positive patients are younger, more frequently male, and have a
15 better outcome and more often a monophasic course.

16

17 In addition to AQP4-Ab seronegative NMOSD¹², MOG-Ab have been detected in other RDS, such as
18 multiphasic disseminated encephalomyelitis (MDEM)¹³, recurrent optic neuritis (RON)¹⁴, and acute
19 disseminated encephalomyelitis, followed by recurrent or monophasic optic neuritis (ADEM-ON)¹⁵.

20

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

29

1 **Methods**

2 *Participants*

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

10

11 *Standard Protocol Approvals, Registrations, and Patient Consents*

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

14

15 *Procedure*

16 All clinical records were reviewed by one YH, who summarized patients' demographics, clinical
17 presentations, demyelinating phenotypes at visits, timing and features at relapses, and expanded
18 disability status scale (EDSS) at 2 years from onset. Onset demyelinating phenotype was determined
19 based on the clinical features and neurological examination according to established criteria³ (without
20 neuroimaging reference) as being optic neuritis (ON), transverse myelitis (TM), neurological deficits
21 associated with encephalopathy (ADEM) or without encephalopathy (a brainstem, cerebellar and
22 hemispheric CIS)

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

26 Within 1 month of an acute event (either onset or relapse), clinically symptomatic children underwent
27 testing for serum AQP4-Ab and MOG-Ab (not CSF), as part of routine assessments of children with
28 demyelinating diseases, performed at the Clinical Neuroimmunology service at the Oxford Radcliffe
29 Hospital Trust, using live cell-based assays^{16, 17}(This laboratory receives samples for antibody testing
30 from all over the world including the US where MOG-Ab testing is not available). Qualitative analyses of
31 serum and CSF oligoclonal patterns were performed by isoelectric focusing on agarose gels followed by
32 immunoblotting¹⁸, and serum IgG-Ab directed against Epstein-Barr virus capsid antigens, nuclear
33 antigens (EBNA1), and early antigens were measured using standard ELISA kits, locally.

34

35 *Clinical review panel*

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

- 4 1. MS, fulfilling the 2013 International Pediatric Multiple Sclerosis Study Group (IPMSSG)
5 consensus criteria³ and the 2010 McDonald criteria²
- 6 2. NMOSD, fulfilling the 2015 Wingerchuk criteria⁶
- 7 3. MDEM and ADEM-ON, fulfilling the 2013 IPMSSG consensus criteria³
- 8 4. Recurrent demyelination in a single CNS area without evidence of clinically-silent disease
9 (e.g., RON)

10 11 *Blinded radiological analysis*

12 A third neuroradiologist (FB) performed a separate analysis to assess whether imaging characteristics
13 alone can support the diagnosis of a specific syndrome, being blind to the clinical features and the
14 antibody results. The following analysis was repeated separately for baseline MRI and follow-up scans.
15 Lesion morphology, distribution, and location were used to support a diagnosis of MS. MS plaques are
16 ovoid and perpendicularly oriented to the ventricular surface, they occur bilaterally, but are typically
17 asymmetrical, and are distributed in both the supra- and infratentorial compartments. Although MS
18 lesions can be located anywhere in the CNS, they frequently seen in the juxtacortical, periventricular,
19 and infratentorial regions¹⁹. MRI scans were grouped into the following categories; (1) not MS, (2) not
20 typical of MS, (3) some MS features, (4) typical of MS.

21
22 For categories 1 and 2, one of the following five main imaging patterns, which included features known
23 to be associated with NMOSD^{6, 20} and features recently reported in patients with MOG-Ab^{13, 21} was
24 chosen by the neuroradiologist as the predominant pattern: (i) disease localized to brainstem and
25 hypothalamus; (ii) predominantly confluent, hazy/poorly marginated lesions involving both grey matter
26 and white matter; (iii) extensive confluent 'leukodystrophy-like' MRI pattern; (iv) sharply demarcated
27 hemispheric white matter lesions (>3cm); (v) TM and/or ON with normal intracranial appearance or non-
28 specific white matter lesions. Additionally, for the follow-up scans, the two following imaging features
29 were looked for: (i) almost or complete resolution of lesions, (ii) destructive lesions, defined as severe
30 rarefaction of tissue leading to central low signal on FLAIR with associated volumes loss.

31 Finally, the presence of lesions in the diencephalon, dorsal brainstem, periependymal area surrounding
32 the lateral ventricles, longitudinally extensive TM (LETM), cortical grey matter, thalamus, basal ganglia,
33 juxtacortical and deep white matter involvement more than periventricular, cerebellar peduncles, pons
34 and optic nerves/tracts, considered to be typical of patients with NMOSD with AQP4-Ab (according to
35 Wingerchuk criteria)^{6, 20} and MOG-Ab associated demyelination, were recorded.

36 37 *Statistical analysis*

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

6 7 **Results**

8 110 consecutive children with RDS were studied. The median length of follow-up (from first clinical
9 presentation) was 4 years (IQR3-7). During this period, a median of 4 repeated MRI scans were
10 performed (range 3-10). All patients had brain MRI at onset and 95/110 (86.3%) had spinal cord MRI
11 too.

12 The panel diagnosed 62/110 (56.4%) children with MS, 28/110 (25.4%) with NMOSD, 14/110 (12.7%)
13 with MDEM, and 6/110 (5.5%) with RON (not fulfilling criteria for chronic relapsing inflammatory optic
14 neuropathy²²).

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

17 18 *Patients with MS*

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

24 25 *Comparison between children with MS and non-MS RDS*

26 48/ 110 (43.6%) children did not have MS; the majority of these patients had NMOSD (28/48, 58.3%),
27 followed by MDEM (14/48, 29.2%), and RON (6/48, 12.5%)(**Table 1, Supplemental Figure 1**). MS
28 patients were older and more likely to present with a brainstem, cerebellar and hemispheric CIS than
29 non-MS RDS; ADEM presentation was only seen in the non-MS group (all p values <0.0001). Brain MRI
30 abnormalities at presentation were more frequently seen in the MS than non-MS RDS (p<0.0001).
31 MOG- and AQP4-Abs were found exclusively in the non-MS group (p<0.0001). All MS patients tested
32 had evidence of remote EBV infection compared to 42.9% of non-MS patients and more frequently
33 showed intrathecal synthesis of OCBs (all p values <0.0001) (**Table 1**).

34
35 The blinded analysis of baseline MRI scans, done in all 110 patients, correctly identified 58/62 (93.6%;
36 52 category 4; 6 category 3) children with MS and all 48 children with non-MS (100%; 38 category 1; 10
37 category 2). Follow-up MRI scans analysis only identified an additional 2 MS cases (The MRI scans of

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

4 *Autoantibodies in children with RDS*

5 Thirty-four out of 41 (82.9%) patients with non-MS RDS (who were tested) were positive to either
6 AQP4-Ab or MOG-Ab. In particular, 30.7% (8/26) of NMOSD cases tested were AQP4-Ab-positive.
7 83.3% (15/18) of AQP4-Ab negative NMOSD cases were MOG-Ab-positive. No patients had antibodies
8 to both antigens. MOG-Ab were found in 100% (9/9) of MDEM tested cases and 33.3% (2/6) of RON
9 tested cases. Seven patients were negative to both antibodies: 4 relapsed with RON and 3 with
10 NMOSD.

11
12 Clinical characteristics of patients with AQP4-Ab NMOSD and all patients with MOG-Ab associated
13 disease are detailed in **Table 2**.

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

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

19
20
21 When the MRI patterns and lesion locations between MOG-Ab-and AQP4-Ab-positive patients were
22 compared, patients with AQP4-Ab were more likely to have disease restricted to the brainstem and/or
23 hypothalamus (at onset 38% vs 0%, $p = 0.0094$, and at follow-up 50% vs 0%, $p = 0.0015$). Destructive
24 lesions at follow-up scans were seen in the majority of AQP4-Ab-positive patients (62.5%) and in none
25 of the MOG-positive patients ($p = 0.002$) (**Table 3, Supplemental Figure 4**). Lesions located in dorsal
26 brainstem were more frequently seen in AQP4-Ab-positive patients (87.5% vs 11.5%, $p = 0.0085$), whilst
27 lesions in the cerebellar peduncles were only seen in the MOG-positive patients at onset and follow-up
28 ($p = 0.03$ and $p = 0.011$) (**Table 3, Figure 1**). Finally, leukodystrophy-like lesions were only seen in MOG-
29 Ab-positive patients (**Table 3, Figure 1**).

30 *Diagnostic algorithm*

31 We propose a diagnostic algorithm (**Figure 2**), applicable to any episode of CNS demyelination, which
32 leads to four main demyelinating syndromes: MS, APQ4-Ab NMOSD, MOG-Ab associated disease and
33 Ab-negative RDS.
34

35

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

4

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

9

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

14

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

17

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36

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 MS² can be applied to children of any age. Current guidance³ 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 NMOSD⁶. 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 cohorts²⁶. This is in keeping with previous studies of AQP4-Ab seropositivity exponentially increasing with age, particularly in women²⁷. 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 cohorts⁷, but not surprising since MOG-Ab are known to be common in children²⁸. Additionally, increasing recognition of this antibody and the application of the latest diagnostic criteria for NMOSD⁶ may also contribute to the observed high number of NMOSD patients²⁹ and hence MOG-Ab-positive cases. CSF testing for AQP4 and MOG antibodies was not performed as in both these

1 conditions it has been shown that the antibodies originate from the periphery and serum testing is more
2 sensitive than CSF^{30, 31}.

3
4 As we found that 100% of patients with MDEM are MOG-positive, and previous investigations detected
5 significant association between ADEM and MOG-Ab^{13, 32}, we feel that performing MOG-Ab testing in all
6 patients presenting with ADEM (both monophasic and relapsing disease) is justified on the bases of
7 expert's opinion consensus. A key question is whether MOG-Ab negative ADEM patients show different
8 clinical and MRI features from MOG-Ab positive ADEM patients. Large ill-defined lesions in the
9 cerebellar peduncle, and a leukodystrophy-like MRI pattern can be a useful clue to this diagnosis.

10
11 Despite the low number of children with AQP4-Ab in this cohort we identify differences between children
12 with AQP4-Ab vs children with MOG-Ab grouped together (independently of their original RDS
13 diagnosis). Children with MOG-Ab were younger, less likely to present with area postrema syndrome,
14 more likely to present with ADEM, lower disability at 2-year and a longer time to relapse than AQP4-Ab
15 NMO/MS. Therefore, we proposed to group all the MOG-Ab-positive patients into a unified phenotype
16 ("MOG-Ab associated disease"). It is possible that the two antigenic targets (AQP4 and MOG) lead to
17 different diseases, via different pathogenic mechanisms, which cause an autoimmune astrocytopathy in
18 AQP4-Ab-associated disease and autoimmune oligodendroglialopathy in MOG-Ab-associated disease.³³
19 Recent studies looking at the effects of MOG-Ab in cell cultures and in mouse models showed loss of
20 the microtubule cytoskeleton of oligodendrocytes when incubated with purified IgG from MOG-Ab
21 positive patients,³⁴ as well as myelin changes and altered expression of axonal proteins when injected
22 directly into mouse brain³⁵. By contrast, AQP4-Ab are thought to produce astrocyte damage by
23 complement-dependent cytotoxicity which leads to blood–brain barrier disruption causing leukocyte
24 infiltration, and cytokine release resulting in damage to oligodendrocytes, myelin and neurons which
25 may explain the more destructive lesions seen in this phenotype³⁶. Abundance of AQP4 in the area
26 postrema³⁷ is likely to explain the increased frequency of nausea and vomiting in the AQP4-Ab positive
27 group, together with imaging abnormalities in the dorsal brainstem and hypothalamus, as previously
28 reported.³⁸

29
30 Overall, the very high percentage (82.9%) of children with non-MS RDS who are positive to one of the
31 two antibodies, suggests that the majority of these children have a known antibody-mediated
32 demyelinating disease. In the rare cases of RDS other than MS, negative to both antibodies, it is
33 important to consider mimics of CNS demyelination.

34
35 The blinded MRI analyses successfully distinguished between MS and non-MS cases already at onset.
36 MRI abnormalities in the brainstem and hypothalamus were typical of AQP4-Ab positive patients, as
37 previously reported,³⁸ whilst ill-defined lesions in the cerebellar peduncle were seen exclusively in the

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

3

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

7

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

14
15
16
17
18
19
20
21
22
23
24

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

	<i>All non-MS (n=48)</i>					
	MS (n=62)	NMOSD (n=28)	MDEM (n=14)	RON (n=6)	Summary <i>of all non-MS</i>	P Value <i>MS vs non-MS</i>
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

3 [^]All these 15 cases were AQP4-Ab negative, so 83.3% (15/18) of AQP4-Ab negative patients were
 4 MOG-Ab positive. * This does not include orbital MRI.
 5

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

3
4

1
2

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)
<i>MRI predominant pattern at onset</i>					
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
<i>Lesion location at onset</i>					
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 matter 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
<i>MRI predominant pattern at follow-up</i>					
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
<i>Lesion location at follow-up</i>					
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.

References

1. Waldman A, Ghezzi A, Bar-Or A, Mikaeloff Y, Tardieu M, Banwell B. Multiple sclerosis in children: an update on clinical diagnosis, therapeutic strategies, and research. *Lancet Neurol* 2014; 936-948.
2. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011;69:292-302.
3. Krupp LB, Tardieu M, Amato MP, et al. International Pediatric Multiple Sclerosis Study Group criteria for pediatric multiple sclerosis and immune-mediated central nervous system demyelinating disorders: revisions to the 2007 definitions. *Mult Scler* 2013;19:1261-1267.
4. Filippi M, Rocca MA, Ciccarelli O, et al. MRI criteria for the diagnosis of multiple sclerosis: MAGNIMS consensus guidelines. *Lancet Neurol* 2016;15:292-303.
5. Sadaka Y, Verhey LH, Shroff MM, et al. 2010 McDonald criteria for diagnosing pediatric multiple sclerosis. *Ann Neurol* 2012;72:211-223.
6. Wingerchuk DM, Banwell B, Bennett JL, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology* 2015.
7. Sepulveda M, Armangue T, Sola-Valls N, et al. Neuromyelitis optica spectrum disorders: Comparison according to the phenotype and serostatus. *Neurol Neuroimmunol Neuroinflamm* 2016;3:e225.
8. Kitley J, Waters P, Woodhall M, et al. Neuromyelitis optica spectrum disorders with aquaporin-4 and myelin-oligodendrocyte glycoprotein antibodies: a comparative study. *JAMA Neurol* 2014;71:276-283.
9. Sato DK, Callegaro D, Lana-Peixoto MA, et al. Distinction between MOG antibody-positive and AQP4 antibody-positive NMO spectrum disorders. *Neurology* 2014;82:474-481.
10. van Pelt ED, Wong YY, Ketelslegers IA, Hamann D, Hintzen RQ. Neuromyelitis optica spectrum disorders: comparison of clinical and magnetic resonance imaging characteristics of

- 1 AQP4-IgG versus MOG-IgG seropositive cases in the Netherlands. *Eur J Neurol* 2016;23:580-
2 587.
- 3 11. Lechner C, Baumann M, Hennes EM, et al. Antibodies to MOG and AQP4 in children
4 with neuromyelitis optica and limited forms of the disease. *J Neurol Neurosurg Psychiatry*
5 2016;87:897-905.
- 6 12. Rostasy K, Mader S, Hennes EM, et al. Persisting myelin oligodendrocyte glycoprotein
7 antibodies in aquaporin-4 antibody negative pediatric neuromyelitis optica. *Mult Scler*
8 2013;19:1052-1059.
- 9 13. Baumann M, Hennes EM, Schanda K, et al. Children with multiphasic disseminated
10 encephalomyelitis and antibodies to the myelin oligodendrocyte glycoprotein (MOG): Extending
11 the spectrum of MOG antibody positive diseases. *Mult Scler* 2016.
- 12 14. Rostasy K, Mader S, Schanda K, et al. Anti-myelin oligodendrocyte glycoprotein
13 antibodies in pediatric patients with optic neuritis. *Arch Neurol* 2012;69:752-756.
- 14 15. Huppke P, Rostasy K, Karenfort M, et al. Acute disseminated encephalomyelitis
15 followed by recurrent or monophasic optic neuritis in pediatric patients. *Mult Scler* 2013;19:941-
16 946.
- 17 16. Hacohen Y, Absoud M, Deiva K, et al. Myelin oligodendrocyte glycoprotein antibodies
18 are associated with a non-MS course in children. *Neurol Neuroimmunol Neuroinflamm*
19 2015;2:e81.
- 20 17. Waters PJ, McKeon A, Leite MI, et al. Serologic diagnosis of NMO: a multicenter
21 comparison of aquaporin-4-IgG assays. *Neurology* 2012;78:665-671; discussion 669.
- 22 18. Freedman MS, Thompson EJ, Deisenhammer F, et al. Recommended standard of
23 cerebrospinal fluid analysis in the diagnosis of multiple sclerosis: a consensus statement. *Arch*
24 *Neurol* 2005;62:865-870.
- 25 19. Aliaga ES, Barkhof F. MRI mimics of multiple sclerosis. *Handb Clin Neurol*
26 2014;122:291-316.
- 27 20. Kim HJ, Paul F, Lana-Peixoto MA, et al. MRI characteristics of neuromyelitis optica
28 spectrum disorder: an international update. *Neurology* 2015;84:1165-1173.
- 29 21. Sepulveda M, Armangue T, Martinez-Hernandez E, et al. Clinical spectrum associated
30 with MOG autoimmunity in adults: significance of sharing rodent MOG epitopes. *J Neurol*
31 2016;263:1349-1360.
- 32 22. Petzold A, Plant GT. Diagnosis and classification of autoimmune optic neuropathy.
33 *Autoimmun Rev* 2014;13:539-545.
- 34 23. Banwell B, Ghezzi A, Bar-Or A, Mikaeloff Y, Tardieu M. Multiple sclerosis in children:
35 clinical diagnosis, therapeutic strategies, and future directions. *Lancet Neurol* 2007;6:887-902.
- 36 24. Banwell B, Bar-Or A, Arnold DL, et al. Clinical, environmental, and genetic
37 determinants of multiple sclerosis in children with acute demyelination: a prospective national
38 cohort study. *Lancet Neurol* 2011;10:436-445.
- 39 25. Pandit L, Asgari N, Apiwattanakul M, et al. Demographic and clinical features of
40 neuromyelitis optica: A review. *Mult Scler* 2015;21:845-853.
- 41 26. Chitnis T, Ness J, Krupp L, et al. Clinical features of neuromyelitis optica in children:
42 US Network of Pediatric MS Centers report. *Neurology* 2016;86:245-252.
- 43 27. Quek AM, McKeon A, Lennon VA, et al. Effects of age and sex on aquaporin-4
44 autoimmunity. *Arch Neurol* 2012;69:1039-1043.
- 45 28. Reindl M, Di Pauli F, Rostasy K, Berger T. The spectrum of MOG autoantibody-
46 associated demyelinating diseases. *Nat Rev Neurol* 2013;9:455-461.
- 47 29. Hyun JW, Jeong IH, Joung A, Kim SH, Kim HJ. Evaluation of the 2015 diagnostic
48 criteria for neuromyelitis optica spectrum disorder. *Neurology* 2016;86:1772-1779.
- 49 30. Jarius S, Ruprecht K, Kleiter I, et al. MOG-IgG in NMO and related disorders: a
50 multicenter study of 50 patients. Part 1: Frequency, syndrome specificity, influence of disease

1 activity, long-term course, association with AQP4-IgG, and origin. *J Neuroinflammation*
2 2016;13:279.

3 31. Majed M, Fryer JP, McKeon A, Lennon VA, Pittock SJ. Clinical utility of testing AQP4-
4 IgG in CSF: Guidance for physicians. *Neurol Neuroimmunol Neuroinflamm* 2016;3:e231.

5 32. Baumann M, Sahin K, Lechner C, et al. Clinical and neuroradiological differences of
6 paediatric acute disseminating encephalomyelitis with and without antibodies to the myelin
7 oligodendrocyte glycoprotein. *J Neurol Neurosurg Psychiatry* 2015;86:265-272.

8 33. Reindl M, Rostasy K. MOG antibody-associated diseases. *Neurol Neuroimmunol*
9 *Neuroinflamm* 2015;2:e60.

10 34. Dale RC, Tantsis EM, Merheb V, et al. Antibodies to MOG have a demyelination
11 phenotype and affect oligodendrocyte cytoskeleton. *Neurol Neuroimmunol Neuroinflamm*
12 2014;1:e12.

13 35. Saadoun S, Waters P, Owens GP, Bennett JL, Vincent A, Papadopoulos MC.
14 Neuromyelitis optica MOG-IgG causes reversible lesions in mouse brain. *Acta Neuropathol*
15 *Commun* 2014;2:35.

16 36. Papadopoulos MC, Verkman AS. Aquaporin water channels in the nervous system.
17 *Nat Rev Neurosci* 2013;14:265-277.

18 37. Ratelade J, Bennett JL, Verkman AS. Intravenous neuromyelitis optica autoantibody in
19 mice targets aquaporin-4 in peripheral organs and area postrema. *PLoS One* 2011;6:e27412.

20 38. Popescu BF, Lennon VA, Parisi JE, et al. Neuromyelitis optica unique area postrema
21 lesions: nausea, vomiting, and pathogenic implications. *Neurology* 2011;76:1229-1237.

22 39. Tintore M, Rovira A, Rio J, et al. Defining high, medium and low impact prognostic
23 factors for developing multiple sclerosis. *Brain* 2015;138:1863-1874.

24 40. Balfour HH, Sifakis F, Sliman JA, Knight JA, Schmeling DO, Thomas W. Age-Specific
25 Prevalence of Epstein-Barr Virus Infection Among Individuals Aged 6-19 Years in the United
26 States and Factors Affecting Its Acquisition. *J Infect Dis* 2013;208:1286-1293.

27
28
29

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

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.