

## MRI and Laboratory Contributions to the Diagnosis of Multiple Sclerosis in Children

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## **SUMMARY:**

*Background:* MRI and laboratory features have been incorporated into international diagnostic criteria for multiple sclerosis (MS). We evaluated the pattern of MRI lesions and contributions of CSF and serum antibody findings that best identifies children with MS, and the applicability of the international diagnostic criteria in the pediatric context.

*Methods:* Detailed clinical evaluations, serum and CSF studies, and MRI scans were performed in youth with incident acquired demyelinating syndromes (ADS). Participants were examined prospectively to identify relapsing disease. All MRI scans were evaluated using a validated scoring tool. A random forest classifier identified imaging and laboratory features that best predicted an MS or monophasic outcome. Performance of the 2001, 2010, and 2017 International “McDonald” MS criteria, 2016 MAGNIMS criteria, and our prior proposed (Verhey) criteria were determined.

*Findings:* We included 324 participants with median follow-up of 73 months (range 6-150); 71 MS, 237 monophasic ADS, 14 relapsing non-MS, and 2 with alternative diagnoses. We scored 2391 brain, 444 spinal, and 67 dedicated orbital MRI scans. One or more T1 hypointense lesions plus one or more periventricular lesions (Verhey criteria) best predicted MS outcome. Performance of the 2017 McDonald criteria was comparable to the 2010 McDonald and was easier to adjudicate. The ability of CSF oligoclonal bands to substitute for the requirement for both enhancing and non-enhancing lesions improved 2017 criteria performance. MOG testing at baseline did not improve performance of the 2017 criteria.

*Interpretation:* The 2017 McDonald criteria for MS, as applied at the time of incident attack, perform well in identifying children and youth with MS- indicating that the same diagnostic criteria for MS apply across the age-span. The presence of “black holes” and periventricular lesions at baseline (Verhey criteria) also effectively distinguish children with MS from children with monophasic demyelination. The presence of CSF OCBs improve diagnostic accuracy. MOG antibodies identify children with ADEM, and those with relapsing non-MS, the vast majority of whom do not meet 2017 criteria at onset.

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## Introduction

The diagnosis of Multiple Sclerosis (MS) requires confirmation of dissemination of inflammatory demyelination of the central nervous system (CNS) over time and across multiple CNS sites. In relapsing-remitting MS, new disease activity is evidenced by focal lesions of the CNS, which may be expressed clinically or solely by MRI. The number of lesions detected by MRI exceeds the frequency of clinical relapses, and more than 80% of adult and pediatric MS patients demonstrate multiple clinically silent brain lesions at the time of their incident clinical attack.<sup>1;2</sup> The value of neuroimaging is emphasized by international MS diagnostic criteria which incorporate MRI findings.<sup>3-6</sup> The 2017 International “McDonald” MS diagnostic panel recently proposed modifications to improve diagnostic accuracy, simplify application of the criteria and enhance timeliness of the diagnosis of MS.(Thompson et al, *Lancet Neurology*, *in press*) The 2017 McDonald criteria re-emphasize the contribution of CSF analysis. The diagnosis of MS also requires “no better explanation”.<sup>4</sup> Serum antibodies against aquaporin-4 (AQP4) are now well accepted as evidence against a diagnosis of MS, while emerging studies suggest that the presence of antibodies directed against myelin oligodendrocyte glycoprotein (MOG) also identify patients with a non-MS clinical phenotype.<sup>7;8</sup>

All versions of the McDonald diagnostic criteria,<sup>3-5</sup> and the 2016 MAGNIMS criteria<sup>6</sup> were developed based on studies of adult-onset MS. Prior studies have shown performance of the 2010 McDonald criteria in children to be comparable to that in adults.<sup>9-11</sup> Caveats to this statement include provisions not to apply the criteria to children with a first attack characterized by encephalopathy and polyfocal deficits (acute disseminated encephalomyelitis, ADEM), and to exercise caution when applying the 2010 criteria to children aged less than 11 years.<sup>4</sup>

Using our previously validated MRI scoring tool,<sup>12,13</sup> and additional MRI and laboratory variables, we identified features most consistent with MS in children. We evaluated the diagnostic performance of the newly proposed 2017 McDonald MS criteria applied at the time of clinical onset in a prospective cohort of children with acquired demyelinating syndromes (ADS), and compared this to performance of prior criteria.

## **Methods**

### **Participants and study design:**

We enrolled participants in a prospective multi-site study as previously detailed.<sup>1</sup> Briefly, children aged less than 16 years were enrolled within 30 days of onset of incident ADS between July 1, 2004 and June 30, 2014. From July 1, 2014 to present, enrollment criteria were broadened to include children aged less than 18 years, presenting within six months of an incident ADS, provided that they met 2010 McDonald criteria at baseline or by demonstration of dissemination of new disease activity within 6 months of onset.

Guardians and participants provided informed consent. Younger children provided verbal assent. The study was approved by ethics boards of participating institutions.

### **Procedures**

All patients were assessed using comprehensive clinical assessments at the time of incident ADS, at 3, 6, and 12 months and annually thereafter and at the occurrence of any subsequent demyelinating attack, according to our published methods.<sup>1</sup> Axial and sagittal T2-, fluid attenuated inversion recovery (FLAIR)- and T1-weighted sequences, and T1-weighted images post-administration of gadolinium obtained at onset were evaluated. All brain, spine, and orbital MRI scans obtained for clinical purposes at any time were de-identified and analyzed centrally. In addition to clinical scans, research scans were offered to all participants at baseline, 3, 6 and 12

months, and annually (annual scans at the three largest sites in Toronto, Calgary, and Philadelphia). All serial scans were evaluated for the presence of new T2 and/or gadolinium enhancing lesions.

Table 1 outlines the analyses performed, and provides detail regarding the number of participants with available data and the rationale for each aspect of the specific analyses. Experienced MRI analysts (GF, RB, BB, GL, DC) were trained and evaluated scans using a modified version of our published MRI scoring tool (Panel 1), blinded to clinical outcome.<sup>1</sup>

The clinical or research scan closest to the date of symptom onset was designated as the baseline scan. If two scans were obtained within 72 hours, both scans were evaluated to ensure maximal lesion identification and to adjudicate the presence of lesion enhancement. For all participants, it was noted whether baseline scans were performed before administration of corticosteroids. When available, MRI studies of the spine or orbits (T2w fat saturated sequences) were also scored, provided they were obtained within 30 days of the presenting symptoms. The presence of lesions in the cervical spine were also scored using the brain MRI, if the images obtained included adequate views of upper cervical spine. We decided not to score cortical lesions separately, given that most MRI studies were performed at 1.5T and did not allow an adequate delineation of cortical lesions.

CSF examination for oligoclonal bands (OCBs) was performed locally, and all study site laboratories utilized isoelectric focusing.

Serum samples obtained at baseline or within 1.5 months of presentation were processed at procurement using our previously published methods,<sup>14</sup> aliquoted, batch-shipped, and analyzed for antibodies directed against myelin oligodendrocyte glycoprotein (MOG) and aquaporin 4 (AQP4) at the Autoimmune Neurology Diagnostic Laboratory, Oxford, UK, blinded to clinical status. In addition, results of serum analyses for MOG and

AQP4 collected as part of clinical care were also evaluated, **given that** the clinical samples were all analyzed by either the same laboratory as used for our research samples, or by a nationally certified laboratory. **Given that** it is widely accepted that the presence of AQP4 antibodies, in conjunction with clinical features, is indicative of neuromyelitis spectrum disorder (NMOSD), we excluded all such participants from our analyses. In contrast, **given that** the clinical implications of anti-MOG antibodies are still uncertain, we did not exclude participants based on anti-MOG status, but included MOG status in our analyses.

### **Outcome:**

We adjudicated diagnostic status as of the last visit conducted before Nov, 2017 (**Table 2**), and only included participants with a minimum of six months observation given our prior work demonstrating the short interval from onset to clinical relapse or MRI evidence for dissemination in time in pediatric MS.<sup>1</sup> We *a priori* defined the MS group based on confirmed clinical relapses consistent with MS (MS-clinical) and/or accrual of new MRI lesions more than 30 days from baseline (MS-MRI). Monophasic ADS was defined by the absence of new MRI lesions or clinical attacks on serial examinations. Relapsing non-MS was diagnosed in children with relapses confined to a single CNS location, or children with ADEM who experienced further disease activity not meeting criteria for MS.<sup>15</sup> Children diagnosed with non-demyelinating disorders or with NMOSD<sup>16</sup> were excluded from the analysis if their diagnoses were conferred <30 days from onset (i.e., before new symptoms or MRI lesions could suggest dissemination in time). Children with an initial presentation consistent with ADEM were required to have  $\geq 1$  non-ADEM relapses and accrual of clinically silent new lesions more than 90 days from initial ADEM presentation to be diagnosed with MS.<sup>15</sup>

### **Statistical Analysis**

Participants were categorized into groups based on outcome (**Figure 1**). Frequency of each of the 26 binary parameters (**Table 3**) was compared by group. To characterize the association of each parameter, in isolation,

with the diagnosis of MS, the difference in frequencies in the *MS All* (including MS-clinical and MS-MRI) was compared to the *non-MS All (including ADEM)* groups, using univariable general linear models with logit link function and binomial distribution, and sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the diagnosis of MS were computed. Finally, to identify features that performed well together, these variables were used as inputs for a random forest classifier (100 trees; Python SKLearn); a backwards elimination process was used<sup>17</sup> to obtain a feature importance ranking accounting for correlation between variables. Supplementary Methods delineate the statistical analyses in detail, including the rationale for not employing Cox models.

The dissemination in space (DIS) and dissemination in time (DIT) criteria were evaluated on baseline images as specified by the McDonald 2017 (Thompson et al, Lancet Neurology, *in press*) and 2010 criteria,<sup>4</sup> the DIS component of the 2001 criteria (DIT requires serial evaluation),<sup>5</sup> and the 2016 MAGNIMS criteria.<sup>6</sup> The contribution of optic nerve involvement to DIS was scored for the MAGNIMS criteria in two manners: (i) clinical evidence of optic neuritis; or (ii) optic nerve T2 lesions seen by MRI (with or without clinical optic neuritis).

To determine whether participants met DIT criteria at baseline using the 2010 and 2017 criteria, we restricted the analysis to 211 participants with gadolinium-enhanced baseline scans. For the 2017 criteria, we allowed CSF OCB data, if available (192 participants), to replace the requirement for enhancing and non-enhancing lesions. We then evaluated the performance of the full (DIS plus DIT) 2010, 2017 and MAGNIMS criteria, and our previously suggested “Verhey” criteria for MS in children<sup>1</sup>, as applied to baseline scans in the same 211 participants (Figure 2). Given that many of these participants were included in our prior work (and thus would be expected to validate our prior findings), we also determined the performance of the Verhey criteria using only data from participants not included in our prior work (Supplementary Table 1). Finally, we investigated the performance of the various criteria stratified by age (<11 versus ≥11 years), given that the 2010 criteria specify caution in application of the criteria under age 11 years (Figure 3).

We compared the performance of the 2010, 2017 and Verhey criteria, as applied at baseline, using a univariable generalized linear model with logit link function and binomial error distribution:

$$sensitivity_{TP=1|FN=0} = g^{-1}[\beta_0 + \beta_1 \cdot criteria_{2010|2017|Verhey}] + \epsilon$$

$$specificity_{TN=1|FP=0} = g^{-1}[\beta_0 + \beta_1 \cdot criteria_{2010|2017|Verhey}] + \epsilon$$

Although the 2010 and 2017 criteria state that they should not be applied at the time of acute ADEM, we included the baseline MRI scans obtained from the 80 children who presented with ADEM in the non-MS group (72 monoADEM, and 7 relapsing non-MS, 1 eventually diagnosed with vasculitis) to determine whether inclusion of children with ADEM influences the performance of the tested criteria.

Figure 4 details the clinical features of participants as stratified by MOG findings, and the criteria performance after the exclusion of the anti-MOG positive participants is provided in Supplementary Table 2.

Analyses were completed using Python ([www.python.org](http://www.python.org), version 2.7.11), Python bridge RPy2 (version 2.7.7), SKLearn (version 0.18.1), and R ([www.r-project.org](http://www.r-project.org), version 3.1.3).

### **Role of the funding source**

The sponsor had no role in the conduct of the study, did not have access to the study data and did not contribute to the writing of the manuscript. GF, RB, and BB had full access to all data and final responsibility for the submission.

### **Results**



Between Sept 1, 2004 and June 30, 2017, 451 children and adolescents were assessed for eligibility, of whom 324 contributed to the analysis (**Figure 1**). Participants were observed for a median of 72 months (range 6-150) (**Table 2**). Of 324 participants, 71 met our *a priori* definition of MS, 48 (68%) on the basis of clinical relapses, 23 (32%) with new MRI lesions only. Of the 23 participants diagnosed with MS on the basis of new lesions in the absence of a second attack, 15 received disease-modifying therapies after confirmation of MS diagnosis on the basis of new MRI lesions. ADEM was the presenting phenotype for 80 participants, none of whom have exhibited clinical and MRI features diagnostic of MS on serial evaluations.<sup>15</sup> Seven children with a presenting diagnosis of ADEM acquired one or more new lesions within the first 12 months following presentation without a second clinical attack. These patients were not considered to meet our gold standard diagnostic criteria, following the guidelines of the International Pediatric Study Group,<sup>15</sup> and were considered as part of the monophasic group for the present study. Four children with non-ADEM first attacks developed isolated non-specific small white matter hyperintensities in the non-periventricular white matter, which were not considered as sufficient for DIT after consensus review by the MRI scoring group. These children were also included in the monophasic cohort. Fourteen participants have experienced recurrent attacks not meeting criteria for MS (5 with recurrent ON, 2 with recurrent TM, 7 with ADEM followed by a single non-ADEM attack). Two additional children were diagnosed with CNS vasculitis more than 30 days from first presentation. The remaining 226 participants remained clinically and radiographically monophasic.

MRI scoring was completed for 2902 scans (2391 brain, 444 spine, and 67 dedicated orbital MRI scans). The cervical spine was scored using 444 brain MRI studies with sufficient visualization of this region. Table 3 describes the number of patients tested for CSF OCBs and serum MOG antibodies. Supplementary Table 3 compares the clinical diagnosis and presentation of children as a function of CSF OCB results. The frequency of MOG antibodies according to clinical presentation is illustrated in Figure 4. Of the 324 included participants, 264 were tested for AQP4 and all were negative. None of the 60 participants who were not tested for AQP4 met clinical criteria for NMOSD.

### ***Frequency of baseline features in children with CNS demyelination***

Even at first attack, children with MS typically had multiple lesions, particularly in the periventricular region (**Table 3**). T2 bright lesions in the thalamus and basal ganglia were notable in children with ADEM. Clinically silent lesions in the optic nerve, as visualized on brain MRI, were rare, but were commonly seen when dedicated orbital views were obtained in the context of clinical optic neuritis. Of 140 participants with dedicated spine imaging, spinal lesions were detected in 106 (75%), 82 of whom had clinical features of transverse myelitis.

The top five baseline features that best discriminated MS patients from non-MS (including ADEM, relapsing non-MS) identified by the random forest were  $\geq 1$  black holes,  $\geq 1$  contrast enhancing lesions,  $\geq 1$  periventricular lesions, the presence of OCBs and MOG antibodies. Given that some lesions would have been scored as both perpendicular and periventricular, and given that adjudication of whether a lesion oriented perpendicular to the long axis of the corpus callosum was more subjective than the determination of whether a lesion abutted to lateral ventricle, we did not include perpendicular lesions as one of the best discriminating features despite such lesions being in the top features (Figure 2, Table 4, Supplementary Tables 1-2 and 4).

### ***Application of MS Diagnostic Criteria to Baseline Presentation:***

**Figure 2** compares the performance of the various criteria at baseline, excluding children presenting with ADEM. Tables 4 and Supplementary Table 4 delineate performance of the DIS and DIT components of the criteria, without and with inclusion of children with ADEM, respectively. The 2017 criteria identified 36 of the 51 MS participants in whom both DIS and DIT components of the 2017 criteria could be applied (as explained in Table 1), 27 of whom also met 2010 criteria at baseline. While the 2017 criteria yielded an increase in

sensitivity of 18% over the 2010 criteria, this difference was not statistically significant ( $p=0.15$ ). The loss of specificity was minimal.

While 57 MS patients had appropriate imaging for evaluation of DIS, 51 had gadolinium-enhanced scans required to evaluate both DIS and DIT. Of the 51, 30 (58.8%) met 2010 criteria for DIT at baseline. Thirty-five MS patients had gadolinium-enhanced scans and CSF OCBs for analysis: 20 (57%) met 2010 criteria and 29 (83%) met 2017 criteria (incorporating OCB results), an increase of 26% in DIT sensitivity. Thus, inclusion of CSF OCBs contributed to a modest increase of the **overall** diagnostic yield of the 2017 criteria (18% increase in sensitivity **overall**) relative to 2010 criteria.

Performance of the 2016 MAGNIMS criteria was comparable to the 2017 McDonald criteria. Allowing the optic nerve as a site (based on clinical or MRI evidence), 47 (82%) MS participants met MAGNIMS criteria for DIS. However, 46 also met 2017 and 47 met 2010 criteria for DIS using only brain or spinal cord lesions, indicating that optic nerve lesion detection did not improve criteria performance. Eight MS participants who met 2017 full criteria failed to meet MAGNIMS criteria.

When considering the full 2010 criteria, 24 of the 51 MS participants did not meet the criteria at baseline (10 with ON, 2 with TM, 1 with ON and TM, 1 with brainstem symptoms, 10 with other features).

The 2001 DIS criteria were met by 45 of the 57 MS participants. The average estimated lesion counts in these 12 participants who failed to meet 2001 DIS criteria was 2.4, which was below the criterion of 9 T2 lesions, and was considerably lower than the average lesion count of 12.5 in the MS participants who did meet DIS at baseline ( $p < 0.0001$ ).

We confirmed the strong association with MS of  $\geq 1$  black holes and  $\geq 1$  periventricular lesions. These “Verhey” criteria are more sensitive than McDonald 2010 ( $p = 0.0033$ ) and approach significance relative to 2017 criteria ( $p = 0.083$ ). Given that the Verhey criteria were derived from a portion of the present cohort, we validated the performance of black holes and periventricular lesions by repeating the analysis in the subset of 50 participants (16 MS vs. 34 non-MS) enrolled before the change of protocol in 2014 who had not contributed to our prior work (Supplementary Table 1).

As shown in **Figure 3** while there was a trend towards higher sensitivity of all criteria when applied to participants older than 11 years at presentation, specificity was uniformly high, irrespective of age at onset.

***Application of MS Diagnostic Criteria in ADEM:*** Supplementary Table 4 evaluates the performance of the criteria tested as applied to baseline scans from all of the children, including those who presented with ADEM. Ten children with ADEM (13%), all with monophasic disease, would have met 2017 criteria at onset if the provision for exclusion of ADEM were not in the criteria.

***Relapsing Non-MS:*** None of the 7 non-ADEM children with relapsing non-MS nor the one with CNS vasculitis met the 2017 or 2010 DIS or DIT criteria. Only one subject with relapsing non-MS and an ADEM presentation had positive CSF OCBs.

***Contribution of MOG antibody status:*** In a subgroup analysis of the 279 children tested for MOG, restricted further to the 188 participants meeting the requirements for the evaluations of all criteria (enhanced imaging at baseline, non-ADEM presentation, enrollment prior 2014), we evaluated whether the performance of the tested criteria would improve upon exclusion of MOG-positive participants (131 remained). As can be seen by comparing Figure 2 and Supplementary Table 2, performance of the various criteria, including the 2017 criteria, were not improved by exclusion of MOG-positive patients. Only 7 of the MOG-positive participants met full

2017 criteria at baseline, 3 of whom also met our gold standard diagnostic criteria for MS and 4 with monophasic disease.

## Discussion

We investigated the MRI and laboratory features that best identify children with MS at the time of first attack, and evaluated the performance of the newly proposed 2017 as well as previous criteria for the diagnosis of MS. First, we sought to define MRI and laboratory features that were particularly common in pediatric MS, and show that the presence of periventricular (as well as lesions perpendicular to the major axis of the corpus callosum), enhancing, and T1 hypointense lesions, and CSF OCBs, and the absence of anti-MOG antibodies are the most robust features to distinguish children with MS from children with non-MS outcomes. Given that periventricular and enhancing lesions and CSF OCBs are also included in the 2017 McDonald criteria, we anticipated that the 2017 criteria would perform well. We previously demonstrated the very high specificity and sensitivity of T1 hypointense lesions and periventricular lesions in identifying children with MS at onset (Verhey criteria),<sup>1</sup> which we re-confirmed in this cohort, and in a subset of this cohort that did not contribute to our prior analysis. As outlined in Panel 1, identification of T1 hypointense lesions employed a well-articulated definition of T1 hypointensity using comparable signal of lesions to cortical gray matter, as can be adjudicated using conventional imaging. The diagnostic contribution of T1 lesion detection by higher field magnets and computerized T1 intensity maps will require future research.

Our major focus was to evaluate the very recently proposed 2017 McDonald criteria in a pediatric population, as these criteria are likely to be adopted as the primary criteria for MS diagnosis in adults. Seventy-one percent of the MS patients evaluated for the full 2017 DIS and DIT criteria would have been diagnosed with MS at onset. The 2017 revisions of the McDonald criteria performed comparably to prior versions. The 2017 criteria no longer require that symptomatic lesions be excluded from the lesion count. This did not increase the number of patients identified at baseline, likely because over 74% of MS participants had periventricular and

juxtacortical lesions (often clinically silent and thus counted by 2010 criteria). However, removal of the requirement to determine which lesions associate with clinical symptoms does render the 2017 criteria easier to apply. The 2017 criteria stipulation that positive CSF OCBs can substitute for the requirement to have enhancing and non-enhancing lesions did increase the diagnosis rate, although the lower rate of positive OCBs in children makes this change less impactful in pediatric, compared to adult-onset MS.

The 2017 criteria are highly specific for MS, driven predominantly by the DIT aspect. The very low frequency of enhancing lesions (9%) or CSF OCBs (15%) in children with monophasic demyelination, including ADEM, explains this finding. The DIS aspect of the criteria is considerably less specific, being met by 79% of children with ADEM, and by 20% of the non-MS, non-ADEM group.

Given the re-emergence of interest in the contribution of CSF OCBs,<sup>18;19</sup> we also evaluated the performance of the 2001 McDonald criteria, as these criteria permit use of CSF OCBs to contribute to the determination of DIS provided that two or more T2 lesions were also present, unlike the 2017 criteria wherein CSF OCBs contribute to the DIT component. The 2001 criteria do not specify where the two lesions must be located, and thus conceptually might be more liberal. Of the 35 MS participants with OCB results, 30 met 2001 DIS; of these, 21 had positive CSF and 5 would not have met 2001 DIS on MRI criteria alone. When using the full 2001 DIS criteria, 45 of the 57 MS participants were identified, which is nearly identical to the 46 of 57 who met 2017 criteria for DIS, which does not consider CSF. The similar frequency of MS patients with >9 lesions (61%) and positive OCBs (70%) explains this observation.

The 2016 MAGNIMS criteria permit optic nerve lesions to contribute to DIS, but inclusion of optic nerve lesions was not endorsed by the 2017 McDonald committee owing to concern that the optic nerve is poorly visualized on brain MRI and that the optimal ways to evaluate optic nerve and pathways involvement for this purpose are not established. Clinically silent lesions of the optic nerves were rarely detected (3 children), but were

visualized (typically by dedicated orbital MRI scans) in almost all of children with clinical optic neuritis. The ability to count the optic **nerve as a site** did not render the MAGNIMS criteria more sensitive. However, to fully evaluate this would require dedicated orbital views in all children. The requirement by the 2016 MAGNIMS criteria for three or more periventricular lesions (detected in 62% of the MS participants), as compared to the 2017 McDonald criteria requirement for one or more periventricular lesion (detected in 89% of the MS participants), led to a slightly reduced sensitivity of the MAGNIMS criteria.

Lesions in the spine contribute to the DIS lesion count in the 2017, 2010, 2001 and MAGNIMS criteria. We analyzed the spine MRI scans performed as part of clinical practice, since spine MRI was not included in our research protocol. Spinal lesions were commonly detected in participants for whom spinal imaging was performed (71% of the MS group, 67% of children with ADEM, and 81% of the non-MS non-ADEM group, largely children with clinical transverse myelitis). Despite the high proportion of abnormal spinal scans in our cohort, which may over-estimate the frequency of spinal lesions in ADS patients as a whole, the presence of a spinal lesion was critical for meeting DIS only for two MS participants. In agreement with what has been reported by other pediatric studies,<sup>10</sup> addition of spinal cord data did not meaningfully change the performance of any of the criteria (data not shown). Spinal imaging is appropriate when clinical concern exists for spinal cord involvement.

It is important to emphasize that while the 2017 (and 2010) criteria are very specific for MS, this high specificity was observed in the context of a carefully delineated cohort of children with ADS. This is appropriate, given that a core tenet of all of the McDonald MS diagnostic criteria is that they be applied only once other diagnostic considerations have been excluded. However, how these diagnostic criteria will fare in general practice may differ, as demonstrated by studies showing a high rate of false MS diagnosis when applying the 2010 criteria to adults with non-specific neurological complaints.<sup>20</sup> In the context of ADEM, neither the 2017 **nor** 2010 criteria should be applied at baseline, and the diagnosis of MS in such children requires at least one

non-ADEM relapse and the accrual of clinically-silent new lesions. Perhaps more germane to the pediatric ADS population is the consideration of MOG antibodies. Several recent studies have demonstrated that MOG antibodies are present in fewer than 10% of children with typical relapsing remitting MS, but are more commonly detected in children with monophasic ADEM (in whom anti-MOG antibodies tend to be transient), and relapsing pediatric patients with AQP4-negative NMOSD, ADEM followed by recurrent ON, and chronic relapsing ON.<sup>7;21;22</sup> We confirm the relatively high frequency of MOG antibodies in children with ADS, show that the presence of such antibodies is largely associated with a non-MS diagnosis, and that MOG antibodies can be present, **although infrequently**, in children with typical relapsing remitting MS. We feel it is premature to conclude that the presence of MOG antibodies excludes a diagnosis of MS in patients with typical MS relapses and MRI features.

We aimed to determine how MOG antibody status influences performance of the 2017 criteria. Exclusion of anti-MOG positive patients had minimal effects on the criteria sensitivity and specificity. Given that application of the 2017 and 2010 criteria excludes children with ADEM at baseline, MOG status in these patients would not influence criteria performance. It is noteworthy that of the 57 MOG positive non-ADEM patients (i) 17 met 2017 criteria DIS; (ii) 3 met DIT as defined by gadolinium-enhancing and non-enhancing lesions; (iii) 8 met DIT when CSF OCBs were allowed to contribute to the adjudication; and only (iv) 7 (12%) would have met both DIS and DIT at baseline (3 of whom met our gold standard for MS, and 4 who have remained monophasic). Of all 99 MOG positive patients, 16 have experienced clinical relapses (Figure 4).

Based on the minimal impact of MOG results on the performance of the 2017 criteria, we do not advocate including MOG testing as a component of the 2017 criteria. Just as is now clinical practice, however, we do endorse testing for MOG and AQP4 antibodies in children with appropriate clinical manifestations, and particularly in children with relapsing disease more consistent with NMOSD, chronic relapsing optic neuritis or ADEM followed by optic neuritis.



Our study has several limitations. We employed a subjective scoring tool. As such, decisions regarding image quality (particularly of the cervical spine on brain scans) and detection of subtle lesions could influence accuracy. Spinal cord and orbital images were largely acquired in children with referable symptoms. Unbiased assessment of lesion frequency would require orbital, brain and spine MRI on all children, which would be difficult to justify from a cost and patient tolerability perspective. Inclusion of the optic nerve as contributory to DIS could also be adjudicated using optical coherence tomography or visual evoked potentials, which were not available for our study cohort. The role of such investigations in supporting the diagnosis of MS has been recognized as a high priority research area by the Panel of 2017 McDonald Criteria (Thompson et al, *in press*). We did not have CSF or serum analyses for all participants, a limitation that is inherent in studies that recruit children after clinical identification. While our research protocol leads to procurement of serum samples over time, we restricted our present analyses only to samples obtained proximate to clinical presentation, as would be mirrored in clinical practice. Strengthening our analyses is the non-biased sampling, as baseline samples were obtained irrespective of clinical presentation.

The 2017 McDonald criteria perform well in children, supporting the applicability of these criteria across the age-span. The new McDonald criteria have the advantage of being considerably simpler compared to previous iterations. We also confirm that, at the time of an incident attack, the presence of one or more T1 hypointense lesions and T2 periventricular lesions is strongly associated with a diagnosis of MS in children. These latter criteria perform as well as the McDonald 2017 criteria and may be of particular utility when there are contraindications for gadolinium contrast imaging (which is valuable given concerns regarding intracerebral accumulation of gadolinium<sup>23</sup>) and when CSF OCBs are not available. Testing for MOG antibodies does not improve performance of diagnostic criteria, but is relevant in the evaluation of children with relapsing disease atypical for MS.

## CONTRIBUTORS (could you put me into the contributors where you see fit please).

GF, RB and BB were responsible for study design, content and writing of the report. GF, RB, GL, DC, JO'M, LV, HMB and BB were responsible for data collection. RB was responsible for data analysis. RAM provided input on statistical analysis. BB, AB-O, DLA, EAY and RAM were principal investigators for the Canadian Pediatric Demyelinating Disease Study, and obtained operating grant funding. SN was responsible for the MRI protocol at all sites. BB, AB-O, DLA, EAY, RAM, LV, HMB, and SN provided editorial comments on the final report. All authors approved the final version.

## CONFLICTS OF INTEREST

AB-O, DLA, EAY, RAM, and BB served as lead investigators, and funds from the study grant (Canadian Multiple Sclerosis Scientific Research Foundation) have supported work done at their institutions. None of the investigators receive personal salary support from the study sponsor. Funds from the study grant have supported travel for presentation at national and international meetings. DC, LHV and JO'M, and HMB have nothing to disclose. G. Fadda has received personal compensation for consulting services from Atara Biotherapeutics INC. R. A. Brown has received personal compensation for consulting services from Biogen Idec and NeuroRx Research. G. Longoni receives training and research support from the National Multiple Sclerosis Society. R. A. Marrie: receives research funding from Canadian Institutes of Health Research, Research Manitoba, Multiple Sclerosis Society of Canada, Multiple Sclerosis Scientific Foundation, National Multiple Sclerosis Society, Rx & D Health Research Foundation, the Waugh Family Chair in Multiple Sclerosis, Crohn's and Colitis Canada, and has conducted clinical trials funded by Sanofi- Aventis. E. A. Yeh has received funds from NMSS, CIHI, CIHR, OIRM, MS Society of Canada, Mario Battaglia Foundation, SickKids Foundation, CBMH Innovation Fund, CMSC, Rare Diseases Foundation and Guthy Jackson Foundation. She serves as a relapse adjudicator for ACI. She has served on a scientific advisory panel for Juno Therapeutics and has received a speaker's honorarium from Novartis. S. Narayanan received a speaker's honorarium from Novartis Canada and

consulting fees from NeuroRx Research for work unrelated to this study. D. Arnold has served on advisory boards, received speaker honoraria, served as a consultant, or received research support from Adelphi, Biogen, Celgene, Genentech, Genzyme, Medday, NeuroRx Research, Novartis, Pfizer, Receptos, Roche, Sanofi, the Canadian Institutes of Health Research, and the Multiple Sclerosis Society of Canada; and holds stock in NeuroRx Research. P. Waters is a named inventor on patents for antibody assays and has received royalties. He has received consulting or speaking fees from Biogen Idec, Euroimmun AG, and Mereo Biopharma and travel grants from the Guthy-Jackson Charitable Foundation. A. Bar-Or has participated as a speaker in meetings sponsored by and received consulting fees and/or grant support from Biogen Idec, Diogenix, Roche/Genentech, Sanofi-Genzyme, GlaxoSmithKline, Medimmune, Novartis, Ono Pharma, Teva Neuroscience, Celgene/Receptos Inc, and Merck/EMD Serono. B. Banwell serves as a central MRI reviewer for Novartis in the context of a clinical trial, and as a non-remunerated advisor on clinical trial design to Sanofi-Aventis, Novartis, Biogen-IDEC, and Teva Neuroscience. Dr. Banwell has received grant support as listed above, as well as from the MS Society of Canada, CIHR, NIH, and NMSS.

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**Table 1: Rationale and Participant Inclusion for each analysis**

Analysis	Objectives	Participants contributing to analysis	Participants not contributing to the analysis	Comments
<b>Baseline lesion frequency on brain MRI</b>				
Baseline lesion frequency on brain MRI <sup>1</sup>	To define the pattern of lesions in ADS  To compare baseline lesion patterns in MS, relapsing non-MS and monoADS	324 with T2/FLAIR scans  297 with Gd+ scans		Goal was to include all participants as reflective of the ADS population- not only those with all sequences acquired, since provision of Gd and standardized sequences are not universal in clinical practice
Upper cervical spine lesion frequency on brain MRI	To define frequency of spinal lesions	244	80 without adequate visualization of C-spine on brain MRI	Referral bias exists as spine MRI ordered primarily in patients with symptoms referable to the spinal cord
Spinal cord lesion frequency on baseline spine MRI	To estimate the contribution of spinal lesions to MS diagnosis	140	184 without spine MRI	
Optic nerve T2 lesion frequency on orbital MRI scans <sup>2</sup>	To define frequency of optic nerve lesions	47	277 without fat-sat orbit dedicated scans	Referral bias exists as ON study was ordered primarily in patients with symptoms referable to the ON
Optic nerve Gd enhancement frequency detected on orbital MRI scans	To estimate the contribution of optic nerve lesions to DIS criteria (per MAGNIMS)	43	277 without orbit dedicated scans <b>plus</b> 4 without Gd administered at baseline	
CSF OCB frequency	To define frequency of +OCBs  To determine contribution of +OCBs to MS diagnosis	192	132 without CSF study at baseline	CSF studies were ordered by treating clinicians and all CSF samples were run using isoelectric focusing at the local hospitals
<b>Performance of diagnostic criteria</b>				
Adjudication of DIS <sup>3</sup>	To determine the performance of DIS criteria	225	22 enrolled after 2014 <sup>4</sup> <b>plus</b> 77 with ADEM presentation <sup>5</sup>	We elected to restrict analysis of DIS to participants with appropriate clinical presentations and for whom full criteria (DIS and DIT) could be applied, as would be done in clinical practice
Adjudication of DIT/full criteria <sup>3</sup>	To determine the performance of DIT/full diagnostic criteria	211	22 enrolled after 2014 <b>plus</b> 77 with ADEM presentation <b>plus</b> 14 without Gd administered at baseline	Same requirements of DIS <b>plus</b> requirement of enhanced images

Additional Analyses				
Impact of age at ADS on 2017, 2010 and Verhey criteria performance	To determine whether criteria should be applied prior to age 11 years	211	22 enrolled after 2014 <b>plus</b> 77 with ADEM presentation <b>plus</b> 14 without Gd administered at baseline	Given that the 2010 criteria explicitly caution application in patients under age 11 years, we evaluated criteria performance in younger children
ADEM analyses: Adjudication of DIS by all criteria  Adjudication of DIT/full criteria	To determine how the criteria perform when applied in all participants, including those presenting with ADEM	302  276	22 enrolled after 2014  26 without Gd administered at baseline	Although ADEM is a clinical diagnosis as defined by international criteria, some children with MS and relapsing non-MS manifest with ADEM at onset. We evaluated criteria performance both within ADEM alone, and when ADEM participants were included in the entire analysis
MOG antibody frequency	To compare MOG status as a function of clinical presentation	279	45	Emerging evidence supports MOG status as a relative predictor of non-MS.
Contribution of anti-MOG antibodies to the performance of McDonald 2017 and 2010, and Verhey criteria	To determine whether consideration of MOG status at baseline alters performance of 2010, 2017, and Verhey criteria	131	22 enrolled after 2014 <b>plus</b> 77 with ADEM presentation <b>plus</b> 14 without Gd administered at baseline <b>plus</b> 23 not tested for anti-MOG <b>plus</b> 57 anti-MOG positives	Given that MOG testing will be commercially available in many countries, we define whether restricting the application of MS diagnostic criteria to “MOG-” patients improves their performance
Validation of Verhey criteria	To validate previous criteria in an independent cohort	50	22 enrolled after 2014 <b>plus</b> 77 pre-2014 with ADEM presentation <b>plus</b> 14 without Gd administered at baseline <b>plus</b> 175 subjects included in prior study (Verhey, 2011)	Restricted to participants enrolled after the previous study and prior to 2014

<sup>1</sup>All MRI scans performed within 30 days from clinical onset (baseline scans) were assessed for the parameters listed in Panel 1. We considered an individual parameter to be “not available” for scoring if the sequence(s) required to adjudicate the parameter were not available (i.e., gadolinium enhanced scans). The total number of T2 lesions was determined manually; lesion numbers exceeding 15 were listed as “greater than 15”.

<sup>2</sup>To identify optic nerve lesions, a lesion was required to be visible either on a dedicated fat-saturated T2 or gadolinium enhanced T1 image. Participants whose baseline imaging did not include dedicated orbital images did not contribute to this analysis.

<sup>3</sup>To compare the performance of the DIS aspect of the 2001, 2010, 2017 McDonald and the Verhey criteria, we limited the analysis to participants able to contribute to all of these criteria evaluation- and thus excluded those with ADEM and those enrolled after 2014. To compare the performance of the DIT aspect of the 2001, 2010 and 2017 McDonald criteria, we restricted our analyses to the same participants as evaluated for DIS provided that they also had Gd-enhanced images.

<sup>4</sup>Participants enrolled after 2014 were required, by the study methods in place, to meet the 2010 criteria for enrolment. As such, these individuals were not felt to be appropriate for evaluation of criteria performance, as they were already pre-selected for key features that are shared between the 2010 and 2017 criteria.

<sup>5</sup>Both the 2010 and 2017 criteria specify that the criteria should not be applied at baseline presentation in the context of ADEM.

**Abbreviations:** ADEM, acute disseminated encephalomyelitis; CSF, cerebrospinal fluid; DIS, dissemination in space; DIT, dissemination in time; fat-sat, fat saturated images as required to optimally visualize the orbital nerves; Gd, gadolinium; Gd+, positive for enhancing lesions after administration of gadolinium; MOG, myelin oligodendrocyte glycoprotein; OCB, oligoclonal bands

Table 2: Clinical and demographic characteristics

	<b>Overall (n 324)</b>	<b>MS (n 71)</b>	<b>Non-MS (n 253 )</b>	<b>p value</b>
<b>Length of clinical observation (years)</b> (IQR) (min-max)	6.03 (3.95 – 8.15) (0.50 – 12.39)	6.35 (3.13 – 9.21) (0.51 – 12.13)	6.00 (3.96 – 7.99) (0.50 – 12.39)	0.23
<b>Age at onset (years)</b> (IQR) (min-max)	10.43 (5.72 – 13.63) (0.46 – 17.87)	14.00 (10.93 – 15.39) (1.90 – 17.86)	8.93 (4.62 – 12.39) (0.46 – 17.87)	<0.0001
<b>Age at onset, n (%)</b> <11 years ≥11 years	176 (46) 148 (54)	18 (25) 53 (75)	158 (62) 95 (38)	<0.0001
<b>Age at 2<sup>nd</sup> clinical attack (if occurred)</b> (IQR) (min-max)	14.97 (11.96 – 16.30) (3.20 – 20.30)	15.04 (12.52 – 16.44) (3.20 – 20.30)	10.89 <sup>1</sup> (8.20 – 15.06) (3.29 – 16.80)	0.05
<b>Time to 2<sup>nd</sup> clinical attack (years)</b> (IQR) (min-max)	0.74 (0.36 – 1.36) (0.08 – 4.88)	0.77 (0.37 – 1.41) (0.08 – 4.88)	0.53 <sup>1</sup> (0.27 – 1.25) (0.08 – 1.92)	0.51
<b>Age at first new MRI lesion (years)</b> (IQR) (min-max)	13.97 (10.90 – 15.67) (1.50 – 21.81)	14.61 (12.07 – 15.76) (4.51 – 18.11)	10.93 (5.18 – 13.05) (1.50 – 21.81)	0.0014
<b>Time to first new MRI lesion (years)</b> (IQR) (min-max)	0.32 (0.25 – 0.63) (0.09 – 6.00)	0.29 (0.25 – 0.55) (0.09 – 3.21)	0.95 (0.33 – 1.80) (0.10 – 6.00)	0.0036
Female, n (%)	168 (51.85)	51 (71.83)	117 (46.25)	<0.0001
<b>Basic Presenting Phenotype, n (%)</b> ADEM Monofocal Polyfocal	80 (25) 195 (60) 49 (15)	0 (0) 48 (68) 23 (32)	80 (32) 147 (58) 26 (10)	<0.0001
ON <sup>2</sup> , n (%) TM <sup>2</sup> , n (%) ON + TM <sup>2</sup> , n (%)	91 (28) 95 (29) 8 (2)	19 (31) 10 (14) 2 (3)	72 (28) 85 (34) 6 (2)	0.116

Continuous variables are presented as median (IQR) (min-max) and categorical variables are presented as n (%)

<sup>1</sup>The time to age at second attack, time to second attack, age at first new lesion and time to first new MRI lesion, for the non-MS group refers patients with relapsing non-MS.

<sup>2</sup>Patients with ADEM may also have experienced ON or TM, or both in conjunction with encephalopathy and possible other deficits. Patients with ON or TM are included in the monofocal category if their symptoms were restricted to the optic nerve or spinal cord, in the polyfocal category if their ON or TM occurred in the context of other neurological deficits.

**Abbreviations:** ADS= acquired demyelinating syndromes, CNS=central nervous system; MS=multiple sclerosis, Non-MS= includes children with monophasic ADS, relapsing non-MS, and the three children diagnosed more than 30 days from onset with CNS vasculitis.



Table 3: Baseline MRI features in children with CNS demyelination

Feature <sup>1</sup>	MS All	MS Clinical <sup>2</sup>	MS MRI <sup>3</sup>	Non-MS All <sup>4</sup>	Non-MS Non ADEM <sup>5</sup>	ADEM <sup>6</sup>	MS All vs non-MS All	Sens.	Spec.	PPV	NPV	Import <sup>7</sup> .
≥ 1 T1 lesion present (Black Hole)	63/71 (89%)	44/48 (92%)	19/23 (83%)	28/251 (11%)	15/172 (9%)	13/79 (16%)	$2.3 \times 10^{-22}$	0.69 (0.59,0.78)	0.97 (0.93,0.98)	0.89 (0.79,0.95)	0.89 (0.84,0.92)	1
Perpendicular to major axis of corpus callosum	49/71 (69%)	33/48 (69%)	16/23 (70%)	22/253 (9%)	12/173 (7%)	10/80 (12%)	$1.9 \times 10^{-20}$	0.69 (0.57,0.79)	0.91 (0.87,0.94)	0.69 (0.57,0.79)	0.91 (0.87,0.94)	2
≥ 1 Contrast enhancing lesion	45/64 (70%)	28/43 (65%)	17/21 (81%)	21/233 (9%)	9/165 (5%)	12/68 (18%)	$5.6 \times 10^{-19}$	0.68 (0.56,0.79)	0.92 (0.87,0.95)	0.70 (0.58,0.81)	0.91 (0.87,0.94)	3
PV N = 0	11/71 (15%)	8/48 (17%)	3/23 (13%)	184/253 (73%)	149/173 (86%)	35/80 (44%)	$6.5 \times 10^{-14}$	0.06 (0.03,0.10)	0.53 (0.45,0.62)	0.15 (0.08,0.26)	0.27 (0.22,0.33)	4
Oligoclonal bands	31/44 (70%)	19/29 (66%)	12/15 (80%)	22/148 (15%)	15/109 (14%)	7/39 (18%)	$8.9 \times 10^{-11}$	0.58 (0.44,0.72)	0.91 (0.85,0.95)	0.70 (0.55,0.83)	0.85 (0.78,0.90)	5
Anti-MOG antibodies	8/57 (14%)	6/39 (15%)	2/18 (11%)	91/222 (41%)	52/150 (35%)	39/72 (54%)	0.00035	0.14 (0.06,0.26)	0.59 (0.52,0.66)	0.08 (0.04,0.15)	0.73 (0.66,0.79)	6
Intracallosal lesion	34/71 (48%)	25/48 (52%)	9/23 (39%)	27/253 (11%)	12/173 (7%)	15/80 (19%)	$7 \times 10^{-11}$	0.56 (0.42,0.68)	0.86 (0.81,0.90)	0.48 (0.36,0.60)	0.89 (0.85,0.93)	7
≥ 15 T2 Lesions	34/71 (48%)	19/48 (40%)	15/23 (65%)	70/253 (28%)	17/173 (10%)	53/80 (66%)	0.0015	0.33 (0.24,0.43)	0.83 (0.78,0.88)	0.48 (0.36,0.60)	0.72 (0.66,0.78)	8
PV N = 2	11/71 (15%)	7/48 (15%)	4/23 (17%)	12/253 (5%)	7/173 (4%)	5/80 (6%)	0.0032	0.48 (0.27,0.69)	0.80 (0.75,0.84)	0.15 (0.08,0.26)	0.95 (0.92,0.98)	9
Gyral projection	6/71 (8%)	5/48 (10%)	1/23 (4%)	27/253 (11%)	6/173 (3%)	21/80 (26%)	0.59	0.18 (0.07,0.35)	0.78 (0.72,0.82)	0.08 (0.03,0.17)	0.89 (0.85,0.93)	10
Other Cerebral white matter lesion	58/71 (82%)	38/48 (79%)	20/23 (87%)	85/253 (34%)	35/173 (20%)	50/80 (62%)	$7.6 \times 10^{-11}$	0.41 (0.32,0.49)	0.93 (0.88,0.96)	0.82 (0.71,0.90)	0.66 (0.60,0.72)	11
Infratentorial	44/71 (62%)	29/48 (60%)	15/23 (65%)	105/253 (42%)	42/173 (24%)	63/80 (79%)	0.0026	0.30 (0.22,0.38)	0.85 (0.78,0.90)	0.62 (0.50,0.73)	0.58 (0.52,0.65)	12
PV N > 3	33/71 (46%)	22/48 (46%)	11/23 (48%)	30/253 (12%)	6/173 (3%)	24/80 (30%)	$1.3 \times 10^{-9}$	0.52 (0.39,0.65)	0.85 (0.81,0.89)	0.46 (0.35,0.59)	0.88 (0.84,0.92)	13
Thalamic lesion	11/71 (15%)	4/48 (8%)	7/23 (30%)	64/253 (25%)	15/173 (9%)	49/80 (61%)	0.087	0.15 (0.08,0.25)	0.76 (0.70,0.81)	0.15 (0.08,0.26)	0.75 (0.69,0.80)	14
≥ 1 Non-enhancing lesion	55/64 (86%)	35/43 (81%)	20/21 (95%)	126/233 (54%)	62/165 (38%)	64/68 (94%)	$1.7 \times 10^{-5}$	0.30 (0.24,0.38)	0.92 (0.86,0.96)	0.86 (0.75,0.93)	0.46 (0.39,0.53)	15
Juxtacortical	55/71 (77%)	36/48 (75%)	19/23 (83%)	96/253 (38%)	33/173 (19%)	63/80 (79%)	$3.2 \times 10^{-8}$	0.36 (0.29,0.45)	0.91 (0.85,0.95)	0.77 (0.66,0.87)	0.62 (0.56,0.68)	16
Internal capsule lesion	19/71 (27%)	12/48 (25%)	7/23 (30%)	42/253 (17%)	11/173 (6%)	31/80 (39%)	0.055	0.31 (0.20,0.44)	0.80 (0.75,0.85)	0.27 (0.17,0.39)	0.83 (0.78,0.88)	17
>9 T2 lesions	43/71 (61%)	28/48 (58%)	15/23 (65%)	84/253 (33%)	24/173 (14%)	60/80 (75%)	$4.7 \times 10^{-5}$	0.34 (0.26,0.43)	0.86 (0.80,0.90)	0.61 (0.48,0.72)	0.67 (0.61,0.73)	18
Spine	17/24 (71%)	11/15 (73%)	6/9 (67%)	89/116 (77%)	65/80 (81%)	24/36 (67%)	0.54	0.16 (0.10,0.24)	0.79 (0.62,0.91)	0.71 (0.49,0.87)	0.23 (0.16,0.32)	19
Optic nerve T2 hyperintensity	8/13 (62%)	3/7 (43%)	5/6 (83%)	19/34 (56%)	18/31 (58%)	1/3 (33%)	0.73	0.30 (0.14,0.50)	0.75 (0.51,0.91)	0.62 (0.32,0.86)	0.44 (0.27,0.62)	20
PV N = 1	9/71 (13%)	8/48 (17%)	1/23 (4%)	16/253 (6%)	8/173 (5%)	8/80 (10%)	0.082	0.36 (0.18,0.57)	0.79 (0.74,0.84)	0.13 (0.06,0.23)	0.94 (0.90,0.96)	21
Basal ganglia lesion	7/71 (10%)	2/48 (4%)	5/23 (22%)	47/253 (19%)	10/173 (6%)	37/80 (46%)	0.087	0.13 (0.05,0.25)	0.76 (0.71,0.81)	0.10 (0.04,0.19)	0.81 (0.76,0.86)	22
PV N = 3	7/71 (10%)	3/48 (6%)	4/23 (17%)	12/253 (5%)	3/173 (2%)	9/80 (11%)	0.11	0.37 (0.16,0.62)	0.79 (0.74,0.83)	0.10 (0.04,0.19)	0.95 (0.92,0.98)	23
Diencephalic lesion	10/71 (14%)	6/48 (12%)	4/23 (17%)	38/253 (15%)	8/173 (5%)	30/80 (38%)	0.84	0.21 (0.10,0.35)	0.78 (0.73,0.83)	0.14 (0.07,0.24)	0.85 (0.80,0.89)	24
≥ 1 T2 lesion present	67/71 (94%)	44/48 (92%)	23/23 (100%)	151/253 (60%)	73/173 (42%)	78/80 (98%)	$4.8 \times 10^{-6}$	0.31 (0.25,0.37)	0.96 (0.91,0.99)	0.94 (0.86,0.98)	0.40 (0.34,0.47)	25
Optic nerve contrast enhancement	4/11 (36%)	2/6 (33%)	2/5 (40%)	16/32 (50%)	15/30 (50%)	1/2 (50%)	0.44	0.20 (0.06,0.44)	0.70 (0.47,0.87)	0.36 (0.11,0.69)	0.50 (0.32,0.68)	26

<sup>1</sup>Data include the number and proportion of patients in each group demonstrating a given MRI feature on baseline scans using sequences evaluable for the given MRI parameter. Panel 1 defines each of the MRI parameters.

<sup>2</sup>**MS clinical** includes all patients who experienced two or more MS attacks

<sup>3</sup> **MS MRI** includes patients diagnosed with MS on the basis of accrual of new lesions on serial imaging in the absence of a second clinical attack to date

<sup>4</sup>**Non-MS all** includes children with monophasic ADS including those with ADEM, relapsing non-MS, and the three children diagnosed more than 30 days from onset with CNS vasculitis

<sup>5</sup>**Non-MS non-ADEM** excludes children with ADEM at onset as well as all children diagnosed with MS

<sup>6</sup>**ADEM** includes all children presenting with encephalopathy and polyfocal deficits, as per international diagnostic criteria. As this analysis was based solely on comparison of baseline MRI features, the ADEM group includes the 8 children who presented with ADEM but were subsequently diagnosed with MS, the 4 children with ADEM at onset followed by relapses not conforming to a diagnosis of MS, and 1 of the children subsequently diagnosed with CNS vasculitis.

<sup>7</sup>**Import.** refers to the ranking from most to least important, obtained through reverse elimination using the random forest classifier, of a given variable in distinguishing MS from non-MS all

<sup>8</sup>Spinal lesions were detected in 112 participants, 106 by spinal MRI, 6 in the cervical spine visualized on brain MRI. Longitudinally extensive lesions (>3 spinal segments in length) were present in 66 children, nearly all of whom were non-MS.

<sup>9</sup>Only 2 children had clinically silent optic nerve lesions, all of the remaining patients with optic nerve lesions had clinical optic neuritis.

<sup>10</sup>CSF OCBs were evaluable at baseline in 192 (59%) of the participants. CSF OCBs were positive in 70% of the 44 MS patients evaluable (median age of the OCB negative MS patients was of 11.75 years (IQR 2.55), median age of the OCB positive MS patients was 14.06 years (IQR 2.44). OCBs were present in 18% of the children with monophasic ADEM.

**Abbreviations:** ADEM=acute disseminated encephalomyelitis, ADS= acquired demyelinating syndromes, CNS=central nervous system; MS=multiple sclerosis; NPV=negative predictive value; PPV=positive predictive value, sens=sensitivity; spec=specificity.

Table 4: Evaluation of MS diagnostic criteria performance

Criteria <sup>1</sup>	MS All N= 57	MS Clinical N= 41	MS MRI N= 16	Non-MS Non-ADEM <sup>4</sup> N=168	Sensitivity	Specificity	PPV	NPV
<b>Baseline DIS Criteria</b>								
<b>McDonald2017</b>	46/57 (81%)	34/41 (83%)	12/16 (75%)	34/168 (20%)	0·81 (0·68,0·90)	0·80 (0·73,0·86)	0·57 (0·46,0·68)	0·92 (0·73,0·86)
<b>McDonald2010</b>	45/57 (79%)	33/41 (80%)	12/16 (75%)	34/168 (20%)	0·79 (0·66,0·89)	0·80 (0·73,0·86)	0·57 (0·45,0·68)	0·92 (0·86,0·96)
<b>MAGNIMS<sup>5</sup></b>	47/57 (82%)	34/41 (83%)	13/16 (81%)	41/168 (24%)	0·82 (0·70,0·91)	0·76 (0·68,0·82)	0·53 (0·42,0·63)	0·93 (0·87,0·96)
<b>MAGNIMS- no ON<sup>6</sup></b>	45/57 (79%)	32/41 (78%)	13/16 (81%)	37/168 (22%)	0·79 (0·66,0·89)	0·78 (0·71,0·84)	0·55 (0·43,0·66)	0·92 (0·86,0·96)
<b>McDonald2001</b>	45/57 (79%)	32/41 (78%)	13/16 (81%)	26/168 (15%)	0·79 (0·66,0·89)	0·85 (0·78,0·90)	0·63 (0·51,0·75)	0·92 (0·87,0·96)
<b>Baseline DIT Criteria</b>								
<b>McDonald2017</b>	39/51 (76%)	24/36 (67%)	15/15 (100%)	9/160 (6%)	0·76 (0·63,0·87)	0·94 (0·90,0·97)	0·81 (0·67,0·91)	0·93 (0·87,0·96)
<b>McDonald2010 or MAGNIMS<sup>7</sup></b>	30/51 (59%)	19/36 (53%)	11/15 (73%)	5/160 (3%)	0·59 (0·44,0·72)	0·97 (0·93,0·99)	0·86 (0·70,0·95)	0·88 (0·82,0·92)

<sup>1</sup>Data include the number and proportion of patients meeting the various criteria. All participants were required to have gadolinium-enhanced images at baseline in order to enter into the analysis for DIT. Criteria performance was evaluated as MS-All vs non-MS non-ADEM

<sup>2</sup>MS clinical includes all patients who experienced two or more MS attacks

<sup>3</sup> MS MRI includes patients diagnosed with MS on the basis of accrual of new lesions on serial imaging in the absence of a second clinical attack to date

<sup>4</sup>Non-MS non-ADEM excludes children with ADEM at onset as required by the 2017 and 2010 criteria

<sup>5</sup>MAGNIMS criteria were evaluated allowed clinical optic neuritis and/or optic nerve swelling, enhancement or T2 lesions in the optic nerve (even in the absence of clinical symptoms) to count as one site in the DIS component.

<sup>6</sup>MAGNIMS-no ON included MRI evidence of optic nerve involvement as one of the sites for DIS, but did not count clinical optic neuritis unless MRI supported optic nerve lesions.

<sup>7</sup> The criteria for DIT are identical for the 2010 criteria and the MAGNIMS criteria. The 2001 criteria do not permit adjudication of DIT using baseline data alone.

**Abbreviations:** ADEM=acute disseminated encephalomyelitis, MS=multiple sclerosis; NPV=negative predictive value; PPV=positive predictive value.

### Legend Figure 1:

All 451 consented study participants were evaluated for eligibility for the present analysis; 29 were excluded due to a diagnosis of non-MS being established within 30 days of presentation, including 4 with NMOSD. AQP4 antibodies were absent in all of the remaining participants tested (n = 264). MRI studies for the 422 eligible participants were evaluated by date of acquisition. 85 participants with first brain MRI scans obtained more than 30 days from presentation 12 with less than 6 months of follow up were excluded. As of the date of most recent evaluation, 71 participants were diagnosed with MS (*termed MS-All*); 48 of whom have experienced 2 or more attacks (*MS-Clinical*); and 23 diagnosed on the basis of accrual of clinical silent lesions on serial images but who have yet to experience a second attack (*MS-MRI*). Of the remaining 253 participants, 237 have experienced a single ADS attack without clinical or MRI evidence of new lesions (*monophasic ADS*); 14 have experienced more than one attack, but without involvement of new regions of the CNS (*Relapsing non-MS*), and 2 children were diagnosed with CNS vasculitis more than 30 days after presentation and were retained given that “no better explanation” for their initial illness was determined at the time of inclusion. Given that the 2010 and 2017 diagnostic criteria specify that baseline MRI features can not be used to determine a diagnosis of MS in children presenting with ADEM, we further divided the *non-MS* group: 173 with *non-MS-nonADEM* and 80 with *non-MS-ADEM*.

Abbreviations: ADEM; acute disseminated encephalomyelitis, ADS; acquired demyelinating syndromes, AQP4; aquaporin 4, CNS; central nervous system, MRI; magnetic resonance imaging, MS; multiple sclerosis, NMOSD; neuromyelitis optica spectrum disorder.

## **Legend Figure 2: MRI Diagnostic Criteria Performance in Children**

Dissemination in space (DIS) and in time (DIT) were adjudicated in each subject first separately, and then combined for the evaluation of the full criteria. The 2017 criteria permit cortical lesions to contribute as 'juxtacortical-cortical'; however, given that most MRI studies were performed at 1.5T we were not able to adequately delineate cortical lesions. Since the 2001 McDonald criteria did not allow for the adjudication of DIT at baseline, these criteria were applied only for the evaluation of DIS.

All participants were required to have gadolinium-enhanced brain MRI sequences at baseline to permit full adjudication of all criteria. Participants enrolled after 2014 were required to meet 2010 criteria as per study enrollment protocol and were excluded. The 2010 and 2017 criteria explicitly exclude application of the criteria at baseline in the context of ADEM, thus the 80 children presenting with ADEM (72 monophasic, 7 relapsing non-MS, and 1 with CNS vasculitis) were excluded from the comparisons. Of the 71 MS participants, 51 had gadolinium enhancing imaging and thus could be evaluated for all of the criteria. Of the 253 participants not diagnosed with MS, 160 had gadolinium enhanced baseline imaging and presented with a non-ADEM phenotype.

**MAGNIMS** criteria were evaluated allowing clinical optic neuritis and/or optic nerve enhancement or T2 lesions in the optic nerve (even in the absence of clinical symptoms) to count as one site in the DIS component.

The **Top five features** represent the five parameters identified by the random forest classifier as best distinguishing MS from non-MS (each of: periventricular, gadolinium-enhancing, T1 hypointense lesions, CSF OCBs, and negative for serum anti-MOG antibodies, the latter two evaluated when available), excluding the parameter perpendicular lesions as discussed.

**MS clin** includes all patients who experienced two or more MS attacks

**MS MRI** includes patients diagnosed with MS on the basis of accrual of new lesions on serial imaging in the absence of a second clinical attack to date

**Non-MS** excludes children with ADEM at onset.

**Abbreviations:** ADEM=acute disseminated encephalomyelitis, MOG=myelin oligodendrocyte glycoprotein; MS=multiple sclerosis; NPV=negative predictive value; PPV=positive predictive value: Sens= sensitivity; Spec=specificity.

**Legend Figure 3: Comparison of Diagnostic Criteria Performance in Children over and below 11 years of age.**

The diagnostic performance of 2010 and 2017 McDonald and Verhey criteria was compared between children younger than 10 years 11 months of age and children older than 11 years. Specificity and negative predictive value (NPV) were comparably high irrespective of age for all the criteria. The sensitivity was lower in children younger than 11 years, although the difference was not statistically significant. Both 2010 and 2017 criteria reported lower positive predictive value (PPV) in younger children, likely driven by both the lower prevalence of MS in this age group and thus the inherently lower pre-test probability of MS.

**Legend Figure 4: MOG antibody status and clinical presentation**

Figure 4 delineates the frequency of MOG antibodies, as measured in serum samples obtained within 1.5 months of presentation, as a function of clinical features. Ninety-nine of 279 tested participants were considered MOG positive, including 8 children meeting our gold standard criteria for MS. As expected, MOG antibodies were common in children with ADEM (36/67 tested, 54%) and in children with relapsing non-MS (10/13 tested, 77%).

## Panel 1: MRI Scoring Tool- Revised Version

Detailed parameter definitions, including an atlas have been included in our prior work.<sup>1</sup> All parameters are binary (i.e. 'present'/'absent'). T2 areas of hyperintensity were required to exceed 3 mm in minimal cross-sectional area in at least one plane to be considered as a potential lesion. A 1 mm or greater boundary between adjacent areas of T2 hyperintensity was required to adjudicate lesions as distinct.

1. **Bilateral Lesion Distribution:** T2 lesions on either side of, or spanning across, the midline in the supratentorial or infratentorial regions
2. **Gyral Projection:** T2 lesion continuously projecting from subcortical white matter at the depth of a sulcus into a gyrus, extending to abut the cortical ribbon at the gyral apex
3. **T1 Hypointensity:** T1 lesion present at incident demyelinating event with all or a portion of the lesion being hypointense to cortical grey matter; should be hyperintense on T2-weighted images
4. **Lesional Contrast Enhancement:** nodular or ring-like hyperintense signal on T1-weighted post-contrast corresponding to a lesion with increased intensity of T2-weighted sequences; not hyperintense on T1-weighted pre-contrast scan
5. **Periventricular Lesion:** white matter T2 lesion abutting any portion of the lateral ventricles; lesions involving the corpus callosal white matter are included, but deep grey matter lesions are excluded.

6. **Periventricular (PV) Lesion counts:** the number of periventricular lesions (PV) were adjudicated as PV=0, PV=1, PV=2, PV=3, or PV>3.
7. **Other cerebral White Matter Lesion:** supratentorial non-juxtacortical and non-periventricular white matter T2 lesion; excludes intracallosal lesions
8. **Juxtacortical Lesion:** supratentorial white matter T2 lesion contiguous with the cortical ribbon, i.e. involves subcortical U-fibers
9. **Intracallosal Lesion:** T2 lesion contained entirely within the margins of the corpus callosum with at least 1 mm of normal appearing white matter surrounding lesion
10. **Thalamic Lesion:** T2 lesion either entirely or partially contained within the thalamus; bi-thalamic lesions counted as two discrete lesions
11. **Basal Ganglia Lesion:** T2 lesion either entirely or partially contained within the caudate (includes head and tail), putamen, or globus pallidus (includes interna and externa)
12. **Diencephalic lesion:** T2 lesion involving the hypothalamus or epithalamus
13. **Internal Capsule Lesion:** T2 lesion centered in the anterior or posterior limb of the internal capsule



14. **Brainstem Lesion:** T2 lesion within the brainstem, which extends from the most inferior aspect of the medulla oblongata (at the level of the pyramidal decussation) to the most superior portion of the midbrain (at the level of the red nuclei); posterior anatomical limits defined in our illustrated appendix.
15. **Cerebellar Lesion:** T2 lesion involving the white or grey matter of any of the following: cerebellar white matter and cortices, dentate nuclei, vermis, flocculus; anterior anatomical limits of the cerebellum.
16. **Cerebellar Peduncle Lesion:** T2 lesion involving the superior or middle cerebellar peduncles.
17. **Tumefactive Lesion:** T2 lesion exceeding 20 mm in maximal cross-sectional diameter, associated with perilesional edema and regional mass effect on adjacent structures.
18. **Lesion perpendicular to the major axis of the corpus callosum:** ovoid T2 lesions with the long axis of the lesion perpendicular to the long axis of the corpus callosum.

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