MRI in the Prediction and Diagnosis of Pediatric-Onset Multiple Sclerosis: Insights from Children with Incident CNS Demyelination

by

Leonard Herman Verhey

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Institute of Medical Science
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Abstract

An acute demyelinating syndrome (ADS) in a child may be a monophasic illness or may represent the incident attack of multiple sclerosis (MS) – an inflammatory demyelinating neurodegenerative disorder affecting the brain, spinal cord and optic nerves. The central objective of this dissertation was to identify MRI parameters present at ADS that predict MS diagnosis. A scoring tool was first created containing 14 parameters identified from the literature and demonstrating substantial inter-rater agreement (Cohen's kappa values ≥0.6). Children aged <16 years were enrolled at incident ADS and are currently followed for five years at 23 Canadian centers. Standardized MRI scans were acquired at onset and serially. MS was defined based on the occurrence of a second demyelinating attack or MRI evidence of new lesions in accordance with McDonald criteria for dissemination in time. Multivariable Cox proportional hazards regression models were used to identify MRI parameters that predicted MS diagnosis. Over 1100 MRI scans in 284 children with ADS were evaluated. To date, 57(20%) children have been diagnosed with MS. For those that developed MS, the median (IQR) time

from incident attack to diagnosis was 6.2 (4.7-11.1) months. The presence of ≥1 T1-hypointense lesion (HR 20.6, 95% CI 5.5-78.0) and ≥1 T2 periventricular lesion (3.3, 1.3-8.8) were associated with an increased likelihood for MS diagnosis (sensitivity 84%, specificity 93%, PPV 76%, NPV 96%). The predictive parameters were validated in an independent Dutch cohort of 45 children with ADS (n=15, 33% MS): sensitivity 93%, specificity 87%, PPV 78%, NPV 96%. Finally, it was determined that the 2010 McDonald criteria are applicable for diagnosis of pediatric-onset MS diagnosis in older children with non-ADEM presentations. The work embodied herein emphasizes the value of MRI in predicting MS diagnosis in children with incident ADS. Early identification of children with MS is important for planning clinical care and will be valuable in future pediatric MS treatment trials.

“If I have seen further,
it is only by standing on the shoulders of giants.”

- Sir Isaac Newton, 1676

To Dad and Mom

For Lauren

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As it is proper etiquette to serve the finest wine first and then the lesser, I wish to take opportunity to mention, at the opening of this dissertation, those who have contributed to the work described herein. While it is a pleasant exercise to recognize the indispensible contributions of many, so too is it a difficult one as I hope to leave no one unrecognized.

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Attributions

Chapter 1: Written in entirety by the doctoral candidate, LH Verhey, with input from the candidate's supervisor, B Banwell.

Chapter 2: LH Verhey conceived the content and wrote the manuscript, with input from B Banwell and MM Shroff.

Chapter 3: LH Verhey conceived the content and wrote the manuscript, with contributions from JG Sled.

Chapter 4: Written in entirety by LH Verhey, with input from B Banwell.

Chapter 5: A Fawcett, S Venkateswaran, and LH Verhey co-conceived the study design; LH Verhey developed the methodology under the guidance of BM Feldman and DL Streiner; HM Branson and S Laughlin performed MRI scoring; LH Verhey was present at each scoring session to receive training and enter data; B Banwell, SM Benseler, BM Branson, S Laughlin, and MM Shroff participated in expert panel meetings which were chaired by LH Verhey; data analysis and interpretation, table and figure preparation, and authoring of the first draft were performed by LH Verhey; B Banwell, BM Feldman, and DL Streiner provided editorial input to the manuscript.

Chapter 6: LH Verhey devised study concept and design (subsequently implemented into B Banwell's existing national research program on pediatric demyelinating disease); LH Verhey created the data entry platform, in collaboration with the Center for Computational Biology (The Hospital for Sick Children, Toronto, Canada); B Banwell, HM Branson, DJA Callen and MM Shroff assisted LH Verhey in scoring MRI scans (512 scans), and LH Verhey independently scored the remaining 627 scans; LH Verhey conceived and performed statistical analyses, interpreted results, prepared figures and tables and drafted the manuscript with editorial input from DL Arnold, B Banwell, A Bar-Or, RA Marrie, S Narayanan, AD Sadovnick, and JG Sled.

Chapter 7: (Study conducted at Sophia Children's Hospital, Rotterdam, the Netherlands) LH Verhey devised study concept and design under the guidance of RQ Hintzen and B Banwell; ED van Pelt-Gravesteijn prepared the MRI data for scoring; LH Verhey scored all MRI scans (approx. 150 in number), conceived and performed statistical analyses, interpreted results, prepared figures and tables, and drafted the manuscript with input from ED van Pelt-Gravesteijn; B Banwell, CE Catsman-Berrevoets, BM Feldman, RQ Hintzen, IA Ketelslegers, RF Neuteboom, JG Sled, and DL Streiner provided editorial input.

Chapter 8: Y Sadaka and B Banwell devised study concept and design, with input from LH Verhey; LH Verhey performed MRI scoring with input from HM Branson and MM Shroff; M McGowan provided input on clinical data; Y Sadaka performed analyses of clinical variables; Y Sadaka performed statistical analyses with input and additional analyses performed by LH

Verhey; B Banwell, Y Sadaka, and LH Verhey interpreted the results, and Y Sadaka and LH Verhey created tables and figures; Y Sadaka and B Banwell authored the first draft of the manuscript, with input and revisions provided by LH Verhey; DL Arnold, A Bar-Or, RA Marrie, S Narayanan, AD Sadovnick, and JG Sled provided editorial input.

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Chapter 10: LH Verhey conceived the content and wrote the manuscript, with editorial input provided by B Banwell and S Narayanan.

Chapter 11: Written in entirety by LH Verhey, with input from B Banwell

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List of Abbreviations

ADEM	acute disseminated encephalomyelitis
ADC	apparent diffusion coefficient
ADS	acute demyelinating syndrome
ATM	acute transverse myelitis
CCSVI	chronic cerebrospinal venous insufficiency
CI	confidence interval
Cho	choline
Cr	creatine
CDMS	clinically definite multiple sclerosis
CNS	central nervous system
CSF	cerebrospinal fluid
DIR	double inversion recovery
DIS	dissemination in space
DIT	dissemination in time
DTI	diffusion tensor imaging
DMT	disease modifying therapy
DWI	diffusion-weighted imaging
EBV	Epstein-Barr virus
EDSS	expanded disability status scale
EPI	echo planar imaging
FA	fractional anisotropy
FLAIR	fluid attenuated inversion recovery
fMRI	functional magnetic resonance imaging
FOV	field of view
FSE	fast spin-echo
GA	glatiramer acetate
GABA	γ-aminobutyric acid
Gd	gadolinium
Gln	glutamine
Glu	glutamate
HLA	human leukocyte antigen
^{1}H-MRS	hydrogen-magnetic resonance spectroscopy
HR	hazard ratio
IFN	interferon
IgG	immunoglobulin G
IPMSSG	International Pediatric Multiple Sclerosis Study Group
IQR	interquartile range
Lac	lactate
LETM	longitudinally-extensive transverse myelitis
LR+	likelihood ratio for a positive test

LR-	likelihood ratio for a negative test
MD	mean diffusivity
MS	multiple sclerosis
MRI	magnetic resonance imaging
MT	magnetization transfer
MTR	magnetization transfer ratio
NA	N-acetyl
NAA	N-acetylaspartate
NAAG	N-acetylaspartylglutamate
NABM	normal appearing brain matter
NAGM	normal appearing grey matter
NAWM	normal appearing white matter
NMO	neuromyelitis optica
NPV	negative predictive value
OCB	oligoclonal bands
ON	optic neuritis
PD	proton density
PPV	positive predictive value
RRMS	relapsing-remitting multiple sclerosis
SD	standard deviation
SE	spin-echo
SLE	system lupus erythematosus
SPGR	spoiled gradient-recalled echo
SPMS	secondary progressive multiple sclerosis
SV-pACNS	small vessel primary angiits of the central nervous system
TE	echo time
TI	inversion time
TM	transverse myelitis
TR	repetition time
TSE	turbo spin-echo

PART I:

INTRODUCTION

Chapter 1:

Scope of Dissertation

1.1 Introduction

Multiple sclerosis is a chronic disorder affecting the brain, spinal cord and optic nerves, and its pathobiology involves immune-mediated inflammatory demyelination and chronic neuroaxonal damage. MS typically follows a clinically relapsing-remitting course, and clinical manifestations include episodes of neurological dysfunction, such as numbness, weakness, diplopia and vision loss. In the early phase of the disease, patients typically recover from episodes of neurological dysfunction, and accrual of physical and cognitive disability is more marked in the later phase. Magnetic resonance imaging reveals multiple discrete foci of T2-weighted hyperintensity representing non-specific areas of edema, demyelination, axonal loss, and gliosis. A subset of lesions apparent on T2-weighted imaging persists as hypointense on *serial* T1-weighted images, representing areas of permanent focal tissue damage. Enhancement of lesions following administration of gadolinium indicates an increase in blood-brain barrier permeability, representing newly formed, actively demyelinating lesions. While the majority of patients are diagnosed with MS in early adulthood, MS also affects children and adolescents. Multi-national collaborative research has led to an increased recognition and awareness of pediatric-onset MS, as well as an increased interest in studying these young patients.

MRI is perhaps one of the most important tools for the diagnosis of MS and for monitoring disease progression and treatment response. The ability of MRI to identify patients at highest likelihood for a future diagnosis of MS has important implications for clinical care. While studies have been performed to document the MRI features of children with a confirmed MS diagnosis, less is known about the utility of MRI in predicting future MS diagnosis. In this thesis, a

programmatic approach is undertaken to develop a systematic method to evaluate MRI scans of children with acute demyelinating syndromes (ADS) of the central nervous system, and to apply this method for evaluating the role of MRI in predicting MS diagnosis in children with ADS.

The core data presented in this thesis emerge from a Canadian national prospective incident study of children with ADS, under the direction of Dr. Brenda Banwell (The Hospital for Sick Children, Toronto, Canada) and funded by the Canadian Multiple Sclerosis Scientific Research Foundation. In total, 298 children with ADS have been enrolled at 23 pediatric tertiary and regional healthcare facilities. In this research program, children are currently being followed for five years, and standardized clinical assessments (including information on genetic family history, neuropsychological functioning, and quality of life) and serum and cerebrospinal fluid samples are collected at onset, 3, 6 and 12 months during the first year and annually thereafter. Standardized MRI scans are obtained according to a research protocol at the same frequency during the first year in all children, and annually during years 2 – 5 only in participants enrolled at the lead site (The Hospital for Sick Children).

1.2 Dissertation Plan

The thesis commences with a general overview of pediatric-onset MS and a more detailed review of the MRI features of pediatric MS **(Chapter 2)**. In **Chapter 3**, the physics and methods of advanced MRI techniques used in the study of MS will be briefly summarized, and the

insights gained from application of advanced imaging to pediatric-onset MS will be comprehensively reviewed. **Chapter 4** provides the rationale for this thesis and outlines the objectives and hypotheses.

Central to this thesis is a semi-quantitative lesion evaluation on over 1100 baseline and serial MRI scans acquired in the prospective study previously described. The consistent and accurate evaluation of MRI scans is predicated on application of a reliable and standardized scoring tool. The methodology to create this scoring tool is the subject of **Chapter 5**. Subsequently, a study was undertaken in which the scoring tool was applied to MRI scans of children with acute CNS demyelination to identify MRI parameters that predict MS diagnosis **(Chapter 6)**. In collaboration with the Dutch Study Group for Pediatric MS (Sophia Children's Hospital, Rotterdam, the Netherlands), the MRI parameters for prediction of MS diagnosis identified in Chapter 6 were validated in an independent cohort of children with acute CNS demyelination **(Chapter 7)**.

During the course of the studies that comprise this thesis, revisions to the McDonald criteria for MS diagnosis were published in 2010. These revisions took into consideration for the first time pediatric-onset MS, under the provision that studies be conducted to test the applicability of the revised criteria for MS diagnosis of children. **Chapter 8** is comprised of a study on the application of the 2010 McDonald criteria to our prospective cohort of children with ADS, and compares its performance to the 2005 McDonald criteria.

There remains a paucity of literature on the MRI features of the spinal cord in pediatric-onset MS patients. **Chapter 9** is a descriptive study of spinal cord MRI in a retrospective cohort of children with MS, none of whom are enrolled in our prospective national program.

Drawing on the findings generated in this thesis and the literature, **Chapter 10** provides a proposed MRI acquisition protocol and reporting scheme for pediatric CNS demyelination. Standardization of MRI in the clinical setting is essential for multi-centered collaborative studies as well as treatment trials that are currently being planned.

The thesis closes with a general discussion and perspective on clinical implications and future perspectives **(Chapter 11)**.

Chapter 2:

Pediatric-Onset MS

Verhey LH, Shroff MM, Banwell B
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2.1 Introduction to Pediatric-Onset MS

Multiple sclerosis is the most common autoimmune demyelinating disease of the CNS, characterized by immune-mediated inflammation and progressive neurodegeneration, causing intermittent and accumulating neurological deficits. Pediatric-onset MS is being increasingly recognized worldwide, and it is estimated that approximately 2.2 – 4.4% of patients are diagnosed with MS prior to age 18 years.[1-6] Given these estimates were derived from MS patient registries,[3,5] incident pediatric MS cases have likely been missed and, therefore, the proportion of pediatric-onset MS may indeed be higher. While the worldwide incidence of pediatric-onset MS is unknown, an American study reported an incidence rate of 0.51 per 100,000 children per year.[7] When considering acute CNS demyelinating events more broadly, two studies have reported an incidence ranging between 0.9-1.56 per 100,000 children per year.[7,8]

Historically, the first documented patient with MS was a girl living in the 14th century who, at 16 years of age, experienced a first attack of MS characterized by gait impairment and later developed optic neuritis, upper extremity paresis, facial weakness and pain.[9] However, it was not until 1958 that a cohort of pediatric-onset MS patients was first described.[10]

That the onset of pediatric MS occurs in close temporal proximity to the inciting events thought to play a role in disease initiation uniquely permits exploration of the earliest aspects of disease pathobiology. The use of MRI as a paraclinical tool to detect disease-related changes in the CNS of pediatric MS patients permits exploration of the earliest aspects of inflammation and

neurodegeneration. As such, the sensitivity of MRI provides valuable information for MS diagnosis, assessment of disease activity and progression, and monitoring of treatment response.

Subsequent to a summary of the pathobiological, clinical and treatment aspects of pediatric-onset MS, this review will discuss the conventional MRI (i.e. T1-weighted, proton-density and T2-weighted imaging) features of MS in children as well as its application in the diagnosis of pediatric-onset MS and in prediction of MS in children with an incident CNS demyelination. Insights gained from studies comparing MRI features of pediatric- and adult-onset MS will be presented. Advanced MRI techniques not yet optimized for clinical application will be discussed separately (Chapter 3).

2.1.1 Pathogenesis: Immunobiology and Risk Factors

MS is largely viewed as an immune-mediated disease that is initiated when a genetically-susceptible host is exposed to environmental risk factors, resulting in a dysregulated immune response targeted at the CNS. T cells and B cells mature in the thymus and bone marrow, respectively and enter the circulation as naïve cells. These naïve cells may encounter various antigens and contribute to memory and effector repertoires. Activation of these peripheral immune cells, such as $CD4^+$ and $CD8^+$ T cells, myeloid cells and B cells, is dysregulated in patients with MS. Such aberrant immune cell activation leads to their more efficient adherence to the blood-brain barrier endothelium and increased trafficking into the CNS via matrix metalloproteinases, where the immune cells become re-activated.[11] The episodic cycles of

immune cell infiltration into and re-activation within the CNS contribute to the development of the multifocal inflammatory lesions within the white and grey matter of patients with MS. Pathological and imaging studies have demonstrated that the CNS injury extends beyond the perivascular cuffs of inflammation and includes extensive neuroaxonal damage within the normal-appearing brain tissue.[12,13] Taken together, the underlying pathophysiology of MS involves a complex interaction of inflammatory and neurodegenerative processes which are interrelated, with chronic inflammation leading to foci of neuronal destruction and neuronal or axonal damage inciting an immune response.

Interactions between genetic and environmental factors contribute to MS risk. The most well-established genetic component of MS susceptibility resides in the major histocompatibility complex (human leukocyte antigen, HLA), and HLA-DRB1 has specifically been associated with MS in adults.[14] A higher frequency of HLA-DRB alleles has been reported in patients with pediatric-onset MS.[15] In a cohort of children with incident CNS demyelination, those diagnosed with MS were more likely to have HLA-DRB1*15 alleles compared to those who remained monophasic, and this association was driven mainly by the presence of European ancestry.[16] In a multivariable analysis of HLA-DRB1*15 genotype, Epstein-Barr virus (EBV) seropositivity and 25-hydroxyvitamin D status in children with acute demyelination, the presence of at least one HLA-DRB1*15 allele was associated with a twofold increased risk of MS.[17]

Vitamin D insufficiency, remote infection with EBV and exposure to cigarette smoke have been proposed as environmental risk factors for MS in both pediatric- and adult-onset patients. Studies in animal models of MS have shown that vitamin D has a role in regulating immune cell activity via the vitamin D receptor on T lymphocytes,[18-20] suggesting the role of vitamin D as a

mediator of immune-mediated inflammation. In a cohort of children with MS, every 10 ng/ml increase in serum 25-hydroxyvitamin D(3) (the active form of vitamin D) was associated with a 34% decrease in relapse rate.[21] When considering several predisposing factors for MS in children with acute demyelinating syndromes, a 10 nmol/L increase in serum 25-hydroxyvitamin D concentration was independently associated with a risk reduction of 0.9.[17] Trials of vitamin D in children with MS are currently being planned. One study has reported that children exposed to parental cigarette smoke are at an increased risk for MS.[22] When comparing serum titres of cytomegalovirus, herpes simplex virus type 1, varicella zoster virus, parvovirus B19 and EBV in children with MS to that of age-matched controls, seropositivity to EBV was associated with a threefold increased likelihood of MS.[23] Several other studies have shown a higher EBV titre in children with MS compared to controls, and this association appears stronger for the Epstein-Barr nuclear antigen (EBNA1) than for the Epstein-Bar viral capsid antigen.[24-27] In children with acute demyelinating syndromes, risk of MS is increased by more than twofold in those with remote EBV infection.[17] Intrathecal EBV antibodies have been reported in children with MS.[28] One study has demonstrated that, in addition to the increased risk of MS in children seropositive for EBV, remote cytomegalovirus infection lowers the odds of MS,[29] suggesting that one's risk for MS depends in part on the viral infections acquired during the early-life window of exposure.

The pathobiology of pediatric-onset MS has many resemblances to that of adult-onset disease. When compared to adults with MS, inflammatory responses in children with MS, measured in studies of T-cell proliferation[30] and B-cell-derived antibodies,[31] are as robust and in some cases stronger than that observed in adult patients.[32] With respect to genetic and environmental risk

factors, presence of HLA-DRB1*15 alleles, remote infection with EBV, vitamin D insufficiency, and cigarette smoke exposure have all also been implicated as risk factors for adult-onset disease.[33]

2.1.2 Chronic Cerebrospinal Venous Insufficiency

Beginning in 2007, the Italian vascular surgeon Paolo Zamboni proposed the theory of chronic cerebrospinal venous insufficiency (CCSVI) as the underlying cause of MS.[34] It was hypothesized that MS results from chronically impaired venous drainage of the CNS due to stenoses in the neck. The investigators implied that this state of CCSVI led to venous reflux, causing extravasation of iron and its accumulation in the CNS parenchyma; they proposed that iron accumulation may represent the inciting event in MS pathobiology, triggering immune-mediated inflammatory injury.[35] Based on ultrasonography of the internal jugular and vertebral veins, CCSVI was defined as the presence of at least two of the following criteria: 1) reflux in the internal jugular veins (IJV) or vertebral veins (VV) in the upright and supine positions, 2) reflux in the deep cerebral veins, 3) high-resolution B-mode evidence of IJV stenoses, 4) lack of Doppler-detectable flow in the IJV or VV, and 5) reverted postural control of main cerebral venous outflow pathways.[36]

Using transcranial colour-coded Doppler sonography and extracranial colour Doppler sonography, Zamboni and colleagues found that MS was associated with venous outflow anomalies[36] and that patients with MS met the criteria for CCSVI with 100% sensitivity, 100% specificity, 100% positive predictive value, and 100% negative predictive value.[37] Interpretation of these results is challenged by the possibility of spectrum bias (sampling conditions for

evaluation of the diagnostic test are not clinically representative), lack of blinding of the sonographer to patient diagnosis, uncertain reproducibility of ultrasonography results given the dependency on patient positioning and angle of insonation, and the possible risk of pulsation artifact from adjacent vascular structures.[38]

Led by Professor Zamboni, an open-label study was conducted in which patients underwent a percutaneous transluminal angioplasty to treat the neck venous stenoses.[39] During the 18-month period of follow-up following the procedure, improvement in clinical outcomes were noted in relapsing-remitting patients, although such improvements were not clearly defined. However, the small sample size, lack of a control group, unblinded neurological evaluations, significant IJV re-stenosis rate (47%), and the confound that treated patients remained on disease-modifying therapies limit interpretation of these findings and make tenuous the interpretation of efficacy.[38]

Using ultrasound and MRI techniques, several international studies have been performed to compare venous structure between patients with relapsing-remitting or progressive forms of MS, those with a first attack of CNS demyelination, patients with other non-demyelinating CNS disorders, and healthy controls. To date, the findings challenge the theory that CCSVI plays a causative role in MS pathogenesis.[40-43] Iron accumulation and iron-mediated CNS injury is not unique to MS and has been reported in many neurological disorders,[44,45] and cerebrospinal ferritin levels have been found to be normal in patients with MS and do not change over time,[46] suggesting that CCSVI is not an inciting factor in MS. For CCSVI to be causative in MS pathobiology, it should be observed in the earliest stages of disease. The presence of anomalous venous drainage patterns in children with MS is currently being evaluated.

Several centers worldwide are conducting endovascular procedures for treatment of CCSSVI. Anecdotal reports indicate serious injury and in some cases death as a result of endovascular procedures to treat CCSVI in patients with MS.[47] It is critical that patient safety remain foremost and that the findings of CCSVI be rigorously replicated in independent cohorts prior to the implementation of trials to evaluate the efficacy of balloon angioplasty as a treatment for MS.

2.1.3 Demographics and Clinical Characteristics

Onset of MS before the age of ten years occurs in only 17% of all pediatric-onset patients.[48] Gender ratios vary according to age at onset. The ratio of girls:boys in children less than six years of age is 0.8:1 and increases to 1.6:1 between the ages of 6 and 10 years. The female preponderance, similar to that seen in adults with MS, only emerges in adolescence, at about 10 years of age[48,49] which may be indicative of a hormonal influence on MS risk or a gender-defined genetic influence on immunologic activity.

An acute demyelinating syndrome (ADS) represents the first presentation of MS in 20-30% of children; for the remaining 70-80%, the demyelinating event occurs as a monophasic illness.[17,50,51] An ADS can be classified into one of several clinical presentations: optic neuritis (ON), acute transverse myelitis (TM), monofocal or polyfocal neurologic deficits extrinsic to the optic nerves or spinal cord, or acute disseminated encephalomyelitis (ADEM). While monofocal presentations are more common in adolescents and adults, children typically present with polyfocal symptoms.[49] A polyfocal presentation of MS, characterized by neurological symptoms referable to multiple areas within the CNS, occurs in 50-70% of children,[2,51,52] whereas 30-50%

present with monofocal symptoms.[48] Approximately 10-23% of children with MS present with ON,[2,50,53] and bilateral ON carries an increased MS risk compared to unilateral ON.[54] Acute isolated TM occurs as a first attack of MS in only 2-14% children;[50,51,55] more frequently, acute TM represents a monophasic illness, or when co-occurring with ON, may represent the heralding features of neuromyelitis optica. Monofocal presentations of motor dysfunction occur in 30% of children with MS, sensory symptoms in 15-30%, axatia in 5-15%, and brainstem symptoms in 25-41%.[48,51]

ADEM, defined by the International Pediatric MS Study Group (IPMSSG) as multifocal neurological deficits and encephalopathy (change in behaviour or consciousness),[56] is the most frequent ADS, occurring in 25-40% of children,[17,51] and represents a monophasic illness in up to 80% of children with symptoms lasting approximately three months.[57] Recurrent and multiphasic forms of ADEM have been described but are rare.[56] As younger children are more likely to experience a first attack of MS characterized by multifocal neurological deficits, the distinction from monophasic ADEM can be very difficult, but is important for prognosis. In studies of children followed for 4-5 years, ADEM has been reported as a first attack of MS in as many as 18% of children,[57] and in as few as 2-6%.[17,50] These discrepant findings may be a result of inconsistent application of criteria for altered mental status or encephalopathy across studies. To mitigate this inconsistency and permit comparison of multiple cohorts, the IPMSSG proposed MS diagnostic criteria for children whose first attack met criteria for ADEM, requiring two non-ADEM events (must exclude encephalopathy) more than three months after first attack.[56]

An MS relapse is defined as neurological symptoms that persist for at least 24 hours and are separated from a previous attack by a minimum of 28 days.[58] Most children with MS experience complete recovery following the first demyelinating episode,[59] remaining clinically stable between subsequent MS relapses. Over 95% of children with MS follow a relapsing remitting disease course;[48,49] primary progressive MS is extremely rare in children. Annualized relapse rate, estimated between 0.38 and 0.87,[3,53] is higher in children compared to adults.[60] On average, the secondary progressive stage of MS occurs 10 years later in children compared to adults, but pediatric-onset patients are 10 years younger than adult-onset patients when they reach this phase of the disease.[49]

2.1.4 Treatment

Corticosteroids are typically used to manage acute symptoms or relapses in children with MS,[61] and intravenous immunoglobulin or plasma exchange may be administered to treat severe or life-threatening symptoms.[62,63] The majority of disease-modifying therapies act on the immune-mediated inflammatory component of the disease. First-line immunomodulatory therapies currently used to treat pediatric MS include glatiramer acetate (GA), intramuscular and subcutaneous interferon (IFN)-β1a and subcutaneous IFN- β1b. At present, most treatment decisions for use of these first-line therapies in children are based on studies performed in adults. Recently, a European consensus for the use of IFN- β and GA in children with MS was published.[64] Initiation of MS-targeted therapy should commence promptly after confirmation of MS diagnosis since efficacy is higher early on in the disease, both IFN- β and GA are safe and

tolerable in children and seem effective at reducing relapse rate in the early stages of the disease, and relapses interfere with school attendance and negatively impact quality of life.[32] Serious adverse effects to first-line therapies are rare in children,[65] and flu-like symptoms, elevated liver enzymes and reduced white blood cell counts are most common.[32] Data on adequate treatment response are lacking, however, a reduction in relapse rate during the first two years of disease from 1.0 to <0.6 would be comparable to the 30-40% relapse rate reductions observed in adults.[32]

Two second-line therapies, natalizumab and fingolimod, have been recently approved for use in adult patients. Natalizumab has shown relapse rate reductions of 68% in adult MS trials and >90% reductions in contrast-enhancing lesions compared to placebo,[66] however, it is associated with an increased risk for infection with JC virus, leading to progressive multifocal leukoencephalopathy (PML).[67] Stringent monitoring and PML management guidelines are now in place. Fingolimod is the first orally-administered disease-modifying therapy and has been shown to reduce MRI activity and clinical relapses in adults with MS.[68,69] Fingolimod acts by binding to sphingosine-1-phosphase receptors on immune cells, impeding their ability to emigrate from the thymus. Whether this sequestration of immune cells in the thymus, a particularly active tissue in children that is responsible for educating the T-cell repertoire, results in premature immune senescence must yet be understood prior to it being considered for treatment of pediatric MS.[32]

2.1.5 Physical and Cognitive Outcome

Long-term outcome in children with MS is largely determined by rate of physical and cognitive disability accrual. The Expanded Disability Status Scale (EDSS)[70] is a widely used measure of physical disability, ranging from 0 to 10 with increasing scores referring to increased disability. Children reach a disability score of 4 (deficits, but able to ambulate 500 meters without aid or rest) a median of 20 years from onset, a score of 6 (able to walk with unilateral aid no more than 100 meters without rest) after 28.9 years, and a score of 7 (able to walk no more than 10 meters without rest) after 37 years.[5] Compared to adults with MS, pediatric-onset patients take 10 years longer to reach irreversible disability scores, but do so at an age of 10 years younger.[5]

Longitudinal studies have shown that over half of children with MS show cognitive impairment at 10 years of follow-up.[71,72] Compared to healthy controls, cross-sectional studies have shown that over 30% of patients have cognitive impairment in the form of deficits in attention, memory, processing speed, expressive language, and visuomotor integration.[73-76] Over a period of two years of follow-up, 70% of children with MS enrolled in a multi-center longitudinal study showed worsening of cognitive impairment.[77] Given that cognitive impairment impacts on quality of life and negatively effects school performance, special attention to school-related accommodations and emotional wellbeing is imperative to the multi-disciplinary care of children with MS.

2.2 MRI Features of Pediatric-Onset MS

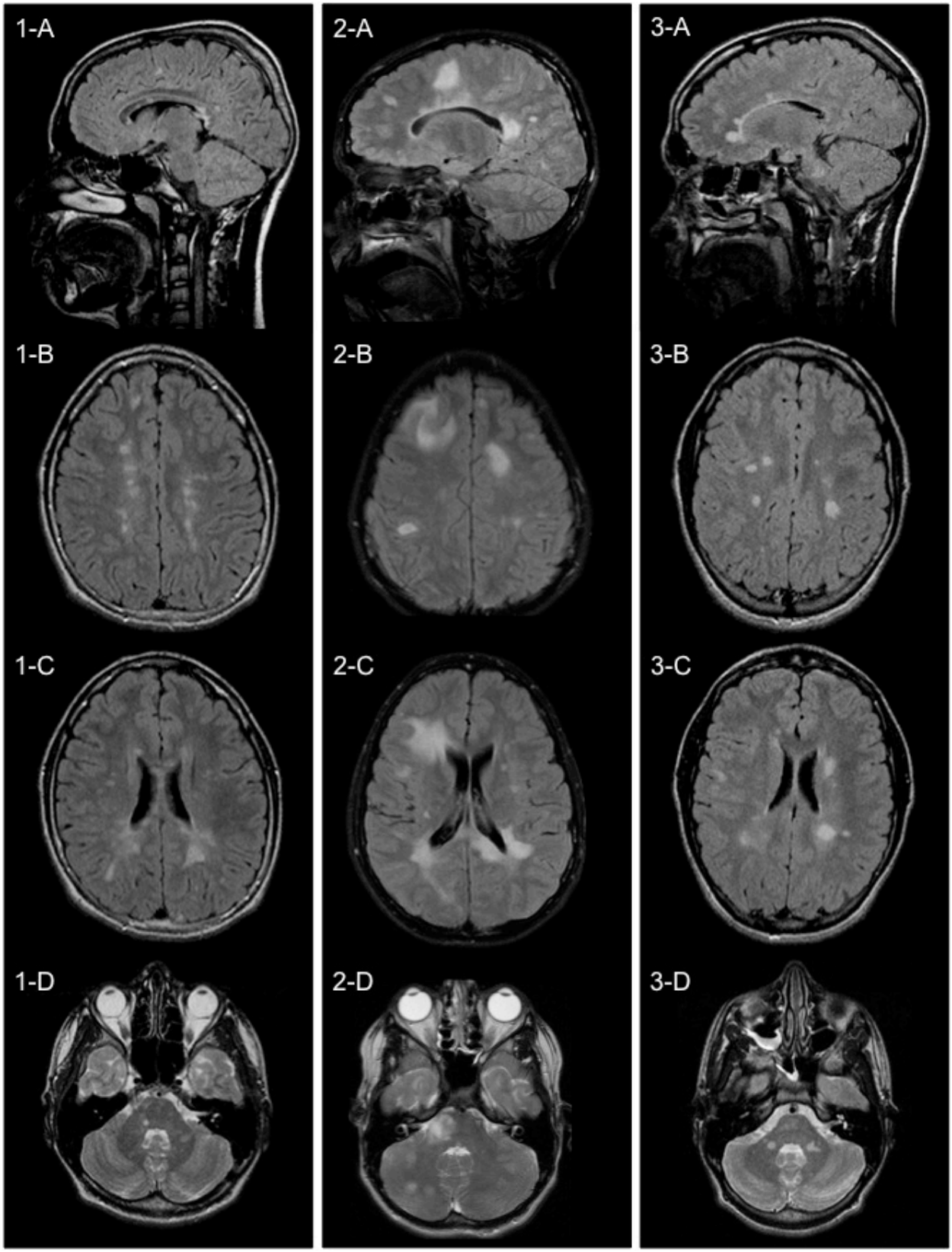
2.2.1 Typical Brain MRI Features

Conventional MRI has high sensitivity for macroscopic tissue abnormalities in MS, but low pathological specificity within the lesions as edema, inflammation, demyelination, remyelination, gliosis and neuro-axonal loss all appear as hyperintense on dual-echo proton-density (PD)/T2-weighted and fluid attenuated inversion recovery (FLAIR) imaging. Dual-echo, FLAIR and pre- and post-contrast T1-weighted sequences provide important information for diagnosing MS, understanding the natural history of lesion accrual and assessing whether treatment reduces accrual of new lesions.

MS lesions typically appear as focal ovoid areas of hyperintensity on dual echo and FLAIR images, ranging from a few millimeters to more than one centimeter in diameter. White matter lesions, such as those involving the juxtacortical and periventricular regions, the corpus callosum, as well as the brainstem and cerebellum can be visualized on conventional T2-weighted or FLAIR images (**Figure 2-1**). In an analysis of supratentorial T2 lesion distribution in children with established MS, lesions have the highest probability of being located in the occipital periventricular white matter followed by the frontal periventricular white matter, while cortical and deep grey matter T2 lesions are less commonly detected (**Figure 2-2**).[78] T2 *hypo*-intensity has been detected in deep grey matter structures of pediatric MS patients, specifically in the head of the caudate nucleus,[79] and may suggest abnormal iron deposition related to diffuse underlying MS pathology. Tumefactive lesions, defined as large lesions (>2 centimeters) with marked perilesional edema, may be difficult to distinguish from malignancy

at onset and therefore biopsy may be required for diagnosis.[80] The presence of tumefactive lesions has been reported in children with acute demyelination who have subsequently been diagnosed with MS (**Figure 2-3**).[81,82]

Gadolinium-enhanced T1-weighted imaging permits differentiation of active or newly formed lesions from inactive ones, since contrast enhancement occurs as a result of increased blood-brain barrier permeability and corresponds to active immune cell transmigration into the CNS.[83] Enhancing lesions are typically focal or ring-like in contour (**Figure 2-4**). In a prospective cohort study of children with ADS, contrast-enhancing lesions were present in 22% of patients (10% of patients who had a monophasic illness and 70% of children subsequently diagnosed with MS).[50] Lesions typically enhance for approximately three weeks,[84] but the duration may be shorter in the context of treatment with methylprednisolone. Both number and volume of new T2 and contrast-enhancing lesions are often used as an outcome measure of *in vivo* inflammatory activity in treatment trials because they capture sub-clinical disease activity with a higher frequency than clinical relapses.


1-A
2-A
3-A
1-B
2-B
3-B
1-C
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1-D
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Figure 2-1: MRI features of children with MS. Sagittal (row A) and axial (rows B and C) FLAIR and axial T2-weighted (row D) images of children with MS. All children were more than 10 years of age at the time of first demyelinating attack. Images acquired four months after onset (Column 1) and at the time of onset (Column 2) are presented from two boys, both of whom presented with polyfocal neurological deficits, without encephalopathy. Column 3 contains images acquired from a boy six months after presentation with brainstem symptoms.

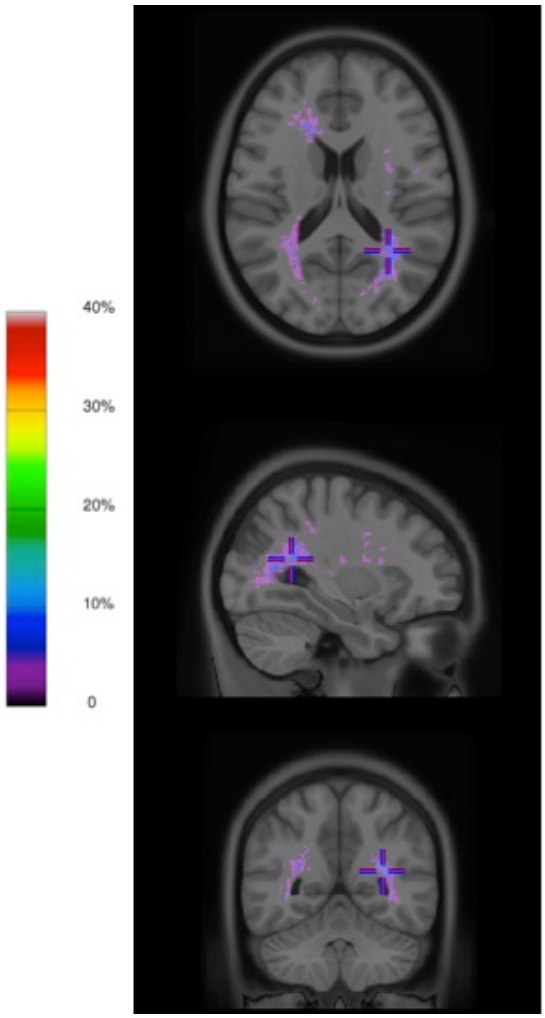

Figure 2-2: T2 lesion probability map of children with MS. The highest probability for lesion accrual in the supratentorial region is in the occipital periventricular white matter followed by the frontal periventricular white matter. (Courtesy of DL Collins, PhD, and B Aubert-Broche,

PhD, McConnell Brain Imaging Center, Montreal Neurological Institute, McGill University, Montreal, Canada)

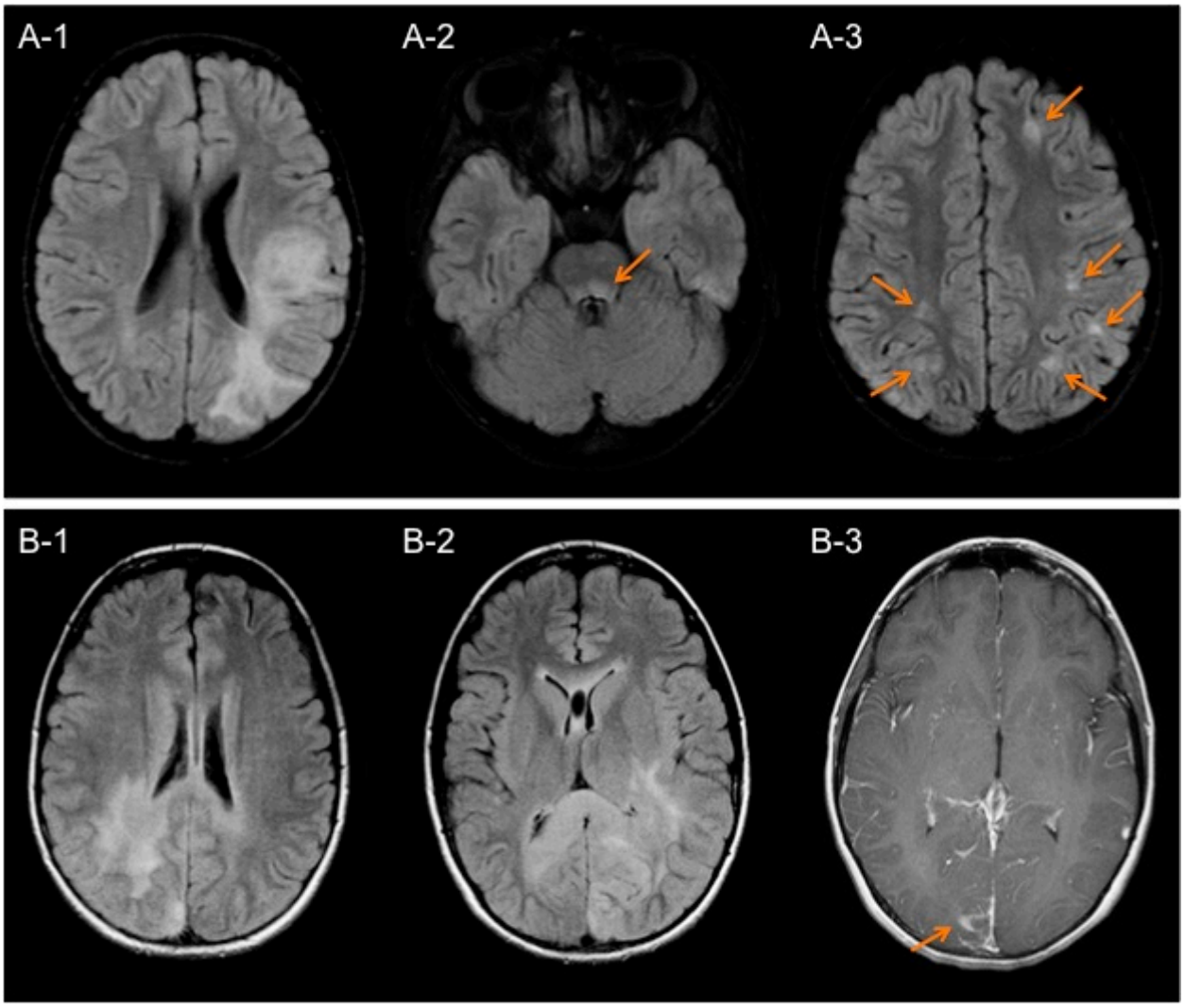

Figure 2-3: MRI features of tumefactive demyelination compared with tumor. (A-1) Axial FLAIR imaging acquired at the time of first attack reveals a tumefactive demyelinating lesion involving the posterior hemispheric white matter in a child with MS; post-contrast T1-weighted imaging was not acquired at the time of onset. (A-2, A-3) Axial FLAIR images acquired 15 months after baseline MRI (A-1) showing new brainstem, juxtacortical and cerebral white matter lesions (arrows). (B-1, B-2) Axial FLAIR imaging shows a tumor involving the cortex, posterior hemispheric white matter and splenium of the corpus callosum in a child initially evaluated for

demyelination. (B-3) Post-contrast T1-weighted imaging reveals enhancement (arrow) of the tumor in the juxtacortical region of the occipital white matter.

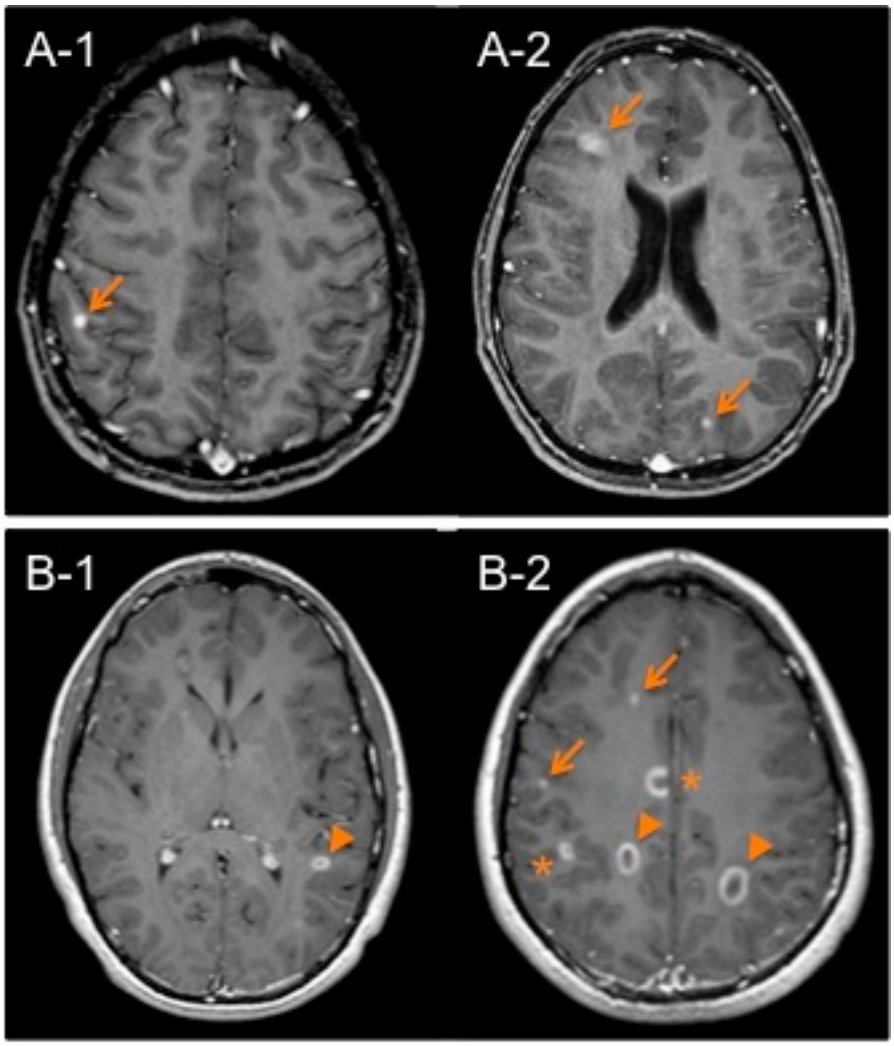

Figure 2-4: Axial post-gadolinium T1-weighted images from two children with MS (panels A and B). Focal enhancing lesions are depicted by arrows in A-1, A-2 and B-2. Closed ring-like enhancing lesions are denoted by arrowheads in B-1 and B-2, and open ring-like enhancing lesions by asterisks in B-2.

Finally, a subset of T2 hyperintense lesions appears hypointense on T1-weighted imaging, and the literature reports T1 hypointensities ranging from isointense to grey matter to isointense to cerebrospinal fluid.[85] T1 hypointensities may initially enhance with gadolinium; those that persist as non-enhancing lesions on *serial* T1-weighted images are termed "black holes" and represent focal areas of tissue damage and irreversible neuroaxonal loss (**Figure 2-5**). The conspicuity of T1 lesions depends on MRI parameter settings (discussed in **Chapter 10**). Decreasing brain volume over time can be observed in children when comparing serial MRI scans (**Figure 2-5**).

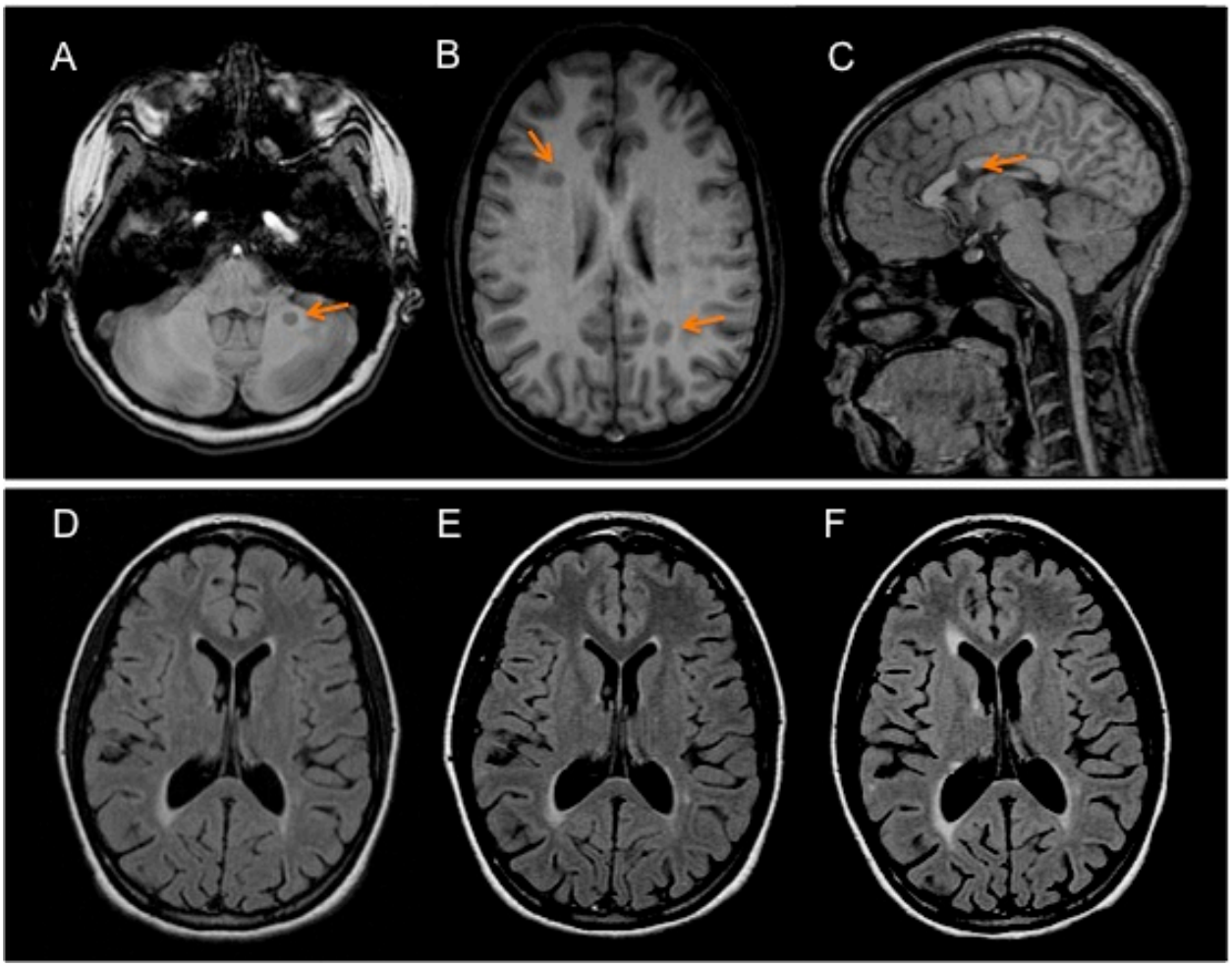

Figure 2-5: "Black holes" and progressive brain volume loss in children with MS. (A-C) T1-weighted images of three children with MS showing T1 hypointense lesions (arrows) in the cerebellar (A) and cerebral (B) white matter and in the corpus callosum (C). (D-F) Serial FLAIR images from a child with MS acquired 4.9 years (D), 5.9 years (E) and 8.4 years (F) from onset; evidence of brain volume loss is depicted by enlargement of the lateral ventricles and widening of the sulci over time.

2.2.2 MRI Features in Pre-Pubertal Children

While the MRI appearance of MS in children >10 years of age may be similar to that of adults, younger children (<10 years of age) present with atypical MRI features. MRI acquired at first attack in young children with MS may show large confluent T2 lesions with poorly defined borders.[86] These large, ill-defined lesions may disappear on serial scans, unlike the more typical ovoid lesions in adolescents and adults that persist on serial imaging. This difference in lesion appearance may relate to differences in an age-related capacity for lesional edema.

2.2.3 MRI Features of the Spinal Cord

Spinal cord lesion features in children with MS resemble that seen in adults. In a study of 36 children with MS (mean onset age 13.1±3.5 years; disease duration 7.5±3.3 years) who had spine imaging acquired due to inter-current spinal cord symptoms or to aid in MS diagnosis, the median lesion count was 1 and lesions preferentially involved the cervical region. Lesions involved only a portion of the transverse diameter of the spinal cord (70% located in the

posterior region), and 90% of lesions were focal – spanning less than three vertebral body segments in the rostral-caudal length. [87] When comparing spinal cord MRI features in children with MS to those with monophasic transverse myelitis, both groups had a median lesion count of 1, but 88% of patients with transverse myelitis had lesions extending ≥3 vertebral body segments compared with only 17% of children with MS. Focal lesions (<3 vertebral body segments) were rare (9%) in patients with monophasic transverse myelitis compared to those with MS (75%).[88] The frequency of clinically silent spinal cord involvement in children with MS is not known, and will be important in determining the added value of spinal cord MRI in MS diagnosis in children. The length of time required to image both the brain and spinal cord, the difficulty for children to lie still for this duration of time without anesthesia, and the poor imaging resolution as a result of a small spinal cord coupled with artifacts from cardio-thoracic motion and cerebrospinal fluid pulsatility challenge the applicability of spine imaging as a routine component of the evaluation of children with MS.

2.3 MRI and the Diagnosis of MS

MRI diagnostic criteria for MS and parameters predictive of MS in patients with acute demyelination are summarized in **Table 2-1**.

Table 2-1: MRI criteria for MS diagnosis

	Pediatric-Onset MS	Adult-Onset MS
Polman, 2005[89]		**DIS*** Three of the following: 1. ≥9 T2 lesions *or* ≥ 1 contrast enhancing lesion 2. ≥1 T2 infratentorial lesion 3. ≥1 T2 juxtacortical lesion 4. ≥3 T2 periventricular lesions **DIT** One of the following: 1. ≥1 contrast-enhancing lesion at least 3 months after clinical onset 2. A *new* T2 lesion at any time compared with a reference scan acquired at least 30 days after clinical onset
Polman, 2011[90]	**DIS** Presence of ≥1 clinically silent T2 lesion in at least 2 of the 4 following CNS regions: 1. Periventricular 2. Juxtacortical 3. Infratentorial 4. Spinal cord **DIT** One of the following: 1. A *new* T2 or contrast-enhancing lesion on any serial scan with respect to a previous scan 2. Simultaneous presence of asymptomatic contrast-enhancing and non-enhancing lesions at any time	
Mikaeloff, 2004[91]	**MRI prognostic factors for MS in children with acute demyelination** Two of the following: 1. T2 lesions perpendicular to the long axis of the corpus callosum 2. Sole presence of well-defined T2 lesions	
Callen, 2009[92]	**MRI criteria to distinguish pediatric MS from relapsing non-demyelinating disease**	

	Two of the following: 1. ≥5 T2 lesions 2. ≥2 T2 periventricular lesions 3. ≥1 T2 brainstem lesion
Callen, 2009[93]	**MRI criteria to distinguish a first attack of MS from monophasic ADEM** Two of the following: 1. Absence of a diffuse bilateral T2 lesion pattern 2. Presence of black holes 3. ≥2 T2 periventricular lesions

*A contrast-enhancing spinal cord lesion is equivalent to an enhancing lesion in the brain; individual spinal cord lesions and brain lesions can together contribute to the requirement of ≥ 9 T2 lesions.

2.3.1 International Pediatric MS Study Group Consensus Definitions

In 2007, the International Pediatric MS Study Group (IPMSSG) met to propose consensus definitions for MS in children – patients younger than 18 years of age at onset.[56] After the exclusion of alternative explanations for the neurological symptoms,[94] the diagnosis of MS in children requires at least two episodes of neurologic dysfunction involving distinct regions of the CNS ("dissemination in space", DIS) and separated by at least 28 days ("dissemination in time", DIT), as is specified for adults.[58] The IPMSSG proposed that the 2005 McDonald criteria for adults[89,95,96] may be used to satisfy the requirements of DIS on MRI in children.[56] The MRI scan must show three of the following four features: 1) ≥1 gadolinium-enhancing lesion or ≥9 T2 hyperintense lesions if there is no gadolinium-enhancing lesion, 2) ≥3 periventricular lesions,

3) ≥1 juxtacortical lesion, and 4) ≥1 infratentorial lesion. The combination of an abnormal cerebrospinal fluid result (presence of oligoclonal bands or an elevated IgG index) and at least two T2 lesions on MRI (one of which is located in the brain) would meet criteria for DIS.

The IPMSSG also agreed with the diagnostic criteria proposed for adult-onset MS, in which MRI can be used to satisfy the criteria for DIT following an initial attack (i.e. ADS), even in the absence of a second clinical attack. According to the 2005 McDonald criteria, DIT on MRI may be demonstrated in one of two ways: 1) gadolinium enhancement ≥3 months after onset of an ADS, or 2) detection of a *new* T2 lesion appearing at any compared with a reference scan done ≥30 days after the onset of an ADS.[89]

2.3.2 2010 McDonald Criteria for MS Diagnosis

In May of 2010, the International Panel on Diagnosis of MS met in Dublin, Ireland to revise the McDonald Criteria.[90] Revisions to the DIS criteria were based on work of the European MAGNIMS multicenter collaborative network, which studies MRI in MS, and indicate that DIS can be demonstrated by the presence of ≥1 clinically silent T2 lesion in at least 2 of the following 4 regions: periventricular, juxtacortical, infratentorial, and spinal cord.[97,98] The DIT criteria can be met by demonstration of either: 1) ≥1 new T2 and/or gadolinium-enhancing lesion on a serial MRI scan, with reference to a baseline scan, irrespective of the timing of the baseline scan, or 2) simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions on any scan.[99,100] These revisions represent the first iteration of the McDonald Criteria where, in some circumstances, both DIS and DIT may be met on the scan

acquired at time of incident demyelination. Importantly, the 2010 McDonald Criteria revisions included a focus on the pediatric population. Consensus of the Panel was that most pediatric MS patients would meet DIS criteria at the time of ADS as most patients have two lesions in at least two of the four specified locations. However, application of the criteria to children with ADEM was not recommended. Validation studies of the revised criteria in the pediatric population, including in children presenting with ADEM, have yet to be performed.

2.3.3 Adult MRI Criteria for MS Diagnosis Applied in Children

The adult McDonald criteria for DIS proposed in 2001 and revised in 2005[89,101] are 93-100% specific for MS diagnosis in children,[92,102] but only about 53-61% of children with MS meet criteria at the time of first attack.[103,104] The sensitivity of the McDonald DIS criteria for MS diagnosis is only 45% in children <10 years of age at onset, but is 67% - similar to that observed in adults - in those >10 years of age,[102] supporting the notion of an atypical lesion pattern in younger children diagnosed with MS.[86] That approximately half of children with MS are not identified as such according to the McDonald criteria suggests the need for new or revised criteria specific to pediatric-onset MS.

2.3.4 Pediatric-Specific MRI Criteria for MS Diagnosis

In a study of 38 children with MS and 45 with non-demyelinating relapsing CNS disease (migraine and CNS lupus), the presence of at least 2 of the following: ≥5 T2 lesions, ≥2

periventricular lesions, and ≥1 brainstem lesion on MRI acquired at second attack distinguished MS from patients with non-demyelinating disease (sensitivity 85%, specificity 98%, positive predictive value [PPV] 97%, negative predictive value [NPV] 90%).[92] These criteria perform well at distinguishing established MS disease from non-demyelinating disease in children; however, the accuracy of these criteria decreases when used to differentiate children with MS from those with monophasic demyelination at the time of first attack.[50,103]

2.4 MRI Predictors of MS Diagnosis in Children at Risk

The utility of MRI in predicting a subsequent MS diagnosis in children with ADS has important implications for identifying children at highest risk for chronic relapsing disease, and therefore early intervention with disease-modifying therapy. The French KIDSEP Study Group evaluated MRI scans of 116 children with ADS, and identified two features on initial MRI that predicted subsequent MS diagnosis (KIDMUS Criteria): ≥1 lesion perpendicular to the long axis of the corpus callosum (hazard ratio [HR] 2.89; 95% confidence interval [CI] 1.65-5.06) and the sole presence of well-defined lesions (HR 1.71; 95% CI 1.29-2.27).[91] Another study which evaluated prognostic factors for MS in children with acute demyelination showed that both the KIDMUS criteria[91] and the 2005 McDonald criteria for DIS[89] had a high positive predictive value and specificity but low sensitivity for distinguishing children with a first attack of MS from those with monophasic ADS.[102] Although the KIDMUS criteria were 100% specific for an MS diagnosis, only 11-21% of children diagnosed with MS had these features at onset.[91,103] The insensitivity of the KIDMUS criteria is likely due to their stringency; while periventricular lesions are common in

children with MS, their orientation perpendicular to the corpus callosum is much less frequently observed, and when applied strictly, the 'sole presence of well defined lesions' is an infrequent MRI feature of pediatric MS. Therefore, applicability of the KIDMUS criteria for predicting MS in the clinical setting of acute demyelination is limited.

In a recent Canadian cohort study of determinants of MS in 302 children with acute demyelination, the presence of one or more T2 lesions on MRI was the most strongly associated with MS diagnosis (HR 37.9; 95% CI 5.25-273.85) when compared with other predictors, such as the presence of HLA-DRB1*15 alleles, previous infection with Epstein-Bar virus, 25-hydroxyvitamin D insufficiency, and cerebrospinal fluid oligoclonal bands.[17] However, the ability of one or more T2 lesions at onset to discriminate between children destined for MS from those with monophasic illness is not sufficiently strong to serve as a diagnostic feature.[50]

2.5 Distinguishing Monophasic ADEM from a First Attack of MS

Up to 18% of children with MS present with an ADEM-like first attack.[57] Therefore, the ability to distinguish children with monophasic ADEM from those destined for MS has important prognostic value. According to the IPMSSG consensus criteria, in a child whose initial ADS is consistent with the clinical features of ADEM (polyfocal neurological deficits and encephalopathy), a second non-ADEM event alone is not sufficient for an MS diagnosis. Rather, further evidence of DIT is required, either by demonstration of new T2 lesions developing at least 3 months subsequent to the second event, or by evidence of a new (third) clinical non-ADEM event.[56] This recommendation is based on the concern that a second non-ADEM episode

in children who initially present with ADEM might still reflect a transient demyelinating illness. Serial clinical and MRI studies are necessary to understand the rate of MS diagnosis in children presenting with ADEM. Biomarkers that distinguish ADEM from a first attack of MS are also needed to identify children destined for recurrent chronic demyelinating disease as opposed to monophasic illness.

MRI features of monophasic ADEM include focal or multifocal T2-weighted lesion(s) without evidence of neuroaxonal degeneration on T1-weighted imaging ("black holes"). Brain lesions are typically large (>1-2 cm in diameter), are confluent through the supratentorial and infratentorial white matter, have ill-defined borders, and involve the deep grey matter with a predilection for the thalami (**Figure 2-6**). In some cases, the spinal cord may also be involved.[105,106] The MRI criteria for pediatric MS diagnosis proposed by Callen and colleagues[92] do not perform well when used to distinguish children with monophasic ADEM from those with MS at the time of first attack.[93,103] To this end, Callen and colleagues conducted a parallel study in which they evaluated MRI scans obtained at first attack in 28 children with MS and 20 with monophasic ADEM, and proposed that any two of the following criteria: 1) absence of diffuse bilateral pattern, 2) presence of black holes, and 3) presence of ≥2 periventricular lesions distinguished patients with MS from those with ADEM (sensitivity 81%, specificity 95%, PPV 95%, NPV 79%).[93] The performance of these criteria have been replicated in an independent Dutch cohort.[103]

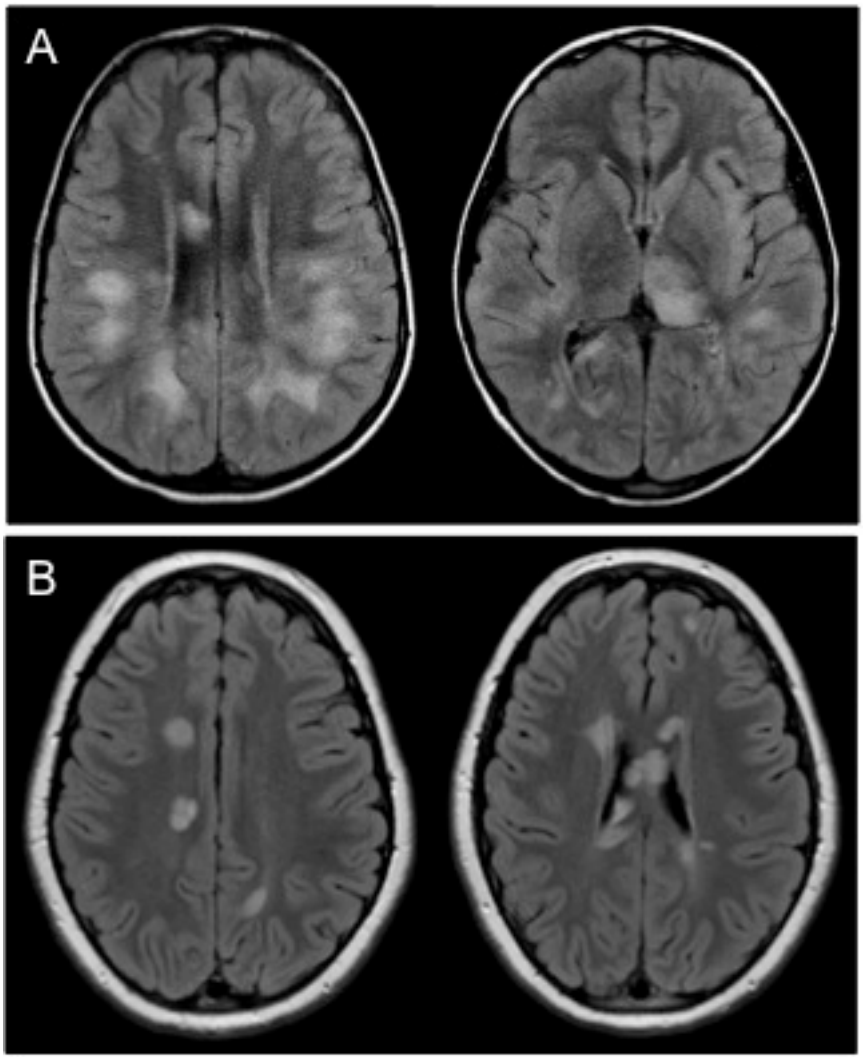

Figure 2-6: Axial FLAIR images acquired at onset in a child with monophasic ADEM (A) and a child diagnosed with MS (B). T2-weighted lesions in ADEM may appear larger and have hazy margins, compared with the focal lesions typically seen in patients with MS.

2.6 Comparison of MRI Features of Adult- and Pediatric-Onset MS

Intuitively, due to the relatively short period of subclinical disease activity in children with MS by virtue of their young age, pediatric-onset patients would be expected to show fewer lesions

on MRI at the time of first attack compared to adults. However, several studies have shown that children show similar or higher levels of disease activity when compared with adults matched for disease duration.[60,107-109] Clinical relapses are more common in the early stages of disease in pediatric-onset MS patients (annualized relapse rate 1.13) compared to that of adult-onset patients (annualized relapse rate 0.40), and this association persists when adjusting for proportion of disease spent on disease-modifying therapy.[60] However, it is important to note that these data were collected from patients at a quaternary MS clinic, and therefore referral bias may have inflated the relapse-rate estimates.

In children with established MS, T2 and gadolinium enhancing lesion volumes are similar to that of adult patients matched for disease duration, and the number of T2 lesions, gadolinium enhancing lesions, and large T2 lesions on initial MRI are higher in children compared to adults.[107-109] The discrepancy between gadolinium lesion counts and volume between adult and pediatric MS patients needs to be interpreted cautiously because, to date, gadolinium-enhancement in pediatric MS has not been investigated in the context of rigorous clinical trials as it has been in adult patients with MS. Considering that gadolinium enhancement occurs in close proximity to clinical attacks, the increased frequency of gadolinium-enhancing lesions in children with MS compared to individuals with adult-onset MS that has been reported[108] may be confounded by a longer duration from onset to MRI in adults than children. Supratentorial T2 lesion distribution is similar and infratentorial (specifically brainstem) lesion count is greater when comparing pediatric- to adult-onset MS patients matched for disease duration, implying that lesion accrual does not require a lengthy subclinical disease phase (**Figure 2-7**).[108,109] The level of T1-hypointense lesion burden may be higher in children than adults early in the disease,

given that the T1-to-T2-lesion volume ratio and the T1-lesion volume-to-brain parenchymal volume ratio are significantly higher in children.[107]

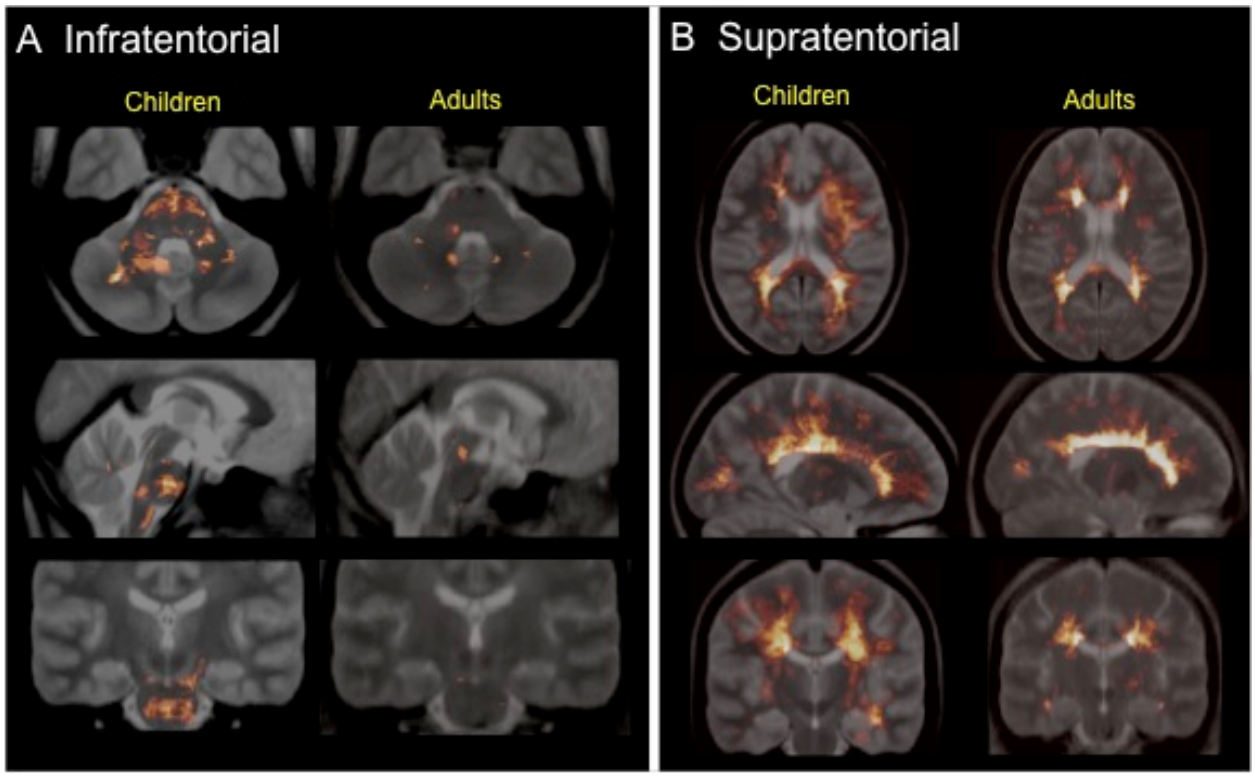

Figure 2-7: Lesion distribution in pediatric- and adult-onset MS patients. Axial (top row), sagittal (middle row), and coronal (bottom row) planes showing infratentorial (A) and supratentorial (B) T2 lesion distribution in patients with pediatric- and adult-onset MS. Lesion maps are superimposed on averaged T2-weighted scans. (Courtesy of R Ghassemi, MSc, McConnell Brain Imaging Center, Montreal Neurological Institute, McGill University, Montreal, Canada)

2.7 Conclusion

Conventional MRI has played a key role in identifying the imaging features of MS in children, and in predicting MS outcome in children at risk. As only 30% of children with an acute

demyelinating episode will herald further attacks leading to a diagnosis of MS, MRI will be important in aiding the clinician to identify children who will not experience relapsing disease. With the mandate of Pediatric Investigation Plans for all new drug trials, treatment trials for pediatric MS are soon to be launched. The ability to identify children at highest risk for MS diagnosis will be important in determining eligibility for such therapeutic trials. There remains a need to understand the natural history of lesion accrual on MRI, as these metrics will inform definitions of optimal therapeutic response in children with MS. Whether MRI can serve as a surrogate marker for prognosis is an important research question that has yet to be addressed and will require long-term clinical follow-up and standardized serial MRI evaluation.

Chapter 3:

Advanced MRI in Pediatric-Onset MS

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Neuroimag Clin N Am, *in press*

3.1 Introduction

The sensitivity of conventional MRI (i.e. proton density, T2-weighted, fluid attenuated inversion recovery and T1-weighted sequences) to detect subclinical disease activity has led to its incorporation into diagnostic criteria for individuals suspected of having MS, and to its use in providing prognostic information for patients in the earliest disease stages. However, the strength of association between conventional MRI findings and clinical parameters in patients with established MS is only modest. This may be explained by the lack of specificity of conventional MRI for the heterogeneous features of MS pathology as well as its inability to quantitatively characterize damage to normal-appearing white matter (NAWM) and normal-appearing grey matter (NAGM). Over the past two decades, advanced MRI techniques have been developed with the objective to identify *in vivo* quantitative pathological substrates of clinical disability and provide more specific or sensitive endpoints for treatment trials. At present, these techniques are not routinely available for clinical use, as they require standardized acquisition, rigorous image analysis pipelines, MRI scanner quality control monitoring, and control or reference data. Multicenter studies on the sensitivity of these techniques to natural history of disease and treatment response are required prior to their implementation into clinical imaging protocols.

This chapter summarizes results from studies that have applied advanced MRI techniques to patients with pediatric-onset MS, and will include a discussion of cortical imaging techniques, volumetry, magnetization transfer (MT) and diffusion tensor (DT) imaging, proton magnetic resonance spectroscopy (^{1}H-MRS), and functional MRI (fMRI). The application of advanced MRI

techniques to pediatric MS is yet in its infancy, and therefore, for several techniques the literature available for review is limited.

3.2 Cortical Imaging

3.2.1 Background

Several histopathological studies have shown that a substantial proportion of the focal cerebral lesion load in adult patients with MS is located within the cortex or at the junction between the cortex and subcortical white matter,[110-112] and that cortical lesions are actively inflammatory and appear early on in the MS disease course.[113] Postmortem studies of adult patients have shown that cortical lesions can be classified according to four subtypes: 1) lesions involving the cortical grey matter and adjacent subcortical white matter, termed “leukocortical lesions”; 2) “intracortical lesions” located solely within the cortex and not involving the subpial cortex or subcortical white matter; 3) lesions beginning in the subpial cortex but that do not reach the boundary between grey and white matter; and 4) lesions involving the entire width of the cerebral cortex, from subpial cortex to the border between grey and white matter.[114,115] These lesions are not typically resolved on conventional MRI sequences due to their relatively small size, their poor contrast against normal appearing cortical grey matter, and the effects of partial voluming from the surrounding cerebrospinal fluid.

The ability to detect and quantify the level of cortical involvement has important implications, as quantitative MRI studies in adults with MS have shown evidence of cortical grey matter

damage in the earliest clinical stages of the disease,[116-118] and cortical grey matter volume loss occurs at a faster rate than that of the white matter.[119] In addition, MRI measures of cortical grey matter damage, specifically accumulation of intracortical lesions, have shown stronger correlations with clinical measures of physical and cognitive impairment[120-123] and with co-occurring epilepsy,[124] compared to measures of white matter lesion burden. Guidelines for the scoring of cortical lesions have recently been proposed.[125] The presence of at least one cortical lesion independently predicts MS diagnosis in adults with a first attack of CNS demyelination.[126] Disease-modifying therapy in adults with MS has also been shown to decrease the number of new cortical lesions and slow the rate of cortical atrophy when compared with untreated patients.[127]

3.2.2 Cortical Imaging Techniques

Several pulse sequences have been shown to substantially improve detection of cortical lesions. Compared to two dimensional T2-weighted spin-echo imaging, the three dimensional (3D) fast FLAIR sequence, which allows acquisition of thinner slices, has an increased sensitivity for cortical lesions.[128-130] However, delineation of cortical lesions on T2-weighted and FLAIR imaging remains a challenge due to poor grey matter-white matter contrast which ambiguates classification of lesions as intracortical, leukocortical or juxtacortical.[131] Limited blood-brain barrier permeability and low inflammatory cell infiltration in cortical lesions[112,132] as well as low myelin density in the upper layers of the neocortex[133] decrease T2 contrast.

Introduction of the double inversion-recovery (DIR) sequence has substantially increased the *in vivo* detection of cortical lesions.[134] By selecting inversion times that can simultaneously achieve T1-based suppression of the signals from both white matter and cerebrospinal fluid, the DIR sequence yields images where T2 variations within grey matter are more easily detected. The result is superior delineation of the grey matter and the boundary between the cortex and subcortical white matter with lesions appearing hyperintense. Using DIR imaging, intracortical lesion detection rates per patient increase by 152% compared to FLAIR imaging, and by 538% when compared to T2-weighted imaging.[131,135] DIR imaging at 3 Tesla results in a 192% increase in cortical lesion detection compared to 1.5 Tesla DIR images.[136] A study of the probabilistic distribution and frequency of cortical lesions in adults with MS confirmed earlier histopathological studies, showing that more than 80% of lesions were found in the frontotemporal cortex, with a predilection for the motor cortex in approximately 35% of patients and anterior cingulate gyrus in 10%.[137] Importantly, there is likely still a large number of cortical lesions not resolved by DIR, as only about 10-20% of cortical lesions identified through postmortem immunohistochemistry are detected on DIR imaging.[110]

Given that DIR imaging is limited by a low cortical lesion detection rate compared to histopathological studies, poor delineation of lesion borders, artifacts caused by cerebrospinal fluid flow and pulsation, and an intrinsically low signal-to-noise ratio, combining DIR imaging with other sequences may improve cortical lesion detection and reliability of cortical lesion classification. Using a combination of phase-sensitive inversion recovery (PSIR) and DIR techniques yields a 337% increase in total number of cortical lesions detected when compared

to FLAIR; intracortical lesion detection was increased by 417%, mixed grey-white matter lesions by 396%, and juxtacortical lesions by 130%.[138]

Three-dimensional spoiled gradient-recalled echo (SPGR) imaging provides high spatial resolution and high signal-to-noise ratio images within a clinically acceptable acquisition time, thereby facilitating accurate classification of cortical lesions.[139,140] One study reported that 30 of the 119 cortical lesions (11 patients) initially identified on DIR and PSIR images were re-classified after reviewing the 3D-SPGR images, with the majority reclassified from mixed grey-white matter lesions to purely intracortical.[139]

3.2.3 Cortical Lesion Detection in Pediatric-Onset MS

To date, only one study has evaluated the presence, frequency and type of cortical lesions in individuals with pediatric-onset MS compared to adult patients.[141] Only 8% of the pediatric patients had cortical lesions, compared to 66% of adults with MS. Although the number and volume of white matter lesions did not differ between pediatric and adult patients with MS, mean cortical lesion count in pediatric patients was only 0.08, compared to 1.99 cortical lesions per adult patient. Median cortical lesion volume was also lower in pediatric versus adult patients. Interestingly, all cortical lesions in the pediatric patients were located at the grey matter-white matter boundary, which is where proliferation of myelin into the peripheral cortical neuropil occurs during childhood and adolescence.[142]

This data suggests that cortical lesion formation is rare in patients with pediatric MS. Whether the formation of cortical lesions is rare in the early stages of MS cannot be determined from this study as the median disease duration was two years longer for adult- compared to pediatric-onset patients. It is possible that the degree of grey matter maturation in pediatric versus adult patients may explain why children with MS have fewer cortical lesions. Quantification of cortical volume by measuring cortical thickness has been conducted in adults with MS.[143-146] Studies to assess whether cortical thinning is more prominent than cortical lesion accrual seen in children with MS have not yet been performed. Additional studies in children with MS are required in order to identify optimal MRI techniques for cortical lesion detection, and to better understand the frequency and localization of cortical lesions and their correlation with clinical parameters such as cognitive impairment.

3.3 Volumetry

3.3.1 Background

MRI measurement of volume loss in MS is appealing as it is thought to reflect a sensitive but non-specific measure of irreversible axonal loss and the neurodegenerative aspect of MS pathobiology.[147] Longitudinal studies in adults with MS have shown that volume loss is evident in the earliest stages of the disease and predicts clinical progression[118,148] and that patients with the highest rate of volume loss early on in the disease reach higher levels of disability than those with a slower rate of volume loss.[148-150] Gray matter volume loss specifically develops at a

faster rate than does white matter volume loss[151,152] and shows stronger correlations with clinical disability than white matter loss and conventional T2 lesion measures.[153-155]

3.3.2 Methodology for Measuring Brain Volume

From a technical point of view, measurement of brain volume is appealing as it can be achieved using a conventional T1-weighted MRI pulse sequence. However, as the typical rate of brain volume loss in adults with relapsing-remitting MS is only between 0.5-1% per year,[147] detection of these subtle volume changes requires quantitative techniques that rely on careful and consistent image acquisition, the use of image analysis algorithms that are not typically available or too intensive to run on MRI consoles, as well the oversight of a trained operator. The technical demands of performing volumetric measurements have to date prevented their use outside of the research setting.

The available methods for estimating brain volume differ depending on whether the intent is to measure volume change from serial images or to measure absolute or normalized volume from individual scans. When measuring absolute brain volume, two broad strategies apply. One is to classify each of the voxel elements of the image as either brain parenchyma or non-brain.[156-159] This classification can be based on image intensity, perhaps with the aid of spatial maps of prior probability, or by evolving a deformable model to find the brain surface. The brain volume is then directly computed by summing the volumes of the constituent voxels. A second strategy is to use image registration to warp the shape of a reference brain image onto the given

image.[160,161] Volume is then computed using the spatial transformation and the brain boundary defined on the reference image.

A challenge to estimating absolute brain volume is that the MRI scanner may not have sufficient geometric accuracy to correctly report the differences on the order of 1% that are anticipated.[147] This limitation can be overcome by careful geometric calibration of the MRI scanner with a specialized phantom or by normalizing the brain volume to another structure. Normalization of brain volume to intracranial volume is a common strategy for analyzing cross-sectional data.[162,163]

Deformation-based and voxel-based morphometry are two methods that allow estimation of brain volume change from serial images. In the case of deformation-based morpometry, images from the time series are warped to align with each other using image registration and the brain boundary is estimated once for the entire series.[164] Compared to estimating brain volume from individual scans, this approach has the advantage that the alignment between consecutive scans following warping tends to be more accurate than the alignment between a given image and the generic reference image. Voxel-based morphometry takes a similar approach except that only linear or coarse scale non-linear alignment is applied.[165-168] The changes between consecutive images are then detected as small changes in grey level intensity that correspond to shifts in the position of the boundary that are smaller than the dimensions of the voxel. These brain shifts can be integrated across cortical regions or averaged across subjects to obtain estimates of volume change. Both deformation-based and voxel-based morphometry are generic methods that apply equally well to analyzing subregions of the brain or to boundaries other than the cortical surface.

3.3.3 Unique Methodological Issues in Children

In healthy individuals, brain volume is nearly constant between the ages of 20 and 55 years,[169] but in children and adolescents the brain is still growing with age and the rate of growth varies between individuals. Thus, choosing appropriate metrics to compare brain volumes among children is challenging. Methods of brain volume measurement that rely on normalization to the inner surface of the skull are inaccurate in children, especially between the ages of 10 and 12 years, as the skull and brain show a differential rate of brain growth. To overcome this challenge, a recent study that compared brain volume loss in children with MS to healthy controls proposed a novel approach of using a z-score at each location in the brain to represent whether the local volume at each given location was larger or smaller than that for a database of age-matched controls (**Figure 3-1**).[78] The z-scores were computed by warping the given brain image to a reference image and computing the Jacobian determinant of this transformation. These Jacobian determinants can then be compared to those found for the age-matched reference population to compute the number of standard deviations the given individual deviates from the mean for that location. A Jacobian determinant greater than one indicates a local volume increase relative to the template and a determinant less than one indicates a local decrease in brain volume.

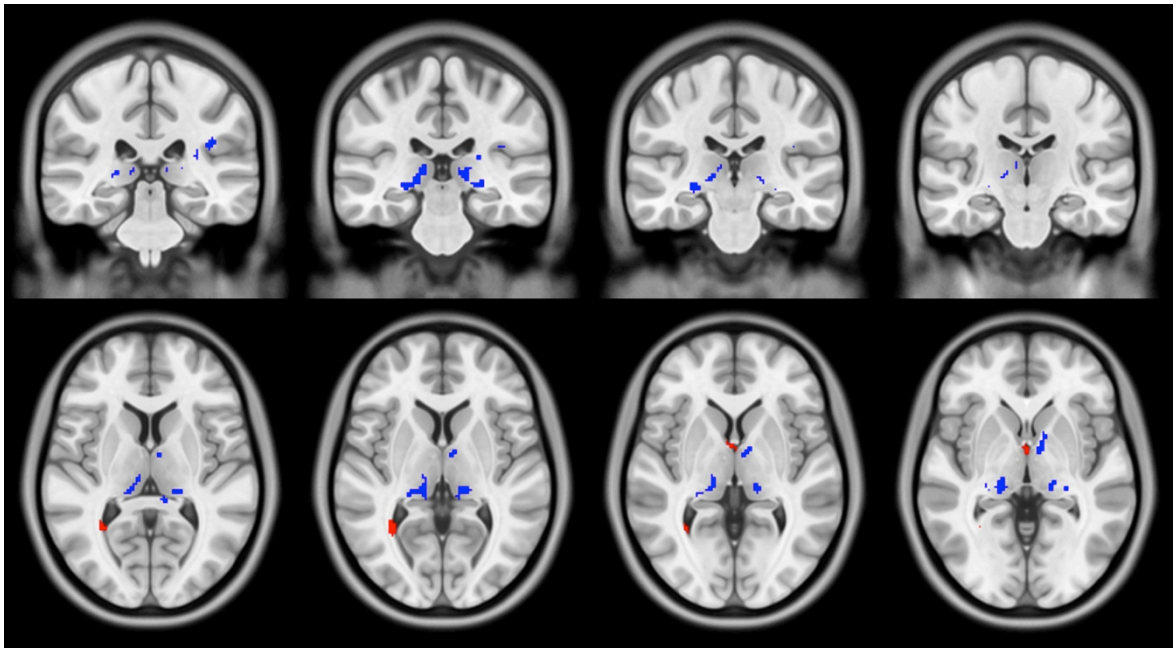

Figure 3-1: Clusters of significant tissue loss (blue) and expansion (red) in children with MS compared to age- and sex-matched healthy individuals ($p<0.05$, corrected for multiple comparisons). (Courtesy of DL Collins, PhD, and B Aubert-Broche, PhD, McConnell Brain Imaging Center, Montreal Neurological Institute, McGill University, Montreal, Canada)

3.3.4 Volumetry in Pediatric-Onset MS

Several cross-sectional studies have been conducted to assess brain volume change in children with MS compared to healthy individuals and correlate these changes with clinical measures of disability and cognitive impairment. One study employed a segmentation technique to calculate the brain parenchymal fraction, which normalizes parenchymal volume to the volume bounded by the inner skull surface; similarly, normalized grey and white matter fractions were also computed. [107] Three key findings can be noted from this study. First, compared to adults with MS matched for disease duration, pediatric patients showed a higher brain parenchymal

fraction and grey matter fraction. Secondly, when comparing adults whose MS was diagnosed in childhood and have had MS for 20 years to adult-onset patients with similar disease duration, to assess the long-term impact of pediatric disease, no differences were found between groups for brain parenchymal fraction or white matter fraction, but adult patients trended towards greater grey matter volume loss compared to pediatric-onset adult patients. Given that aging causes brain volume to decrease by about 0.2% per year between age 30-50 years, a greater brain parenchymal fraction would be expected for the pediatric-onset group due to their younger age; that the brain volume was similar between groups suggests that pediatric patients have a more aggressive disease course. And lastly, when assessing whether brain volume differed between adults with pediatric-onset MS and adult-onset patients who were of similar age but had a shorter disease duration, the adult pediatric-onset group showed a lower brain parenchymal fraction compared to adult-onset patients, and this was driven largely by a decrease in the grey matter tissue compartment.

Another study employed voxel-based morphometry to evaluate the pattern of grey matter loss in pediatric patients with MS compared to a control group matched on age and sex.[170] No differences were detected in normalized brain and grey matter volume or intracranial volume between healthy individuals and children with MS, but patients showed locally reduced grey matter volume in the right and left thalami compared to controls. While T2 lesion load was significantly negatively correlated with both left and right thalamic volume, no correlations were found with disability or disease duration. It is important to note that children included in this study had a mean disease duration of only three years, and the accrual of disability in patients with pediatric-onset MS typically occurs 15-20 years after onset.[48,171] The association

between T2 lesion load and thalamic volume suggests that focal white matter lesions may cause retrograde or anterograde transynaptic degeneration of the afferent and efferent neuronal connections that relay through the thalamus. Evidence for this is further given by a study that used deformation-based morphometry to evaluate the spatial distribution of brain volume loss in children with MS in a cross-sectional manner (**Figure 3-1**).[78] Significant volume reductions were seen in the pulvinar and anterior nuclei of the left and right thalami as well as in the splenium of the corpus callosum and globus pallidus in pediatric patients compared to age- and sex-matched controls. T2 lesion load was negatively correlated with volume loss in the splenium of the corpus callosum, supporting the notion of Wallerian degeneration of the crossing nerve fiber tracts caused by axonal transection within hemispheric white matter focal lesions.[172] Loss of volume within the optic tract (from the chiasm to the lateral geniculate bodies, and the anterior portion of the optic radiations) was associated with disease duration but not T2 lesion volume; however, *post hoc* analyses did not show any correlation between history of optic neuritis and optic pathway volume loss.

Using a region-of-interest method to analyze brain volume in children and adolescents with MS compared to healthy individuals, a significant reduction in global brain volume was detected in patients with MS.[173] After correction for global brain volume, an even greater reduction in thalamic volume was observed in MS patients compared to control subjects. Reduced normalized brain volume correlated moderately with increased T1 and T2 lesion volumes, suggesting an important contribution of focal MRI-visible lesions to the neurodegenerative process of MS. The authors also observed a reduction in head size of the pediatric-onset MS patients compared to matched healthy control subjects. Given that skull size is largely

determined by brain growth during the first ten years after birth,[174] these findings suggest that pediatric-onset MS may affect primary brain and skull growth.[173]

In a study of MRI correlates of cognitive impairment in children with MS, thalamic volume and corpus callosal area were positively correlated with an index of global intellectual function, mental processing speed and confrontation naming.[73] These findings support the notion that the neurodegenerative aspect of MS pathobiology is operative in the earliest stages of the disease, and that immune-mediated damage of immature neural networks may have significant deleterious consequences on cognitive function. Longitudinal studies are needed to determine whether resiliency of developing neural networks will be able to protect children with MS from long-term cognitive deficits.

3.4 Magnetization Transfer Imaging

3.4.1 Background

MRI studies have been pivotal in showing that pathological changes are present not only within lesions but also in the normal-appearing brain matter (NABM) of patients with MS. Magnetization transfer (MT) imaging is a technique that can be used to quantify the level of microstructural damage within T2 visible lesions as well as NAWM and NAGM.[175-178]

On MTR maps, demyelinating lesions (T2 visible lesions) appear as hypointense (**Figure 3-2**). Post mortem studies of patients with MS have demonstrated the relationship between low

MTR and neuraxonal damage and myelin loss both within lesions as well as in NABM.[179-183] Remyelinated lesions have a higher MTR than demyelinated lesions,[181] suggesting the potential role of MT imaging in monitoring remyelination.

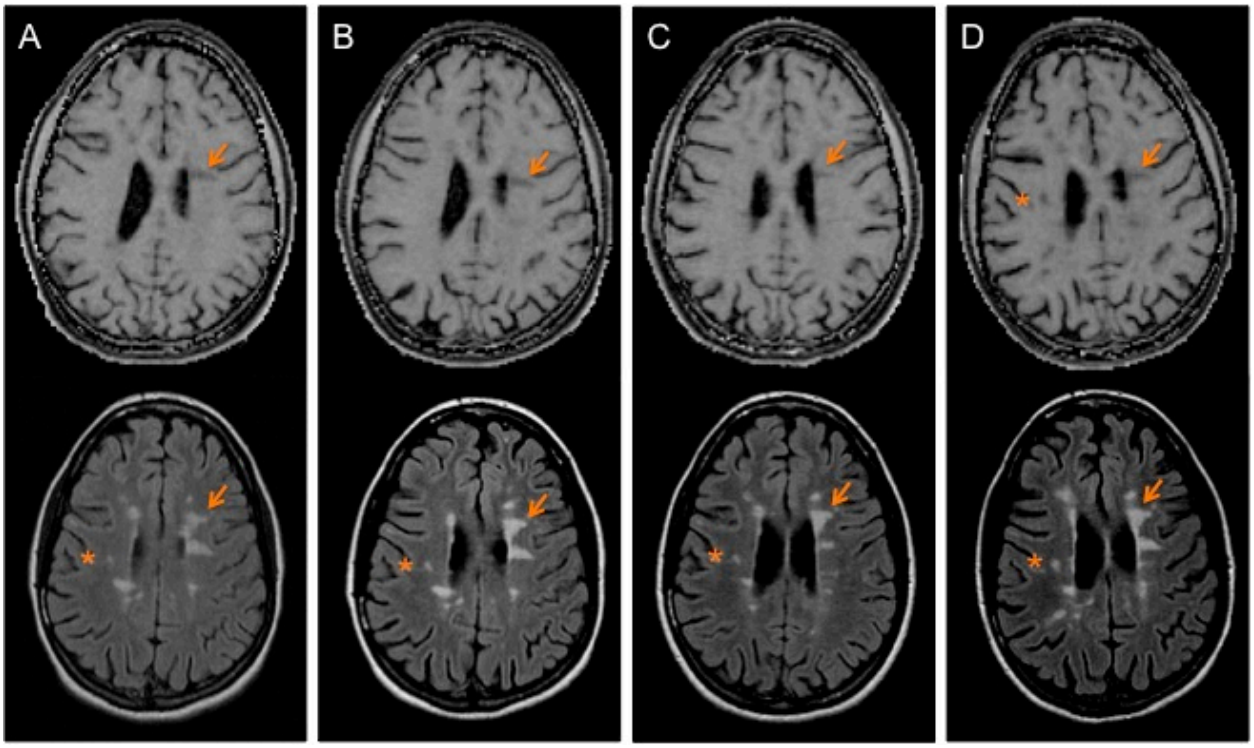

Figure 3-2: Axial MTR maps (top row) and corresponding axial FLAIR images (bottom row) of a girl who presented with a first attack of MS at age 11 years. Scans were acquired at 5 years (Panel A), 6 years (Panel B), 7 years (Panel C) and 8 years (Panel D) post-onset.

A subset of the T2 lesions is visible on the corresponding MTR maps. The periventricular T2 lesion denoted by the arrow on FLAIR images demonstrates decreased MTR on corresponding MT images across all time points. The T2 lesion demarcated by an * on all serial FLAIR images shows a decreased MTR at the 8 year follow-up scan.

3.4.2 Physics of Magnetization Transfer Imaging

Conventional MRI pulse sequences exploit the properties (i.e. T1 and T2 relaxation behaviour as well as spin density) of free water protons to produce tissue contrast. There is a pool of non-aqueous protons associated with proteins and macromolecules, such as myelin, that may permit quantification of myelin integrity in the context of MS. However, due to the short transverse (T2) relaxation times of these bound proton spins, signal decays too rapidly and prohibits acquisition of an MRI signal. Another challenge to imaging bound protons is that the frequency of the signal from aqueous protons is near that of the signal from the non-aqueous proton pool.

Using MT imaging, the protons bound to macromolecules can be probed via their exchange or transfer of magnetization with free-water protons.[184,185] To achieve this, a selective radiofrequency pulse that is offset from the water resonance is applied to excite primarily the bound proton pool, thereby partially saturating its magnetization. Magnetization exchange between the partially saturated bound proton pool and the more mobile pool of spins causes a reduction in the signal measured by MRI. The MT ratio (MTR) represents the efficiency of this magnetization exchange, and is calculated as a difference in signal between the images acquired with and without the off-resonance radiofrequency pulse. MTR maps are computed at a voxel level according to the following equation:

$$MTR = (1-MT_{on}/MT_{off}) \times 100\%$$

in which MT_{on} refers to the signal intensity with the off-resonance radiofrequency pulse and MT_{off} refers to the intensity without the off-resonance pulse.

3.4.3 Magnetization Transfer Imaging Analytical Methods

Several methods exist for analyzing MTR maps. These include computing average MTR within regions of interest such the NAWM or NAGM or within lesions; computing histograms of MTR for the whole brain; or voxel-by-voxel analysis. The latter is more technically demanding as image registration is required to identify corresponding voxels in multiple scans from a time series or across scans of different individuals.

3.4.4 Magnetization Transfer Imaging in Pediatric-Onset MS

To date, only three studies have evaluated microstructural tissue abnormalities in children with MS using MT imaging.[107,186,187] A preliminary study evaluated the average MTR and histogram peak height (i.e. the most frequently occurring MTR value) in both the NABM and cervical spinal cord of 13 children with MS compared to age- and sex-matched healthy controls. However, no significant differences were found.[186] In a follow-up study of 23 children with MS and 16 age- and sex-matched healthy controls, average MTR and histogram peak height in both the NAWM and NAGM were similar.[187] In a study comparing 33 adults with pediatric-onset MS to 381 adults with adult-onset disease, MTR values tended to be lower within T2 lesions, NAWM and NAGM,[107] suggesting a greater degree of microstructural abnormality in pediatric-onset adult patients than adult-onset patients, perhaps explained by a longer disease duration. Further studies using voxel-based methods and longitudinal MTR analyses are required to determine

the extent and time-course for neuroaxonal and myelin injury in children and whether children demonstrate enhanced reparative capacity.

3.5 Diffusion Tensor Imaging

3.5.1 Background

Diffusion imaging is a powerful and sensitive technique used to non-invasively measure restriction of water diffusion *in vivo* within an image voxel, thereby providing information on the structural organization of the brain and spinal cord, as well as pathological changes not visualized on conventional MRI scans. In diffusion imaging, the displacement (on the order of 10-15 μm[188]) of thermally driven, randomly moving (i.e. Brownian motion) water molecules is measured. This distance is sufficient for restriction caused by subcellular and cellular structure to affect the measured signal; however, establishing which aspects of cellular structure account for observed changes is difficult. In white matter, the axonal membrane, intra-axonal structures such as neurofilaments, as well as the ensheathing layers of myelin are all thought to affect the diffusion signal observed by MRI.[188]

3.5.2 Physics of DTI

The addition of a pair of strong gradient pulses to a spin-echo sequence is the most common strategy to create a diffusion-weighted imaging sequence, and is typically combined with an

echo planar imaging (EPI) readout for rapid acquisition. The amplitude, duration, and interval between the gradient pulses determine the diffusion weighting of the sequences and can be summarized by single variable termed the *b*-value. The exponential rate of signal loss with increasing *b*-values is used to define the apparent diffusion coefficient (ADC) for a tissue and reflects both the diffusivity of water as well as the restrictions on water motion imposed by cellular structures. For the modest b-values that are obtainable using clinical MRI systems, ADC shows little variation with *b*.

A limitation of reporting ADC alone is that for tissue with oriented structure, such as white matter tracts, the coefficient varies with the direction of the diffusion weighting gradients. A useful way to represent this directional dependence is to imagine an ellipsoidal region that describes where a given water molecule is likely to be found a short time later. While this ellipsoidal confidence region needs to grow with time to allow for larger and larger displacements of the water molecule, its shape remains the same. The ellipsoidal region can be represented mathematically by a 3 by 3 tensor where the eigenvectors of the tensor describe the axes of the ellipsoid and the eigenvalues the diffusivity along each axis. A minimum of six different diffusion-weighting directions is needed to estimate the diffusion tensor for a given tissue.

Where water freely diffuses in all directions, such as in cerebrospinal fluid, this confidence region is spherical – representing isotropic diffusion. In tissue, where there are obstacles to water diffusion in certain directions, the confidence region could be ellipsoidal – representing anisotropic diffusion. In myelinated tracts of the brain, for example, diffusion within each voxel would occur principally parallel to the axons, with relatively little diffusion perpendicular to the

axon possibly due to the hydrophobic nature of myelin.[189] The principal eigenvector ($\lambda_{,1}$) represents the diffusion direction of greatest magnitude within the voxel, and is parallel to the white matter fibers. The secondary ($\lambda_{,2}$) and tertiary ($\lambda_{,3}$) eigenvectors are perpendicular to $\lambda_{,1}$ and transverse to white matter fiber tracts.

Four diffusion parameters derived from the diffusion tensor are typically reported in DTI studies: 1) mean diffusivity (MD) is the arithmetic average of the three diffusivities; 2) fractional anisotropy (FA) is a measure of the eccentricity of the ellipsoidal confidence region;[190] 3) parallel (axial) diffusivity ($\lambda_{||}$) is the same as $\lambda_{,1}$; and 4) transverse (radial) diffusivity ($\lambda_{\perp}$) is the average of $\lambda_{,2}$ and $\lambda_{,3}$ (**Figure 3-3**).

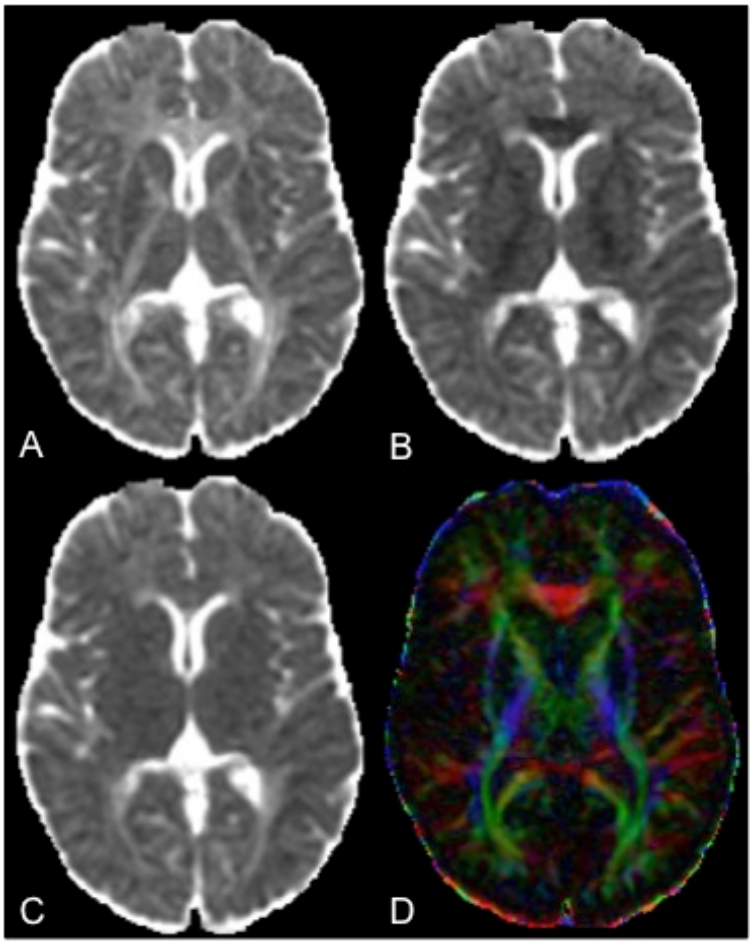

Figure 3-3: Axial diffusivity (A), radial diffusivity (B), mean diffusivity (C), and fractional anisotropy (D) maps of a child with MS. In (D), fibers oriented right-left are in red, fibers oriented superior-inferior are in blue, and fibers oriented anterior-posterior are in green.

3.5.3 DTI Analysis Methods

Several DTI techniques have been developed to selectively localize white matter tracts and evaluate their diffusion related changes in individuals with MS. Using a voxel-wise comparisons (VWC) method, several studies have shown correlations between FA and clinical parameters such as physical disability and cognitive impairment.[165,191,192] Voxel-wise methods rely on rigorous spatial registration and spatial smoothing, both of which can affect the consistency of results and the influence of partial volume.[193,194]

A relatively new technique that permits whole-brain evaluation without *a priori* knowledge of location of white matter tract damage, known as tract-based spatial statistics (TBSS), eliminates the requirements for spatial smoothing and robust nonlinear image registration.[195] When conducting TBSS, an initial approximate nonlinear FA image registration is first performed and images are then projected onto a group-mean white matter tract FA skeleton, permitting coregistration between subjects or patient groups. Voxelwise statistics can then be computed across subjects on the data projected to the FA skeleton.

Another DTI analysis technique is to characterize white matter pathways through fiber tracking.[196-198] Fiber tracking techniques can be summarized as deterministic or probabilistic. Deterministic methods involve propagation of a path from a seed point to another coordinate

in the brain by interpolating diffusion tensors or following orientations of primary eigenvectors.[198] Applying this technique in patients with MS has some limitations as decreased FA values within lesions lead to uncertainty about orientation of the primary eigenvector or deterministic tracts may terminate altogether within a lesion.[199] A variation on this method, probabilistic fiber tracking, is to consider multiple tract trajectories emanating from a given voxel and weight these by the probability density function for water diffusion at that location.

3.5.4 DTI in Pediatric-Onset MS

The earliest DTI studies conducted in children with MS report histogram analyses of lesions, NAWM and NAGM.[186,187] In pediatric-onset MS patients, the NABM average MD is lower compared to healthy controls.[186] More specifically, the microstructural abnormalities seen in the NABM of children with MS may be specific to the NAWM, given the increased average MD and decreased MD histogram peak height observed in the NAWM when compared to controls.[187] The NAWM also shows lower average FA and higher peak height, compared to healthy children.[187,200] Increased MD and decreased FA in the NAWM of children with MS suggests the presence of white matter tract disorganization, and may support the hypothesis of glial proliferation since glial cells do not have the same anisotropic morphology as myelinated fibers.[187] When compared to adults with a first attack of CNS demyelination, the FA and MD of NAWM, NAGM or lesions was not different for children with acute demyelination.[200] However, adult patients with relapsing-remitting MS (RRMS) had higher average MD values both within lesions and the NAGM compared to pediatric relapsing-remitting MS patients.[200] Average lesion

FA and MD as well as FA and MD of NAWM correlate with T2 lesion volume,[187,200] suggesting that NAWM damage may be a phenomenon of Wallerian degeneration of axons that pass through visible T2 lesions.

To date, one study has used a tract-based approach to characterize diffusion abnormalities of the callosal, projection, and association pathways in children with MS.[201] In lesional white matter, mean apparent diffusion coefficient (ADC) values within corpus callosal, posterior limb of the internal capsule and long association fiber regions of interest are higher in children with MS compared to healthy individuals and FA values are lower. When considering only NAWM, higher ADC and lower FA values are observed.[201] Tract-based measures of NAWM fibers show higher ADC and lower FA values in children with MS compared to healthy controls.[201]

Recently, a study was conducted to assess the functional consequence of loss of microstructural integrity in children with MS using DTI and cognitive measures of processing speed.[202] In a cohort of 36 children with MS and 30 age- and sex-matched healthy controls, children with MS showed lower FA values in the NAWM of both the genu and splenium of the corpus callosum as well as in the NAWM of the bilateral temporal, parietal and occipital lobes, compared to controls.[202] FA and MD values of the averaged right-left thalami did not differ between patients and controls. For children with MS, T2 lesion volume was associated with corpus callosal FA, specifically the genu and splenium, and NAWM hemispheric FA, but not with thalamic FA. In children with MS, processing speed, measured with the *Symbol Digit Modalities Test (SDMT)* and the *Visual Matching* subtest of the Woodcock-Johnson III Test of Cognitive Abilities, was faster in children with higher FA values within the lobar NAWM, particularly in the right hemisphere. Faster processing speed on the SDMT was correlated with higher FA in the genu of

the corpus callosum, whereas faster performance on the Visual Matching subtest was associated with higher FA in all corpus callosal regions. Lower MD values in the corpus callosum were correlated with faster processing speed on both tests. FA of the thalamus in children with MS was positively correlated with performance on the SDMT but not Visual Matching subtest.

These studies together suggest that disruption of normal white matter structure occurs early in the disease, that the WM integrity disruption is widespread, and that this early damage to the NAWM has functional consequences.

3.6 ^{1}H-Magnetic Resonance Spectroscopy

3.6.1 Background and Summary of Technique

^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) is similar in technique to MRI, but instead of using the signal from water protons to provide structural information, ^{1}H-MRS acquires information from hydrogen nuclei of molecules or metabolites present in tissues, and this metabolic information has pathological specificity not obtainable from water proton signals.[203] Magnetic resonance spectra are identified by their resonance frequency and expressed as a shift in frequency in parts-per-million (ppm) relative to a reference standard. Two major factors determine whether metabolite resonances can be usefully studied by ^{1}H-MRS: 1) only freely mobile molecules yield well-defined, discrete resonances, and 2) only those molecules with high concentrations (i.e. on the order of millimoles per liter) will produce sufficient signal-to-noise.[204]

The concentration of water in brain tissue is much greater than that of metabolites, and therefore produces a much greater signal compared to the signals produced by metabolite protons. Therefore, ^{1}H-MRS studies use MRI sequences that suppress the signal from water protons. The echo time in a water-suppressed ^{1}H-MRS study determines the number of observed spectral peaks as well as their amplitude. Four major metabolite resonances are revealed in the brain at long echo times (e.g. TE=144 ms): 1) the methyl resonance of tetramethylamines, especially the choline-containing phospholipids at 3.2 ppm that include choline (Cho), phosphocholine, glycerophosphocholine and betaine; 2) methyl resonance of creatine and phosphocreatine (Cr) at 3.0 ppm; 3) methyl resonance of N-acetyl-containing compounds (NA), especially N-acetylaspartate (NAA) at 2.0 ppm; and 4) methyl resonance of lactate (Lac) as a doublet at 1.3 ppm, which is normally difficult to visualize above baseline noise and mobile lipids and other macromolecules.[205] At shorter echo times, signals from molecules with short T2 relaxation times can be obtained. These metabolite resonances include amino acids such as γ-aminobutyric acid (GABA) and glutamate, and sugars such as myo-inositol. Short-echo time ^{1}H-MRS measurements can be complicated by gradient-induced distortions and a poorly defined baseline.

Obtaining estimates of absolute metabolite concentrations from ^{1}H-MRS is challenging because the spectral peak area depends on acquisition parameters as well as the T1 of the metabolite. While reference phantoms can aid in calibrating such studies,[206] many studies use another endogenous metabolite as a reference and report a metabolite ratio. The source of metabolite signals collected is spatially localized to a given brain region by using either a single-voxel (^{1}H-MRS) or multi-voxel (^{1}H-MR spectroscopic imaging) technique. Single voxel techniques often

use larger volume elements (e.g. 2-3 cm^3) than multi-voxel techniques (e.g. 0.5-1 cm^3 per voxel) and obtain a proportionately higher signal to noise ratio.[205]

3.6.2 The ^{1}H-MRS Resonances

To understand in vivo measures of chemical pathology associated with impairments in axonal metabolic and structural integrity, the normal physiology of metabolite resonances commonly studied in MS will be briefly reviewed.

1. The Methyl Resonance from N-acetyl Groups: The NA resonance represents N-acetylaspartate (NAA) primarily and N-acetylaspartylglutamate (NAAG) to a lesser degree. NAA is synthesized by neuronal mitochondria and localizes almost exclusively in neurons and neuronal processes. It is important in myelin lipid synthesis, water pump function in myelinated neurons, and as a precursor for NAAG.[207,208] Decreases in NAA can result from any of the following intravoxel changes: 1) decrease in axonal density secondary to axonal loss or atrophy, 2) mitochondrial metabolic dysfunction within neurons and axons, or 3) dilution of NAA secondary to edema or non-NAA-containing cells. NAA as a specific marker of axonal integrity in patients with MS has been demonstrated by strong correlations between in vivo ^{1}H-MRS measurements of NAA resonance and immunohistochemical analyses of brain biopsy specimens.[209]

2. The Creatine Resonance: The resonance of creatine (Cr) reflects the presence of creatine and phosphocreatine which play roles in energy metabolism and homeostasis. Although present in neurons and glial cells, its concentration is highest in astrocytes and oligodendrocytes.[210]

Creatine is often used as an intravoxel standard for other metabolites due to its constant concentration and resistance to change throughout the brain. In the study of patients with MS, the intravoxel NAA/Cr ratio is commonly used as an index of neuronal integrity.[211-213]

3. The Glutamate Resonance: Glutamate (Glu), produced from glutamine (Gln) in neurons, is the primary excitatory neurotransmitter in the CNS. The Glu resonance is of interest in ^{1}H-MRS studies of patients with MS because glutamate excitotoxicity is associated with neurodegeneration.[214] Glutamate, is also produced by lymphocytes and microglia,[215,216] and in excess, is associated with axonal and oligodendrocyte damage.[215] Oligodendrocyte-mediated clearance of glutamate in the extrasynaptic white matter of the CNS in adult patients with MS is impaired, and therefore glutamate levels are elevated in acute lesions and NAWM, but not in chronic lesions.[217-219]

3.6.3 The NA Resonance: a Marker of Axonal Injury in MS

Owing to its prominence in the ^{1}H-MR spectrum and its localization within neurons, decreases in NAA are often used as indicators of MS pathology and disease progression.[205,212,220] The presence of low NAA, measured both as an absolute concentration and as a ratio of NAA/Cr, has been confirmed within lesional white matter, NAWM and normal-appearing cortical grey matter of adult patients with MS relative to healthy controls.[209,213,221-228] Decreases in NAA resonance intensity of up to 50% can be observed in the NAWM of adult patients[229] and of up to 80% in white matter regions of T2-hyperintensity.[230] The loss in neuronal integrity measured by decreased NAA/Cr ratios follows a gradient around T2 lesions, with greater injury proximal

to the lesion relative to the more distal NAWM.[223] Based on brain biopsy and post mortem spinal cord analyses of adults with MS, decreases in NAA resonance intensities are associated with decreases in axonal density.[209,231,232] Loss of neuronal integrity as measured by MRS has been demonstrated even in the earliest stages of disease.[233,234] The NAA/Cr ratio in adult patients with relapsing-remitting MS shows a strong negative correlation with measures of clinical disability,[235-237] a negative correlation with cerebral atrophy measures,[238] and a positive correlation with functional measures of adaptive reorganization.[239] In a study evaluating multiple MRI techniques, cerebral NAA/Cr values were most strongly associated with clinical disability measures.[235]

3.6.4 ^{1}H-MRS in Pediatric-Onset MS

Figure 3-4 illustrates single-voxel ^{1}H-MRS spectra from the NAWM of a child with MS and healthy control. To date, the application of ^{1}H-MRS to children with MS has been limited to three studies. The first study included eight individuals with pediatric-onset MS and the findings mimic that seen in patients with adult-onset disease, showing decreased NAA and Cr resonances and increased resonances of choline and myo-inositol within lesions relative to healthy age-matched controls.[240] In contrast to observations in adults, two studies have shown that the ^{1}H-MR spectra of the NAWM of pediatric patients are not different than white matter of healthy controls.[240,241] However, according to unpublished work by the authors of this chapter, a decreased NAA/Cr can be observed in the NAWM of children with MS compared to healthy controls (**Figure 3-4**).

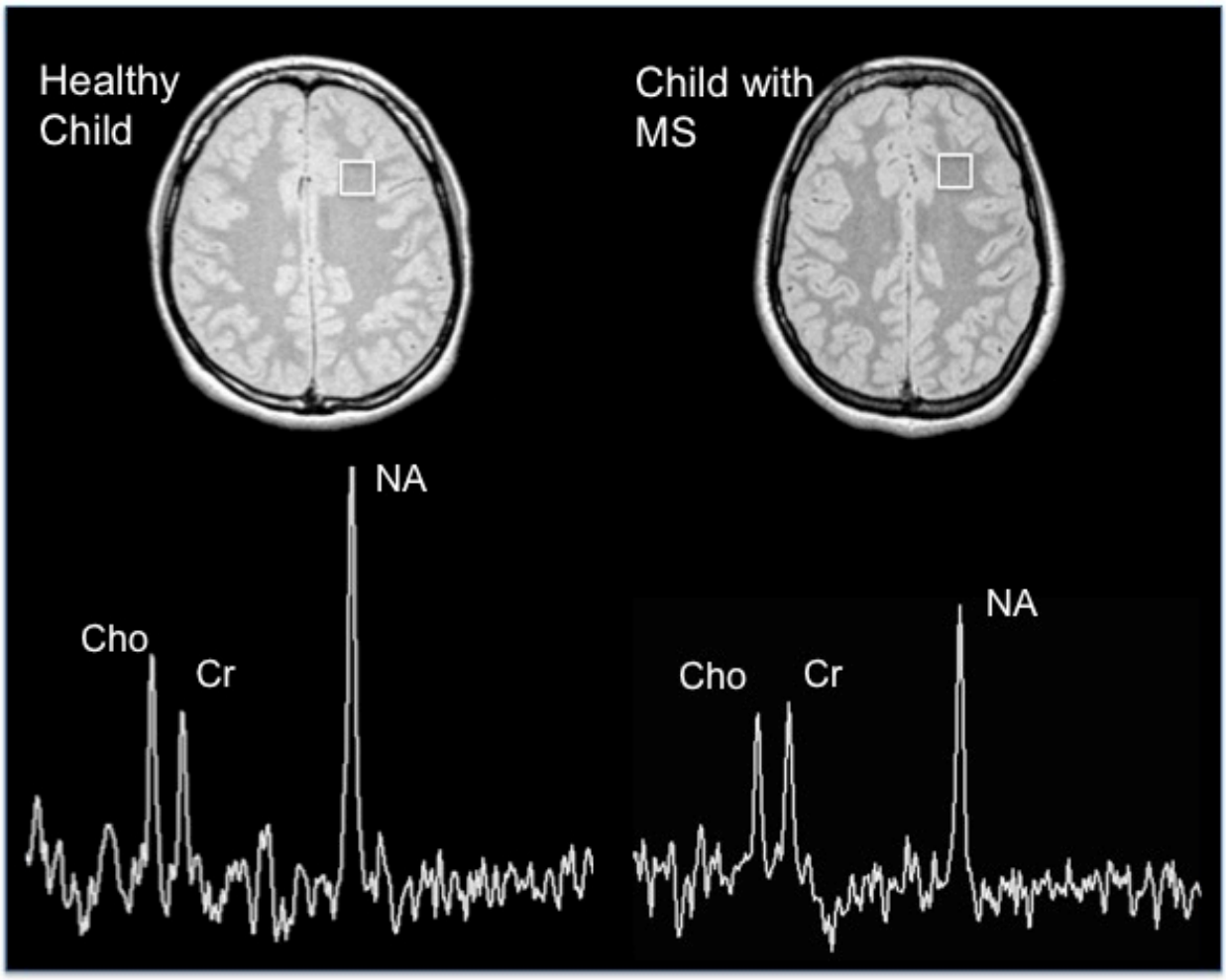

Figure 3-4: ^{1}H-MRS resonances demonstrating a reduced NA/Cr ratio in the left frontal NAWM of child with MS compared to a healthy child. The NA peak includes methyl resonances from N-acetyl groups of NAA and NAAG. The Cr resonance includes creatine and phosphocreatine. The Cho peak includes methyl resonances of all choline-containing compounds (choline, phosphocholine, glycerphosphocholine, and betaine). (Courtesy of S Narayanan, PhD, McConnell Brain Imaging Center, Montreal Neurological Institute, McGill University, Montreal, Canada)

A second study evaluated the resonance of citrulline in the brains of 27 children with MS compared to 23 control subjects.[241] Citrullination is a posttranslational modification of myelin

basic protein (MBP), the only essential structural protein for myelin formation, where arginine is converted to citrulline via deamination. Increased citrullination of MBP diminishes its ability to organize lipid bilayers into compact multilayers, resulting in myelin instability.[242,243] Increased levels of citrullinated MBP have been shown in brain specimens from MS patients,[244] suggesting that citrullination may predispose the white matter to demyelination. This hypothesis was tested in children with MS with ^{1}H-MRS using spectral narrowing to search for the citrulline resonance.[241] A total of 44% of children with MS showed a citrulline peak compared to only 13% of control subjects who were imaged for headache or syncope. A citrulline peak was observed in both NAWM and T2 hyperintense lesions, and conforms with histopathological evidence of increased citrullinated MBP in the white matter of patients with MS.[245] The NAA/Cr and Cho/Cr ratios were not different between pediatric-onset patients and controls, raising the possibility of better metabolic neuroaxonal recovery in children. However, mean myo-inositol/Cr ratio was significantly higher within lesions than NAWM of patients and the white matter of controls, suggesting significant glial proliferation as suggested by other studies.[246]

The third study included seven children with acute disseminated encephalomyelitis (ADEM) with the goal of identifying an MRS signature of monophasic ADEM that is distinct from that reported in MS.[247] ADEM represents a monophasic inflammatory disorder characterized by large edematous lesions within the CNS. The authors report a substantial reduction in the myo-inositol/Cr ratio in children with ADEM,[247] which contrasts with the increased intralesional myo-inositol/Cr ratio reported in MS patients.[248] Myo-inositol is an osmolyte and a precursor of myelin phophatidyl inositol found in astrocytes, and is considered a glial marker.[249] Functioning as an osmolyte, decreases in myo-inositol could indicate decreases in regulatory volume

necessary to reduce cell swelling and normalize edema.[250] Reduced myo-inositol resonance in children with ADEM could therefore be explained as a compensatory mechanism to counter-act edema.

3.7 Functional MRI

3.7.1 Background and Summary of Technique

The concept that regional cerebral blood flow reflects neuronal activity is the basis for functional MRI (fMRI). A focal increase in neuronal synaptic and electrical activity triggers a local increase in cerebral blood flow, cerebral blood volume, cerebral metabolic rate of oxygen use and cerebral metabolic rate of glucose rate consumption. Increased neuronal activity leads to a local increase in blood flow, which exceeds the heightened demand for oxygen thereby causing a net increase in blood oxygenation in that region. As deoxyhemoglobin is paramagnetic, the reduction in the deoxyhemoglobin concentration that occurs with increased blood flow results in a reduction in T2* and a local increase in the signal observed in T2* weighted MRI scans. This blood oxygenation dependent contrast or BOLD effect is the basis of most functional MRI studies. The BOLD response is closely coupled with neuronal firing rate,[251] and functional localization derived from fMRI agrees with the localization obtained in electrophysiological studies.[252]

3.7.2 Methods of fMRI Acquisition and Analysis

Several experimental designs are used when acquiring fMRI data. Task-dependent paradigms can be used to address several questions of interest in MS related to visual or motor function, cognition, fatigue, and depression. Another paradigm, resting state fMRI, is unique in that it does not require a stimulus presentation. The objective of this paradigm is to detect signal fluctuations (up to 3%) that are correlated between different parts of the brain. These fluctuating patterns are analyzed to determine network connectivity.

Several factors can confound the detection of activation patterns in patients with MS. For example, it has been shown that the disease-modifying therapy interferon β-1a increases basal ganglia blood flow,[253] and benzodiazepines and baclofen which are used to relieve hypertonia reduce cerebral metabolism.[254] The BOLD signal can also be affected by caffeine intake,[255] circadian rhythm[256] and menstrual cycle.[257] These potential confounders must be mitigated against in the design of fMRI studies.

3.7.3 fMRI in Pediatric-Onset MS

To date, two studies have evaluated cortical activation patterns and functional connectivity using fMRI in children with MS.[258,259] Natural history studies have shown that time to reach physicial disability in children with MS is on average 10 years longer than that seen in adults.[2,5,48,260] One plausible explanation for this is the enhanced capacity for brain network reorganization in pediatric- versus adult-onset patients. To test this hypothesis, the first study

evaluated the movement-associated pattern of cortical activations and motor network connectivity in 17 children with MS compared to 9 age- and sex-matched healthy controls.[258] Increased activation was observed in the contralateral sensorimotor cortex compared to controls, which was correlated with T2 lesion volume. The authors suggested that the increased contralateral activation represents an adaptation to the presence of tissue disruption. In a functional connectivity analysis, the investigators found reduced connectivity between the left primary sensorimotor cortex and left thalamus, left insula and left secondary sensorimotor cortex, supplementary motor area and the left secondary sensorimotor cortex, left thalamus and left insula, and the left thalamus and left secondary somatosensory cortex, when compared to controls. The authors speculated that this connectivity "downregulation" could be compensatory, representing a functional reservoir which may be upregulated when irreversible structural damage accumulates.

The authors expanded on these findings in a second study by evaluating effective connectivity changes within the motor network in children with MS compared to adult-onset patients, and considered the influence of structural damage to the corpus callosum and corticospinal tracts on connectivity.[259] No changes in effective connectivity were detected between pediatric MS patients and age- and sex-matched healthy controls, suggesting that the adaptive properties or plasticity of the cortex may be preserved in children. The effective intra- and inter-hemispheric motor network connectivity was increased in adults with a first attack of CNS demyelination and more markedly so in adults with MS compared to children with MS. These connectivity changes were associated with microstructural changes within the corpus callosum and corticospinal tracts as measured by MD and FA. Taken together, the incremental recruitment of

networks in adult- compared to pediatric-onset MS and the association between this recruitment and structural damage suggests that the propensity for brain plasticity or the functional reservoir may deplete over time, and manifest as accrual of physical disability.

3.8 Conclusion

The application of advanced MRI techniques to MS undoubtedly enhances understanding of disease pathophysiology, and holds promise in its ability to resolve the discrepancy between conventional MRI findings and clinical disability – a necessary step to identifying imaging markers of prognosis and therapeutic response. While application of these modern techniques to pediatric MS is still in its infancy, several challenges unique to the pediatric population are currently being resolved, such as optimization of brain atrophy measurement in individuals where brain and skull growth is ongoing, and standardization of MRI acquisition and image analysis to permit multi-centered studies necessary for obtaining sufficient sample sizes to address unique questions. Several techniques have not yet been applied to pediatric MS or at most have only been piloted, including multicomponent T2 (myelin water content) imaging, MT imaging, susceptibility weighted imaging, cortical imaging, fMRI, high resolution imaging of the optic nerve and spinal cord, and imaging at very high field strengths (>3 Tesla). Children with MS represent a unique cohort in which these techniques can be applied to aid in elucidating the earliest signatures of MS disease, given the short window of time between potential inciting exposures and disease onset. Further work is required to determine whether these advanced techniques can be used in treatment trials. Implementation and optimization of multi-centered

standardized protocols for advanced imaging techniques is timely given that pediatric trials of MS-related therapies are soon to be launched.

Chapter 4:

Dissertation Rationale and Objectives

4.1 Rationale

Approximately 20-30% of children with an incident ADS are at risk for subsequent demyelinating events, or MS.[17,50,51] In the remaining 70-80% of children, ADS represents a monophasic illness with no serial clinical or magnetic resonance imaging (MRI) evidence of further disease. A role for MRI in identifying children with ADS who are at an increased likelihood for a future diagnosis of MS is supported by several observations. First, MRI is an established tool for the diagnosis and management of individuals with MS, and MRI features associated with MS have been well defined.[261] Second, features of MS are well visualized on 1.5 Tesla MRI scanners, and such scanners have become widely accessible as a diagnostic tool in pediatric healthcare centers. Finally, the utility of MRI to identify patient populations suited for clinical trials has served as a key component of pharmaceutical research in adult-onset MS, and will soon be a component of pediatric MS clinical trials.

MRI criteria for diagnosis of MS in children[92,93,103] and for prediction of subsequent MS diagnosis in children with ADS[91,262] have been published. The ability to compare the predictive utility of these criteria for subsequent MS diagnosis is limited for several reasons: i) lack of uniform pediatric populations in which the MRI characteristics were developed or tested; ii) predictive parameters or diagnostic criteria that emerged from the studies were highly specific for MS, but failed to recognize a large proportion of children with MS (i.e. low sensitivity), especially in children less than ten years of age at onset; and iii) lack of well-described and consistently applied definitions for the MRI parameters evaluated.

The present dissertation is focused on defining the predictive value of MRI characteristics for subsequent MS diagnosis in children with ADS. Prior to commencement of the core analyses, a methodological study was conducted in which an evidence-based standardized MRI scoring tool was developed. The scoring tool was subsequently applied to the Canadian pediatric cohort to define MRI features predictive of MS diagnosis, and the MRI predictive parameters were then validated in an independent Dutch pediatric cohort. MRI scans were also scored according to the recently revised international McDonald criteria for MS diagnosis.[90] Finally, we evaluated the MRI features of the spinal cord in a cohort of children with established MS.

4.2 Specific Objectives

1) Develop a standardized MRI scoring tool for evaluation of MRI scans of children with acute CNS demyelination that contains the following properties: acceptable inter-rater reliability, specificity for demyelinating disorders, and straightforward to use in the clinical setting.

2) Determine MRI features that predict subsequent MS diagnosis in children with acute CNS demyelination enrolled in a prospective national cohort study.

3) Validate the MRI parameters for prediction of MS diagnosis identified under Objective 2 in an independent Dutch cohort of children with acute CNS demyelination.

4) Evaluate the predictive value of the 2010 McDonald criteria for MS diagnosis in a prospective national cohort study, and compare their performance with the 2005 McDonald criteria.

5) Describe the MRI characteristics of spinal cord lesions in a selected cohort of children with MS who were imaged either for inter-current spinal cord symptoms or to aid in confirmation of MS diagnosis.

4.3 Hypotheses

1) A scoring tool consisting of binary-response parameters and accompanied by a manual with clear parameter definitions and an anatomical atlas will permit systematic and reliable scoring of MRI scans acquired in the Canadian cohort (Objective 2), and will also have applicability as a clinical tool.

2) Specific MRI features (i.e. T2-weighted juxtacortical, periventricular, brainstem and intracallosal lesions – characteristic locations for lesion formation in MS patients, T1-hypointense lesions – suggestive of established tissue injury and thus chronic disease, and contrast-enhancing lesions – indicative of blood-brain barrier permeability and immune cell activation) are highly predictive of subsequent MS diagnosis in children with ADS.

3) The high sensitivity and specificity of the predictive parameters identified in our Canadian cohort are replicable in other pediatric ADS cohorts.

4) The 2010 McDonald criteria demonstrate good sensitivity and predictive value for MS diagnosis when applied to scans of children with ADS. When compared to the 2005 McDonald criteria, the 2010 criteria permit an earlier MS diagnosis.

5) In pediatric-onset MS patients, spinal cord lesions are typically focal rather than longitudinally-extensive, are most frequently located in the cervical spinal cord, and are not associated with permanent neurological deficits.

PART II:

MRI AS A TOOL FOR PREDICTION OF PEDIATRIC-ONSET MS DIAGNOSIS

Chapter 5:

Development of a Standardized MRI Scoring Tool for CNS Demyelination in Children

Verhey LH, Branson HM, Laughlin S, Shroff MM, Benseler SM, Feldman BM, Streiner DL, Sled JG, Banwell B

Am J Neuroradiol, *in press*

5.1 Summary

Background The degree to which magnetic resonance imaging (MRI) is useful in the diagnosis of multiple sclerosis (MS) is predicated on standardized and reliable evaluation of MRI parameters. We aimed to devise items for an MRI scoring tool that had high inter-rater agreement and were straightforward to apply.

Methods Based on a literature search and consensus of an expert panel, we identified 48 parameters that describe acute central nervous system (CNS) demyelination, predict MS diagnosis, or characterize demyelinating disorder mimics. MRI scans of children with clinically-confirmed MS, monophasic acute disseminated encephalomyelitis (ADEM), and angiography-negative biopsy-positive small vessel primary angiitis of the CNS (SV-cPACNS) were scored by two neuroradiologists independently, using the preliminary 48-parameter tool. Parameters with Cohen's kappa ≥0.6 and deemed important in predicting diagnosis were retained**.** Parameters not visualized on routine clinical imaging, or not important in differentiating between MS, ADEM and SV-cPACNS were discarded.

Results Of 65 eligible patients, 55 children were enrolled (16 monophasic ADEM, 27 MS, 12 SV-cPACNS); 10 were excluded (6 had hardcopy films, 4 did not meet MRI quality requirements). Of the 48 parameters, 16 were retained in the final scoring tool. The remaining 28 parameters were discarded (4 had kappa <0.6 and were not deemed useful in predicting diagnosis; 9 were not visible on routinely acquired clinical scans; and 15 had inter-rater agreement ≥0.6, but were not useful in differentiating between monophasic ADEM, MS and SV-cPACNS).

Interpretation We propose a 16-parameter MRI scoring tool that is straightforward to apply in the clinical setting and demonstrates high inter-rater agreement.

5.2 Introduction

Owing to its high sensitivity, MRI is an invaluable tool for the diagnosis and management of patients with MS. MRI plays a key role in confirming an MS diagnosis prior to a second clinical attack in patients with an incident CNS demyelinating event,[89,90,101] in excluding alternate diagnoses, in monitoring response to MS-targeted therapy by evaluation of lesion accrual on serial imaging, and in monitoring disease progression by formation of confluent lesions and atrophy. The value of MRI in MS diagnosis and in informing clinical management largely rests on the extent to which standard acquisition protocols and consistent terminology is used. The Consortium of MS Centers published a consensus-based MRI acquisition protocol recommended for patients with MS.[263] A standard lexicon of the MRI features of CNS demyelination would further increase the consistency with which MRI scans are reported, and would facilitate pooling of data sets in multi-centered studies. We aimed to devise items for a standard MRI scoring tool for pediatric-onset CNS demyelination that demonstrated high inter-rater reliability and was straightforward to apply in the clinical setting.

5.3 Patients and Methods

Participants and definitions Children and adolescents less than 18 years of age with MS and monophasic ADEM were identified from the Pediatric Demyelinating Disease Registry (The Hospital for Sick Children – SickKids, Toronto, Canada), and those with SV-cPACNS were identified from a single-center prospective cohort of children followed at SickKids.[264,265]

Participant selection was based on the following inclusion criteria: 1) availability of an MRI scan acquired within 30 days of initial clinical presentation; 2) children with MS had at least two demyelinating episodes,[58] and were followed from first attack for a minimum of two years; 3) children with monophasic ADEM (defined as polyfocal neurological deficits *and encephalopathy*)[56] have been followed for at least three years without further clinical or MRI evidence of new or recurrent demyelination; and 4) children with SV-cPACNS were required to meet the Calabrese diagnostic criteria (no evidence of systemic vasculitis, normal cerebral angiography *and* brain biopsy confirmation of isolated small vessel vasculitis).[266] Participants with SV-cPACNS were included in this study as their presenting clinical and MRI features often overlap with that of acute CNS demyelination, and thus were felt to be informative in delineating MRI features suited to future studies in which the MRI tool will be evaluated for specificity across different CNS disorders. Ethics approval was obtained for this study.

MRI scoring tool A comprehensive PubMed search for articles published between January 1, 1980 and January 1, 2012 was performed by using combinations of the following search terms: "magnetic resonance imaging" OR "MRI", "multiple sclerosis" OR "MS", "pediatric", "inflammatory demyelination", "acute disseminated encephalomyelitis" OR "ADEM", and "small vessel CNS vasculitis". The search was restricted to English language publications. References cited in original and review articles were also assessed. A total of 51 articles were reviewed from which 48 MRI parameters were identified. **Appendix 1** lists the MRI parameters that formed the initial iteration of the scoring tool. A panel of experts in pediatric CNS demyelinating and inflammatory disease that included a neurologist (BB), a rheumatologist (SMB), and three neuroradiologists (HMB, SL, MMS) was convened (moderated by LHV) to

create working definitions for each of the 48 parameters, based on the literature and panel consensus. As detailed in **Appendix 1**, all parameters with the exception of lesion count were binary ('present'/'absent'). Following the panel session, a dictionary was created to document the definition of each parameter. A second follow-up panel meeting was held to further revise the parameters and definitions.

Two investigators (HMB, SL) independently applied the 48-parameter scoring tool, blinded to clinical information, to a training set of nine randomly ordered MRI scans (n=3 MS, n=3 ADEM, n=3 SV-cPACNS). These nine scans were not included in the test set described subsequently.

MRI analysis MRI scans were acquired at 1.5 Tesla according to clinical protocol and archived at SickKids. At minimum, T1-weighted and T2-weighted or FLAIR images were required for each patient. Post-contrast T1-weighted and diffusion-weighted images were evaluated when available. MRI scans were reviewed for image quality by a pediatric neuroradiologist (HMB or SL) blinded to clinical information; hardcopy films and scans degraded by dental hardware or patient motion artifact were not evaluated. All MRI scans were copied from the GE Centricity Picture Archiving and Communication System (version 3.2.0.2), anonymized, and subsequently analyzed on an eFilm (version 1.5.3) DICOM viewer workstation.

The MRI scan acquired at presentation was evaluated by the two trained raters (HMB, SL) independently and blinded to presenting symptoms and diagnosis. One individual (LHV) was present during all scoring sessions to monitor and record discrepancies in the use of the tool by the two trained raters and to perform data entry. A dictionary of the 48 parameters was

available to both raters during the scoring sessions. A Microsoft Access (version 2003) database was created, into which the two trained raters' responses to the 48 parameters were entered.

A lesion was defined as a T2-weighted or FLAIR hyperintensity with a minimal diameter of 3 mm in either the axial, sagittal or coronal plane. Adjacent lesions were classified as distinct when separated by at least 1 mm of normal-appearing tissue. T1-hypointense lesions were defined as hypointense to cortical grey matter on T1-weighted imaging, and were correlated with hyperintense lesions on T2-weighted imaging. T1-hypointense lesions were confirmed as non-enhancing on post-contrast T1-weighted imaging.

Statistical analysis The level of inter-rater agreement for each of the 48 parameters was determined by calculating Cohen's kappa (κ)[267] or the intraclass correlation coefficient (ICC)[268] as appropriate. Strength of agreement was arbitrated as: ≤0 = poor, 0.01-0.2 = slight, 0.21-0.40 = fair, 0.41-0.60 = moderate, 0.61-0.8 = substantial, 0.81-1=almost perfect.[269] The expert panel proposed the following *a priori* rules for determining whether parameters were retained or discarded: 1) parameters with inter-rater agreement ≥0.6 and the lower limit of the 95% CI ≥0.5 would be retained, provided the parameters were deemed diagnostically useful based on the panel expertise and relevant literature; 2) parameters deemed by the panel as important, based on the literature, in discriminating between monophasic ADEM, MS and SV-cPACNS for which κ or ICC was <0.6 or the lower limit of the 95% CI was <0.5, would be re-evaluated independently by both raters on all scans after refinement of the parameter definition; if inter-rater agreement increased to ≥0.6 after re-scoring, the parameter would be retained; 3) parameters not visualized on routine clinical MRI sequences would be discarded, irrespective of

the level of inter-rater agreement; and 4) parameters not contributory to differentiating between ADEM, MS and SV-cPACNS would be discarded.

Following completion of MRI scoring by both raters, the panel was reconvened to review the inter-rater agreement of each parameter and decide on which parameters were retained, based on the *a priori* rules defined previously. When there was disagreement among the panel members, one individual (MMS), expert in neuroimaging and not involved in the scoring, served as arbitrator.

A one-way ANOVA with *post hoc* Bonferroni pairwise comparisons was used to compare age at first attack in children with monophasic ADEM, MS and SV-cPACNS.

Statistical analyses were performed using Stata 12.0 (Stata Corp, College Station, TX) and SPSS 20.0 (IBM SPSS Statistics, Armonk, NY).

5.4 Results

Participants Sixty-five children and adolescents were assessed for eligibility. MRI scans for 10 participants were excluded either because they were of insufficient quality (n=4) or the images were on hardcopy film (n=6). MRI scans were evaluated for the 55 children (n=16 ADEM, n=27 MS, and n=12 SV-cPACNS) included (**Figure 5-1**). Children with ADEM were younger (mean ± SD, 6.2 ± 4.4 years) at the time of first attack than those with MS (12.8 ± 3.5 years; $p<0.0001$) and SV-cPACNS (10.3 ± 4.3 years; $p=0.028$). Onset age did not differ between children with MS and SV-cPACNS ($p=0.209$).

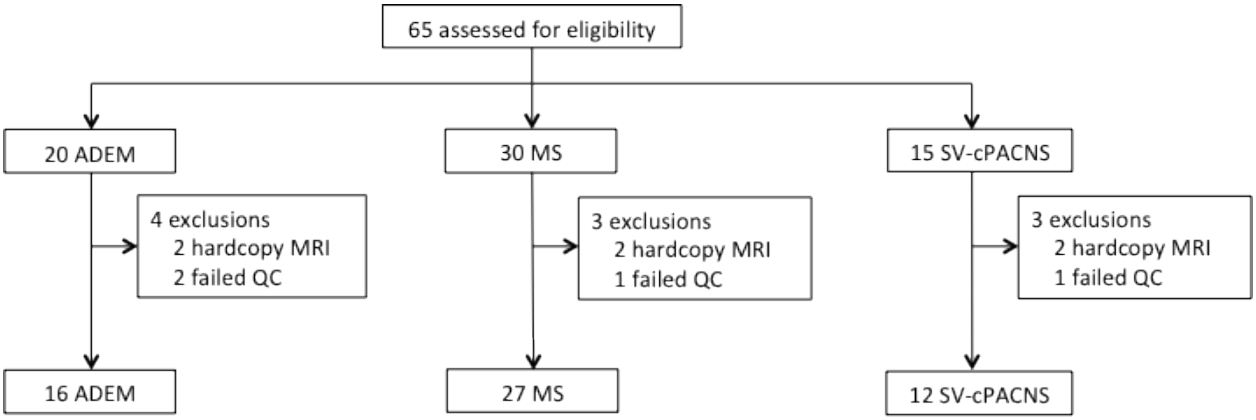

Figure 5-1: Study profile

Retained Parameters Of the 48 parameters, 10 demonstrated an inter-rater agreement statistic ≥0.6 with a lower limit of the 95% CI ≥0.5, could be readily evaluated on routine clinical sequences, and were deemed diagnostically useful based on panel consensus and the literature (**Table 5-1**). A *post hoc* decision was to collapse 'midline brainstem', 'left brainstem' and 'right brainstem' into one parameter ('brainstem') as any one lesion commonly co-occurred in all three locations. The panel agreed to rename the parameter, 'finger-like projections' as 'gyral projections', a more accurate descriptor of the feature. Of the ten parameters, two that were not deemed by the panel to be MRI features of acute CNS demyelination were retained for their perceived utility in distinguishing between CNS demyelination and SV-cPACNS or other mimics: 1) leptomeningeal enhancement (κ 0.85, 95% CI 0.55-1.0) was deemed by the panel as an important feature in differentiating CNS inflammatory demyelination from SV-cPACNS in cases where presenting symptoms are non-discriminatory; 2) diffusion restriction (κ 1.0), visible on diffusion weighted imaging, was deemed by the panel as a specificity parameter given its reported sensitivity for arterial ischemia.

Table 5-1: Inter-rater agreement of parameters retained

Parameter	κ	95% CI
Lesion Count	0.81[Φ]	0.68 – 0.89
Gyral Projection*	0.74	0.54 – 0.93
Diffusion Restriction of Lesion	1.0	1.0 – 1.0
Lesion Enhancement	0.92	0.81 – 1.0
Leptomeningeal Enhancement	0.85	0.55 – 1.0
Black Hole	0.76	0.54 – 0.98
Periventricular Lesion	0.80	0.66 – 0.94
Internal Capsular Lesion	0.74	0.58 – 0.91
Brainstem Lesion[Ψ]	0.72	0.57 – 0.88
Cerebellar Lesion	0.73	0.56 – 0.91

[Φ]Intraclass Correlation Coefficient reported; *Formerly 'Finger-like Projection'; [Ψ]'Brainstem' collapsed from 'Right Brainstem' (κ 0.71, 95% CI 0.53-0.88), 'Midline Brainstem' (κ 0.78, 95% CI 0.61-0.95), 'Left Brainstem' (κ 0.78, 95% CI 0.61-0.95)

The panel attributed the low inter-rater agreement of 3 of the 48 parameters ('caudate', κ 0.49, 95% CI 0.25-0.73; 'putamen', κ 0.44, 95% CI 0.16-0.71; 'globus pallidus', κ 0.39, 95% CI 0.13-0.65) to the challenge of precisely delineating the borders of the lentiform nuclei on conventional MRI. The panel also deemed that the radiologic distinction between the nuclei was not relevant to acute CNS demyelination. Therefore, the panel agreed to collapse these three parameters into one, 'basal ganglia'.

Of the 48 parameters, six (one of which included the collapsed parameter, 'basal ganglia') were deemed by the panel as important in predicting chronic demyelination as opposed to a monophasic demyelinating illness, but had inter-rater reliability statistics <0.6 following first-pass scoring (**Table 5-2**). The panel agreed that ambiguity in the definitions of five of the six parameters hindered their reliable application across raters. Revisions were made to the

definition of the five parameters as follows: 1) 'bilateral lesion distribution': specified inclusion of both supratentorial and infratentorial regions; 2) 'juxtacortical': specified that a lesion must involve the subcortical U-fibers to be scored as juxtacortical; 3) 'intracallosal': refined through provision of anatomical landmarks to define borders of corpus callosum in the transverse plane (**Appendix 2**), and through specification that 1 mm of normal appearing white matter surrounding a lesion was required in order to define a lesion as being intracallosal (in order to distinguish such lesions from periventricular lesions); and 4) 'thalamic' and 'basal ganglia': clarified such that these parameters encompassed lesions that involve the thalamus or basal ganglia even if the lesions were not entirely contained within these regions. The poor inter-rater agreement of the sixth parameter, 'subcortical lesions', was due to a difference in opinion between raters on what represented subcortical white matter. One rater referred to all supratentorial white matter extending between the cortical ribbon and lateral ventricles as subcortical, and therefore a lesion located in the supratentorial white matter that did not abut the cortex or lateral ventricle was scored as subcortical. The other rater viewed the supratentorial white matter as divided into deep (adjacent to the lateral ventricles) and superficial (adjacent to the cerebral cortex) white matter, and therefore scored only those lesions in the white matter that were adjacent to, but not contiguous with, the cortex as subcortical lesions. To ensure the 'subcortical lesion' parameter was interpreted as involving all supratentorial (non-juxtacortical and non-periventricular) white matter, the definition was revised and the parameter renamed to 'cerebral white matter-other'.

After the definitions were revised to eliminate ambiguity, the six parameters were independently re-evaluated on all scans by both trained raters. Inter-rater agreement increased to ≥0.6, permitting their retention in the final tool (**Table 5-2**).

Table 5-2: Inter-rater agreement of parameters retained following re-scoring

	First-Pass Scoring		Re-Scoring	
Parameter	**κ**	**95% CI**	**κ**	**95% CI**
Bilateral Distribution	0.66	0.45 – 0.87	0.73	0.50 – 0.95
Intracallosal	0.65	0.42 – 0.87	0.90	0.76 – 1.00
Thalamic	0.60	0.40 – 0.80	0.85	0.71 – 0.99
Basal Ganglia$^{\Phi}$	0.37	0.16 – 0.58	0.77	0.60 – 0.94
Cerebral White Matter – Other*	0.38	0.16 – 0.59	0.69	0.46 – 0.91
Juxtacortical	0.46	0.28 – 0.63	0.64	0.41 – 0.86

$^{\Phi}$'Basal Ganglia' collapsed from 'Caudate' (κ 0.49, 95% CI 0.25-0.73), 'Putamen' (κ 0.44, 95% CI 0.16-0.71), 'Globus Pallidus' (κ 0.39, 95% CI 0.13-0.65); *Formerly 'Subcortical Lesion(s)'

In total, 16 parameters were retained in the final tool (**Tables 5-1 and 5-2**). A textual and pictographic atlas of the final 16-parameter scoring tool was created and published in our recent work (**Appendix 2**).[50] In the atlas, anatomical landmarks have been delineated for parameters that rely on accurate identification of anatomical structure.

Parameters Discarded As shown in **Table 5-3**, of the 48 parameters, 28 were excluded: (i) 4 due to low inter-rater agreement; (ii) 9 due to poor visualization of the parameter on routine clinical imaging; and (iii) 15 that the expert panel deemed to be not diagnostically valuable.

Specifically, 'symmetrical pattern', 'ependymal enhancement', 'finger-like + projection', and 'proportion of discrete lesions' had $\kappa<0.6$ (or the lower limits of the 95% CI were <0.5), and even if re-defined, were not deemed by the panel to be diagnostically useful.

Nine parameters could not be accurately scored on routine clinical sequences. Although well-recognized as features of MS, the evaluation of 'intracortical', 'cervical spinal cord' and 'optic nerve' lesions requires targeted cortical, spinal cord or orbital imaging sequences – all of which are not routinely acquired in a clinical brain MRI protocol. One parameter ('dot-dash sign') has been described in one study as an early MRI feature of MS; however, scoring the parameter requires thin sagittal T2-weighted or FLAIR imaging through the midline. The remaining five parameters ('optic nerve enhancement', 'optic nerve sheath enhancement', 'extra-optic fat enhancement', 'extra-optic muscle enhancement', and 'perineural enhancement') require specialized fat-suppressed orbital imaging.

Finally, fifteen parameters, despite demonstrating acceptable inter-rater agreement, did not aid in differentiating between ADEM, MS and SV-cPACNS and were therefore excluded (**Appendix 3**). The formation of demyelinating and SV-cPACNS lesions does not respect lobar (four parameters) or vascular territory (four parameters) boundaries and the location of contrast enhancing lesions (i.e. supratentorial and infratentorial) was not discriminatory. While the presence of leptomeningeal enhancement was retained in the scoring tool, the more specific parameters ('nodular leptomeningeal enhancement', 'linear leptomeningeal enhancement', and 'dural enhancement') were too infrequently noted to permit computation of inter-rater reliability, and were not deemed by the panel to be discriminatory. The parameter 'cerebellar peduncle lesions' was discarded due to its co-occurrence with that of 'brainstem lesions' and

'cerebellar lesions', and also given the challenge of deciphering the margins of the cerebellar peduncles. 'Target lesions' was excluded as the panel could not reach consensus on a consistent definition.

Table 5-3: Inter-rater agreement of parameters not included in the scoring tool

Parameter	κ	95% CI
Inter-rater agreement <0.6 or lower limit of 95% CI <0.5		
Symmetrical Pattern	-0.04	-0.08 – -0.01
Ependymal Enhancement	0.38	-0.15 – 0.91
Finger-like + Projection	0.46	0.10 – 0.82
Proportion of Discrete Lesions	0.39$^{\Phi}$	0.24 – 0.54
Not Visualized on Routine Clinical Brain Imaging		
Cervical Spinal Cord	0.72	0.42 – 1.0
Dot-Dash Sign	0.71	0.46 – 0.97
Cortical Grey Matter	0.36	0.15 – 0.58
Optic Nerve Lesion	0.48	-0.03 – 0.98
Optic Nerve Enhancement	0.82	0.50 – 1.0
Optic Nerve Sheath Enhancement*	--	--
Extra-optic Fat Enhancement*	--	--
Extra-optic Muscle Enhancement*	--	--
Perineural Enhancement*	--	--
Not Contributory to Differentiating Between ADEM, MS and SV-pACNS		
Frontal Lobar Location	0.60	0.36 – 0.85
Temporal Lobar Location	0.81	0.67 – 0.94
Parietal Lobar Location	0.80	0.66 – 0.94
Occipital Lobar Location	0.70	0.53 – 0.86
ACA Vascular Territory	0.56	0.37 – 0.75
MCA Vascular Territory	0.80	0.58 – 1.0
PCA Vascular Territory	0.64	0.47 – 0.82
Vertebrobasilar Vascular Territory	0.73	0.57 – 0.89
Cerebellar Peduncle	0.76	0.59 – 0.93
Target Lesion	0.78	0.62 – 0.94
Nodular Leptomeningeal Enhancement*	--	--
Linear Leptomeningeal Enhancement*	--	--
Dural Enhancement*	--	--
Supratentorial Lesion Enhancement	0.76	0.59 – 0.94
Infratentorial Lesion Enhancement	0.78	0.58 – 0.98

ACA, anterior cerebral artery; MCA, middle cerebral artery; PCA, posterior cerebral artery; $^{\Phi}$Weighted κ reported; *κ invalid due to low frequency of occurrence in ≥1 cell of the 2x2 table

5.5 Discussion

We created an MRI scoring tool consisting of sixteen parameters that demonstrate substantial inter-rater reliability. Rationale for each of the parameters included in the tool is detailed in **Appendix 4**, and is based on evidence for their utility in characterizing the MRI features of acute demyelination, and for their utility in discriminating between acute CNS demyelination and SV-cPACNS. For a clinically useful tool to be generally accepted, it must be practical to use in the clinical setting without the need for rigorous training. Thus, we intentionally created the tool to be binary-response (with the exception of lesion count, which was limited at counts greater than 15) in order to minimize the quantitative requirement and maximize its efficient use in clinical practice.

The first component of developing the MRI tool was to devise the parameters themselves. We used established methods of item identification, including a literature search and expert consensus,[270] to formulate a comprehensive list of potential parameters. The rationale for performing a literature search was so that the tool would be comprised of parameters that have empirically been demonstrated to be MRI characteristics of acute CNS demyelination and relapsing-remitting MS, or features that might discriminate between demyelination and SV-cPACNS. This method of devising tool parameters has been used in other instances, as in a scale for differentiating between irritable bowel syndrome and organic bowel disease in which the

parameters represented clinical features and laboratory values that were found to distinguish the two patient groups.[271]

We also used expert consensus as a second method for devising potential parameters. We selected the expert panel from neuroradiology, neurology and rheumatology staff working in our established pediatric demyelinating disease and CNS vasculitis programs, ensuring extensive clinical and radiological experience with pediatric-onset MS and SV-cPACNS. The expert panel played a key role in defining the comprehensive list of parameters identified from the literature, and in devising definitions for each parameter that were objective and straightforward to apply. Similar utility of expert opinion has been reported elsewhere, such as the Patient-Reported Outcomes Measurement Information System initiative funded under the National Institutes of Health, in which experts contribute potential parameters to be used in evaluating patient-reported outcomes across multiple health conditions.[272]

Following identification of relevant MRI parameters for the diseases under evaluation, we created definitions for each parameter to enable consistency in application of the parameters between raters.[273,274] Therefore, a key component of our work was to assess the inter-rater reliability of the potential parameters identified by the literature search and expert opinion; only parameters that demonstrated 'substantial' or 'good' inter-rater agreement (kappa ≥0.6)[267,269] were retained in the final tool. Several MRI parameters demonstrated poor inter-rater agreement on first evaluation but were deemed important discriminatory parameters by the panel. These parameters were re-defined and then all scans were re-scored, and only when the inter-rater agreement increased to ≥0.61 was the parameter retained. A second noteworthy aspect is that we focused on the inter-rater reliability of our parameters, and not intra-rater

reliability. As the error contributing to intra-rater reliability is considered to be contained within inter-rater reliability, demonstration of high inter-rater reliability is sufficient.[273] Of the 16 parameters retained in the final tool, 14 demonstrated inter-rater agreement that was considerably higher (≥0.72) than our *a priori* cut-point of 0.6. The use of inter-rater agreement to guide the selection of parameters included in a scoring tool is not unique to our study. Similar methodology was employed during the creation of the well-established Glasgow Coma Scale, a clinical scale for evaluating the depth and duration of impaired consciousness and coma,[275] as well as the American Spinal Injury Association's International Standards for Neurological Classification of Spinal Cord Injury, a practice guideline for classifying the degree of neurological impairment due to spinal cord injury.[274,276,277]

A key aspect of the diagnostic criteria for multiple sclerosis is the exclusion of mimics of CNS demyelination.[94,278] In creating our proposed tool, we included MRI scans of children with SV-cPACNS, as this disease was felt to have a similar but potentially also distinct inflammatory pattern on MRI, and due to the clinical challenge of distinguishing SV-cPACNS from MS. We also included parameters that have been reported in CNS infection and malignancy. Specifically, leptomeningeal enhancement has been described in children with SV-cPACNS[265] and is well-documented in infectious[279,280] and neoplastic[281-283] processes. Leptomeningeal enhancement is not a feature of MS, and its presence was highlighted by an international consensus panel as a "red flag" to consider other, non-demyelinating etiologies.[278] In contrast, the MRI parameter of acute diffusion restriction, while highly characteristic of vascular occlusion such as stroke,[284-286] has also been a feature recently reported in acute demyelinating lesions, particularly tumefactive lesions,[287,288] and therefore was retained in the final tool.

In developing the MRI scoring tool, we gave consideration to the challenges of MRI acquisition in the pediatric context. Parameters that required sequences beyond a standard clinical brain protocol for accurate scoring were not included in the final tool. For example, while optic nerve and spinal cord lesions are well-documented features of MS, focused spinal cord or fat-suppressed orbital imaging is required to adequately resolve these lesions. Adding these sequences to a standard clinical brain MRI protocol significantly increases scan time, rendering such acquisitions intolerable in children who are not sedated, and yielding higher costs in the context of research studies. The yield of orbital or spinal imaging in the absence of clinical evidence of acute or remote optic nerve or spinal cord involvement has not been established in children subsequently diagnosed with MS; further studies will be required, with appropriate consideration of scan time.

We created a manual that contains an atlas and definition for each parameter and highlights important anatomical delineations in order to ensure that the tool can be accurately utilized by radiologists and clinicians who were not part of the tool's creation. Not only will this aid in use of the tool across clinical centers, it also serves as a model for MRI scoring tools to be applied in pediatric MS clinical trials where inclusion into the trial will be predicated on accurate MS diagnosis.

The intended future application of our proposed MRI tool is multifaceted and requires validation. The first priority will be to determine the ability of the MRI tool to identify distinct features of different CNS inflammatory disorders, such as monophasic CNS demyelination, MS, ADEM and SV-cPACNS, as such disorders share clinical features rendering accurate diagnosis challenging at onset. The MRI tool will then be evaluated for specificity in identifying non-

inflammatory CNS disorders, such as inherited or metabolic diseases that also onset during childhood and impact CNS white matter. Finally, we need to evaluate user satisfaction with the tool, as application in busy clinical environment will require endorsement of utility and applicability.

5.6 Conclusion

We developed an MRI scoring tool consisting of sixteen items that demonstrate substantial inter-rater agreement. Using established methods of item identification, including a literature search and expert consensus, the parameters are based on evidence for their utility in characterizing the MRI features of acute CNS inflammation and demyelination. The binary-response nature of the parameters as well as the tool manual will facilitate utility of the tool in clinical practice without need for rigorous training. The scoring tool will inform the creation of structured reporting that is increasingly being proposed for use in diagnostic radiology.

5.7 Acknowledgements

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Chapter 6:

MRI Parameters for Prediction of MS Diagnosis in Children with Acute CNS Demyelination: a Prospective National Cohort Study

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6.1 Summary

Background Multiple sclerosis (MS) diagnostic criteria incorporate MRI features that predict MS in adults with acute central nervous system (CNS) demyelination. To identify MRI predictors of a subsequent MS diagnosis in a pediatric population, a standardized scoring tool was created and applied to MRI scans of a national prospective incident cohort of children with CNS demyelination.

Methods Clinical and MRI examinations were performed at onset of acute CNS demyelination, quarterly in the first year thereafter, and at the time of a second demyelinating attack. A total of 1139 MRI scans were evaluated in 284 children who have been followed for a mean (SD) of 3.9 (1.7) years. MS was diagnosed based on clinical or MRI evidence of relapsing disease. Baseline MRI scans were evaluated for the presence of 14 binary-response parameters. Seven parameters with multiple tetrachoric correlation coefficients of $r<0.70$ were retained and entered into univariate analyses and multivariable Cox proportional hazards models to determine predictors of MS diagnosis.

Findings Fifty-seven (20%) children were diagnosed with MS after a median (IQR) of 188 (192) days. The presence of both ≥1 T1-weighted hypointense lesion (HR 20.6, 95% CI 5.46-78.0) and ≥1 periventricular lesion (HR 3.34, 95% CI 1.27-8.83) were associated with an increased likelihood of MS diagnosis (sensitivity 84%, specificity 93%, positive predictive value 76%, negative predictive value 96%). Risk for MS diagnosis was highest when both parameters were present (HR 34.27, 95% CI 16.69-70.38). Although the presence of contrast-enhancement,

cerebral white matter, intracallosal, and brainstem lesions were associated with MS in the univariate analyses, these parameters were not retained in the multivariable models.

Interpretation Specific MRI parameters can predict MS in children with incident demyelination of the CNS.

Funding Canadian Multiple Sclerosis Scientific Research Foundation

6.2 Introduction

Magnetic resonance imaging detects lesion dissemination in time (DIT) and space (DIS) within the CNS. Prospective studies of adults with incident demyelination of the CNS have identified specific MRI features at baseline that predict MS,[97] and these findings are incorporated into the McDonald MS diagnostic criteria.[89,90,101] In our prior work, using MRI scans obtained at the time of MS diagnosis (second attack), we demonstrated that two of: (i) ≥5 T2 hyperintense lesions, (ii) ≥2 periventricular lesions, and (iii) ≥1 brainstem lesion better characterized the MRI appearance of MS in children than did the 2005 McDonald criteria for DIS.[92] In a second study, we also showed that two of: (i) ≥2 periventricular lesions, (ii) the presence of ≥1 T1 hypointense lesion, and (iii) the absence of diffuse bilateral T2 lesions distinguished children with MS from children with monophasic acute disseminated encephalomyelitis (ADEM).[93] The French KIDSEP group demonstrated that the "sole presence of well-defined lesions" and "lesions perpendicular to the long axis of the corpus callosum" were predictive of MS in children.[91] However, as only 11-21% of pediatric MS patients have these MRI features at onset,[91,103] applicability of these MRI criteria in the clinical setting of acute demyelination is limited. We aimed to define MRI parameters that predict MS diagnosis in children by applying a standardized scoring tool to scans acquired in a prospective cohort of children with incident demyelination.

6.3 Patients and Methods

Participants and study design In a national 23-site prospective inception cohort study of patients <16 years of age with acquired demyelinating syndromes (ADS) of the CNS, comprehensive clinical data were collected at onset, 3, 6, and 12 months and annually for 5 years. Clinical features at onset were categorized according to *a priori* criteria, as previously described.[17] Clinical deficits at most recent evaluation were scored using the Expanded Disability Status Scale (EDSS)[70] score. Guardians and participants (old enough to comprehend the study fully) provided informed consent, and younger children provided assent. Ethics approval was obtained at each participating site.

Procedures A standardized 1.5 Tesla brain MRI protocol was optimized at each site (**Appendix 5**). Participants were scanned at baseline, 3, 6, and 12 months, and at a second demyelinating episode if it occurred. At The Hospital for Sick Children (Toronto, Canada), participants were also imaged annually after the 12-month scan. Brain MRI scans performed in addition to research protocol scans were also obtained. MRI scans were reviewed for image quality and archived at the McConnell Brain Imaging Centre at the Montreal Neurological Institute (Montreal, Canada).

A standardized MRI scoring tool was designed for this study (**Appendix 2**), and parameters included in the tool represent MRI features of MS or characteristics that may aid in differentiating MS from non-demyelinating CNS inflammatory disease.[92,95,96] MRI scans were analyzed at The Hospital for Sick Children by experienced evaluators (LHV, HMB, MMS, BB, DJAC) trained in the application of the MRI scoring tool. Raters were blinded to clinical data.

Scans from children enrolled at The Hospital for Sick Children were visualized on a GE Centricity Picture Archiving and Communication System (version 3.2.0.2) workstation. Scans acquired at the other participating centers were visualized using the OsiriX (version 3.9.2) viewing platform.

Four *a priori* rules were used when applying the scoring tool: 1) a lesion was defined as a T2-weighted or fluid attenuated inversion recovery (FLAIR) hyperintensity ≥3 mm in diameter in either the axial, sagittal, or coronal plane; 2) lesions required visualization in ≥2 planes; 3) adjacent lesions were considered distinct when separated by ≥1 mm of normal-appearing white or gray matter; and 4) T1-weighted hypointense lesions were confirmed as hyperintense on T2-weighted images. The 14 parameters specified in the MRI scoring tool (**Appendix 2**) were evaluated on all baseline scans. Serial scans were evaluated for new T2-weighted or FLAIR lesions, new T1-weighted hypointense lesions, and new contrast-enhancing lesions, and were adjudicated for 2005 McDonald criteria for DIT.[89] Baseline scans were also scored using the 2005 McDonald criteria for DIS[89], pediatric KIDMUS criteria[91], and our previously proposed pediatric MS[92] and pediatric ADEM-MS criteria.[93]

The primary outcome was MS diagnosis, based on either a confirmed second demyelinating episode (occurring more than 28 days following an incident attack)[58] or by the accrual of new MRI lesions as per the 2005 McDonald DIT criteria.[89] Children with an initial diagnosis of ADEM were diagnosed with MS if they experienced two or more non-ADEM attacks or if they developed new MRI lesions on serial studies, provided that the subsequent events occurred more than 90 days following their initial illness and that more than 28 days separated the two further events.[56] Participants not meeting clinical or MRI criteria for MS were classified as having a monophasic ADS as of data closure.

Statistical analysis We estimated a sample size of approximately 300 participants with ADS for the primary study.[17] Assuming that 25% of the children with ADS would be diagnosed with MS, β=0.80 and α=0.05, five-to-seven independent MRI parameters of interest would be permitted entry into a Cox proportional hazards model, using the rule of 10 outcome events for each independent variable.[289]

The 14 scoring tool parameters were evaluated for frequency of occurrence, inter-rater reliability, and multiple correlations between parameters using a tetrachoric correlation matrix.

Categorical data are summarized as frequency (%), and continuous data as mean (SD) or median (IQR) as appropriate. Univariate analyses were conducted using Fisher's exact tests, χ^2 tests, t-tests, and Mann-Whitney U tests as appropriate. Ability of the MRI parameters to predict MS diagnosis was assessed using Kaplan-Meier curves.

Cox proportional hazards models were constructed with zero time defined as ADS onset, and the event of interest as date of MRI DIT[89] or second demyelinating episode, whichever occurred first. Participants were censored at the date data were locked for analyses, or last study visit date prior to withdrawal or loss-to-follow-up. The proportional hazards assumption was tested using graphical methods and the time-dependent covariates extension of the Cox model.[290] Hazard ratios (HRs) with 95% confidence intervals (CIs) are reported as measures of association between MRI parameters and MS. As MRI features of MS in children may differ by age of onset,[86] and given the difference in distribution of male and female patients in pre- and post-pubertal onset populations,[48] we report unadjusted HRs and HRs adjusted for onset age

(modeled as a continuous variable) and sex. Harrell's *c* statistic is reported as a measure of model discriminating ability.[291]

We evaluated the performance of MRI parameters identified in the multivariable analysis and the performance of published MRI criteria for MS by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).[292] Youden's J, an index that equally weights sensitivity and specificity, was also calculated as: (sensitivity + specificity) -1.[293]

We performed a series of sensitivity analyses to evaluate whether the performance of our MRI parameters was influenced by clinical presentation, method of MS diagnosis (confirmed clinical relapse or MRI evidence of DIT alone), and frequency of MR imaging.

Statistical analyses were performed using Stata version 11 and SPSS version 12.0.

6.4 Results

Between September 1, 2004 and June 30, 2010, 332 children and adolescents were assessed for eligibility (**Figure 6-1**), 298 participants were enrolled in the current study, and 14 (5%) were subsequently excluded either because a baseline MRI scan was unavailable or compromised by artifact. The mean (SD) period of observation for the final cohort of 284 children was 3.9 (1.7) years (**Table 6-1**). Six children (2%) with a monophasic ADS (median [IQR] onset age 13.3 [1.7] years) withdrew (Figure 1) after a median (IQR) of 142 (79) days of follow-up.

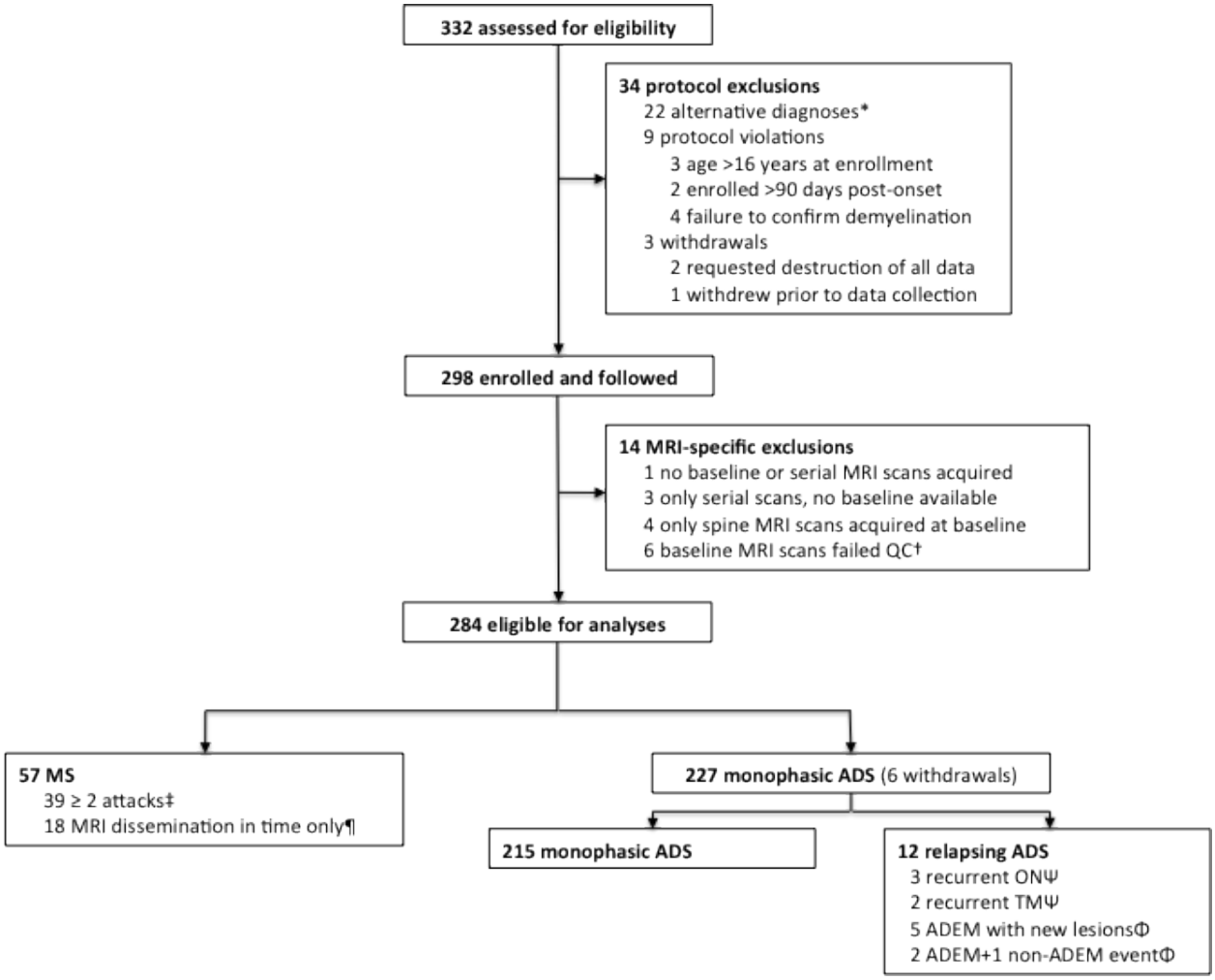


Figure 6-1: Study profile. *2 were diagnosed with neuromyelitis optica on the basis of optic neuritis and/or longitudinally-extensive (spanning ≥3 vertebral body segments) transverse myelitis and serum positivity for antibodies against aquaporin-4.[294] †Scans do not pass QC if: 1) degraded by motion or dental hardware artifact, 2) missing axial T2-weighted and/or Fluid Attenuated Inversion Recovery sequences, or 3) scans are only available on film. ‡Attacks are separated by at least 28 days and localize to distinct regions of the central nervous system.[58,89] ¶Gadolinium enhancement at least 3 months after clinical onset if not at site corresponding to initial event, or a new T2 lesion at any time compared with a reference scan done at least 30 days after clinical onset.[89] 12 children had relapsing demyelinating episodes, but, based on International Pediatric MS Study Group consensus criteria,[56] did not meet criteria for MS: ΨRecurrence of isolated TM or ON in the absence of T2 lesions in the brain, and without antibodies to aquaporin-4; ΦChildren either had new lesions >3 months from ADEM onset

without subsequent clinical attacks or a non-ADEM attack >3 months from ADEM onset but no subsequent non-ADEM events.

Table 6-1: Clinical and demographic characteristics of children with ADS

	Overall (n=284	**Monophasic ADS (n=227)**	**MS (n=57)**	**p value**
Length of clinical observation (years)	3.94 (1.65)	3.86 (1.70)	4.25 (1.38)	0.07
Age at onset	9.57 (4.55)	8.75 (4.5)	12.8 (3.07)	<0.0001
Age at onset				
<10 years	137 (48%)	129 (57%)	7 (12%)*	<0.0001
≥10 years	147 (52%)	98 (43%)	50 (88%)*	--
Age at MS diagnosis (years)†	--	--	13.46 (3.04	--
2nd clinical attack	--	--	39 (68%)	--
Female	145 (51%)	107 (47%)	38 (67%)	0.01
Female:male ratio	1.1	0.9	2.0	--
Phenotype of ADS				
ADEM	72 (25%)	71 (31%)	1 (2%)	--
Transverse myelitis	58 (20%)	54 (24%)	4 (7%)	--
Optic neuritis	69 (24%)	56 (25%)	13 (23%)	--
Monofocal – other	35 (12%)	17 (7%)‡	18 (32%)	--
Polyfocal	50 (18%)	29 (13%)	21 (37%)	--

Data are mean (SD) or number (%).*MS diagnosis was confirmed within a median (IQR) of 184 (352) days of symptom onset, which did not differ from children with MS whose incident attack occurred after age 10 years (188 [202] days, p=0.95). †MS diagnosis based on 2005 McDonald Criteria for MRI dissemination in time: gadolinium enhancement at least 3 months after clinical onset if not at site corresponding to initial event, or a new T2 lesion at any time compared with a reference scan done at least 30 days after clinical onset.[89] ‡One child presented with seizures and likely had ADEM but, due to post-ictal state, encephalopathy could not be assessed.

Of 284 participants, 183 (64%) had a baseline and at least 3 serial scans, 35 (12%) had a baseline and 2 serial scans, 44 (15%) had a baseline and one serial scan, and 22 (8%) had a baseline scan

only, yielding 1139 scans for analysis. A median of 4 scans were available for evaluation from the monophasic ADS patients and those diagnosed with MS.

Fifty-seven children (20%) were diagnosed with MS (**Table 6-1**). Median (IQR) time to DIT on MRI or a second clinical attack for children with MS was 188 (192) days. Of 57 children with MS, 43 (75%) demonstrated DIT on MRI within 12 months of symptom onset; the remaining 14 children demonstrated DIT within 36 months of onset. Thirty-nine children (68%) experienced a second demyelinating episode a median (IQR) of 259 (314) days from onset. Median (IQR) annualized relapse rate for the MS group was 0.4 (0.6). At most recent clinical assessment (median [IQR] time from onset 3.1 [2.0] years), all but 5 of the 57 children with MS remained fully ambulatory without aid. One child is wheelchair dependent (EDSS=8), one requires a cane for ambulation (EDSS=6), two children have sufficient ataxia as to limit gait but do not require ambulatory aids (EDSS=4), and one has mild ataxia with no limitation of function (EDSS=2).

Of the 14 candidate MRI parameters, we excluded two that occurred rarely and were not associated with MS diagnosis (gyral projections n=30, 11%, p=0.81; leptomeningeal enhancement n=8, 3%, p=1.0), four (bilateral lesion distribution and basal ganglia, internal capsular, and cerebellar lesions) that were highly correlated (multiple tetrachoric correlation coefficients of r ≥0.7) with the 7 parameters retained, and one (juxtacortical lesions) that was not collinear but had an inter-rater reliability coefficient less than our *a priori* threshold of Cohen's kappa = 0.7. The seven retained parameters had inter-rater reliability coefficients ≥0.7, with lower limits of the 95% CIs ≥0.5.

All seven retained MRI parameters were independently associated with MS in the univariate analyses (**Table 6-2**). As detailed in **Table 6-2**, baseline MRI scans were normal in 98 of 284 children (35%). The remaining 186 children (65%) had ≥1 T2-weighted lesions at ADS, which strongly predicted subsequent MS diagnosis (*c* statistic 0.67; sensitivity 96%; specificity 42%). Two children diagnosed with MS had normal brain MRI scans at onset; both presented with optic neuritis and demonstrated brain lesions over time. Of the 227 children diagnosed with a monophasic ADS to date, 131 (58%) had T2 lesions at baseline. Of these 131 participants, 68 (52%) had an initial presentation meeting criteria for ADEM, 13 (10%) presented with clinically monofocal deficits, and 20 (15%) with polyfocal deficits. Excluding children with monophasic ADEM, children currently classified with a monophasic ADS for whom initial MRI revealed T2 lesions might be considered at particularly high risk of a future MS diagnosis. The mean (SD) age of onset of these children (10.8 [4.12] years) was younger than that of children diagnosed with MS (12.8 [3.1] years). The mean (SD) duration of observation of these children (4.0 [1.6] years) did not differ from that of the entire cohort (3.9 [1.7] years).

Table 6-2: Baseline MRI features: univariate analyses

	Overall (n=284)	**Monophasic ADS (n=227)**	**MS (n=57)**	**Hazard ratio**
≥1 T2 lesion¶	186 (65)	131 (58)	55 (96)	16.9 (4.12-69.31)
Cerebral white matter	111 (39)	69 (30)	42 (74)	5.13 (2.85-9.27)
Periventricular lesion	112 (39)	63 (28)	49 (86)	12.05 (5.7-25.46)
Intracallosal lesion	51 (18)	20 (9)	31 (54)	7.29 (4.31-12.33)
Thalamic lesion	62 (22)	56 (25)	6 (11)	0.39 (0.17-0.90)
Brainstem lesion	112 (40)	77 (34)	35 (61)	2.68 (1.57-4.57)
≥1 T1-hypointense lesion	80/282 (28)†	26/225 (12)†	54 (95)	72.77 (22.69-233.37)
Contrast enhancement	56/254 (22)‡	21/204 (10)*	35/50 (70)§	11.88 (6.47-21.82

Data are number (%) of participants positive for each MRI feature at baseline or hazard ratio (95% CI). Hazard ratios refer to prognostic value of each MRI parameter on rate of MS versus monophasic ADS. ¶Brain MRI was normal at baseline in 41 children with isolated optic neuritis, 41 with isolated transverse myelitis, 4 with other monofocal neurological deficits, 3 with acute disseminated encephalomyelitis (defined clinically by neurological deficits and encephalopathy),[56] and 9 with polyfocal deficits. Presence of one or more T2 lesions was not one of the seven parameters entered into the model. †For 2 participants, a T1-weighted sequence was not acquired at baseline. ‡For 30 participants with T2 lesions present at baseline, a contrast-enhanced scan was not acquired or was of insufficient quality to be assessed. *For 23 participants with T2 lesions present at baseline, a contrast-enhanced scan was not acquired or was of insufficient quality to be assessed. §For seven participants with T2 lesions present at baseline, a contrast-enhanced scan was not acquired or was of insufficient quality to be assessed.

The presence of ≥1 T1 hypointense lesion at baseline was the strongest of the seven MRI parameters at predicting MS in the univariate analyses (**Table 6-2**), with 95% sensitivity and 88% specificity. Overall, 54 of the 80 patients with T1 hypointense lesions at baseline were diagnosed with MS (68% PPV). Of the 202 children without T1 hypointense lesions at baseline, 199 were not diagnosed with MS (99% NPV). The association between T1 hypointense lesions and MS was stronger when considering T1-weighted lesions present at baseline that *also* persisted ≥3 months on serial scans (“persisting T1 hypointense lesions”; HR 116.85 95% CI 36.31-376.08). Only 11 of 26 children with a monophasic ADS who had T1 hypointense lesions had these confirmed as persisting T1 hypointense lesions on serial images, while all 54 children with MS having T1 hypointense lesions at baseline had persisting T1 hypointense lesions.

The presence of ≥1 periventricular lesion at baseline corresponded to a greater than 10-fold increased likelihood of MS diagnosis (**Table 6-2**). Of the 57 children diagnosed with MS, 49 had ≥1 periventricular lesion at baseline (86% sensitivity), while periventricular lesions were absent in 164 of the 227 children not diagnosed with MS (73% specificity). Of 112 children with baseline periventricular lesions, 49 were diagnosed with MS (44% PPV); 164 of the 172 without periventricular lesions were not diagnosed with MS (95% NPV).

Lesions involving the thalamus were more likely to be seen in children with a monophasic ADS than those diagnosed with MS (p=0.02).

When considering contrast-enhancing lesions, 50 of 57 children diagnosed with MS had gadolinium administered, and 35 (70%) had contrast-enhancing lesions at baseline. In comparison, only 21 of 204 (10%) children with a monophasic ADS (who received gadolinium) had enhancing lesions. Baseline gadolinium-enhancing lesions were associated with a greater than 10-fold increase in MS risk (**Table 6-2**).

Of the 284 children, 32 did not have either a contrast-enhanced scan or T1-weighted images acquired at baseline and were not entered into the multivariable models (**Table 6-3**). The presence of ≥1 periventricular lesion and ≥1 T1 hypointense lesion at baseline were independently associated with an increased risk of MS diagnosis. Contrast-enhancing lesions were also independently associated with MS but did not contribute to the discriminating ability of the model. The *c* statistic for the model including periventricular and T1 hypointense lesions was 0.89, and remained unchanged when contrast-enhancement was added. After adjustment

for onset age and sex, contrast-enhancing lesions were no longer statistically significantly associated with MS diagnosis, but the hazard ratio was only slightly attenuated.

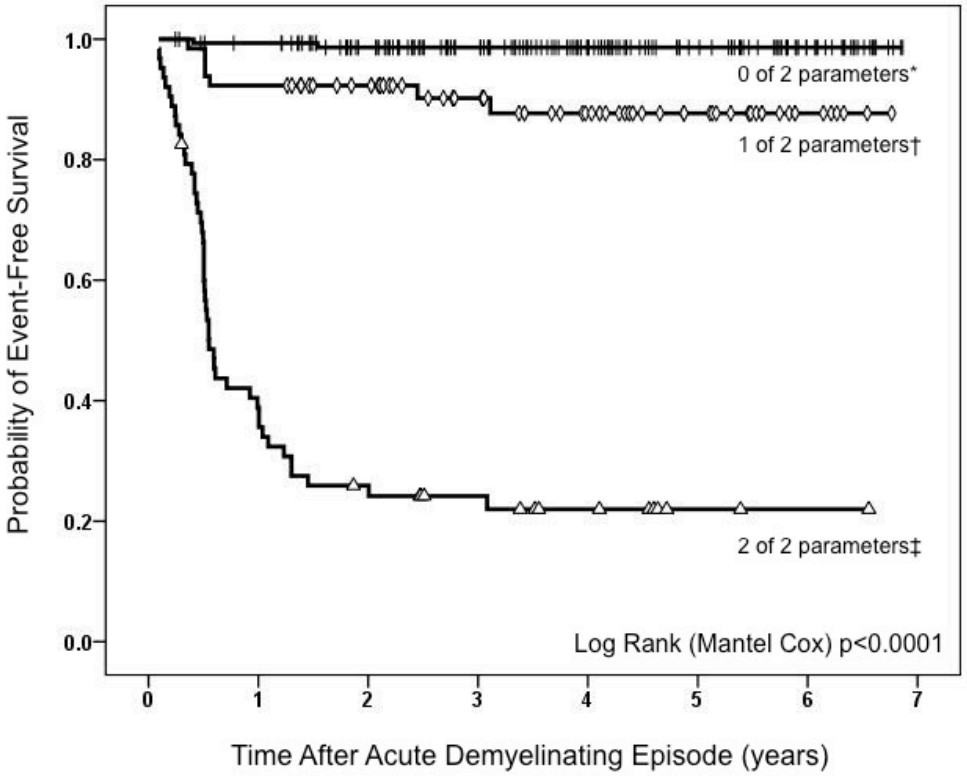
Table 6-3: MRI predictive model for MS in children with ADS: multivariable analyses

	Hazard ratio (95% CI)
Prognostic factors; ADEM included*	
Periventricular lesion	3.55 (1.42-8.92)
T1-hypointensity	24.82 (6.89-89.25)
Contrast enhancement	2.03 (1.06-3.87)
Prognostic factors adjusted for onset age and sex; ADEM included†	
Onset age	1.21 (1.10-1.33)
Sex	1.66 (0.88-3.16)
Periventricular lesion	3.34 (1.27-8.83)
T1-hypointensity	20.31 (5.52-74.72)
Contrast enhancement	1.7 (0.87-3.32)
Prognostic factors; ADEM excluded‡	
Periventricular lesion	4.64 (1.82-11.83)
T1-hypointensity	20.64 (5.46-78.04)
Contrast enhancement	1.38 (0.71-2.67)
Prognostic factors adjusted for onset age and sex; ADEM excluded§	
Onset age	1.13 (1.02-1.26)
Sex	1.74 (0.91-3.36)
Periventricular lesion	4.42 (1.65-11.83)
T1-hypointensity	17.84 (4.5-70.8)
Contrast enhancement	1.34 (0.68-2.66)

*50 of 254 patients had MS. *c* statistic 0.91. Seven patients diagnosed with MS did not have contrast-enhanced scan acquired at baseline. †50 of 254 patients had MS. *c* statistic 0.93. ‡49 of 196 patients had MS. *c* statistic 0.90. §49 of 196 patients had MS. *c* statistic 0.92. One child with MS who met criteria for ADEM at initial episode was excluded.

The presence of *both* ≥1 periventricular lesion and ≥1 T1 hypointense lesion at baseline strongly predicted MS (HR 34.27, 95% CI 16.69-70.38, **Figure 6-2**). Forty-eight of the 57 children

subsequently diagnosed with MS met both parameters (84% sensitivity, **Table 6-4**), 7 (12%) met one of the two parameters, and two (4%) had neither periventricular lesions nor T1 hypointense lesions. Of the nine children with MS whose baseline MRI did not have both ≥1 T1 hypointense lesion and ≥1 T2 periventricular lesion, 2 had a normal brain MRI at onset, 6 had T1 hypointense lesions and T2 lesions but no periventricular lesions, and 1 had periventricular and other T2 lesions but no T1 hypointense lesions. Of 227 children not diagnosed with MS, 212 did not have both T1 hypointense lesions and at least one periventricular T2 lesion (93% specificity, **Table 6-4**). Given that only five children in our MS cohort have measurable physical disability, correlation of disability with MRI features was not feasible.



Number at risk				
0 of 2 parameters	156	129	72	23
1 of 2 parameters	65	52	29	6
2 of 2 parameters	63	15	7	1

Figure 6-2: Time to MS diagnosis with proposed predictive MRI parameters. Kaplan-Meier curves of time to MS diagnosis according to presence of proposed predictive parameters on MRI acquired at onset of acute demyelination. *Patients with no T2 periventricular lesions or T1-hypointense lesions. †Patients with either one or more T2 periventricular lesions or one or more T1-hypointense lesions. ‡Patients with one or more T2 periventricular lesions or one or more T1-hypointense lesions.

Table 6-4: Comparison of published MRI criteria with proposed predictive parameters for diagnosis of MS

	Proposed Parameters	**2005 McDonald DIS*[89]**	**Pediatric MS†[92]**	**Pediatric MS-ADEM‡[93]**	**KIDMUS§[91]**
Sensitivity	48/57 (**84**)	32/57 (**56**)	42/57 (**74**)	54/57 (**95**)	23/57 (**40**)
Specificity	212/227 (**93**)	164/227 (**72**)	154/227 (**68**)	205/227 (**90**)	222/227 (**98**)
PPV	48/63 (**76**)	32/95 (**34**)	42/115 (**37**)	54/76 (**71**)	23/28 (**82**)
NPV	212/221 (**96**)	164/189 (**87**)	154/169 (**91**)	205/208 (**99**)	222/256 (**87**)
Youden's J	**0.77**	**0.28**	**0.42**	**0.85**	**0.38**

Data are number (%). *Three of the following: ≥9 T2 lesions or ≥1 contrast-enhancing lesions, ≥3 periventricular lesions, ≥1 juxtacortical lesion, ≥1 infratentorial lesion; †Two of the following: ≥5 T2 lesions, ≥2 periventricular lesions, ≥1 brainstem lesion; ‡Two of the following: ≥2 periventricular lesions, ≥1 hypointense lesions, absence of diffuse bilateral lesion pattern; §Two of the following: Dawson fingers (lesions perpendicular to the long axis of the corpus callosum), and sole presence of well-defined lesions.

The presence of ≥1 periventricular lesion and ≥1 T1 hypointense lesion identified children diagnosed with MS on the basis of confirmed clinical relapses (sensitivity 90%, specificity 93%, PPV 71%, NPV 98%) with comparable diagnostic performance as found in our primary analysis in which MS diagnosis was conveyed by clinical or MRI evidence of DIT.

Of all 57 children diagnosed with MS to date, only 5 children who have not yet experienced a clinical relapse were diagnosed based on MRI scans obtained more than 12 months post-onset. The diagnostic utility of ≥1 periventricular lesion and ≥1 T1 hypointense lesion in the entire cohort (**Table 6-4**) did not differ from the performance of these parameters in the 108 participants who had annual MRIs (sensitivity 83%, specificity 92%, PPV 68%, NPV 97%) or the 176 children who did not have annual MRI scans (sensitivity 85%, specificity 94%, PPV 80%, NPV 96%).

Given that children with ADEM have multifocal brain lesions, we performed a sensitivity analysis to determine whether children with ADEM influenced our findings. Of all 57 children diagnosed with MS, 1 was diagnosed with ADEM at onset but was subsequently diagnosed with MS after experiencing two non-ADEM attacks.[56] Of the 72 children who met criteria for ADEM, 43 (60%) had periventricular lesions, 13 (18%) had T1 hypointense lesions, and only 11 of 58 (19%) with gadolinium-enhanced scans had contrast-enhancement. When we excluded the ADEM patients, the associations between the presence of ≥1 T1 hypointense lesion and ≥1 periventricular lesion at baseline and MS diagnosis in the multivariable models were essentially unchanged (**Table 6-3**). However, the diagnostic performance of ≥1 T1 hypointense lesion and ≥1 periventricular lesion improved slightly: sensitivity 84%, specificity increased to 97%, PPV increased to 90%, and NPV decreased to 94%.

We then evaluated the performance of the MRI parameters when excluding children with transverse myelitis, as this population in general also has a lower rate of subsequent MS diagnosis.[48] Our overall findings were not altered (sensitivity 83%, specificity 91%, PPV 75%, NPV 95%).

Performance of published MRI criteria in the present cohort is summarized in **Table 6-4**.

6.5 Discussion

We define MRI parameters present at baseline that predict MS in an *unselected* population-based cohort of children with incident demyelination. The presence of ≥1 T1-hypointense lesion and ≥1 periventricular lesion identified children destined for clinical or MRI evidence of early relapse with a sensitivity of 84% and specificity of 93%.

The presence of ≥1 T1-hypointense lesion and ≥1 periventricular lesion was more sensitive and specific than the 2005 McDonald criteria for DIS, [89] less specific but far more sensitive than the KIDMUS criteria,[91] and slightly more sensitive and specific than our prior criteria proposed to distinguish children with MS from children with relapsing non-demyelinating disease.[92] That the presence of ≥1 T1-hypointense lesion and ≥1 periventricular lesion emerged as predictors of MS supports our prior work in which we found such lesions to be important distinguishing features of MS and ADEM in children.[93] The predictive MRI parameters identified here are easily adjudicated, eliminate the subjective criterion "diffuse bilateral distribution" defined in our previous work,[93] are simpler than the 2005 McDonald criteria for DIS,[89] and complement the recently proposed 2010 McDonald criteria.[90] We provide the summary in **Appendix 6** which places our findings in context of the literature.

Nine of the 57 children with MS (16%) did not have ≥1 T1 hypointense lesion and ≥1 periventricular lesion, two of whom had no lesions at baseline. Although these nine children

were younger at onset (median [IQR] 11.8 [5.6] years) relative to the 48 MS patients who met both MRI parameters (14.2 [2.6] years, $p<0.05$), it is important to note that ≥1 T1 hypointense lesion and ≥1 periventricular lesion was present in 5 of 7 children (71%) with onset <10 years of age, a similar frequency to children with older onset age (43 of the 50 children ≥10 years at onset, 86%, $p=0.30$).

The presence of T1 hypointense lesions at baseline emerged as the single strongest predictor of MS diagnosis. Conceptually, T1 hypointense lesions present at baseline could indicate established tissue injury, and thus chronic disease. To confirm that these lesions represented foci of chronic tissue destruction rather than transient inflammation, we assessed the persistence of T1 hypointense lesions at least 3 months post-onset. When this "persistent T1 hypointensity" parameter was entered in the place of baseline T1 hypointense lesions into the multivariable model, it was indeed strongly associated with MS (HR 33.35, 95% CI 8.87 – 125.47). The presence of T1 hypointense lesions on baseline images, or persistent T1 hypointense lesions on serial imaging, has not been proposed as a criterion in any of the iterations of the McDonald criteria, and T1 lesions were not evaluated by the MAGNIMS studies of adults with acute demyelination.[98,99]

Although contrast-enhancing lesions at onset are important in predicting MS in adults with acute demyelination,[295] this did not emerge as a useful predictor in our multivariable analyses as they did not increase the models' discriminating ability. All children with ADS have active brain inflammation, and might thus be expected to demonstrate increased blood-brain barrier permeability and lesion enhancement. Arguably, the most "inflammatory" of all ADS phenotypes is ADEM characterized by large, bilateral, multifocal CNS lesions and a hyperacute

clinical presentation. Of 58 children with ADEM given gadolinium, only 11 (19%) demonstrated contrast-enhancing lesions. The paucity of enhancing lesions in children with monophasic ADS, and specifically with ADEM, was not influenced by corticosteroid therapy (data not shown), nor by time from symptom onset to first MRI. Thus, that contrast-enhanced imaging did not aid in prediction of MS in our cohort, is perhaps a fortuitous finding given that contrast-enhanced imaging requires insertion of an intravenous catheter, a procedure not always acceptable to children or their parents.

Interestingly, the presence of thalamic lesions was associated with a 61% decreased risk of MS diagnosis. The frequent presence of thalamic lesions in children with ADEM has been noted previously.[103,106]

Our study methods were established prior to the recently published 2010 McDonald MS diagnostic criteria,[90] application of which will require re-analysis of all baseline images, correlating lesion location with clinical features. However, the presence of periventricular, brainstem, and contrast-enhancing lesions were each associated with MS in our univariate analyses, and thus we anticipate that the 2010 McDonald criteria will perform well when applied to our cohort.

Our study has several limitations. Although all 284 participants had baseline scans, which served as the key scan for predictive models, serial imaging was not always obtained at every time point, and budget constraints necessitated that only children enrolled at the Hospital for Sick Children (124 of 284 participants) were offered annual research scans in years 2-5. However, we obtained over 1100 MRI scans, and 65% participants had ≥4 MRI scans for

analysis. Given that MRI DIT criteria were met by 43 of the 57 children (75%) within 12 months of onset, our quarterly imaging protocol in the first year following incident demyelination captured most of such occurrences. Fourteen children were diagnosed with MS more than 12 months following onset: nine based on confirmed second attack, and only five based on serial MRI scans performed after the first year. Nonetheless, as is inherent in any prospective cohort, some children destined to experience new clinical and MRI evidence of disease are currently classified as monophasic ADS. One might predict that the 15 of 227 children in the monophasic ADS group (7%) who had ≥1 T1 hypointense lesion and ≥1 periventricular lesion are at highest risk.

As a component of our ongoing funded program, we will enroll an additional 100 children with incident demyelination using the same MRI protocol. These children will serve as an independent cohort for validation of our findings. As the performance of any criteria is inherently higher when evaluated in the cohort from which the criteria were derived, evaluation in a new cohort will also be important to confirm whether the performance of our proposed MRI parameters are indeed superior to published criteria.

We developed an MRI scoring tool designed to characterize CNS inflammation suitable for use by clinicians. We then demonstrate that specific baseline MRI features are predictive of pediatric MS, supporting the recent 2010 McDonald criteria which have been controversial with respect to confirmation of MS at an incident demyelinating event. Finally, it is both timely and imperative that the MRI features of MS in children be clearly defined. Pediatric trials of MS-related therapies are soon to be launched, and are now mandated through regulatory Pediatric Investigation Plans for all new therapeutic agents. MRI metrics will serve as key outcome

measures in these clinical trials, and thus documentation of the natural history of baseline and lesion accrual must be understood in order to evaluate therapeutic impact.

6.6 Acknowledgements

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Chapter 7:

Validation of MRI Predictors of Multiple Sclerosis Diagnosis in Children with Acute CNS Demyelination

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Multiple Sclerosis and Related Disorders, *submitted*

7.1 Summary

Background: In a recent Canadian prospective study of children with acute demyelinating syndromes (ADS), we demonstrated that the presence of T2 periventricular and T1-hypointense lesions predicted MS diagnosis. We aimed to validate these predictors in a Dutch cohort of children with ADS.

Methods: Participants with ADS were identified from a prospective cohort or archived dataset. MS was diagnosed based on clinical or MRI evidence of relapsing disease. Baseline MRI scans were evaluated for the presence of the two predictive parameters. Sensitivity, specificity, positive (LR+) and negative likelihood ratios (LR-), and positive (PPV) and negative predictive value (NPV) were calculated to evaluate the performance of the MRI parameters at classifying children as having MS or monophasic demyelination.

Findings: Of 118 children identified with ADS between December 1993-December 2009, MRI scans from 87 children (45 prospective; 47 archived) were evaluated; scans of 28 children were excluded due to incomplete or poor quality imaging. Mean duration of observation was longer in the archived group (7.1 years, SD 3.5) than the prospective cohort (3.3 years, SD 1.4). Thirty children were diagnosed with MS. Performance of the parameters was not statistically different between the prospective cohort (sensitivity 93.3% [68.1-99.8]; specificity 86.7% [69.3-96.2]; LR+ 7.0 [2.8-17.6]; LR- 0.08 [0.01-0.5]; PPV 77.8% [52.4-93.6]; NPV 96.3% [81.0-99.9]) and archived group (sensitivity 66.7% [38.4-88.2]; specificity 85.2% [66.3-95.8]; LR+ 4.5 [1.7-11.9]; LR- 0.4 [0.2-0.8]; PPV 71.4% [41.9-91.6]; NPV 82.1% [63.1-93.9]).

Interpretation: In an independent Dutch cohort, we confirm that the presence of ≥1 T2 periventricular and ≥1 T1-hypointense lesions reliably identifies children with MS.

Funding: Dutch MS Research Foundation

7.2 Introduction

The ability of MRI to aid in identifying children at risk for MS is important for early diagnosis and prompt initiation of MS-targeted therapies. With treatment trials for pediatric-onset MS currently being planned, tools to stratify children with ADS of the CNS into groups at highest risk for MS and those at low risk for recurrent demyelination has become increasingly valuable. We recently proposed MRI parameters associated with MS diagnosis in children with incident ADS. The presence of at least one T2 periventricular lesion and one or more T1-hypointense lesions at onset were predictive of MS diagnosis (sensitivity 84%, specificity 93%).[50] In collaboration with the Dutch Study Group for Pediatric MS, our aim was to validate the predictive MRI parameters in an independent cohort of children with ADS.

7.3 Patients and Methods

Participants and definitions We included patients younger than 17 years of age with ADS. Participants were identified from either an archived group or a prospective cohort under the Dutch Study Group for Pediatric MS, a nationwide network of 15 pediatric healthcare centers in the Netherlands.

Archived group: Children with ADS between 1990 and 2007 inclusive were identified from hospital records at the 15 participating centers. Demographic, clinical and laboratory data collected at onset of ADS and at any follow-up assessments were extracted from health records by a trained individual using standardized case report forms, and subsequently entered into a

centralized database. Duration of follow-up was determined based on the last clinic visit or telephone contact with the site neurologist or pediatrician. MRI scans acquired at onset, and any serial scans were retrieved from each hospital for centralized analysis.

Prospective Group: Beginning in 2008, children with incident ADS are consecutively enrolled into a prospective study at each of the 15 participating centers. Uniform definitions for ADS[56] and clinical assessments are conducted at onset and annually by trained neurologists at each site using standardized case report forms. In the instance that patients are unable to attend a clinic visit, neurologists at the lead site perform a telephone interview with the patient and site neurologist. MRI scans are not acquired according to a standardized research protocol at all sites; however, recommended sequences and scanner settings were provided to each site to increase the consistency of image acquisition. MRI scans are acquired at the time of incident ADS, at three months following ADS, and annually.

Acute demyelinating syndromes were defined according to International Pediatric Multiple Sclerosis Study Group consensus definitions,[56] and included optic neuritis (ON), transverse myelitis (TM), monofocal neurological deficits other than ON or TM, polyfocal deficits, and acute disseminated encephalomyelitis (ADEM) – defined by the presence of polyfocal neurological deficits *and* encephalopathy. Eligible children had a brain MRI scan acquired within 90 days of the incident demyelinating attack. Each participant had at least two years of clinical observation following acute demyelination. Children with neuromyelitis optica were excluded.[294]

Participants or their guardians in the prospective cohort provided written informed consent. This study was conducted under research ethics approvals obtained previously at each participating center for the archived group and prospective cohort.

The outcome was defined as MS diagnosis, which was adjudicated by neurologists with expertise in demyelinating disorders (RFN, CEC-B, RQH) independently of the individual (LHV) who performed the analyses of baseline MRI scans. MS diagnosis was based on either a confirmed second demyelinating attack occurring at least 28 days after the incident episode,[58] or on the accrual of new lesions according to the 2005 McDonald criteria for dissemination in time (DIT) in patients who had not yet experienced a second attack at the time of data analysis.[89] To adjudicate whether participants met MRI criteria for DIT, available serial scans were evaluated by neurologists at the lead site (RFN, CEC-B, RQH). Children initially diagnosed with ADEM were diagnosed with MS if they had two or more non-ADEM attacks, provided that the subsequent events occurred at least 90 days after their initial ADS and the two subsequent attacks were separated by at least 28 days.[56] Participants not meeting clinical or MRI criteria for MS diagnosis were classified as having monophasic demyelination at the time of data analysis.

MRI analysis MRI scans were acquired at each participating site on 1.0 or 1.5 Tesla scanners in the archived group and 1.5 Tesla scanners in the prospective study. Scans were archived as either electronic images or hardcopy films at Erasmus MC or Sophia Children's Hospital (Rotterdam, the Netherlands). For inclusion, T1-weighted and T2-weighted or fluid attenuated inversion recovery (FLAIR) images acquired at the time of ADS were required for each patient. An individual (LHV) with expertise in neuroimaging excluded MRI scans with artifact due to

patient motion or dental hardware, inadequate coverage of the whole brain, or poor grey matter–white matter contrast, according to pre-defined quality criteria.[50]

The MRI scan acquired at the time of incident ADS for each participant was evaluated by a trained rater (LHV) blinded to clinical data. Lesions were evaluated, measured, and localized according to *a priori* rules previously described.[50] Two parameters were scored on each baseline scan as described in our original work:[50] i) *T1-hypointense lesions* were defined as lesions hypointense relative to cortical grey matter and non-enhancing on post-contrast T1-weighted imaging, and ii) *T2 periventricular lesions* were defined as lesions hyperintense on T2-weighted images abutting any portion of the lateral ventricles (including lesions in the corpus callosal white matter, but excluding deep grey matter lesions). Each scan was classified as meeting the MRI parameters when at least one T2 periventricular and one or more T1-hypointense lesions were present.

Statistical analysis Categorical data were summarized as frequency (%) and continuous data as mean (standard deviation, SD) or median (interquartile range, IQR). Univariate statistics were computed using Fisher's exact tests, X^2 tests, t tests and Mann-Whitney U tests as appropriate. We evaluated the performance of the MRI parameters against confirmed MS diagnosis by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).[292]

In addition, we also calculated likelihood ratios for a positive test (LR+) and negative test (LR-) in order to evaluate our parameters using a metric that is independent of the prevalence of the outcome of interest,[292] although the relative proportion of children diagnosed with MS

following an ADS event was actually similar in the Canadian and Dutch cohorts (approximately 30% during the periods of observation).[17,50,91,102]

For the estimates of diagnostic performance, 95% confidence intervals were computed from the binomial distribution.

We performed a sensitivity analysis to assess whether the performance of the MRI parameters was influenced by method of MS diagnosis (based on occurrence of a second clinical attack[58] or MRI evidence of DIT alone[89]).

Statistical analyses were conducted using Stata (version 11).

7.4 Results

Of 115 children and adolescents presenting with an incident ADS between December 1993 and December 2009 who were eligible, 87 were analyzed (**Figure 7-1**). Twenty-eight children were excluded because one or more of the required brain MRI sequences was not acquired at onset (n=9), or one or more of T1-weighted, T2-weighted, and FLAIR sequences were not of sufficient quality (n=19). As detailed in **Appendix 7**, the 28 children excluded from the present analysis did not differ from the 87 participants retained in the study with respect to sex (females included 49 (56%), females excluded 12 (43%), p=0.23), ADEM versus non-ADEM presentations (non-ADEM included 59 (68%), non-ADEM excluded 21 (75%), p=0.48), mean age at onset (included 9.3 (5.2) years, excluded 9.0 (4.5) years, p=0.77), or proportion of patients diagnosed with MS (included 30 (34%), excluded 6 (21%), p=0.20).

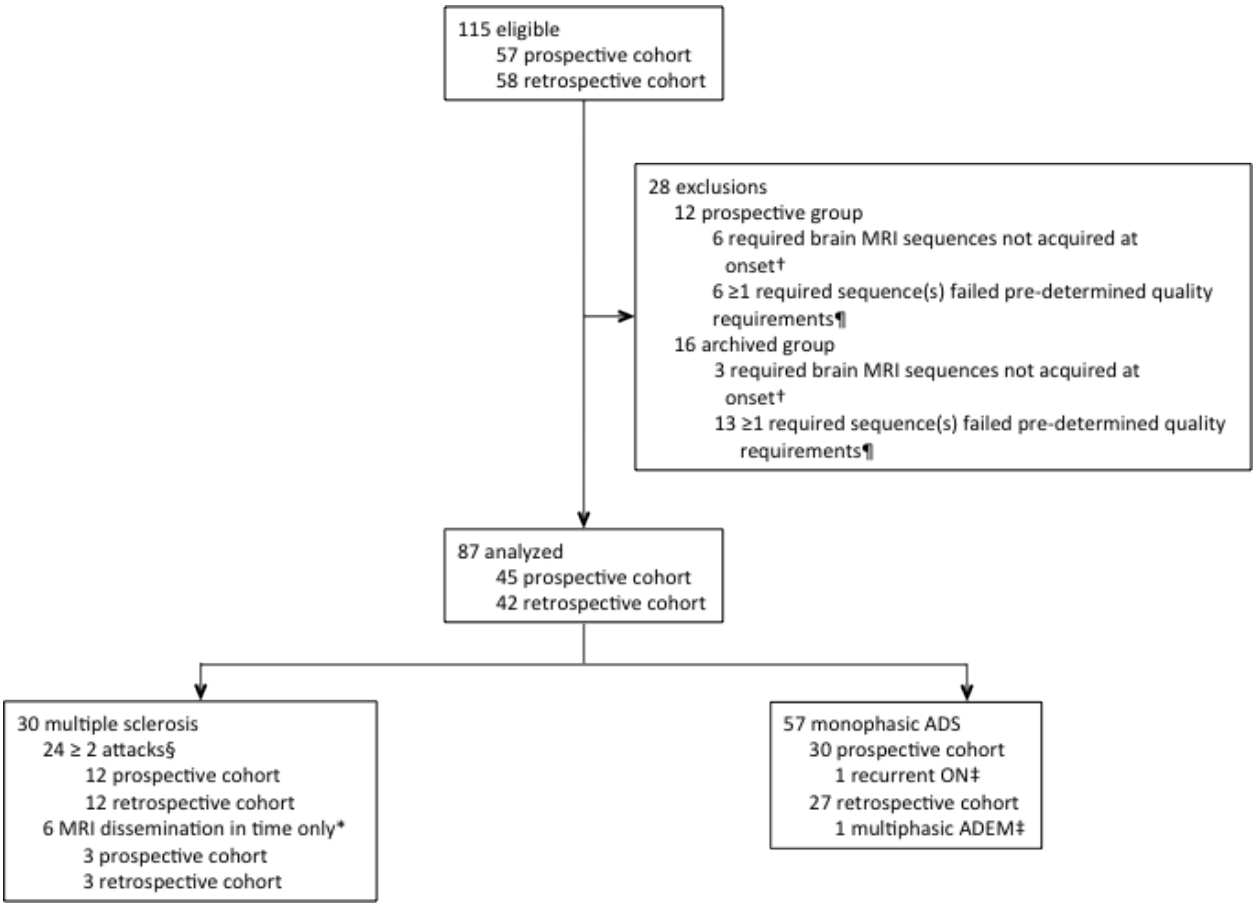

Figure 7-1: Study profile. †Both T1-weighted and T2-weighted or fluid attenuated inversion recovery (FLAIR) imaging of the brain are required to evaluate the presence of the predictive MRI parameters for each participant. ¶MRI scans do not pass quality control if they are degraded by motion or dental hardware artifact, and if images have poor grey matter-white matter contrast or inadequate coverage of the brain. §Attacks separated by at least 28 days and localize to distinct regions of the central nervous system.[58] *Gadolinium enhancement at least 3 months after clinical onset if not at site corresponding to initial event, or a new T2 lesion at any time compared with a reference scan done at least 30 days after clinical onset.[89] ‡Two children had relapsing demyelinating episodes, but did not meet criteria for MS in the following circumstances: (1) recurrence of optic neuritis in the absence of T2 lesions in the brain; (2) occurrence of a second episode of ADEM six years after a first attack of ADEM, with no subsequent non-ADEM attacks or accrual of new lesions on MRI.[56]

Mean duration of clinical observation was longer for the 42 children in the archived group (7.1 years, SD 2.5) than for the 45 participants enrolled in the prospective cohort (3.3 years, SD 1.4; p<0.0001) (**Table 7-1**). Serial MRI scans were evaluated only for evidence of MRI DIT. At least one serial scan was acquired in 59 (69%) of the 87 patients (24 had one serial scan, 23 had two, and 12 had at least three); the remaining 28 (32%) children did not have any serial imaging.

Of 87 children, 30 (34%) children were diagnosed with MS, and 57 (66%) have experienced monophasic ADS to date (**Table 7-1**). Of the 30 children diagnosed with MS, 24 (80%) experienced two or more clinical attacks, whereas the remaining 6 (20%) patients were diagnosed with MS based on MRI evidence of DIT[89] alone. The median time from incident demyelination to MS diagnosis in those that developed MS was shorter in the prospective group (3.6 months, IQR 2.4-8.4) than the archived group (14.4 months, IQR 6.0-20.4; p=0.02). One child in the archived group, who presented with transverse myelitis, was diagnosed with MS after 8 years and has subsequently experienced three relapses. Five (17%) children with MS experienced their first attack prior to ten years of age. Of the 28 children who met criteria for ADEM at onset, only one was diagnosed with MS after experiencing two non-ADEM attacks.[56] Of the 27 children with clinically monophasic ADEM, 17 had serial MRI scans and none had new lesions.

Table 7-1: Clinical and demographic characteristics of participants

	Archived Group			Prospective Group			
	Overall (n=42)	**MS¶ (n=15)**	**Monophasic ADS (n=27)**	**Overall (n=45)**	**MS¶ (n=15)**	**Monophasic ADS (n=30)**	**p-value***
Length of clinical observation (years)	7.1 (3.5)	8.0 (3.5)	6.6 (3.4)	3.3 (1.4)	3.3 (1.3)	3.3 (1.5)	<0.0001
Age at onset (years)	9.5 (4.9)	12.1 (3.5)	8.0 (5.0)	9.1 (5.5)	15.1 (1.9)	6.1 (4.1)	0.73
Age at onset							
<10 years	19 (45%)	4 (27%)	15 (56%)	26 (58%)	1 (7%)	25 (83%)	0.29
≥10 years	23 (55%)	11 (73%)	12 (44%)	19 (42%)	14 (93%)	5 (17%)	
Female	22 (52%)	10 (67%)	12 (44%)	27 (60%)	7 (47%)	20 (67%)	0.52
Female : male ratio	1.1	2.0	0.8	1.5	0.9	2.0	--
Age at MS diagnosis¶ (years)	--	13.8 (3.9)	--	--	15.6 (1.8)	--	0.12§
Time to MS diagnosis¶ (years)	--	1.2 (0.5-1.7)	--	--	0.3 (0.2-0.7)	--	0.02§
2nd clinical attack	--	12 (80%)	--	--	12 (80%)	--	1.0§
ADS phenotype							
ADEM	13 (31%)	1 (7%)	12 (44%)	15 (33%)	0 (0%)	15 (50%)	0.61
TM	5 (12%)	1 (7%)	4 (15%)	2 (4%)	1 (7%)	1 (3%)	
ON	5 (12%)	2 (13%)	3 (11%)	4 (9%)	1 (7%)	3 (10%)	
Monofocal-other	8 (19%)	7 (47%)	1 (4%)	7 (16%)	5 (33%)	2 (7%)	
Polyfocal	11 (26%)	4 (27%)	7 (26%)	17 (38%)	8 (53%)	9 (30%)	
Abnormal MRI scan	34 (81%)	14 (93%)	20 (74%)	38 (84%)	15 (100%)	23 (77%)	0.78

Data are mean (standard deviation, SD), median (interquartile range, IQR) or number (%). *Comparison between the overall archived and prospective groups. §Comparison between children with MS in the archived group and those with MS in the prospective group. ¶MS diagnosis based on either a confirmed second clinical attack at least 28 days after incident demyelination,[58] or 2005 McDonald criteria for MRI dissemination in time: gadolinium enhancement occurred at least 3 months after clinical onset if not at the site corresponding to the initial event, or a new T2 lesion was seen at any time compared with a reference scan acquired at least 30 days after clinical onset.[89]

T2 lesions were present in 73 (84%) children; 14 (16%) had normal brain imaging at onset (1 with ADEM, 5 with monofocal ON, 5 with monofocal TM, and 3 with polyfocal neurological deficits). Of the 30 children with MS, 29 had T2-weighted lesions present on brain MRI at ADS.

The one child without brain lesions presented with transverse myelitis, but subsequently developed clinical and MRI evidence of brain involvement.

Table 7-2 demonstrates the performance of the MRI parameters. Comparing the prospective cohort to the archived group with regards to performance of the MRI parameters at correctly identifying children diagnosed with MS according to the occurrence of a second clinical attack or MRI evidence of DIT, sensitivity (93.3% vs. 66.7%; p=0.07) differed between the two groups, although this difference was not statistically significant. Specificity (86.7% vs. 85.2%; p=0.87), PPV (77.8% vs. 71.4%; p=0.68), and NPV (96.3% vs. 82.1%; p=0.09) were not statistically different. Similarly, the 95% confidence intervals overlapped considerably when comparing positive and negative likelihood ratios of the prospective cohort (LR+ 7.0, 95% CI 2.8-17.6; LR- 0.08, 95% CI 0.01-0.5) to the archived group (LR+ 4.5, 95% CI 1.7-11.9; LR- 0.39, 95% CI 0.2-0.8).

In total, MRI scans of six patients with MS (5 in the archived group; 1 in the prospective cohort) did not demonstrate the MRI parameters on baseline images (20% false negative rate). Of these, one child who presented with transverse myelitis did not have brain lesions at onset. Of the remaining five children (three of whom were under ten years of age at ADS), three children had T2 brain lesions, but no T2 periventricular or T1-hypointense lesions, and two had T2 periventricular lesions but no T1-hypointense lesions.

Table 7-2: Performance of MRI parameters at identifying children with MS

	MS diagnosis according to occurrence of 2nd attack[58] *or* 2005 McDonald DIT[89]			**MS diagnosis according to occurrence of 2nd attack[58] alone**		
	Archived Group (N=42)	**Prospective Group (N=45)**	**p-value**	**Archived Group (N=42)**	**Prospective Group (N=45)**	**p-value**
True Positives	10	14		8	11	
True Negatives	23	26		24	26	
False Negatives	5	1		4	1	
False Positives	4	4		6	7	
Sensitivity, % (95% CI)	66.7 (38.4-88.2)	93.3 (68.1-99.8)	0.07	66.7 (34.9-90.1)	91.7 (61.5-99.8)	0.13
Specificity, % (95% CI)	85.2 (66.3-95.8)	86.7 (69.3-96.2)	0.87	80.0 (61.4-92.3)	78.8 (61.1-91.0)	0.90
PPV, % (95% CI)	71.4 (41.9-91.6)	77.8 (52.4-93.6)	0.68	57.1 (28.9-82.3)	61.1 (35.7-82.7)	0.82
NPV, % (95% CI)	82.1 (63.1-93.9)	96.3 (81.0-99.9)	0.09	85.7 (67.3-96.0)	96.3 (81.0-99.9)	0.17
LR+, (95% CI)	4.5 (1.7-11.9)	7.0 (2.8-17.6)		3.3 (1.5-7.6)	4.3 (2.2-8.5)	
LR-, (95% CI)	0.39 (0.2-0.8)	0.08 (0.01-0.5)		0.42 (0.2-0.9)	0.1 (0.02-0.70)	

Eight children (4 in the archived group; 4 in the prospective cohort) were predicted to have MS based on presence of both MRI parameters, but to date have not experienced MRI or clinical evidence of relapsing disease (14% false positive rate). Of the eight participants, 6 were female and 6 were less than 10 years of age at onset. Three children presented with ADEM, 2 with monofocal deficits, and 3 with polyfocal neurological deficits.

We performed a sensitivity analysis to evaluate the effect of method of MS diagnosis on the performance of the MRI parameters. As illustrated in Table 2, when MS diagnosis was defined on the basis of clinical relapses only,[58] estimates of diagnostic performance were not significantly different from the estimates obtained when MS was diagnosed on the basis of a second attack or MRI evidence of DIT (statistical analyses not shown).

In a *post hoc* analysis, we evaluated the performance of the MRI parameters at correctly classifying children with ADEM as having monophasic demyelination. Of the 28 children with monophasic ADEM, 25 were correctly classified as not having MS and three children were incorrectly predicted to have MS. Of the 25 children with ADEM who were correctly classified as having a monophasic illness, 15 had neither T2 periventricular nor T1-hypointense lesions, 8 had T2 periventricular but no T1-hypointense lesions, and 2 had T1-hypointense but no T2 periventricular lesions. Three children with clinically and radiographically monophasic ADEM had both T1-hypointense and T2 periventricular lesions at onset, and therefore were falsely classified as having MS. All three children were female, less than ten years of age at onset, and have been followed clinically for 4.9 years, 3.2 years, and 2.4 years.

7.5 Discussion

We confirm the high sensitivity and specificity of T1 hypointense lesions and T2 periventricular lesions as predictive of MS outcome in children with ADS, validating our initial work.[50] That the presence of periventricular lesions – a characteristic location for MS lesion formation, and T1-

hypointense lesions at ADS – an indicator of established tissue injury – are robust early predictors of MS diagnosis is in line with current concepts of MS pathobiology.

The first aspect of our validation study was performed in the Dutch prospective cohort, an independent but methodologically similar study population to the Canadian Demyelinating Disease program[17] from which the MRI parameters were originally developed.[50] Specifically, both studies utilized the International Pediatric MS Study Group consensus definitions[56] to characterize ADS phenotype and MS diagnosis, both studies utilized the same standardized clinical reporting form, and the MRI protocols utilized were sufficiently similar to permit consistency in evaluation of commonly acquired sequences. The Dutch and Canadian cohorts are also similar clinically: (i) the proportion of children with ADS subsequently diagnosed with MS was 36% in the Dutch prospective cohort and 20% in Canadian prospective study; and (ii) the diagnosis of MS in children with an initial ADEM presentation was extremely rare in both cohorts (none in the Dutch cohort and one in the Canadian). Thus, in cohorts of children with typical ADS, we demonstrate robust sensitivity (Dutch study 93%, Canadian study 84%) and specificity (Dutch study 87%, Canadian study 93%) of the MRI parameters.

We then applied the MRI parameters in a dataset of archived scans of children with ADS in the Netherlands, in order to evaluate the MRI parameters in patients not enrolled in prospective research protocols. MRI scans in this archived group were obtained at either 1.0 or 1.5 Tesla, and were not obtained according to a consistent protocol. The sensitivity of the MRI parameters was qualitatively higher in the prospective cohort (93%) than in the archived group (67%), a decrease driven by an increase in the number of false negatives in the archived cohort. Although all participants included in the prospective and archived groups had scans that passed

our quality requirements, scans from the archived dataset were less likely to conform to parameter standards than the scans acquired in the prospective cohort. Specifically, FLAIR imaging was either less frequently acquired or acquired in the coronal rather than axial plane, whole brain coverage was not achieved, and the contrast between grey matter and white matter on T1-weighted imaging was poorer, rendering detection of T2 lesions in the periventricular region and T1-hypointense lesions more challenging. Since common practice as well as a published MRI protocol for MS[263] includes axial FLAIR and T2 sequences with contiguous three millimeter slices, we anticipate that the sensitivity of our MRI parameters will be higher when such sequences are obtained.

We have not evaluated the predictive value of the proposed MRI parameters in children with incident ADS from world regions where MS outcome following ADS is rare. Such studies will require multi-national involvement, and will be challenged by the essential inclusion of countries where not only is MS rare, but so too is availability of MRI. However, such analyses will be important to ensure that the MRI parameters are universally predictive of MS outcome.

The ability to predict whether children with incident CNS demyelination will be diagnosed with MS is important for counseling of families and for planning of patient care. A key challenge in pediatric demyelination is the ability to predict MS diagnosis across the full range of ADS presentations, particularly in the context of ADEM. Given that ADEM is particularly more common in children than adults,[105,106,296] MRI parameters that effectively stratify unselected groups of children with ADS, including those with ADEM, into high and low risk for MS have significant clinical value.

Eight children (14% false positive rate) would have been predicted by our MRI parameters to be diagnosed with MS, yet have experienced a clinically monophasic disease course to date (three with ADEM, three with polyfocal and two with monofocal ADS). Whether these eight children are particularly likely to reach a diagnosis of MS in the future remains to be determined as standardized serial imaging is not performed in the Dutch study, and thus MRI evidence of DIT was potentially underappreciated. Thus, while the ability to predict MS diagnosis based on initial MRI has obvious value to the immediate care of the child, it remains important to emphasize that long-term monitoring of children with ADS is essential to capture either new lesions on serial MRI scans or a second clinical attack.

Evaluation of the predictive validity of the MRI parameters at baseline is influenced by the method of MS diagnosis. As expected, given that MRI evidence of new lesions is often independent of clinical relapses,[297,298] when restricting our analysis to only children with confirmed clinical relapses, the positive predictive value of the MRI parameters was slightly but not significantly reduced. A further confound is that six children in the current study initiated MS-targeted therapy after MRI confirmation of DIT, four of whom have not had a second clinical attack to date.

Overall, we confirm that T2 lesions in the periventricular white mater, a region well-recognized as targeted in MS, and the presence of T1-hypointense lesions, typically indicative of established pathology, are robust predictors of a future diagnosis of MS in children with ADS.

7.6 Acknowledgements

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PART III:

MRI AS A TOOL FOR DIAGNOSIS OF PEDIATRIC-ONSET MS

Chapter 8:

2010 McDonald Criteria for Diagnosing Pediatric MS

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8.1 Summary

Background The diagnosis of Multiple Sclerosis (MS) rests on confirmation of central nervous system inflammatory disease which is disseminated in space (DIS) and time (DIT), as evidenced clinically or by MRI. The 2010 McDonald criteria simplified MRI requirements, and newly proposed that the criteria are also suitable for the diagnosis of pediatric MS.

Methods In a national prospective incident cohort study of children with acute demyelination observed for a minimum of 24 months, baseline and serial clinical and MRI examinations were used to retrospectively evaluate the 2010 and 2005 McDonald criteria using clinically relapsing disease as the gold standard.

Findings Of 212 eligible participants, 34 experienced two or more clinical attacks, 58 met the 2010 criteria and 42 met 2005 McDonald criteria. The 2010 criteria demonstrated high sensitivity (100%), specificity (86%), positive predictive value ([PPV] 76%) and negative predictive value ([NPV] 100%) for children older than 11 years with non-ADEM presentations, as did the 2005 McDonald criteria. In younger children with a non-ADEM presentation, PPV of the 2010 criteria was only 55%. None of the 50 children with ADEM met clinical criteria for MS, but 10 met 2010 and 4 met 2005 criteria.

Interpretation Both 2005 and 2010 McDonald criteria identify children with relapsing-remitting MS, although caution is suggested when applying these criteria in younger children. The 2010 McDonald criteria are simple and enable an early diagnosis of MS, but are not suited for application in the context of ADEM-like presentations.

Funding Canadian Multiple Sclerosis Scientific Research Foundation

8.2 Introduction

The diagnosis of MS rests on confirmation of CNS-directed inflammation that occurs across multiple regions of the CNS and over time.[299] Clinical criteria for MS diagnosis require that relapses persist for a minimum of 24 hours, and that discrete attacks be separated by more than 30 days.[58] The advent of MRI as a sensitive tool for detecting clinically silent lesions led to further modification of diagnostic criteria such that clinically silent lesions in the CNS could serve as evidence of dissemination of disease in space (DIS), and lesions accruing over time could serve as evidence of disease dissemination in time (DIT).[95,96,300] The diagnostic algorithm for MS proposed in 2001,[101] and further refined in 2005[89] stipulated specific lesion locations for DIS[95,96] and delineated specific requirements for new T2-weighted or gadolinium-enhanced lesions to fulfill DIT.

In 2010, the McDonald criteria were revised further.[90] These revisions were based partly on findings of the MAGNIMS group who demonstrated that the presence of two or more lesions in specific CNS regions yielded simplified, yet highly sensitive criteria for MRI DIS, and that even a single MRI scan demonstrating both enhancing and non-enhancing lesions at baseline could serve as evidence for DIT.[97,99] New T2 lesions, irrespective of the timing of MRI studies, were also sufficient to fulfill MRI evidence of DIT; thus the 2005 criteria requirement that the reference scan be acquired more than 30 days from the incident clinical attack was not retained in the 2010 criteria.

The 2010 McDonald panel felt that the new criteria were applicable for the diagnosis of pediatric MS, but emphasized the need to validate this assertion.[90] The 2010 McDonald panel

specifically stated that the criteria for MS diagnosis should not be applied in the context of acute disseminated encephalomyelitis (ADEM), even though 5-18% of such children will ultimately be diagnosed with MS.[17,57]

We evaluated the ability of the 2010 and previous 2005 McDonald criteria to accurately predict clinical relapses consistent with MS in a prospective cohort of children with acute demyelinating syndromes (ADS) of the CNS.

8.3 Patients and Methods

Participants and study design Participants 15 years 11 months of age or younger with neurological deficits and MRI findings consistent with ADS, as defined in our prior publications,[17,50] were enrolled within 90 days of symptom onset. All participants were evaluated using standardized clinical assessments and all site investigators were provided a list of investigations appropriate for exclusion of children with non-demyelinating disorders.[17] Enrolment occurred across 23 Canadian health care facilities (including all 16 pediatric health care facilities in Canada), and all participants and their families provided informed consent. Assent was obtained from younger children. Clinical data and brain MRI scans were acquired at baseline, 3, 6, and 12 months at all 23 study sites, annually thereafter for children enrolled at the Hospital for Sick Children (Toronto, Canada), and at the time of a second clinical event (if it occurred) for all participants. Clinical data and the results of cerebrospinal fluid analysis for oligoclonal bands (OCBs) were collected in a standardized manner. NMO IgG antibodies were assayed by indirect immunofluorescence using primate cerebellar sections with diluted

sera (1/50) following the manufacturer's instructions (Instrumentation Laboratory, Lexington, Massachusetts, USA), and quantified using a cell-based assay.[301]

Data were entered into a central database. As previously described,[17] the presenting phenotype was characterized without consideration of the MRI features as clinically monofocal ADS (all symptoms and neurological findings localizable to a single CNS location with the specific CNS location noted), clinically polyfocal ADS (more than one CNS site required to explain neurological findings), or as ADEM (polyfocal deficits plus encephalopathy).[56] All children meeting entry criteria for ADS enrolled prior to January 2009 were included in our primary evaluation of the 2010 and 2005 criteria to ensure sufficient duration of observation to capture a second attack (CDMS).

Research MRI scans were acquired for all patients on 1.5 Tesla MRI scanners according to a defined protocol that included pre- and post-contrast axial T1-weighted, proton density and T2-weighted, and sagittal and axial turbo fluid attenuated inversion recovery (FLAIR) images. Any additional brain and spine scans performed for clinical purposes were also reviewed. All scans were anonymized and analyzed centrally. Scans that failed quality evaluation (ie: movement or dental hardware artifact, missed sequences) were excluded. Raters were blinded to clinical outcome.

Outcome Children were diagnosed with one of the following outcomes: (i) clinically defined MS (CDMS), defined by a second demyelinating attack, separated by more than 30 days from the incident attack and with objective findings indicating involvement of a new CNS location.[58] Children with ADEM at onset were required to experience two, non-ADEM attacks with the first

non-ADEM event occurring more than 90 days following the initial illness;[56] (ii) relapsing demyelination not meeting criteria for CDMS, as defined by recurrent episodes in the same CNS location without clinical evidence of new lesions in other CNS locations. Children with an initial diagnosis of ADEM who experienced subsequent symptoms but did not experience the two non-ADEM attacks required for MS diagnoses according to international diagnostic criteria [56] were also included in this group; (iii) neuromyeltiis optica (NMO) and NMO spectrum disorders were diagnosed according to published criteria [294]; (iv) non-ADS diagnoses were conferred when subsequent clinical, laboratory, or MRI findings indicated an alternative diagnosis; and (v) all other children were considered to have experienced monophasic ADS at the time of data analysis.

Evaluation of 2010 and 2005 McDonald criteria Although both the 2005 and 2010 McDonald criteria can be met if patients experience two or more clinical relapses (clinical DIS and clinical DIT) irrespective of MRI features, our work focuses on adjudication of 2005 and 2010 McDonald "positivity" as indicated in **Figure 8-1.**

The 2005 McDonald criteria for DIT require that the reference scan be more than 30 days from onset of the first attack.[89] Baseline MRI scans in our study were obtained at onset and thus the first MRI obtained often could not serve as the reference scan for the 2005 McDonald criteria, but these scans were acceptable as the reference scan for the 2010 McDonald criteria.[90] Thus, adjudication of the 2005 criteria often utilized the three month scan in our protocol as the reference scan. For adjudication of the 2010 criteria, correlation between clinical features at presentation and lesion localization on baseline MRI was performed in order to determine whether lesions (specifically brainstem or spinal cord) were clinically silent or not.

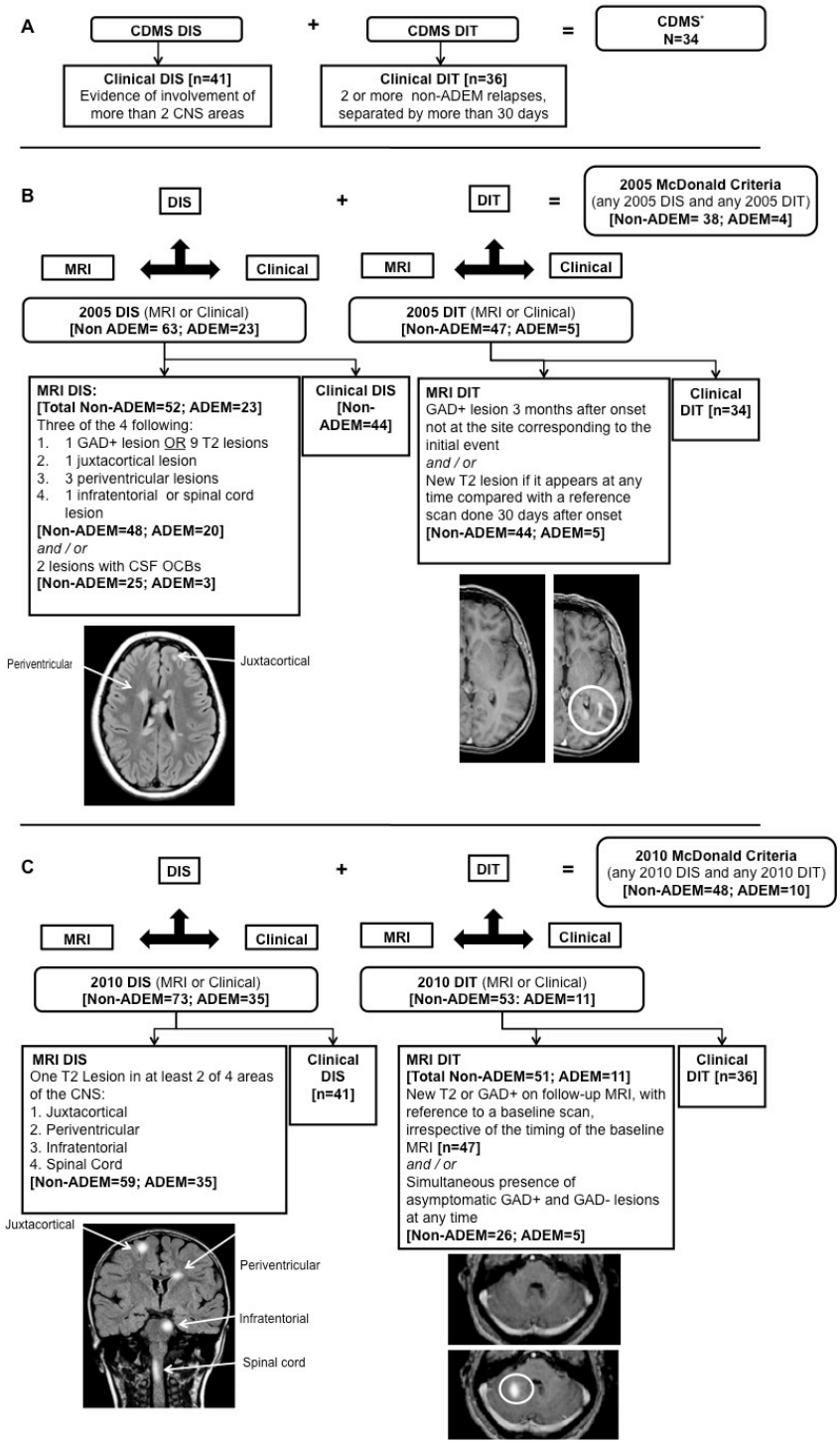

Figure 8-1: Methods of MS diagnosis

Figure 8-1 continued: Numbers presented refer to number of children meeting diagnostic criteria under each condition. (**A**) Diagnosis of clinically-defined MS required clinical DIS

(involvement of at least two discrete areas of the CNS at baseline and/or involvement of a new CNS location at the time of a second attack), and clinical DIT (two attacks separated by more than 30 days). Patients with clinical DIS and clinical DIT served as the "gold standard" for the present manuscript and were termed "clinically diagnosed MS, CDMS". *For children with ADEM, we did not score clinical DIS at baseline given that polyfocal deficits are a component of the ADEM illness and precise localization is often difficult in the presence of encephalopathy. For children with ADEM, clinical DIS was met if the patient experienced neurological deficits referable to two or more CNS areas on subsequent non-ADEM attacks, and clinical DIT in these patients required two non-ADEM attacks with the second demyelinating event (first non-ADEM attack) required to be more than 90 days from initial ADEM illness, as per international consensus definitions.[56] (**B**), DIS and DIT criteria are adjudicated according to the 2005 McDonald criteria.[89], and in (**C**) the DIS and DIT criteria are illustrated according to the 2010 McDonald criteria.[90] It is relevant to note that 188 patients (90%) of 209 included participants had enhanced images available at onset, and 117 children had CSF OCB analysis. As application of the McDonald 2010 for DIS at baseline stipulates that lesions located in the brainstem or spinal cord be discounted in the presence of clinical brainstem or spinal cord attack features, we reviewed all clinical features for each child to exclude those lesions from analysis. The presence of encephalopathy and the young age of many children with ADEM limited clinical delineation of silent and clinically-apparent lesion localization, and thus all lesions were counted, irrespective of CNS location.

Statistical analyses Categorical variables were summarized as frequency (%), and continuous variables as mean (standard deviation [SD]) or median (interquartile range [IQR]) as appropriate. CDMS served as the gold standard against which the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the full 2005 and 2010 McDonald criteria and the DIS and DIT components of each set of criteria were measured. Youden's J, an index that equally weights sensitivity and specificity, was also calculated as:

(sensitivity + specificity) – 1.[293] Sensitivity analyses were performed to evaluate whether MRI frequency after year one influenced findings. Time from initial presentation to fulfillment of (i) "clinically-defined MS", (ii) 2005 or (iii) 2010 McDonald MS criteria was recorded for each non-ADEM participant.

Statistical analyses were performed using Stata (version 11).

8.4 Results

As delineated in **Figure 8-2**, of 337 children with incident ADS, 212 met our entry criteria. Three children (none of whom had ADEM at onset) were started on MS disease-modifying treatment (DMT) following MRI confirmation of new lesions but in the absence of a second clinical attack. Given that such therapy modifies time to second attack (and thus confirmation of CDMS according to our criteria), these three children were excluded from analysis. Of the 209 participants, all were evaluated for the 2010 criteria and 181 had MRI studies appropriate for evaluation of 2005 McDonald criteria. **Table 8-1** describes the clinical and CSF characteristics of the cohort, and delineates the proportion that met criteria for CDMS, 2010 and 2005 criteria. The mean (SD) number of MRI studies obtained for each participant was 4.5 (1.9), and the mean (SD) duration of observation of the entire cohort is 4.2 (1.3) years.

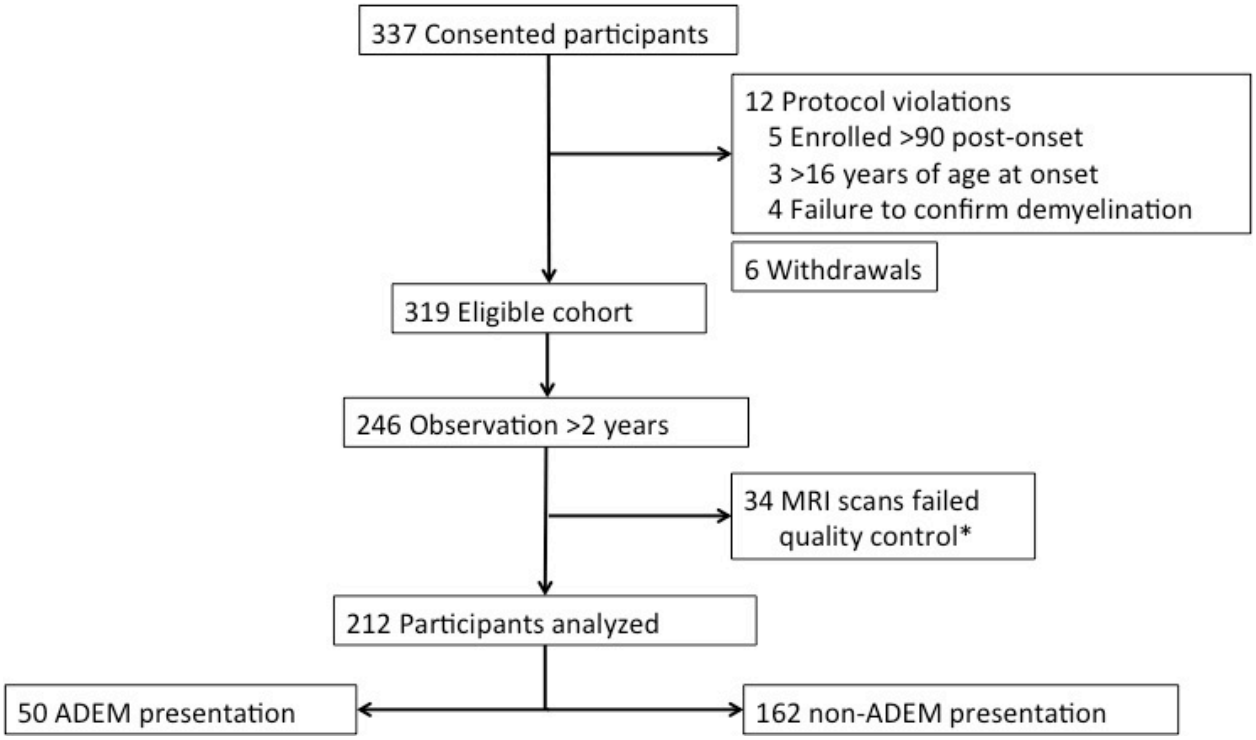

Figure 8-2: Study profile

Table 8-1: Characteristics of children meeting CDMS, and 2010 and 2005 McDonald criteria

Characteristic	Children Analyzed	Non-ADEM at Onset	ADEM at Onset	Clinically Relapsing MS	Not Diagnosed with Clinically Relapsing MS	McDonald 2010 Positive	McDonald 2010 Positive but No 2nd AttackΨ		McDonald 2005 Positive	McDonald 2005 Positive but No 2nd Attack	
							Non-ADEM	ADEM at Onset		Non-ADEM	ADEM at Onset
Number	212*	162*	50	34	175¶§	58	14†	10‡	42	9	4
Age at onset, years; mean (SD)	10.1 (4.2)	11.1 (3.7)	6.8 (4.3)	13.2 (2.4)	9.4 (4.2)	13.2 (2.4)	11.8 (2.6)	5.7 (4.9)	12.8 (3.1)	12.4 (2.6)	7.5 (6)
Female, n (%)	114 (54)	91 (56)	23 (46)	26 (76)	85 (49)	39 (67)	9 (64)	6 (60)	30 (71)	6 (67)	2 (50)
ADS Phenotype											
Monofocal TM	37 (17)	37 (23)	--	2 (6)	34 (19)	3 (5)	1 (7)	--	2 (5)	0	--
Monofocal ON	53 (25)	53 (33)	--	7 (21)	46 (26)	14 (24)	6 (43)	--	10 (24)	3 (33)	--
Monofocal, other	28 (13)	28 (17)	--	10 (29)	16 (9)	9 (16)	0	--	8 (19)	1 (11)	--
Polyfocal	44 (21)	44 (27)	--	15 (44)	29 (17)	22 (38)	7 (50)	--	18 (42)	5 (56)	--
ADEM	50 (24)	--	--	0	50 (29)	10 (17)	--	--	4 (10)	--	--
MRI scans per patient, mean (SD)	4.5 (1.9)	4.7 (1.8)	3.8 (1.8)	4.8 (1.7)	4.4 (1.9)	4.9 (2.2)	6.1 (2.9)	3.3 (1.1)	4.2 (2.2)	6.7 (3.2)	4.3 (2.6)
OCB-positive/all with OCB testing (%)	35/117 (30)	31/98 (32)	4/20 (20)	15/23 (65)	18/92 (21)	21/37 (57)	5/9 (56)	1/5 (20)	20/26 (81)	5/6 (83)	1/1 (100)
Length of observation, mean (SD)	4.2 (1.3)	4.2 (1.3)	4.2 (1.2)	4.2 (1.2)	4.2 (1.3)	4.9 (2.2)	4.4 (1.1)	4.1 (0.8)	4.3 (1.2)	4.5 (1.2)	4.6 (1.4)

OCB, oligoclonal bands; ΨThe clinical and MRI features and current diagnosis of these participants are further detailed in **Appendix 8**. *Three children of the 212 evaluated were treated with disease-modifying therapy prior to second clinical attack, and were excluded from analysis of the 2005 and 2010 criteria. The clinical features are included here for completeness. ¶Of 175 children not diagnosed with CDMS, 2 experienced relapses in the same anatomical location, 2 with ADEM experienced a second non-ADEM attack, 1 was diagnosed with a neuromyelitis optica spectrum disorder, 11 have been diagnosed with non-demyelinating disease, and 159 are considered to have monophasic ADS.

Table 8-1 continued: §Comparing the 34 children diagnosed with clinically relapsing MS with the 175 children who have not experienced clinical relapses (50 ADEM and 125 non ADEM at presentation), CDMS patients are older (all non-relapsing $p<0.0001$, non ADEM $p<0.0001$, ADEM $p<0.0001$). The female: male ratio is higher in patients with CDMS (all non-CDMS $p<0.0001$, non- ADEM $p<0.0001$, ADEM $p<0.0001$), and they tend to have more positive OCB results (all non-CDMS $p<0.0001$, non- ADEM $p<0.0001$, ADEM $p<0.0001$). However, there was no difference between these groups in the number of MRI scans ($p=0.3$) or in the length of observation ($p=0.96$). The frequency of the different presenting phenotypes differs between children with CDMS and those who have not experienced a second attack ($p<0.0001$). Specifically, polyfocal presentations and monofocal attacks (most localized to the brainstem or cerebellum) were more common in children with CDMS, while ADEM, monofocal ON and TM were more likely to occur in children who have not had clinical relapses. †In a comparison of 34 children diagnosed with clinically relapsing MS with 14 non-relapsing McDonald 2010-positive children who had non- ADEM presenting phenotype, no differences were found in age at onset ($p=0.09$), positive OCB results ($p=0.07$), number of scans ($p=0.06$) or length of observation ($p=0.72$). ‡In contrast, comparing 34 CDMS children with 10 non-relapsing McDonald 2010 positive children who had ADEM presenting phenotype, CDMS children were older ($p<0.0001$), had more positive OCB results ($p<0.0001$) and had higher female;male ratio.

Clinical Outcome Thirty-four children met criteria for CDMS, none of whom had ADEM at onset. Four children experienced relapsing demyelination not meeting criteria for CDMS, two with recurrent episodes in the same CNS location without clinical evidence of new lesions in other CNS locations, and two children presented with ADEM and experienced a single non-ADEM attack (none experienced recurrent episodes of ADEM). One child was diagnosed with a NMO spectrum disorder, and 11 were diagnosed with non-demyelinating disorders based on

investigations performed subsequent to their initial study inclusion. The remaining 159 children were classified as clinically monophasic ADS at the time of data closure.

The 34 children who met criteria for CDMS were older, more likely to be female, and more likely to have CSF OCBs when compared to the 175 participants who were not diagnosed with CDMS (**Table 8-1**).

2010 McDonald criteria Fifty-eight children met the 2010 criteria (at baseline or on serial scans; **Figure 8-1**); 10 of these children were diagnosed with ADEM at presentation. Of these 58 children, 34 (none of whom manifested with ADEM at onset) ultimately experienced two or more relapses consistent with CDMS. All 34 children who met both CDMS and 2010 criteria showed clinical DIT (two or more attacks) and MRI DIT. Ultimately, all 34 had lesion patterns either at baseline or on serial scans that conformed to the 2010 McDonald MRI DIS requirements. **Appendix 8** details the clinical and MRI features and outcome of the remaining 24 children who met 2010 criteria (observed for a median [IQR] of 4.2 [0.95] years) but who have not been diagnosed with CDMS. The duration of observation and the number of MRI scans analyzed did not differ between the 34 clinically-relapsing patients and the 24 patients positive for 2010 criteria who have not met criteria for CDMS.

Unique to the 2010 McDonald revisions, patients with ADS can meet 2010 criteria on a single baseline scan if DIS criteria are fulfilled together with the simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions. Twenty-nine of the 58 patients that met 2010 criteria did so based on baseline images. Eighteen of these 29 children have experienced clinical relapses (CDMS), 5 presented with ADEM (none of whom meet

criteria for CDMS), and 6 have remained clinically monophasic (four of whom have developed new MRI lesions on serial scans).

Although spinal cord imaging was not acquired as part of our study protocol, we obtained spine MRI studies from 81 participants (18 with ADEM) who underwent spinal MRI at the time of first demyelinating event. One or more spinal lesions were detected in 60 of the children, 44 of whom had symptoms of spinal cord involvement. For the 16 children with clinically silent spinal cord lesions, eleven had already met the 2010 DIS criteria based on brain imaging, three did not meet 2010 or 2005 criteria for DIS even with the spinal lesion considered, and in the remaining two children the presence of a clinically silent spinal cord lesion led to confirmation of 2010 DIS criteria. However, these patients eventually also developed MRI DIS criteria on subsequent MRI scans.

2005 McDonald Criteria Forty-two children met the 2005 McDonald criteria (**Figure 8-1**), of whom 29 were also diagnosed with CDMS, and 13 have not been diagnosed with CDMS (as described in **Table 8-1**). Of five children with CDMS who did not meet 2005 McDonald criteria, two did not have appropriately timed reference scans for evaluation, and three did not meet 2005 DIS criteria on any MRI.

According to the 2005 McDonald criteria, MRI DIS can be achieved based solely on MRI criteria or through the combination of OCB with two T2 lesions. Of the 42 children meeting 2005 criteria (28 with CSF obtained for analysis), 20 children met DIS criteria based on CSF OCBs and at least two brain lesions. Of these 20 children, 19 ultimately met 2005 criteria even if the OCB results were not considered.

All but one of the children who met the 2005 McDonald criteria also met the 2010 criteria. The one child who met 2005 but not 2010 criteria presented with two lesions (one symptomatic brainstem lesion) and positive OCBs (meeting 2005 DIS but not 2010 DIS), and then developed a new infratentorial lesion on serial imaging (2005 and 2010 DIT, but still did not meet 2010 MRI DIS).

McDonald criteria performance and clinical features The performance of the 2010 and 2005 McDonald criteria, as well as the DIS and DIT components of each set of criteria, in identifying children with CDMS are shown for all 209 children in **Table 8-2**. **Table 8-3** and **Table 8-4** describe the non-ADEM and ADEM groups separately.

Table 8-2: Analysis of the McDonald criteria for prediction of clinically defined MS

	Children with ADS					
	McDonald 2005 (n=181)	**DIS 2005**	**DIT 2005**	**McDonald 2010 (n=209)**	**DIS 2010**	**DIT 2010**
True Positives	29	30	31	34	34	34
True Negatives	136	104	131	151	115	147
False Positives	13	45	18	24	60	28
False Negatives	3	2	1	0	0	0
Sensitivity% (95% CI)	91 (75-98)	94 (79-99)	97 (84-100)	100 (90-100)	100 (90-100)	100 (90-100)
Specificity% (95% CI)	91 (86-95)	70 (62-77)	88 (82-93)	86 (80-91)	66 (58-73)	84 (78-89)
PPV % (95% CI)	69 (53-82)	40 (29-52)	63 (48-77)	59 (45-71)	36 (27-47)	55 (42-68)
NPV % (95% CI)	98 (94-100)	98 (93-100)	99 (96-100)	100 (98-100)	100 (97-100)	100 (98-100)
Youden's J	0.82	0.64	0.85	0.86	0.66	0.84

Table 8-3: Analysis of the McDonald criteria for prediction of clinically defined MS excluding children with ADEM at onset

	Children with non-ADEM ADS					
	McDonald 2005 (n=142)	**DIS 2005**	**DIT 2005**	**McDonald 2010 (n=159)**	**DIS 2010**	**DIT 2010**
True Positives	29	30	31	34	34	34
True Negatives	101	88	97	111	100	108
False Positives	9	22	13	14	25	17
False Negatives	3	2	1	0	0	0
Sensitivity% (95% CI)	91 (75-98)	94 (79-99)	97 (84-100)	100 (90-100)	100 (90-100)	100 (90-100)
Specificity% (95% CI)	92 (85-96)	80 (71-87)	88 (81-94)	89 (82-94)	80 (72-87)	86 (79-92)
PPV % (95% CI)	76 (60-89)	58 (43-71)	70 (55-83)	71 (56-83)	58 (44-70)	67 (52-79)
NPV % (95% CI)	97 (92-99)	98 (92-100)	99 (94-100)	100 (97-100)	100 (96-100)	100 (97-100)
Youden's J	0.83	0.74	0.85	0.89	0.8	0.86

Table 8-4: Analysis of the McDonald criteria for prediction of clinically defined MS in children presenting with ADEM

	ADEM at Onset					
	McDonald 2005 (n=39)	**DIS 2005**	**DIT 2005**	**McDonald 2010 (n=50)**	**DIS 2010**	**DIT 2010**
True Positives	0	0	0	0	0	0
True Negatives	35	16	34	40	15	39
False Positives	4	23	5	10	35	11
False Negatives	0	0	0	0	0	0
Sensitivity % (95% CI)	0	0	0	0	0	0
Specificity % (95% CI)	90 (64-88)	41 (26-58)	87 (73-96)	80 (66-90)	30 (18-45)	78 (64-88)
PPV % (95% CI)	0	0	0	0	0	0
NPV % (95% CI)	100 (91-100)	100 (79-100)	100 (90-100)	100 (91-100)	100 (78-100)	100 (91-100)
Youden's J	-0.11	-0.59	-0.13	-0.2	-0.7	-0.22

Given that ADEM is a common manifestation of ADS in childhood, we evaluated the performance of the 2010 criteria in this group because of the suggestion to exclude such patients stated in the 2010 criteria manuscript.[90] Of the 50 ADEM patients, none met criteria for CDMS, although two experienced a single subsequent non-ADEM attack. Five ADEM patients met 2010 criteria on the baseline scan, only one of whom developed new MRI lesions over time, while another five ADEM patients met 2010 criteria when serial images were considered. The new lesions detected were small and juxtacortical in location in all five children. **Appendix 9** illustrates the new lesions noted in two children with ADEM, and contrasts this with the MRI appearance at the time of new lesion formation in two children with CDMS. Only four children with ADEM met the 2005 McDonald criteria.

Since the MRI features of very young children with MS may be more likely to differ from adult-onset MS in appearance,[86] we explored the performance of the 2010 and 2005 criteria in young children (arbitrarily defined as age <11 years) versus adolescent (≥11 years) children (**Table 8-5**)**.** The 2010 criteria demonstrated high sensitivity (100%), specificity (86%), PPV (76%) and NPV (100%) for children older than 11 years with non-ADEM presentations, as did the 2005 McDonald criteria in these children. In younger children with a non-ADEM presentation, specificity and NPV of both criteria remained robust, sensitivity was high for the 2010 criteria (100%) and lower for the 2005 criteria (67%), but PPV was reduced to 55% (2010 criteria) and 67% (2005 criteria).

Table 8-5: Evaluation of diagnostic criteria as a function of age at first attack

	Age-Dependent Analysis							
	<11 Years		**≥11 Years**		**<11 Years Excluding ADEM**		**≥11 Years Excluding ADEM**	
	McDonald 2005 n=85	**McDonald 2010 n=108**	**McDonald 2005 n=96**	**McDonald 2010 n=101**	**McDonald 2005 n=54**	**McDonald 2010 n=67**	**McDonald 2005 n=88**	**McDonald 2010 n=92**
True Positives	4	6	25	28	4	6	25	28
True Negatives	74	89	62	62	46	56	55	55
False Positives	5	13	8	11	2	5	7	9
False Negatives	2	0	1	0	2	0	1	0
Sensitivity % (95% CI)	67 (22-96)	100 (54-100)	96 (80-100)	100 (88-100)	67 (22-96)	100 (54-100)	96 (80-100)	100 (88-100)
Specificity % (95% CI)	94 (86-98)	87 (79-93)	89 (79-95)	85 (75-92)	96 (86-99)	92 (82-97)	89 (78-95)	86 (75-93)
PPV % (95% CI)	44 (14-79)	32 (13-57)	76 (58-89)	72 (55-85)	67 (22-96)	55 (23-83)	78 (60-91)	76 (59-88)
NPV % (95% CI)	97 (91-100	100 (96-100)	98 (91-100)	100 (94-100)	96 (86-99)	100 (94-100)	98 (90-100)	100 (94-100)
Youden's J	0.61	0.87	0.85	0.85	0.63	0.92	0.85	0.86

Time to diagnosis The time from first attack to meeting criteria for MS diagnosis was shortest when the 2010 criteria were applied compared to the 2005 criteria, and was longest when using time to CDMS (**Figure 8-3**).)

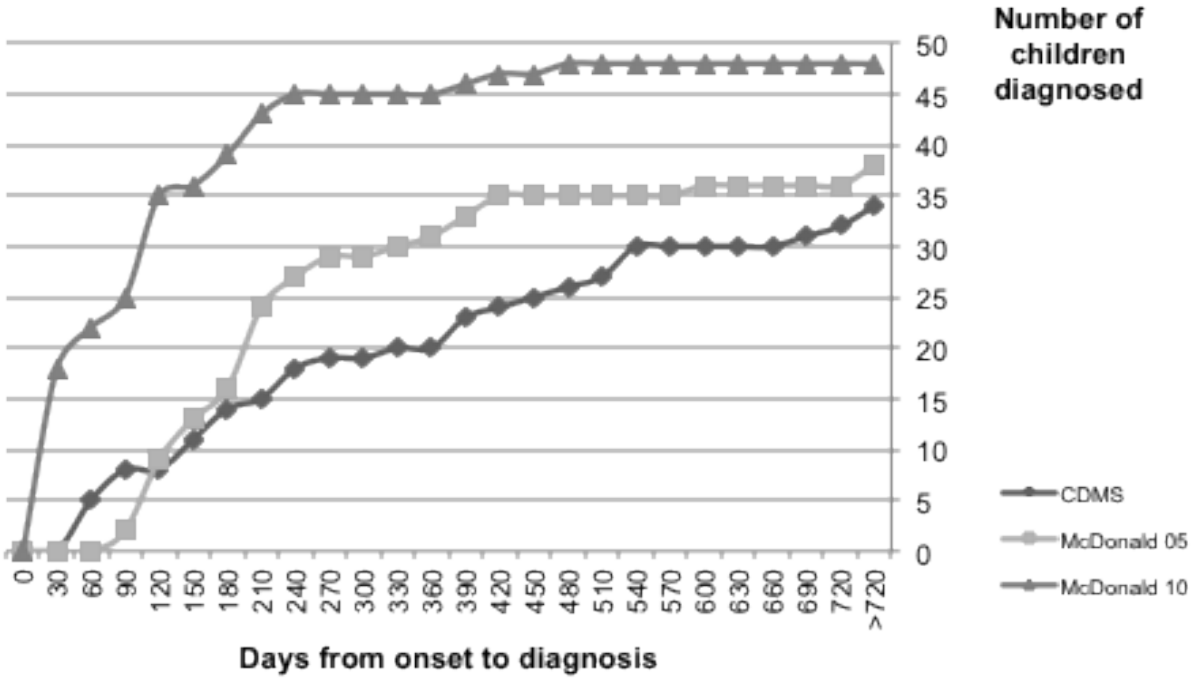

Figure 8-3: Time to MS diagnosis. Time course from presentation with ADS to second attack (clinically defined MS, CDMS) or to the first time point when both the DIS and DIT components of the 2010 and 2005 McDonald criteria were met. As none of the children with ADEM at onset met criteria for CDMS, these patients were not included in the analysis. Time from first attack to meeting criteria for MS diagnosis was a median of 76 days (IQR 116) when 2010 McDonald criteria were applied (n=48 children), 185 days (IQR 139) for the 2005 McDonald criteria (n=38 children), and 231 days (IQR 325) when using CDMS (n=34 children).

2010 McDonald criteria and non-CDMS diagnoses To explore the specificity of the 2010 criteria, we performed a *post-hoc* evaluation of the performance of the 2010 criteria in the 11 children with non-demyelinating disease and one child with a NMO spectrum disorder identified from our core group of 209 participants, and 8 additional children diagnosed with other CNS conditions (seven with non-demyelinating diseases and one with NMO) who were enrolled in our national study but were not included with the 209 children analyzed formally either because they were enrolled after 2009 or did not have sufficient number of MRI scans. The presenting features, initial ADS diagnosis, and final diagnosis of the 18 children eventually diagnosed with non-demyelinating diseases and the two NMO patients are described in **Appendix 10**. Both children with NMO spectrum disorders met the 2010 criteria, as did two of four patients with isolated small vessel CNS vasculitis, and one patient with viral encephalitis.

8.5 Discussion

In a prospective cohort of children diagnosed with ADS, we demonstrate that the new 2010 McDonald criteria are sensitive and specific for the diagnosis of pediatric MS, provided that the criteria are not applied in the context of ADEM, and with consideration of age at onset. The PPV of the 2010 criteria for CDMS in children with non-ADEM presentations above the age of 11 years was 76%, similar to the 79% PPV found by Swanton et al in their evaluation of the MRI DIS and serial MRI DIT aspect of the 2010 criteria (DIT on baseline scan was not considered) in adults with incident demyelination.[97]

In younger children with ADS (excluding those with ADEM), the PPV was only 55%. Some studies have suggested a longer duration between ADS and subsequent MS-confirming attack in younger children,[302] and thus our ongoing observation of the this subgroup is required.

The 2010 criteria perform poorly in children manifesting with ADEM, as was anticipated by the 2010 McDonald panel.[90] The international consensus criteria for ADEM require polyfocal neurological deficits, and most children have multifocal lesions on MRI. Thus, ADEM patients typically fulfill both clinical and MRI 2010 DIS at onset. The simultaneous presence of both enhancing and non-enhancing lesions occurred in 10% of our ADEM patients, who thus were considered 2010-positive at baseline. As shown in **Appendix 9**, five (14%) of our ADEM patients met 2010 DIT criteria based on development of small, often juxtacortical isolated new lesions on serial imaging. While such lesions were of sufficient size to meet our *a priori* lesion criteria, such lesions developed in the context of an MRI that typically demonstrated resolution of initial ADEM lesions, or faint regions of residual signal intensity, but not in the context of multifocal lesions typical of MS.

Whereas none of the children with ADEM in the cohort analyzed experienced two non-ADEM attacks required to confirm MS (one child with ADEM-like presentation in our full cohort has met criteria for MS, but was enrolled after 2009 and thus did not meet the present inclusion criteria), an ADEM-like first MS attack has been reported in up to 18% of some pediatric MS cohorts.[57] Potential reasons for the rarity of MS diagnosis following ADEM in our cohort, as well as MRI features (one or more periventricular T2 lesions and T1 hypointense lesions) that may aid in predicting MS outcome in children with non-ADEM and ADEM at onset are presented in our recent work.[50]

Spinal images did not impact McDonald 2010 criteria performance. However, as spinal MRI was acquired only when clinically indicated, we cannot comment on whether MRI detection of silent spinal cord lesions at baseline would have increased the number of children meeting 2010 criteria at onset.

An unexpected result from the present study was the robust performance of the 2005 McDonald criteria. Prior application of the 2005 criteria in pediatric MS populations suggested that these criteria performed relatively poorly. It is noteworthy, however, that these studies only evaluated the MRI DIS aspect of the 2005 criteria (summarized in **Appendix 11**).[103,104] When evaluating the lesion distribution on baseline scans alone, only 21 of the 34 (62%) CDMS patients in our cohort met 2005 criteria for MRI DIS, similar to results obtained from our prior work in which 53% of children with established relapsing remitting MS met the 2005 DIS criteria on their baseline MRI scan.[104] Similarly, only 61% of baseline scans obtained from 28 pediatric MS patients enrolled in a prospective cohort from the Dutch pediatric MS study group met 2005 DIS criteria.[103] To our knowledge, evaluation of the full 2005 DIS criteria (clinical DIS, Barkhof DIS and DIS as defined by >2 lesions plus CSF OCBs) and 2005 DIT criteria in a prospective incident pediatric population has not been previously performed previously. A likely explanation for the superior performance of the 2005 criteria in our pediatric population is that we applied the full DIS criteria together with DIT criteria in our cohort.

Even with consistently applied inclusion criteria and structured investigations to exclude children with acute non-ADS illness, 20 of the 337 children enrolled in our national study were subsequently diagnosed with other CNS disorders. Five of these children met 2010 criteria, four of whom had either NMO spectrum disorders or CNS vasculitis; illnesses that have a clear

inflammatory insult to white matter. The availability of serum NMO IgG and the performance of brain biopsy led to the correct diagnosis. As emphasized by both the 2005 and 2010 McDonald criteria panels, clinicians must remain alert to clinical or paraclinical features that prompt consideration of non-MS diagnoses.[89,90]

The present study must be viewed in light of several limitations. Annual scans beyond the 12-month time point were acquired only at the lead site or at the time of a second attack (all sites). However, as 14 of the 16 children at the lead site and 32 of the 33 children followed at other centers meeting the 2010 criteria did so within 12 months of first attack, further serial imaging would not have changed our results. Inherent in all prospective studies is the possibility that children currently classified as having monophasic demyelination will develop new attacks over time. To mitigate this concern, we specifically restricted our study population to those children enrolled prior to 2009 (mean [SD] period of observation of 4.3 [1.3] years), based on published data regarding the frequency of early relapse in pediatric MS patients.[32,60] Of all 34 children with relapses, 32 experienced their second attack less than two years from onset (median [IQR]= 222 [287] days) and only 2 children experienced their second attack more than two years from onset (740 and 810 days, respectively). Thus, we feel our monophasic delineation to be as accurate as can be reasonably determined. It is important to note that our data reflect application of the diagnostic criteria in a well-characterized cohort of ADS patients evaluated by specifically trained pediatric neurologists using standardized clinical definitions for ADS. Whether the 2010 criteria will fare as well when applied in clinical practice is unknown.

We demonstrate that the 2010 McDonald criteria accurately identify children destined for MS relapses within the first few years after incident demyelination. Caution is advised when

applying the 2010 criteria in younger children with ADS, and the criteria are not suited to predict MS outcome in children with ADEM. The diagnosis of MS in children whose initial ADS event is consistent with ADEM should be based on serial evidence of new non-ADEM attacks, as was originally proposed by an international panel.[56]

8.6 Acknowledgements

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PART IV:

MRI OF THE SPINAL CORD IN PEDIATRIC MS

Chapter 9:

Magnetic Resonance Imaging Features of the Spinal Cord in Pediatric Multiple Sclerosis: a Preliminary Study

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*Contributed equally to the present work

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9.1 Summary

Background Spinal cord lesions in adults with MS are thought to contribute to disability. The MRI appearance and clinical correlates of spinal cord lesions in children with MS have not been reported.

Methods T1-weighted pre- and post-gadolinium and T2-weighted TSE/FSE spine MR images of 36 children (age: 14.3 ± 3.3) with relapsing-remitting MS (annualized relapse rate: 0.7; disease duration: 7.5 ± 3.3 years) were analyzed for total lesion count, lesion location and length, intramedullary extent, and gadolinium enhancement. Clinical, demographic, laboratory and MRI data were correlated.

Findings Lesions preferentially involved the cervical region, were predominantly focal and involved only a portion of the transverse cord diameter. However, 10 of 36 patients demonstrated longitudinally-extensive lesions. Children with the highest clinical relapse rate also tended to have more spinal cord lesions, and were more likely to accrue new lesions on serial spinal scans.

Interpretation These preliminary data suggest that MS lesions of the spinal cord in children are radiographically similar to that of adult-onset MS – supporting a common biology of pediatric- and adult-onset disease. However, children with relapsing-remitting MS can also develop longitudinally-extensive lesions, suggesting that such lesions may be less specific for diseases such as neuromyelitis optica in pediatric patients. All patients recovered well from spinal cord attacks, and the presence of spinal cord lesions in the first few years of disease did not correlate

with physical disability. Measures of spinal cord atrophy and longer periods of observation are required to determine the impact of spinal cord involvement in pediatric-onset MS.

Funding Multiple Sclerosis Society of Canada

9.2 Introduction

Multiple Sclerosis is a chronic neurological disease characterized by recurrent episodes of neurological deficit associated with inflammatory lesions of the central nervous system. MS lesions involving the spinal cord are considered to contribute substantively to physical disability, both during acute relapse and cumulatively over time.[303] The prevalence of spinal cord lesions in adults with established multiple sclerosis (MS) ranges from 70-90%.[304-307]

Magnetic resonance imaging not only aids in the confirmation of clinically-suspected spinal lesions, but also in the identification of the clinically-silent lesions. Clinically-silent lesions in the spine are present in 30-40% of adults at the time of a first demyelinating attack, (reviewed in[308]) and can provide evidence for disease dissemination in space.[89,307] MRI and pathological studies of MS have both demonstrated a propensity for lesions to be located in the cervical spine with preferential localization to the dorsal and lateral columns as well as central spinal grey matter.[306,307,309,310] Spinal lesions in MS are typically well circumscribed and extend less than two vertebral body segments in maximal dorsal-caudal length.[304,307,310] Longitudinally-extensive lesions, defined as extending more than 3 vertebral body segments in length form a core component of the diagnostic criteria for neuromyelitis optica.[294] Given the important differences between NMO and MS in their management and outcome,[311] defining the MRI features of spinal lesions in pediatric MS patients has clinical relevance.

The neuroimaging features of spinal cord involvement in pediatric-onset MS have not been previously described. Studies have demonstrated important distinct aspects of MS lesions in the brain of children with MS as compared to those of adults,[91,92] and thus it is possible that the

lesion appearance of MS in the spine may also differ between pediatric and adult-onset patients. Here we describe the spinal MRI features of children and adolescents with relapsing-remitting MS, and correlate these findings with clinical deficits and number of clinical relapses.

9.3 Patients and Methods

Participants and definitions This retrospective study included patients cared for in the Pediatric Multiple Sclerosis Clinic at The Hospital for Sick Children (Toronto, Canada) between 1999 and 2008. Inclusion criteria were: (i) first demyelinating attack experienced prior to age 18 years; (ii) MS diagnosis made on the basis of clinical relapse or MRI evidence of disease dissemination in time,[89] and (iii) T2-weighted FSE spinal MR imaging at 1.5 Tesla performed during the course of care. Patients with relapsing or multi-phasic ADEM,[56] clinically-diagnosed NMO[294] or children with serum antibodies against aquaporin-4, irrespective of clinical features, were excluded.

Spinal MRI exams were acquired on 1.5 T MRI and assessed for image quality. All patients had sagittal T2-weighted FSE scans of the spinal cord. Axial T2-weighted FSE scans and axial and sagittal T1-weighted post-gadolinium scans were also reviewed when available. The scans of four patients were excluded due to significant motion artifact. Thirty-six patients had one spinal MRI available for analysis. Eighteen patients had two or more MRI scans for a total of 41 follow-up spinal MR scans.

MRI analyses All scans were scored by one pediatric neuroradiologist (HMB), blinded to clinical outcome, using a pre-defined standardized scoring tool (**Appendix 12**). A subset of scans (n=7)

was scored independently by a second pediatric neuroradiologist (MMS) for assessment of inter-rater reliability. Total spinal cord lesion number on each scan was counted. The following characteristics were recorded for each lesion: location (involving one or more of cervical, thoracic or lumbar regions), rostral-caudal length (as measured relative to adjacent vertebral body segments), and presence of lesion enhancement (if contrast images were available). Based on rostral-caudal length, lesions were also categorized as focal (lesions extending ≤2 vertebral body segments) or longitudinally-extensive (lesions spanning ≥3 contiguous vertebral segments in length). The number of lesions in the cervical and thoracic spinal cord was divided by the respective number of vertebral body segments in each of the cervical (n=7) and thoracic (n=12) regions to determine the number of lesions per vertebral body segment, given that the thoracic spinal cord spans more of the total cord length compared to the cervical. Lesion extent in the transverse plane was categorized as involving one or more of the following cross-sectional regions: anterior, posterior, central or all (complete). Clear involvement of the anterior horn cell region was specifically noted. **Figure 9-1** illustrates how the intramedullary regions of the spinal cord were defined in the axial plane. For children with serial spinal imaging, each scan was evaluated for the presence of new lesions, persistence of prior lesions, and for lesion resolution. The brain MRI scan acquired closest in time to the initial spine MRI was assessed for concomitant brain lesion involvement.

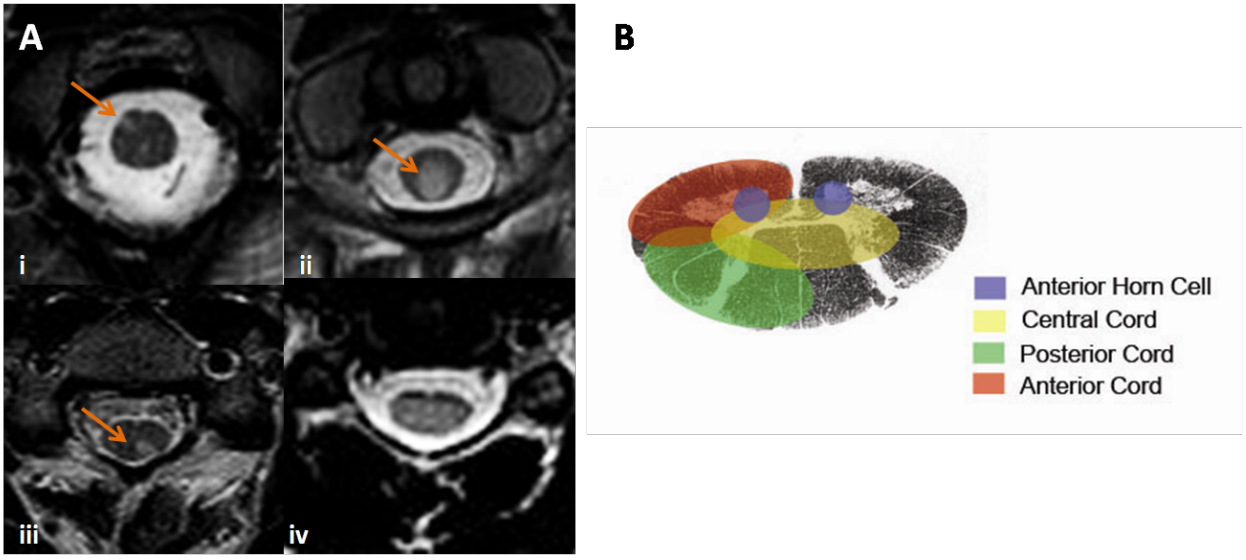

Figure 9-1: Axial plane anatomical atlas for spine cord lesion scoring. (A) Axial T2-weighted FSE MRI of the spinal cord showing intramedullary regions of hyperintensity: (i) anterior, (ii) centro-posterior, (iii) posterior, and (iv) complete. (B) Cross-sectional atlas of the spinal cord defining intramedullary regions in the transverse plane.

Clinical and demographic variables Demographic information (country of birth, ancestry, sex, age at first attack, age at second attack, age at spinal MR imaging), reason for spinal imaging (confirmation of concurrent clinical symptoms; performed to aid in MS diagnosis or as a component of overall MS disease activity assessment, or serial scan for documentation of resolution of prior spinal cord lesions), and spinal cord symptoms at the time of imaging were recorded for all patients. In addition, total number of clinical relapses, annualized relapse rate, Expanded Disability Status Scale score (EDSS) [70] at most recent assessment, total disease duration (as of the date of data analysis, December 17, 2009) and the presence of cerebrospinal fluid oligoclonal bands was also recorded.

Statistical analyses The study is descriptive. Continuous variables were analyzed using means and standard deviations, and medians and inter-quartile ranges when appropriate; frequencies were calculated for nominal and categorical variables. Total lesion count at initial MR scan, lesion localization, and categorization of lesions as focal or longitudinally extensive were scored independently by both neuroradiologists (HMB and MMS). Inter-rater agreement was assessed by computing Cohen κ values. All statistical analyses were conducted using SAS V9.1.3.

The Research Ethics Board at The Hospital for Sick Children approved this study.

9.4 Results

Table 9-1 shows the demographic and clinical characteristics of the 36 patients. The mean time from first clinical attack to initial spinal MR scan was 1.3 years (SD: 1.9). For 17 of the patients, spinal cord symptoms were present and initial spine MRI was performed at the time of their first MS attack.

Table 9-1: Demographic and clinical characteristics

	Summary Statistic
Mean age at first attack, y ± SD (range)	13.1 ± 3.5 (5.4-17.3)
<10 years, n (%)	7 (19)
≥10 years, n (%)	29 (81)
Mean age at second attack (MS diagnosis), y ± SD (range)	14.0 ± 3.5 (6.6-18.2)
<10 years, n (%)	6 (17)
≥10 years, n (%)	30 (83)
Mean age at spinal MRI, y ± SD (range)	14.3 ± 3.3 (5.4-18.6)
Sex, Female:Male	3:1

Child's country of birth, n (%)	
Canada	28 (78)
Other	8 (22)
Child's ethnicity, n (%)	
White, not Hispanic	21 (58)
Asian	4 (11)
Other	11 (31)
MS phenotype, n (%)	
Relapsing-remitting MS	36 (100)
2 or more clinical attacks, CDMS	33 (92)
1 clinical attack, with MRI DIS and DIT	3 (8)
MS disease course (from first attack to date of data analysis)	
Total number of relapses, mean ± SD; median (range)	4.9 ± 3.5; 4 (1-16)
Disease duration (years), mean ± SD; median (range)	7.5 ± 3.3; 7.2 (2.0-16.8)
Annualized relapse rate (relapses/year), mean ± SD; median (range)	0.7 ± 0.4; 0.6 (0.2-1.8)
Spinal cord symptoms at the time of first spinal MRI, n (%)	
None	11 (31)
L'Hermitte's symptom	9 (25)
Motor	12 (33)
Bladder	5 (14)
Sensory level	16 (44)
Brainstem	10 (28)
CSF OCB-Positive*, n (%)	18 (72)
EDSS (most recent clinical visit), median (IQR), range	1 (0.5), 0-3

CSF, cerebrospinal fluid; OCB, oligoclonal bands; EDSS, expanded disability status scale. *25 children had CSF OCB results available.

For all 36 patients, MRI scans of the brain obtained at acute first presentation were scored to determine Barkhof/Tintore criteria for dissemination of lesions in space.[101] We specifically determined whether the presence of spinal cord lesions would contribute to the criteria, either based on total lesion count >9, single enhancing spinal lesion, or in the category of infratentorial. Twenty-two patients (61%) had both brain and spine MR imaging within a 30-day epoch, of which 15 (68%) met McDonald criteria based on brain MRI alone. Of the 7 patients

that did not meet McDonald criteria when considering the brain MRI in isolation, two met criteria after inclusion of spinal cord lesions. Fourteen patients did not have a spine MRI performed within 30 days of the brain MR scan; of these, 8 (57%) met criteria for dissemination in space.

At the time of spinal imaging, 12 (33%) children had partial motor or bilateral lower limb weakness, 9 (25%) reported L'Hermitte's symptom, 16 (44%) had a spinal sensory level, 5 (14%) were experiencing impaired bladder function, and 10 (28%) had brainstem signs. Concurrent with spinal cord symptoms, 4 (11%) children had clinical findings of optic neuritis. None of these four children had LETM, and all experienced subsequent attacks typical of RRMS. Two children (5%) had encephalopathy as well as a polyfocal clinical feature involving the brain and spine (meeting criteria for an initial diagnosis of ADEM.[56] All 36 children have experienced two or more non-ADEM clinical attacks, all had brain MRI imaging at first attack and at multiple time points, and all have demonstrated new T2 lesions on serial brain imaging. Relapse data is presented in **Table 9-1**.

At the time of first spine MRI, 29 patients (81%) had at least one lesion visualized in the cord (**Figure 9-2**): 21 (58%) had symptoms localizable to the spinal cord and brainstem, 2 had brainstem symptoms only, and 6 had clinically-silent lesion(s)—in 5 children spinal imaging was performed to evaluate lesion dissemination as part of their diagnostic evaluation, and 1 child had an initial spine MRI to evaluate of possible prior episode of mild transverse myelitis. Of the remaining seven patients whose initial spine MRI was negative, four had neurological deficits referable to the spinal cord (L'Hermitte's symptom, spinal sensory level, bilateral lower limb weakness, bladder dysfunction) but no corresponding visible lesions, two patients were imaged

to search for clinically-silent lesions to assist in MS diagnosis, and one patient had a prior history suggestive of spinal cord involvement but for whom no prior spine imaging was available for analysis.

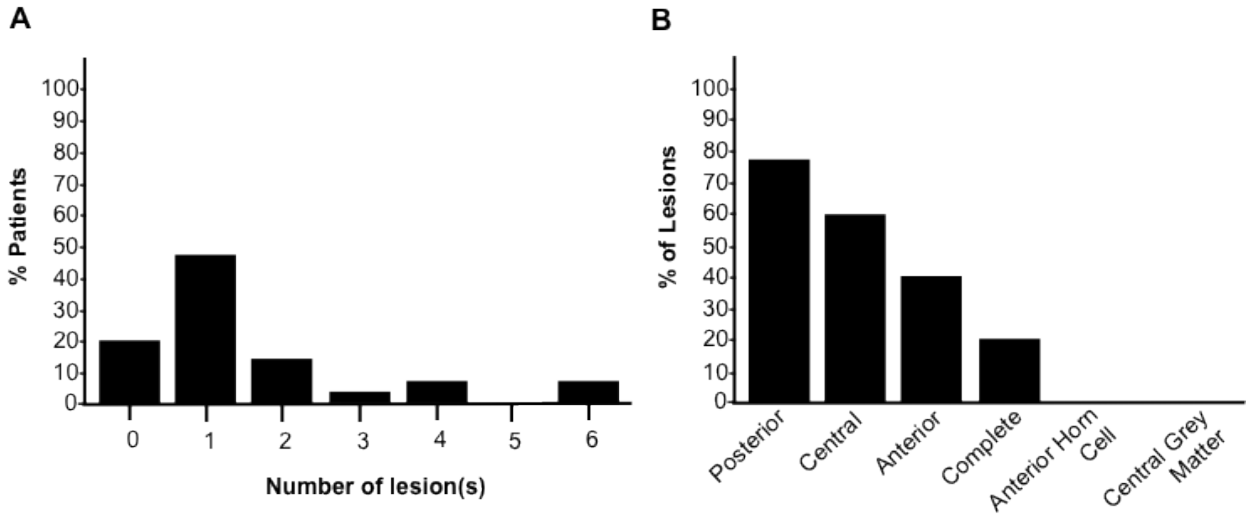

Figure 9-2: Spinal cord lesion count and intramedullary extent. (A) Proportion of children categorized by the total number of spinal cord lesions per patient; (B) Proportion of lesions (n=44) involving each intramedullary region (each lesion may involve more than one intrameduallary region)

Features of spinal cord lesions are described in **Table 9-2** and illustrated in **Figure 9-3.** A total of sixty lesions were detected within the spinal cord of the 29 children, with a similar number of lesions located in the cervical (n=30, 50%) and thoracic (n=27, 45%) regions; lumbar lesions were not observed (it should be noted that the spinal cord usually terminates in the upper lumbar region). Three lesions (5%) were localized to both the cervical and thoracic regions. There were 4.7 lesions per vertebral body segment in the cervical cord and 2.5 in the thoracic

region. Ninety percent of all lesions were focal and spanned fewer than 3 vertebral body segments in rostral-caudal length. Longitudinally-extensive lesions were rare, accounting for only 6 of the lesions (10% in n=6 patients).

Table 9-2: Spinal cord lesion features at initial MRI scan

	Summary Statistic
Number of lesions per patient, median (IQR, range)	1 (1, 1-6)
Number of children with, n (%)	
Focal lesions	23 (64)
Longitudinally-extensive lesions	3 (8)
Both	3 (8)
Number of children with lesions in each region, n (%)	
Cervical	11 (31)
Thoracic	9 (25)
Cervical and thoracic	9 (25)
Lumbar	0
Number of children with gadolinium-enhancing lesions,* n (%)	5 (31)
Number of lesions detected in 36 children, n (%)	60 (100)
Number of, n (%)	
Focal lesions	54 (90%)
Longitudinally-extensive lesions	6 (10%)
Number of lesions in each region, n (%)	
Cervical	30 (50)
Thoracic	27 (45)
Cervical and thoracic	3 (5)
Lumbar	0
Number of lesions in each intramedullary axial location,¶ n (%)	
Posterior	34 (77)
Central	26 (59)
Anterior	18 (41)
Complete	8 (18)
Anterior horn cells	0
Central grey matter	0

*Intravenous contrast injection was refused, the patient had a prior contrast agent allergy, or contrast was not given by the supervising radiologist in 20 children, leaving 16 children in which gadolinium-enhanced images could be analyzed. Five children (14%) were receiving IV-

methylprednisolone at the time of their initial MRI. Only two of these children had a contrast-enhanced scan and both appeared normal with no MRI evidence of spinal cord lesions. The corticosteroid status of 6 children (17%) at the time of their MRI was unknown. ¶44 (73%) lesions could be assessed for intramedullary axial extent due to absence of axial spinal cord imaging for 16 lesions

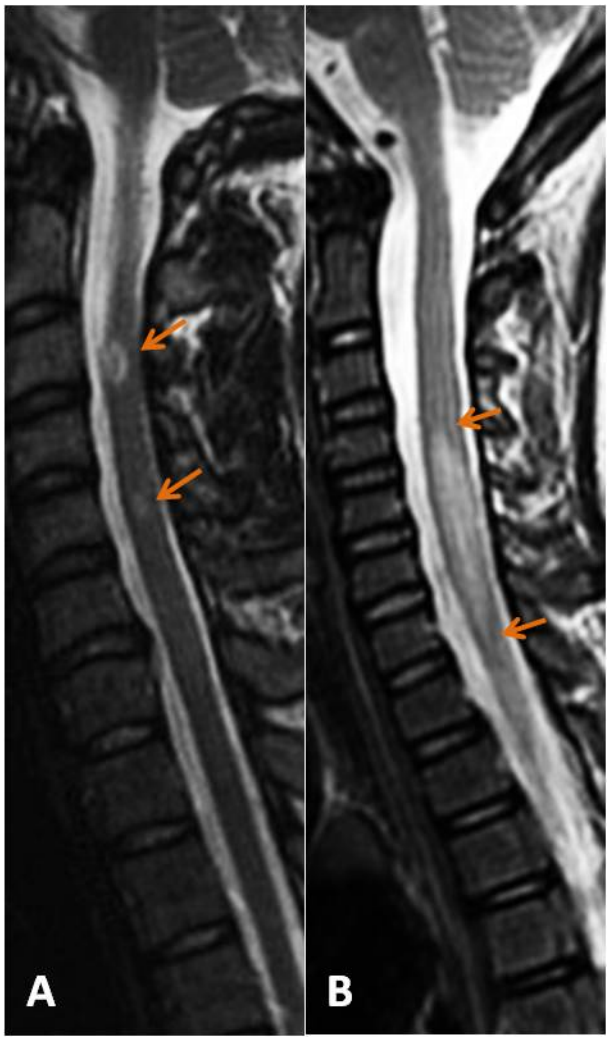

Figure 9-3: Sagittal T2-weighted FSE images of the spinal cord. Two children with MS demonstrating spinal cord involvement: (A) one child with two focal cervical T2-hyperintense lesions (arrows) and (B) one child with a cervical longitudinally-extensive lesion spanning four vertebral bodies (bounded by arrows) with associated spinal cord swelling.

Axial images permitted evaluation of the intramedullary characteristics for 44 (73%) of the lesions. Lesions were more likely to involve the posterior region of the spinal cord (n=34, 77%) and were least likely to involve the complete horizontal intramedullary extent (n=8, 18%) (**Figure 9-1 and 9-2B**). The complete cord was involved in two of the children with longitudinally-extensive lesions.

Eighteen of the 36 children (50%) had two or more spine MRI scans: 7 children had a total of 2 spinal MRI scans; 6 had 3 scans, 2 had 4 scans, 1 had 5 scans, 1 had six scans, and 1 child had 8 spine MRI scans, totaling 41 serial scans in the 18 children. Twelve children (67%) had one or more new lesions evidenced on at least one serial scan (mean 1.1 (range: 0.2-4) new lesions per scan per patient). One child had normal imaging at the time of first scan but developed two lesions over the course of seven serial scans acquired over the span of approximately three years. Five children (28%) had only residual lesions at any serial time point. No child with serial imaging demonstrated complete resolution of all lesions. **Table 9-3** delineates the lesion features of serial MR scans.

Table 9-3: Lesion features on serial spinal cord MRI scans

	Summary Statistic
Number of children with ≥1 serial scan, n (%)	18 (50)
Number serial scans analyzed, n (%)	41 (100)
Number of scans with only residual lesions, n (%)	21 (51)
Number of scans with complete lesion resolution, n (%)	0
Number of scans with new lesions	17 (41)
1 new lesion	10 (24)

2 new lesions	5 (12)
3 new lesions	1 (2)
6 new lesions	1 (2)
Number of normal scans, n (%)	3 (7)
Features of new lesions on serial scans	
Number of new lesions, n (%)	29 (100)
Number of new lesions in each region, n (%)	
Cervical	11 (38)
Thoracic	15 (32)
Cervical and thoracic	3 (10)
Lumbar	0
Number of scans in which new lesions are, n (%)	
All focal	12 (71)
All longitudinally-extensive	4 (24)
Both focal and longitudinally-extensive	1 (6)
Number of new lesions in each axial region,* n (%)	
Posterior	5 (29)
Central	8 (47)
Anterior	1 (6)
Complete	3 (18)
Anterior horn cells	0
Central grey matter	0
Number of scans with new gadolinium-enhancing lesions¶	3 (43)

*10 serial scans (n=17 lesions) had axial imaging available for analysis; ¶7 of the 17 serial scans had a post-contrast T1-weighted sequence available for analysis.

As stated above, 12 children demonstrated new spinal cord lesions on follow-up. Seventeen scans from these 12 children revealed 29 new lesions (mean (SD), 1.7 (1.3) new lesions per patient; median 1). The 29 new lesions were distributed in the cervical (n=11, 38%) and thoracic (n=15, 52%) spinal cord regions. Seventy-one percent of the new lesions were focal. Four of the 12 children with new lesions on serial imaging had longitudinally extensive lesions, none of whom had longitudinally extensive lesions on their first spinal MRI scan.

Ten of the 17 serial scans (59%) showing new lesions (n=17 lesions) had axial T2-weighted FSE/TSE sequences for analysis of intramedullary involvement. Similar to initial MR scans, complete intramedullary involvement was rarely seen (n=3 lesions, 18%). Central cord (n=8 lesions, 47%) and posterior regions of the cord (n=5 lesions, 29%) were more frequently involved.

Figure 9-4 illustrates the relationship between clinical relapses and spinal imaging features. Patients with the highest rate of clinical relapses were more likely to have spinal lesions on baseline and serial spinal images, although the number of patients limited statistical power.

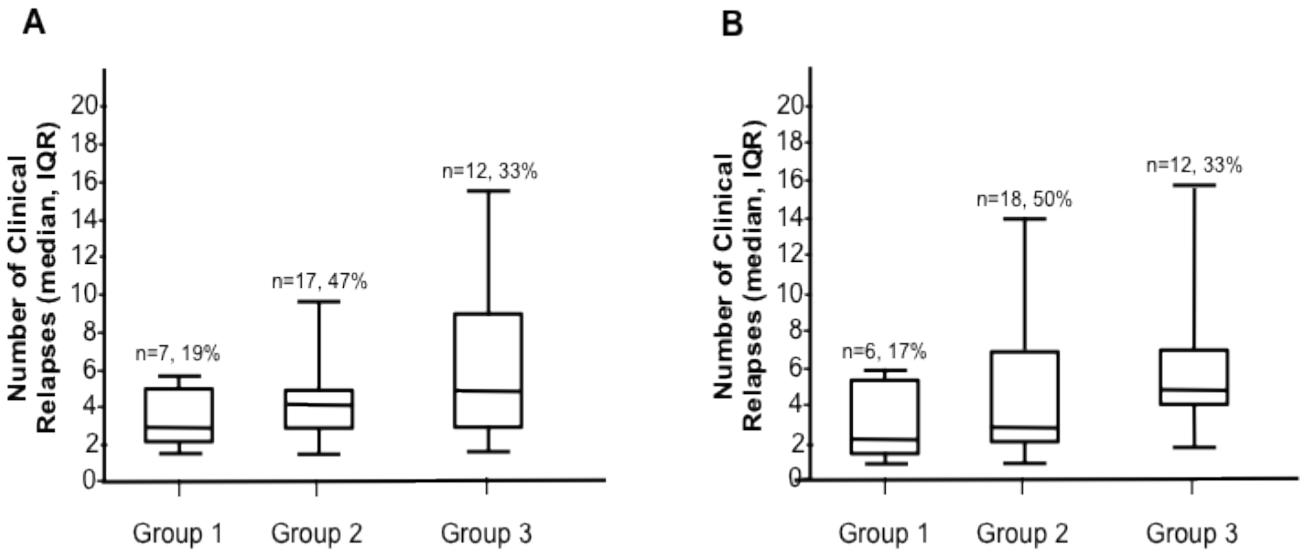

Figure 9-4: Association between clinical relapses and presence of spinal cord lesions. (A) Association between clinical relapses and spinal lesion features at initial MRI scan. *Group 1* Children with no lesions on their initial spine MRI; *Group 2* Children with one lesion on initial spine MRI; *Group 3* Children with >1 lesion on initial spine MRI. (B) Association between clinical relapses and spinal features on serial scans. *Group 1* Children with no spinal cord lesions at any

time point; *Group 2* Children with spinal cord lesions at only one time point (initial or follow-up scan); *Group 3* Children with spinal cord lesions at more than one time point (initial or follow-up scans).

Of the 25 children with OCB testing, 18 (72%) were positive. Spinal fluid analyses differed by referring laboratory: immunofixation was used for 10 (40%), isoelectric focusing for 7 (28%), and electrophoresis for one patient. The method for OCB detection was not specified for nine patients. Of the 18 OCB-positive patients, all had brain lesions and 14 (78%) also had spinal cord lesions. Of the seven MS patients with negative CSF evaluation for OCBs, all had brain lesions, five demonstrated one or more focal spinal lesions on initial MRI, and four developed new focal lesions on serial spinal imaging. Of the remaining two patients with negative CSF OCBs, one had LETM of the entire cervical and thoracic segments on initial imaging, but has subsequently experienced seven relapses typical of MS as well as focal spinal cord lesions. The other child had no visible lesions on spine MRI, but experienced motor and sensory deficits referable to the spinal cord, and had multiple focal T2-weighted FLAIR lesions supratentorially.

Spinal MR imaging was also evaluated as a function of age. At the time of spine MRI, five children were less than 10 years of age, and two had LETM. The other four patients with LETM evident on their initial spine MRI were 15 (n=1), 18 (n=2) and 19 (n=1) years of age at the time of spinal imaging (note: all had MS onset prior to age 18 years). The four patients with LETM were 10 (n=1), 16 (n=2) and 19 (n=1) years of age at the time of the serial image.

Ten patients had LETM on spinal MRI, prompting the consideration of NMO. The one patient for whom serum anti-aquaporin 4 antibodies was evaluated was negative. The other nine children

were not tested, either due to the LETM episode occurring prior to the availability of antibody evaluation, or due to clinical features that do not conform to the diagnostic criteria for NMO.[294] Specifically, these 9 children have had a median of 7 clinical relapses (range: 2 – 16), with the majority of clinical attacks not involving the spinal cord or optic nerves; none have experienced permanent visual loss or paraplegia, and all have brain MRI features consistent with MS. Of these 9 children, 1 also had CSF positive for OCB.

A second neuroradiologist independently scored a subset (n=7) of randomly selected spine MRI scans. There was perfect agreement between both raters for lesion location and lesion length (i.e., longitudinally-extensive vs. focal). Lesion count was discrepant in 3 cases, with the lesion number differing by only one lesion per case. The final lesion count was determined by re-scoring the discrepant cases by consensus of both neuroradiologists.

9.5 Discussion

Spinal cord lesions in pediatric MS patients tend to be focal, are partial in cross-sectional intramedullary extent, and are preferentially localized to the cervical spinal cord. Longitudinally-extensive lesions, however, do occur in children with relapsing-remitting MS both at the time of initial attack and at relapse.

This retrospective analysis shows that the majority of spinal cord lesions in pediatric-onset MS patients closely resemble that of adults with RRMS, at least early in the MS disease course. Specifically, the cervical cord has a predilection for lesions in both children and adults,[304,307] and

this observation held true after controlling for the fact that the cervical cord accounts for less of the total cord length compared to the thoracic segments. The median spinal cord lesion count in our pediatric cohort was one, which appears lower than that reported for adults with RRMS imaged within the first six months of MS diagnosis (median of three spinal cord lesions).[307] It could be hypothesized that the duration of subclinical disease in pediatric-onset patients is shorter (based on the inherent limitations imposed by young age) compared to adult-onset MS and thus time-related accrual of lesions is lower. Against this hypothesis, however, is the recent data demonstrating a similar total brain T2 lesion count[108] and total brain T2 lesion volume[109] in pediatric and adult patients.

The infrequent finding of longitudinally extensive and complete intramedullary involvement in pediatric MS patients, and the paucity of complete TM in adult patients with relapsing-remitting MS,[304,306,309] suggests that extensive cord lesions are characteristic of idiopathic acute transverse myelitis (ATM) or NMO, rather than MS. However, longitudinally extensive lesions have been noted in some adults with secondary progressive MS (SPMS).[304] Of our 10 pediatric MS patients with at least one longitudinally-extensive lesion, none have entered the secondary progressive stage of MS, and mean disease duration is 8.7 years (median: 7.4; range: 4.4-16.8). Thus, it is possible that the substrate for longitudinally-extensive lesions differs between pediatric MS patients for whom such lesions reflect extensive acute inflammation, and adult MS patients for whom such lesions reflect coalescence of multiple focal lesions over time.

The presence of LETM prompts consideration of NMO. None of the children with LETM in the present study meet diagnostic criteria for NMO.[294] While the spectrum of NMO continues to

expand,[312] it is important to note that the LETM lesions in the present cohort occurred in the context of clinical and brain MRI features typical of RRMS.

LETM has been reported more commonly in Asian MS patients.[313] Of the 10 children with at least one LETM lesion, 5 were White with northern European heritage, one was Black, one was Hispanic, one was from Pakistan, one was south Asian and one was of mixed ethnicity. Larger, multinational series are required to determine if Asian ancestry influences the predilection for LETM lesion patterns in pediatric MS patients.

The goal of our work was to describe the MRI features of MS lesions in a pediatric MS population. We cannot comment on the absolute frequency of spinal cord involvement in children with MS, as all children in our study were imaged either due to inter-current spinal symptoms, or to aid in their MS diagnosis. The length of scan time required to image both the brain and spine and the limited ability of young children to lie still without anesthesia leads to practical limitations in obtaining spinal imaging routinely in all pediatric MS patients.

All 36 children remain ambulatory and none report persistent bladder dysfunction – a markedly more favorable outcome than is seen in children with idiopathic TM. In a series of 47 patients with pediatric-onset idiopathic TM, 21% were left with residual moderate to severe limitations in ambulation and 68% reported residual bladder impairment.[55] It is likely that the pathobiological processes operative in isolated TM are more destructive.

No association was found between spinal cord involvement (i.e. lesion count analyzed as a continuous and dichotomous variable) and clinical disability at most recent evaluation (median (IQR) EDSS for the 36 MS patients, 1 (0.5)). The limited accrual of physical disability in the first

10 years of disease in pediatric-onset patients has been previously reported.[3,5,48] Our findings suggest that children recover well from MS attacks involving the spine, as they do from attacks involving the brain early in their disease.[61] Given that none of the children with spinal lesions demonstrated radiographic evidence of complete lesion resolution on serial imaging, however, we might hypothesize either that pediatric MS patients experience functional recovery within lesions, or that the partial lesion extent permits neural transmission in the normal-appearing cord sufficient for neurological improvement. We also explored the associations between the frequency of lesions seen at the time of a first spine MRI and on serial imaging with the frequency of clinical relapses. Although we were underpowered to evaluate these relationships statistically, children with more frequent clinical relapses (involving brain or spinal cord) appeared more likely to have spinal cord lesions at baseline and were also more likely to demonstrate spinal cord lesions at multiple time points (Figure 4). Limitations to these exploratory analyses include: (i) spinal MRI scans were not performed in all patients at first demyelinating attack, and were not performed at consistent intervals over time; (ii) the absence of consistent serial imaging of the spine precludes evaluation of the contribution of accrual of clinically-silent spinal lesions to overall clinical relapse rate; and (iii) duration of observation from first attack varies across our participants—a larger cohort is required to perform time to event analyses.

9.6 Conclusion

We provide a preliminary view into the value of spinal cord imaging as a measure of MS disease activity in pediatric MS patients. Whether children with spinal lesions are at greater risk for future disability relative to pediatric-onset MS patients who do not experience early spinal cord involvement requires longitudinal observation into adulthood.

9.7 Acknowledgements

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PART V:

TOWARDS STANDARDIZED MRI ACQUISITION AND REPORTING

Chapter 10:

Standardized MRI Acquisition and Reporting in Pediatric-Onset MS

Verhey LH, Narayanan S, Banwell B
Neuroimag Clin N Am, *in press*

10.1 Introduction

Magnetic resonance imaging is perhaps one of the most important paraclinical tools for the diagnosis of MS and for monitoring disease progression and treatment response. However, the value of MRI in informing clinical management of MS patients largely depends on the use of a consistent and standard imaging protocol. The goals of MRI in MS include: confirmation of an MS diagnosis prior to a second clinical attack[58] in individuals with an acute demyelinating syndrome according to dissemination in time and space criteria,[89,90] exclusion of alternative diagnoses,[278] and prediction of outcome. In addition, clinicians caring for patients with MS rely on comparison of serial scans to qualitatively evaluate rate of new lesion accrual for diagnosis, to inform on treatment decisions and to monitor disease evolution apparent by formation of confluent lesions and atrophy. A standard MRI acquisition protocol and reporting method will improve the accuracy and reliability of MRI evaluation among radiologists and clinicians within and across centers and will aid the clinician in managing patients with MS.

An expert panel of adult MS radiologists and neurologists within the Consortium of MS Centers published a consensus-based MRI protocol for use in the investigation and monitoring of patients with MS.[263] However, the panel indicated that the applicability of the protocol to pediatric-onset MS requires further study. Although many of the MRI features of MS in children may overlap with that of adults, specific considerations unique to the imaging of children and the distinct MRI features of MS in pre-pubertal children[86] may necessitate a revised MRI protocol for pediatric MS.

The goal of this chapter is to provide clinicians and neuroradiologists caring for children with demyelinating disorders with a suggested standard MRI acquisition and reporting protocol, and to define a standard lexicon for lesion features typical of MS in children. Only conventional MRI sequences used in routine clinical practice and standard to most scanners will be discussed; advanced MRI techniques are being increasingly applied in MS research, but are not yet applicable or feasible in the clinical setting. As considerable overlap exists between the features of pediatric- and adult-onset MS, these recommendations may be of relevance to adult clinicians and radiologists. The recommendations are based on recent MRI studies conducted in pediatric MS, as well as the expertise of pediatric MS neurologists and neuroradiologists at The Hospital for Sick Children (Toronto, Canada) – the lead center for the Canadian Pediatric Demyelinating Disease Network, a prospective cohort study in which standardized clinical and MRI data and biological samples are collected at onset and serially in children with incident demyelination.

10.2 Unique Considerations in MRI of Children

There are several considerations to be mindful of when imaging the child being investigated for MS:

1. In very young children (i.e. up to 3-4 years of age), primary myelination is not yet complete. Use of T2-weighted sequences, and to a greater extent, FLAIR imaging, are not optimal to assess the presence of inflammatory demyelinating lesions. The normal T2-weighted appearance of ongoing primary myelination in very young children may mimic the lesion

appearance in children with a first attack of MS[86] or children with ADEM,[106] where lesions are large, hazy and ill-defined.

2. Dental hardware, such as braces and retainers, can cause significant susceptibility artifact. Sequences especially prone to artifact are diffusion weighted imaging (DWI) and spoiled gradient-recalled echo imaging.
3. Tolerability of MRI scan length is a challenge in pediatrics, and often results in motion artifact-laden images. However, with the assistance of MRI-compatible movie goggles and headphones, children can tolerate lying still for longer durations of time. In the very young children, sedation may be necessary to obtain sufficient imaging. Advances in MRI technology, such as parallel imaging, have significantly decreased scan time. However, situations may arise where a child has difficulty lying still for the total duration of the scan protocol; in these cases, an abbreviated set of images without motion artifact is preferable to a complete protocol laden with motion artifact.

10.3 Standard MRI Protocol for Pediatric Demyelination

Table 10-1 describes the suggested standard protocol for pediatric demyelinating disorders, and indicates a recommended minimum set of scans for situations where a child cannot tolerate the complete protocol. Consistent prescription of slice angulation is important for evaluation of new lesion formation across serial scans; oblique axial images, where the acquisition plane is parallel to the subcallosal line joining the inferior margin of the genu and inferior margin of the splenium, are recommended. Contiguous slices of 3 mm thickness are

recommended to permit accurate lesion detection, given that minimum MS lesion diameter criteria are approximately 3 mm.[50,86] Attention should be given to ensure complete head coverage. To avoid distortions or edge blurring sometimes present in the first or last slice of a slab when the whole brain is not covered, acquisition of 1-2 slices of air outside the skull is recommended. This is especially important when new lesions are present in regions of the brain omitted when whole brain coverage is not achieved, such as the juxtacortical region or brainstem (**Figure 10-1**) – both of which are important components of the 2010 revisions to the McDonald criteria for dissemination in space.[90]

Table 10-1: Proposed MRI protocol for pediatric demyelinating disease

Order	Sequence	Recommendation	Comment
		Brain Imaging	
1	3-plane localizer	Recommended	Prescribe oblique axial images†
2	3D T1-weighted spoiled gradient-recalled echo imaging	Recommended	By increasing TR to 30 milliseconds, contrast is comparable to the contrast on SE imaging – important in assessment of T1-hypointense lesion formation
3	Sagittal FLAIR	Recommended*	
4	Axial T2-weighted FSE or TSE	Recommended*	
5	Axial DWI	Recommended	In children presenting with acute symptoms, DWI is important to rule out arterial ischemic stroke
6		***Contrast Administration***	
7	Axial FLAIR	Recommended	Acquired during 5-minute delay between contrast injection and post-contrast imaging
8	Axial T1-weighted post-contrast spoiled gradient-recalled echo imaging	Recommended	By increasing the TR to 30 milliseconds, the conspicuity of gadolinium enhancement is more comparable to that of SE imaging

	Optional Orbital Imaging *(acquired following contrast administration)*		
1	3-plane localizer	If clinically indicated	Prescribe oblique axial images†
2	Coronal and axial T2-weighted fat saturated imaging	If clinically indicated	Should include imaging of the optic nerves through to and including the optic chiasm
3	Coronal and axial T1-weighted post-contrast fat saturated imaging	If clinically indicated	If acquired with brain protocol, acquire after sequence #8 under Brain Imaging
	Optional Spinal Cord Imaging *(acquired following contrast administration)*		
1	3-plane localizer	If clinically indicated	
2	Sagittal T2-weighted FSE or TSE¶	If clinically indicated	Acquire in superior, middle and inferior sections
3	Axial T2-weighted FSE or TSE	If clinically indicated	Acquire only through regions of interest
4	Sagittal T1-weighted post-contrast SE	If clinically indicated	If acquired with the brain protocol, acquire after sequence #8 under Brain Imaging

TR, repetition time; SE, spin-echo; FSE, fast spin-echo; TSE, turbo spin-echo. †Axial images parallel to the genu-splenium subcallosal line. ¶A short inversion time recovery (STIR) sequence may be acquired either in place of or in addition to T2-weighted FSE images to enhance ability to detect inconspicuous intramedullary lesions.[314] *May be omitted when child or adolescent cannot tolerate the scan.

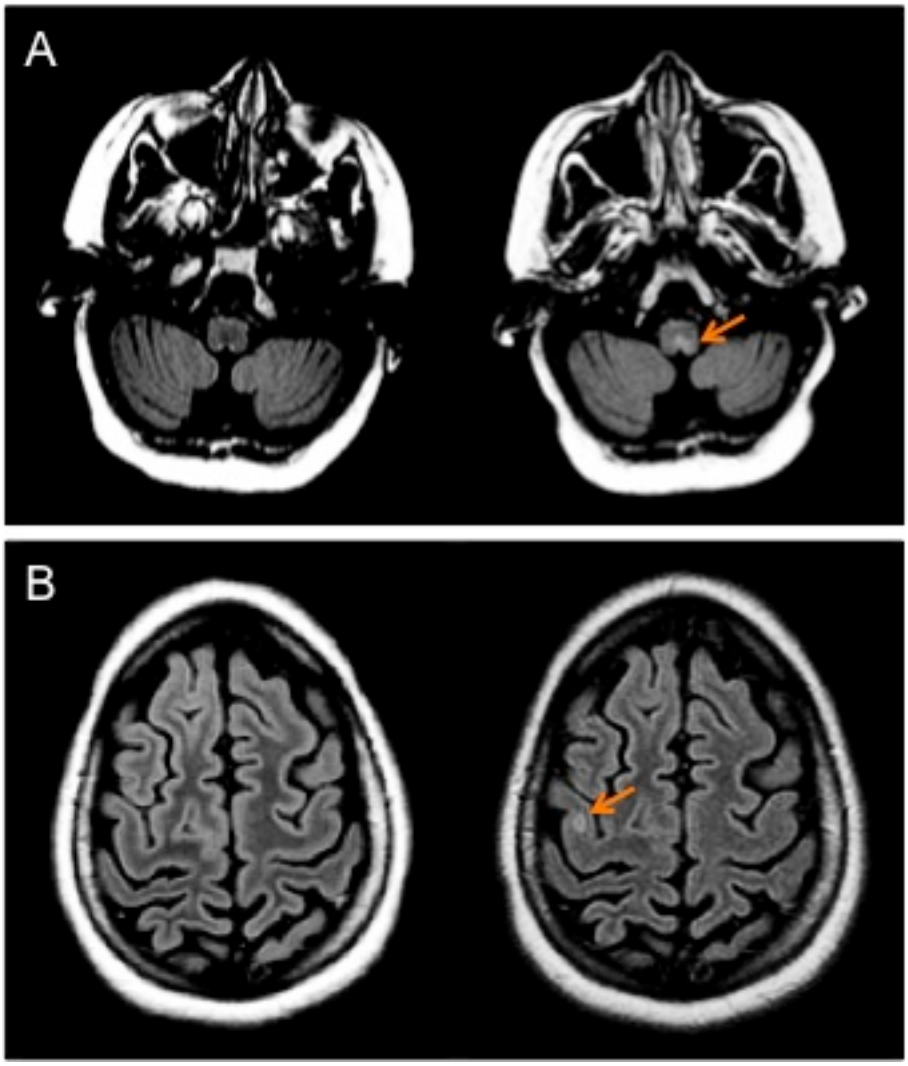

Figure 10-1: Detection of new lesions in portions of the brain often not imaged in clinical MRI protocols. Axial FLAIR images of a girl with MS who presented with a first attack at age 11 years. (A) A new T2 lesion is visible in the medulla when compared to the previous scan acquired 6 months earlier. (B) In the same patient, a new juxtacortical lesion is visible when compared to the previous scan 2 years prior.

The rationale for the sequences included in **Table 10-1** is summarized as follows:

1. *3D T1-weighted imaging*: A spoiled gradient-recalled echo sequence is recommended for its efficiency; i.e. one can achieve a higher signal-to-noise ratio (SNR) with a shorter

acquisition time compared to spin-echo (SE) imaging. Three-dimensional imaging also enables reformatting of the data into axial, coronal, or sagittal planes, and is often used in centers performing volumetric analyses. Acquiring T1-weighted imaging permits evaluation of black hole accrual over time, the presence of which is highly predictive of MS in children with acute demyelination.[50] Black holes initially were defined on SE imaging.[85] However, increasing the TR of a T1-weighted 3D spoiled gradient-recalled echo sequence from the typical 20 ms used for high contrast, high resolution applications to 30 ms mutes the contrast to a level similar to a SE sequence. Lastly, T1-weighted imaging performed prior to contrast injection permits confirmation of lesion enhancement when present.

2. *Axial T2-weighted imaging*: Fast or turbo spin echo imaging with a TE between 80-120 ms is recommended; a longer TE increases lesion conspicuity, at the cost of lower SNR. T2-weighted imaging provides better contrast than FLAIR for infratentorial lesion detection (**Figure 10-2**). Infratentorial lesions and specifically brainstem lesions are important features of MS in children.[92,109] The presence of a "false lesion" in the pons caused by a deep interpeduncular cistern leading to a partial volume effect has been reported[315] and warrants consideration when interpreting pontine lesions. An intermediate-weighted or proton density is not felt to contribute to lesion detection beyond that of combined T2-weighted and FLAIR imaging, but may be used in some centers as it provides another contrast for automated lesion detection techniques.

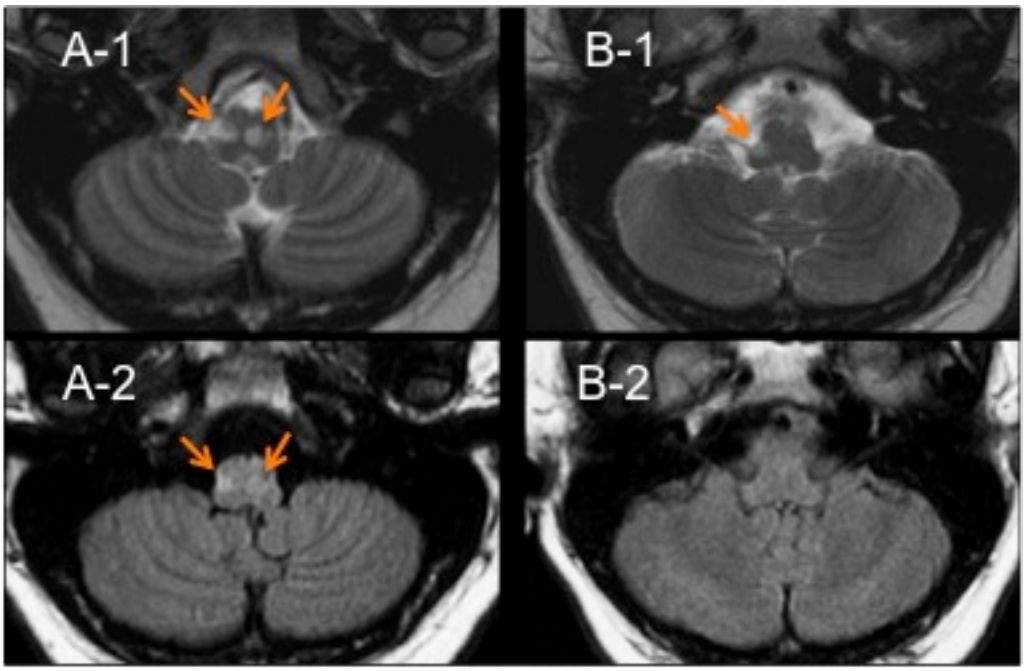

Figure 10-2: Comparison of lesion conspicuity on axial T2-weighted (top row) and FLAIR (bottom row) imaging. Two brainstem lesions (arrows) are visible on T2-weighted imaging in A-1, whereas both lesions (arrows) are more inconspicuous on FLAIR (A-2). At another level of the brainstem, the T2 lesion in B-1 (arrow) is not visible on FLAIR (B-2).

3. *Axial and sagittal FLAIR*: Juxtacortical and periventricular lesion detection may be obscured by the partial volume effect on T2-weighted imaging caused by cerebrospinal fluid. FLAIR imaging effectively suppresses the signal from cerebrospinal fluid, and therefore is a preferred sequence for MS lesion detection. Sagittal FLAIR imaging permits visualization of intracallosal lesions and lesions perpendicular to the long axis of the corpus callosum – a feature demonstrated to be predictive of MS in children.[91,316,317] A recommendation to reduce total examination time would be to acquire the axial FLAIR scan during the 5 minute delay time following gadolinium injection and prior to

collection of contrast-enhanced imaging.[318] Caution should be taken when leptomeningeal hyperintensity is detected on FLAIR imaging as this can be a normal finding in children imaged under sedation or general anesthetic.

4. *Axial DWI*: Given the broader differential in pediatric demyelination compared to adults, the ability to promptly rule out mimics of demyelinating disease is crucial. A short (<1 minute) DWI sequence provides sufficient information to assess the presence of a vascular occlusive disorder such as arterial ischemic stroke.
5. *Axial contrast-enhanced T1-weighted imaging*: "Muted-contrast" spoiled gradient-recalled echo imaging (i.e. long TR), matched to the pre-contrast 3D T1-weighted spoiled gradient-recalled echo sequence described above, is recommended as it permits direct comparison with the pre-contrast 3D T1-weighted spoiled gradient-recalled echo images, and provides conspicuity of enhancing lesions that is roughly comparable to standard SE imaging. The contrast-enhanced T1-weighted scan should be the last sequence of the protocol, as gadolinium injection is sometimes not well-tolerated in children and leads to motion artifacts. Although higher gadolinium doses, use of magnetization transfer contrast and a longer post-injection delay before collecting the data may increase enhancement conspicuity,[319,320] these tactics are not essential for routine clinical imaging.[321] The presence of contrast-enhancing lesions indicates new lesion formation, which is important in establishing an MS diagnosis according to dissemination in time.[89,90] Along with markers of disease activity such as annualized relapse rate and new T2 lesion formation, contrast-enhancement is also used as a surrogate marker of therapeutic efficacy in MS treatment trials. Contrast-enhanced imaging is pivotal in excluding other diagnoses such as malignancy, infectious processes

or small-vessel primary angiitis of the CNS[266] which may show leptomeningeal enhancement. Standard pediatric gadolinium dose (0.1 mmol/kg) is recommended, with a 5-minute delay to acquisition of contrast-enhanced images. Auto-injection is not essential. For patient safety reasons, a maximum of 5 mmol of contrast is recommended during a given MRI acquisition. Adherence to site-specific protocols around contrast administration in children with impaired renal function is necessary.[322]

Spinal cord and optic nerve imaging Imaging of the spinal cord or optic nerves is recommended only to investigate symptoms referable to the optic nerves or spinal cord, or to aid in the diagnosis of MS when brain MRI findings are equivocal. The additional scan time required may be tolerable for adolescent patients, but is often prohibitive in younger children. Considering that the spinal cord terminates at T12-L1 in children, sagittal T2-weighted superior and middle images are sufficient and axial T2-weighted region of interest images are recommended. Both T1- and T2-weighted short-tau inversion recovery (STIR) imaging may be more sensitive to spinal cord demyelinating lesions than fast spin-echo and FLAIR imaging.[314,323-326] The increased sensitivity is due to the synergistic effect of prolonged T1 and T2 relaxation times in abnormal tissue, which increases lesion conspicuity. Compared to spin-echo, STIR image quality tends to be noisy, but the contrast-to-noise ratio is high.[327] The STIR sequence may be more susceptible to motion and cerebrospinal fluid flow artifacts.[327] With respect to imaging of the optic nerves, axial and coronal fat-suppressed orbital imaging (i.e. spectral attenuated inversion recovery [SPAIR]) is recommended. When spinal cord or optic nerve imaging and the brain MRI protocol are acquired simultaneously, additional gadolinium is not required.

10.4 Standard MRI Lexicon for Pediatric Demyelination

Standardization of terms used to describe MRI features of demyelination in children is important to permit comparison of findings across studies and to engage in multi-center collaborative studies. As pediatric-onset MS represents a smaller entity compared to adult-onset MS, multi-centered studies are increasingly important to achieve sufficient power. Use of a consistently applied operative lexicon will facilitate the merging of data from multiple centers to inform on key questions related to the natural history of MS on MRI in children, evaluation of MRI surrogates of efficacy in treatment trials, and the study of MRI indicators of prognosis.

The proposed lexicon is applicable to children with incident CNS demyelination as well as those with established MS. It consists of parameters identified based on a review of the literature for MRI features of MS and other demyelinating disorders, and characteristics that would aid in excluding the diagnosis of MS. An expert panel of pediatric neurologists and neuroradiologists at The Hospital for Sick Children (Toronto, Canada) met to refine the parameter definitions. The lexicon and accompanying atlas has been published as part of the work in Chapter 6 (**Appendix 2**).[50] The fourteen parameters included describe the distribution and features of inflammatory demyelinating lesions on T1-weighted pre- and post-contrast and T2-weighted or FLAIR imaging. Use of this lexicon by other groups will be essential to further refine or extend the parameters.

10.5 Standardized MRI Reporting in Pediatric MS

A recommended template of an MRI report for children presenting with acute demyelination or those with established MS is provided in **Table 10-2**. MRI reports should be consistent to permit comparison of serial scans and should conform with current diagnostic criteria for pediatric MS.[56] Published MRI diagnostic criteria for MS in children and adults are summarized in **Table 10-3**.

Table 10-2: MRI reporting template for pediatric demyelinating disease

Report Contents
1. Description of findings on a single scan
a. Number of T2 lesions*
b. T2 lesions located in regions considered characteristic for pediatric MS
i. Periventricular
ii. Juxtacortical
iii. Corpus callosum
iv. Brainstem or cerebellum
v. Spinal Cord (if acquired)
c. Number of contrast-enhancing lesions
d. Presence of T1-hypointense lesions, defined as hypointense to cortical grey matter on T1-weighted imaging
2. Summarizing change over serial scans
a. Timing between scans being compared
b. Number of *new* T2 lesions*
c. Persistence of T1-hypointense lesions
d. Number of *new* contrast-enhancing lesions
e. Changes in global brain volume
3. Statement of whether MRI features meet published MRI scoring criteria (refer to **Table 10-3**)
a. McDonald MS criteria for DIS and DIT[89,90]
b. Pediatric MS criteria for DIS[92]
c. Pediatric criteria for differentiating MS from ADEM[93]
d. Parameters predictive of MS in children with acute demyelination[50]

*T2 lesion count is a component of the DIS criteria for MS diagnosis. The number of T2 lesions required to meet DIS has decreased as revisions to the diagnostic criteria have been published (refer to **Table 10-3**). However, it remains an important parameter for evaluating new T2 lesion accrual for dissemination in time. Therefore, T2 lesion count, as much as practically possible, should be included when reporting.

Table 10-3: MRI criteria for MS diagnosis

	Pediatric-Onset MS	Adult-Onset MS
Polman, 2005[89]		**DIS*** Three of the following: 1. ≥9 T2 lesions *or* ≥ 1 contrast enhancing lesion 2. ≥1 T2 infratentorial lesion 3. ≥1 T2 juxtacortical lesion 4. ≥3 T2 periventricular lesions **DIT** One of the following: 1. ≥1 contrast-enhancing lesion at least 3 months after clinical onset 2. A *new* T2 lesion at any time compared with a reference scan acquired at least 30 days after clinical onset
Polman, 2011[90]	**DIS** Presence of ≥1 clinically silent T2 lesion in at least 2 of the 4 following CNS regions: 1. Periventricular 2. Juxtacortical 3. Infratentorial 4. Spinal cord **DIT** One of the following: 1. A *new* T2 or contrast-enhancing lesion on any serial scan with respect to a previous scan 2. Simultaneous presence of asymptomatic contrast-enhancing and non-enhancing lesions at any time	
Mikaeloff, 2004[91]	**MRI prognostic factors for MS in children with acute**	

	demyelination Two of the following: 1. T2 lesions perpendicular to the long axis of the corpus callosum 2. Sole presence of well-defined T2 lesions
Callen, 2009[92]	**MRI criteria to distinguish pediatric MS from relapsing non-demyelinating disease** Two of the following: 1. ≥5 T2 lesions 2. ≥2 T2 periventricular lesions 3. ≥1 T2 brainstem lesion
Callen, 2009[93]	**MRI criteria to distinguish a first attack of MS from monophasic ADEM** Two of the following: 1. Absence of a diffuse bilateral T2 lesion pattern 2. Presence of black holes 3. ≥2 T2 periventricular lesions
Verhey, 2011[50]	**MRI parameters that predict MS in children with acute demyelination:** Two of the following: 1. ≥1 T2 periventricular lesion 2. ≥1 T1-hypointense lesion

*A contrast-enhancing spinal cord lesion is equivalent to an enhancing lesion in the brain; individual spinal cord lesions and brain lesions can together contribute to the requirement of ≥9 T2 lesions.

10.6 Conclusion

A standardized algorithm for MRI acquisition and reporting in pediatric MS that is based on the current literature and published diagnostic criteria is proposed. Use of this algorithm by other centers is vital for making consensus revisions and further refinement that will permit its implementation as a standard protocol. Adopting a standard lexicon and reporting scheme will inform the creation of structured reporting that is increasingly being proposed for diagnostic radiologists. Standard acquisition methods will be key in the collection of surrogate MRI treatment response data in pediatric clinical trials of disease modifying therapy for MS.

10.7 Acknowledgments

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PART VI:

GENERAL DISCUSSION

Chapter 11:

Discussion, Clinical Implications and Future Perspectives

11.1 General Summary

Our work contributes to the body of evidence on the important role of MRI in the diagnosis of pediatric-onset MS. The uniqueness of our work is predicated on the derivation of predictors of MS from MRI scans acquired according to a standardized protocol in a national prospective incident cohort of children with ADS. There are four key highlights to our work. (i) We devised a scoring tool consisting of MRI parameters representative of the literature and expert opinion and that demonstrate substantial inter-rater reliability. (ii) Using this tool to systematically evaluate over 1100 MRI scans, we defined parameters present at ADS that are predictive of subsequent MS diagnosis. Subsequently, we validated the parameters in an independent cohort of children with ADS, in collaboration with the Dutch Study Group for Pediatric MS (Rotterdam, the Netherlands). (iii) We also determined that the recently revised 2010 McDonald criteria for MS diagnosis were highly sensitive and specific for MS diagnosis in older children with non-ADEM ADS presentations. (iv) The radiographic features of the spinal cord in children with MS are similar to that of adult-onset MS patients, and spinal cord lesions do not correlate with physical disability in children in the first few years of the MS course.

The central objective of our work was to develop a predictive algorithm for MS diagnosis that was applicable in the clinical setting. Thus, we gave careful consideration when devising the scoring tool to include only parameters that were straightforward to apply on conventional MRI sequences typically acquired in a clinical brain protocol (i.e. T1-weighted pre- and post-contrast, T2-weighted, and FLAIR images). In addition to the conventional MRI sequences acquired in our Canadian Pediatric Demyelinating Disease Program, high-resolution volumetric images,

magnetization transfer MRI, diffusion tensor MRI and ^{1}H-MRS were also collected. These techniques are not routinely available for clinical use as their interpretation is dependent on rigorous analysis pipelines and control or reference data; therefore, advanced imaging is not the focus of this thesis. Advanced MRI has, however, significantly increased our ability to quantitatively elucidate *in vivo* the underlying mechanisms of tissue injury, as well as repair and functional reorganization, in children with MS. Insights gained from application of these techniques in pediatric-onset MS has been extensively reviewed in **Chapter 3**.

In the present chapter, the findings and limitations of the research presented herein will be placed in context of the literature; clinical implications as well as future perspectives will also be discussed. Our work provides key data on stratification of children into groups at highest and lowest risk for MS, which will be important for sufficiently powering pediatric trials of MS-targeted therapies currently being planned. Further work is required to improve our understanding of the natural history of MRI lesions in pediatric MS in order to define expected therapeutic response, and to define MRI features relevant to clinical trials. Large cohorts are required and are likely only to be achieved in multinational collaborative initiatives.

11.2 Semi-quantitative Evaluation of MRI: A Systematic Approach

The core analyses of this dissertation are based on the manual characterization of lesions on more than 1100 baseline and serial MRI scans acquired in our national study. To accurately and

reliably score the large number of scans, we required a standard scoring method. We developed a scoring tool **(Chapter 5)** to serve two aims: i) to aid in distinguishing acute CNS demyelination from non-demyelinating disorders (discussed in **Chapter 5**), and ii) to predict subsequent MS diagnosis in children with confirmed ADS (**Chapter 6**). As the application of the tool in this dissertation was to identify MRI features that are predictive of MS, the present portion of the discussion focuses on the second aim.

To permit adoption of this tool into clinical practice, we included parameters that were straightforward to use and reliably scored across raters. We surveyed the literature and consulted expert opinion, both established methods for tool development,[270] to identify potential parameters for inclusion in the tool. By searching the literature, we identified parameters that have been empirically shown to be characteristics of acute demyelination or MS. Considering that the tool has potential applications in both the clinical and research setting by multiple individuals with varying degrees of training, we purposefully did not include parameters that could be interpreted subjectively, such as 'absence of diffuse bilateral lesion pattern'[93] or 'sole presence of well-defined lesions'.[91] With respect to 'absence of diffuse bilateral lesion pattern', *diffuse* could refer to lesions that have a hazy appearance or borders that are not clearly delineated and that coalesce with adjacent lesions, or it could refer to the situation in which typical focal lesions are scattered throughout the supratentorial and infratentorial regions of the brain. With reference to 'well-defined lesions', while many MS lesions have clearly-defined lesion margins, it is rare that patients have *solely* well-circumscribed lesions. In addition, we found when reviewing the literature that even some MRI parameters that were shared across different studies were not well described or were

inconsistently defined across studies. Therefore, we assembled an expert panel to review all proposed MRI parameters and devise or refine definitions. In addition, a scoring manual was created consisting of definitions and an atlas which will permit the reliability of the tool to be maintained over time, and could serve as a reference for MRI scoring in future studies as well as clinical practice. Further validation of the tool is required in future studies that might include: differentiation between acute demyelination and non-demyelinating disorders such as infection, arterial ischemic stroke, neoplasm, and SV-pACNS; differentiation between patients with monophasic ADEM and those with ADEM who are diagnosed with MS; and application to adults with acute demyelination and MS.

Evaluation of MRI scans also requires that operational rules for what defines a lesion be applied consistently to all scans. As detailed in the methods of **Chapter 6**, we define the minimum size required to consider an area of T2-hyperintensity as a lesion. While we defined a minimum size criterion, we did not measure the actual size of lesions, as automated methods of lesion segmentation have superior accuracy to the semi-quantitative methods available for manual scoring, and such tools are already the focus of another aspect of our national program.[109,328]

Our model for prediction of MS diagnosis was based on MRI features present at baseline. We utilized the serial scans to identify new lesions, as new lesions over time are sufficient to meet the dissemination in time component for MS diagnosis.[89] During our evaluation of serial scans, we identified a small group of children who developed a single, tiny 3-5 millimeter new lesion on a single scan over time. Although the presence of these single small lesions technically qualifies the scan as meeting criteria for DIT, concern was raised as to whether a single small lesion is clinically significant in absence of clinical evidence of relapsing disease. To determine

whether such children have been correctly classified as having MS, prospective studies with prolonged periods of clinical observation and MRI scanning are required.

11.3 Novel Insights on Predicting *Early* MS Diagnosis in Children

The MRI parameters for predicting MS diagnosis in children with ADS identified in our Canadian cohort **(Chapter 6)** and validated in an independent Dutch cohort **(Chapter 7)** provide novel insight for stratification of children into groups at high and low risk for MS. The presence of at least one T1-hypointense lesion and one or more T2 periventricular lesion in children with ADS are highly sensitive and have good positive predictive value for MS. In our national cohort, the duration of observation at the time of data analysis was only 3.9 years and children with MS were diagnosed a median of 6.2 months after ADS. Therefore, our criteria predict the diagnosis of MS within the first few years of ADS, and whether the same MRI parameters will perform as well in predicting MS diagnosis in children who do not demonstrate relapsing disease for several years following ADS will require the prolonged serial evaluation of these children.

The MRI parameters identified were not only robust in their predictive value, but are also straightforward to apply to MRI scans acquired under a non-research protocol, as was demonstrated in our validation study (**Chapter 7**). As such, the parameters are well suited for application in the clinical setting, and across multiple centers. Evaluation of the inter-rater reliability of the tool when applied by experts in other centers (i.e. Rotterdam, the Netherlands) is a subject of ongoing work.

Finally, it is important to consider the biological plausibility of the MRI parameters as informative to MS underlying pathobiology. Periventricular lesions emerged as one of our MRI predictive parameters. The periventricular region is a consistently documented location for MS lesion formation,[91,316,317,329] and thus the periventricular parameter represents a common occurrence in MS. The presence of T1-hypointense lesions at the time of first attack was the second MRI parameter we identified. T1-hypointense lesions are consistent with chronic tissue injury and, when present at the time of a first clinical event, suggest that this first attack is really the first clinical evidence of an already established disease process.[85]

Our work **(Chapter 6)** builds on what is known about the utility of MRI in the diagnosis of pediatric-onset MS (literature review in **Appendix 6**). We evaluated published MRI DIS criteria for adult- and pediatric-onset MS (Table 11-1) in our cohort of children with ADS. The Canadian national study was designed prior to the publication of the 2010 revisions to the McDonald criteria; therefore, we evaluated the 2005 McDonald criteria, and the application of the 2010 revisions to our cohort is the subject of a separate study (**Chapter 8**).

Table 11-1: MRI criteria for adult- and pediatric-onset MS diagnosis

	Pediatric-Onset MS	Adult-Onset MS
2005 McDonald criteria[89]		Three of the following: 1. ≥9 T2 lesions *or* ≥ 1 contrast enhancing lesion 2. ≥1 T2 infratentorial lesion 3. ≥1 T2 juxtacortical lesion 4. ≥3 T2 periventricular lesions
KIDMUS criteria[91]	**MRI prognostic factors for MS in children with acute demyelination**	

	Two of the following: 1. T2 lesions perpendicular to the long axis of the corpus callosum 2. Sole presence of well-defined T2 lesions
Pediatric MS criteria[92]	**MRI criteria to distinguish pediatric MS from relapsing non-demyelinating disease** Two of the following: 1. ≥5 T2 lesions 2. ≥2 T2 periventricular lesions 3. ≥1 T2 brainstem lesion
Pediatric MS-ADEM criteria[93]	**MRI criteria to distinguish a first attack of MS from monophasic ADEM** Two of the following: 1. Absence of a diffuse bilateral T2 lesion pattern 2. Presence of black holes 3. ≥2 T2 periventricular lesions

When evaluating the performance of published MRI DIS criteria (**Table 11-1**) using baseline scans acquired in our national study, we note several limitations. First, the 2005 McDonald criteria for DIS[89] were developed for patients with adult-onset MS, and when applied to baseline scans of an unselected cohort of children with ADS, fail to identify about half of the children with MS.[50,102,104] Second, the French KIDMUS criteria,[91] while highly specific, are not met by about 60% of children with MS; this is an expected finding given that the presence of well-defined lesions and lesions perpendicular to the corpus callosum are features of established MS and likely have a lower prevalence in patients at the time of first attack. Finally, a prior study by our group proposed pediatric MS diagnostic criteria based on a comprehensive

semi-quantitative delineation of the MRI lesion features of children with MS in relation to patients with non-relapsing CNS disorders[92]; however, these criteria were developed using archived clinical scans from patients with established MS (none of whom were in our Canadian national study).

The predictive parameters we propose herein provide a unique twofold contribution to the literature: i) they were derived from an unselected cohort of children with incident CNS demyelination, and ii) MRI scans from which the parameters were derived were acquired according to a standardized research MRI protocol. Our parameters are similar in content and have similar sensitivity and specificity to criteria developed earlier by our group to distinguish children with ADEM from those with MS.[93] An advantage of the predictive parameters we propose is that the subjective parameter 'absence of diffuse bilateral lesion pattern' is not included.

That we have replicated our work in an independent Dutch cohort **(Chapter 7)** suggests that our MRI parameters are robust predictors of MS diagnosis, at least in pediatric ADS populations enrolled in world regions where MS outcome following ADS occurs with reasonable frequency.

11.4 Two Sub-Populations for Further Study: ADEM and Pre-Pubertal Children

In the present dissertation, the role of MRI in evaluating MS risk has been evaluated in an unselected cohort of children with ADS. The next important questions pertain to whether the

predictors we have identified are applicable across all epochs of onset age and ADS phenotypes. These questions are relevant when considering that evaluation of MS risk in children with ADEM and in children younger than ten years of age at presentation is particularly challenging.

Predicting MS Diagnosis in Children with ADEM ADEM represents about 30% of all ADS presentations, and is particularly common in children less than ten years of age.[48] Typically, ADEM represents a fulminant but monophasic inflammatory demyelinating illness, that occurs following a viral prodrome.[105] Complete recovery occurs in a approximately 80% of children.[106] However, presentation with ADEM may represent a first attack of MS in 5-20% of children.[17,57,330]

In the present work, we conducted sensitivity analyses to evaluate the effect that inclusion of patients with ADEM had on the performance of our proposed predictive parameters as well as the 2010 McDonald criteria **(Chapter 8)**.

Evaluation of the 2010 McDonald criteria in children with ADEM. The positive predictive value of the 2010 McDonald criteria was low (59%, 95% CI 45%-71%) when applied to all children with ADS; in comparison, our proposed MRI predictive parameters **(Chapter 6)** performed better at identifying children with MS when children with ADEM were included in the ADS cohort (positive predictive value 76%, 95% CI 64%-86%; p=0.039). This observation is in keeping with the 2010 McDonald panel's suggestion that the criteria were likely not applicable in the context of ADEM.[90]

Evaluation of our MRI parameters in predicting MS diagnosis following ADEM presentation. We demonstrated that our MRI parameters for predicting MS diagnosis are applicable across all

ADS presentations, including ADEM. What we were not able to evaluate was the ability of our MRI parameters to predict the rare child who is diagnosed with MS but for whom the initial ADS event was considered as ADEM. As this sub-group represents a very small proportion of all children diagnosed with MS, a large sample would be required – achievable only by a multinational collaborative effort. A more feasible study is the identification of parameters that distinguish children confirmed to have monophasic ADEM from those with MS; this is a subject of ongoing work from this dissertation.

Predicting MS Diagnosis in Pre-Pubertal Children Children greater than ten years of age at the time of incident ADS are at increased risk for MS, compared to younger children.[51] The time from first attack to second MS-confirming relapse may be longer in children younger than ten at onset (median 6 years), compared with that in older children (median 1 year).[51] Baseline MRI scans of younger children with MS do not demonstrate the typical ovoid and well-defined lesions that are characteristic of MS; rather, lesions appear large and ill-defined.[86] Taken together, diagnosis of MS in children younger than ten years of age is rare (only 7 of 284 children [7 of 57 diagnosed with MS] in our national study) and may be challenging, delaying the time to initiation of MS-targeted therapies.

We evaluated the performance of the MRI predictive parameters (**Chapter 6**) and 2010 McDonald criteria **(Chapter 8)** as a factor of age at onset. In children aged <10 years at onset, the positive predictive value for MS diagnosis was low (predictive parameters: 29%, 95% CI 10%-56%; 2010 McDonald criteria: 32%, 95% CI 13%-57%), and increased when applied only to children ≥10 years of age at onset (predictive parameters: 93%, 95% CI 82%-99%; 2010 McDonald criteria: 72%, 95% CI 55%-85%). Children enrolled in our national study have been

followed for approximately 4-5 years; ongoing observation of young children in our cohort is necessary to determine the proportion of young children, currently classified as having monophasic disease, who will yet be diagnosed with MS. Given that the diagnosis of MS in children under 10 years of age is relatively rare, pooling of data from multiple cohorts will be necessary to sufficiently power a study to evaluate predictors of MS diagnosis in children less than 10 years of age.

11.5 MRI of the Spinal Cord to Aid in Predicting MS Diagnosis

MRI diagnostic criteria for MS indicate that the presence of spinal cord lesions can be used to demonstrate dissemination in space.[89,90,101] In a cohort of adults newly diagnosed with MS (median time from first attack to diagnosis, 18.4 months), 66% of patients met dissemination in space criteria based on brain MRI acquired at first attack; the proportion of patients meeting criteria for DIS at onset increased to 85% when spinal cord lesions were considered in addition to brain imaging.[307] These findings exemplify the added value of spinal cord MRI in MS diagnosis when brain MRI findings are equivocal.

Spinal cord MRI is challenging in the pediatric setting. The length of time required to acquire both brain and spinal cord images without sedation poses practical limitations in obtaining standard spinal cord imaging in the context of a pediatric prospective study. By virtue of their young age, children have small spinal cords relative to adults, and coupled with the MRI artifacts from cardiac and respiratory motion, poor image resolution may challenge the

detection of small lesions in pediatric cohorts. Given the paucity of literature on the topic, we performed an exploratory study to characterize the MRI features of the spinal cord in children with MS **(Chapter 9)**.

Considering the impracticalities of standard spinal cord imaging in children, we did not include spinal cord MRI in the protocol of our national prospective study. However, we did assess whether spinal cord MRI was contributory to fulfillment of the 2010 McDonald criteria for those children in our national study who had *clinically indicated* spinal cord imaging at ADS **(Chapter 8)**. The majority of children with clinically-silent spinal cord lesions had already met criteria for MS based on brain MRI findings. Only two children were diagnosed with MS following MRI demonstration of clinically-silent spinal cord lesions; however, these children did develop MRI DIS on subsequent brain scans. While it appears that spinal cord MRI was not contributory to confirming MS diagnosis, spinal cord MRI was not systematically acquired in our national study. Therefore, we cannot determine whether the presence of clinically-silent spinal cord lesions at baseline would have increased the number of children meeting 2010 McDonald criteria at the time of ADS.

11.6 MRI as a Predictive Tool: Clinical Implications

Planning Care for Patients and their Families The incidence of pediatric ADS ranges from 0.66-1.56/100,000 children per year,[7,331,332] and was found to be 0.9/100,000 per year in our Canadian national cohort.[8] Approximately 20-30% of children with ADS are subsequently

diagnosed with MS,[17,51,102] whereas the remaining 70-80% of children experience a monophasic illness. Stratification of children with ADS based on likelihood for subsequent MS diagnosis has important clinical implications including, re-assuring patients at low risk for MS diagnosis, and counseling of families and early consideration of initiation of MS-targeted therapies for children at high risk for chronic disease. While the timing of treatment initiation is a patient-specific decision, prompt development of a comprehensive multidisciplinary patient- and family-centered care plan is necessary for managing the complex clinical issues experienced by children and adolescents with MS and for planning career and lifestyle goals.

Prediction of Physical and Cognitive Disability Relapsing-remitting MS in children rarely associates with physical disability within the first ten years of disease onset.[2,4,5,53,302] In the French KIDMUS cohort of children with ADS, 83% of the children had minimal to no disability after approximately 4.5 years of observation, and none of the MRI features evaluated on baseline MRI were predictive of disability.[91] In our national prospective study, all but five of the 57 children with MS were fully ambulatory without aid at the time of data analysis (mean observation of 4.3 years). As a result, we were unable to evaluate whether our MRI parameters predict MS-related disability.

Known as the "clinico-radiological paradox in MS," MRI measures of lesion burden are at best only modestly positively correlated with physical disability in individuals with MS.[333-335] This is likely due to the lack of specificity of T2-weighted imaging to the heterogeneous pathological features of MS. Recent applications of advanced MRI techniques have given insight into the diffuse damage and grey matter pathology that occurs beyond the T2-weighted-visible lesions (reviewed extensively in **Chapter 3**). One such technique is brain volumetry. Recent studies by

our group and others' have shown reduced head and brain size and disproportionately smaller thalami in children with MS relative to healthy individuals.[78,107,170,173] Brain volume is also associated with cognitive impairment in children with MS. Thalamic volume has been shown to be inversely associated with global IQ, processing speed and expressive vocabulary.[73] In children with MS, the ability to learn word-lists is positively correlated with hippocampal volume.[336]

Diffusion tensor MRI is another technique, and is used to evaluate the integrity of brain tissue microstructure by measuring the diffusion of water molecules. DTI has shown that the white matter not affected by T2-lesions (i.e. normal appearing white matter) is actually abnormal in children with MS when compared to healthy children.[187,200] While DTI metrics do not correlate with physical disability in children with MS, impaired microstructure of the corpus callosum (measured with fractional anisotropy) is associated with arithmetic performance and tasks of cognitive speed.[202,337]

These studies provide robust evidence for an association between advanced MRI measures of brain volume and microstructural tissue integrity and disability. Future work needs to focus on longitudinal analyses to evaluate the predictive value of MRI for long-term physical and cognitive outcome, and should include a multi-parametric assessment of brain volume, DTI, magnetization transfer or myelin water fraction imaging, ^{1}H-MRS and functional MRI.

11.7 MRI as a Predictive Tool: Implications for Treatment Trials

Both the Food and Drug Administration and the European Medicines Agency have mandated Pediatric Investigation Plans for all new therapies. Such requirements will serve to increase the number of emerging therapies available to pediatric patients, but consideration must be given to how such therapies will be evaluated in children. To date, no randomized-controlled trials in children with MS have been conducted. While double-blind placebo-controlled trials have been the gold standard for current MS therapies in adults, the required sample size to sufficiently power such trials is prohibitive in the pediatric MS population. To achieve sufficient sample sizes for pediatric MS trials, multi-national collaborations will be essential. The MRI predictors identified in this work are indicative of established MS pathobiology in children with ADS, and therefore, these predictors have potential applicability in identifying children for eligibility in pediatric MS treatment trials. Multi-centered trials that include secondary MRI outcomes will require standardized MRI acquisition, evaluation, and reporting **(Chapter 10)**.

The primary outcome of treatment trials in adult MS patients is typically relapse rate reduction. Given that the relapse rate in the first few years of disease in children with MS seems to be higher compared with adult patients,[60] a feasible outcome in pediatric MS trials could be relapse rate reduction.

Typical secondary outcome measures in adult MS trials include number of new T2 and contrast-enhancing lesions. Pediatric- and adult-onset MS patients matched for disease duration demonstrate similar lesion burden.[107,109,338] T2 and contrast-enhancing lesion formation is related to relapses in adults with MS.[297,339] Accrual of new lesions while on immunomodulatory

therapy is associated with poor outcome in adult MS patients.[340,341] To date, no serial prospective MRI evaluation of lesion accrual in children with MS has been conducted, but is needed for planning pediatric trials to define an expected treatment response on MRI. The natural history of MRI lesion evolution is currently being evaluated in our national cohort as a continuation of the work presented herein. Parameters being evaluated include the number of new T2 lesions and contrast-enhancing lesions over time. Given that lesion accrual is more active during the early phase of the disease, these metrics must account for time from ADS onset. While lesion volume would permit evaluation of enlarging lesions in addition to newly forming lesions, such analyses are more rigorous using semi-automated lesion detection methods rather than manual scoring. The comparison of manual and semi-automated lesion detection is the focus of an ongoing collaboration with the McConnell Brain Imaging Center at the Montreal Neurological Institute (Montreal, Canada).

11.8 Conclusion

The present work contributes to the current body of evidence for the role of MRI in pediatric MS diagnosis. The derivation of a standard scoring MRI scoring tool and its subsequent application to MRI scans acquired in a national prospective incident cohort of Canadian children and adolescents with ADS has led to the identification of parameters that predict subsequent MS diagnosis, and these parameters have been validated in an independent Dutch cohort. We confirm that the 2010 McDonald diagnostic criteria are applicable in pediatric patients, but also caution that these criteria do not apply equally across all ADS phenotypes and epochs of onset

age. Future research should focus on longitudinal analyses evaluating the role of advanced MRI techniques in predicting disease severity in children with MS. Based on evidence from case-control studies, MS-targeted therapies are safe in children, reduce relapse rate early in the disease phase, and are more effective if initiated at disease onset or ADS.[32,342] Therefore, it is timely that randomized controlled trials are now being planned for pediatric-onset MS. The work presented herein will is unique in its ability to inform on identification of eligible participants as well as selection of MRI metrics for pediatric MS trials.

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APPENDICES

Appendix 1: MRI parameters considered for inclusion in the MRI scoring tool (Chapter 5)

Number	Parameter(s)	Type	Definition
1	Lesion Count	Continuous	T2 lesions counted discretely to 15; if scan has more than 15 lesions, lesion count binned as ">15"
2	Bilateral Distribution	Binary	Supratentorial or infratentorial T2 lesions located in both hemispheres; or, in the case of one brain lesion, the lesion crosses midline
3	Symmetrical Pattern	Binary	Symmetric T2 lesion pattern on either side of midline
4-7	Lobar Location	Binary	T2 lesions located within 'Frontal', 'Temporal', 'Parietal', or 'Occipital' lobes
8-11	Vascular Territory Location	Binary	T2 lesions involving the territory of the 'Anterior Cerebral Artery', 'Middle Cerebral Artery', 'Posterior Cerebral Artery', or 'Vertebrobasilar Arteries'
12	Cortical Grey Matter	Binary	T2 lesions located within the cerebral cortical ribbon
13	Juxtacortical	Binary	White matter T2 lesions abutting the cortical ribbon
14	Periventricular	Binary	White matter T2 lesions abutting the lateral ventricle(s)
15	Subcortical	Binary	Non-juxtacortical and non-periventricular white matter T2 lesions
16	Intracallosal	Binary	T2 lesions located within the confines of the corpus callosum
17	Internal Capsular	Binary	T2 lesions involving the anterior or poster limbs of the internal capsule
18-21	Deep Grey Matter	Binary	T2 lesions involving the 'Caudate', 'Putamen', 'Globus Pallidus', 'Thalamus'
22	Cerebellar	Binary	T2 lesions involving the cerebellar white or grey matter
23	Cerebellar Peduncle	Binary	T2 lesions involving the superior, middle, or inferior cerebellar peduncles
24-26	Brainstem	Binary	T2 lesions involving the 'Right', 'Left', or 'Midline' brainstem
27	Cervical Spinal Cord	Binary	T2 lesions involving the visible region of the cervical spinal cord on brain MRI
28	Black Hole	Binary	Lesions isointense or hypointense to cortical grey matter on T1-weighted imaging that are confirmed as T2-hyperintense[92]
29	Finger-like Projection	Binary	T2 lesion projecting continuously into a gyrus from the subcortical white matter to juxtacortical white matter at apex of a gyrus
30	Finger-like + Projection	Binary	Finger-like projection extending into cortical grey matter at gyral apex
31	Dot-Dash Sign	Binary	A T2-weighted irregularity of the ependymal stripe on the undersurface of the corpus callosum, defined as at least 2 "dots" connected by a "dash". The "dot" is a round hyperintense irregularity of the ependymal undersurface with a diameter larger than the thickness of the "dash" adjacent to it. The "dash" is the remaining normal ependymal stripe.[317]
32	Target Lesion	Binary	T2 lesion with a more hyperintense centre relative to the penumbra
33	Diffusion Restriction	Binary	Restricted diffusion on DWI and ADC, correlating with a T2 lesion

34	Optic Nerve Lesion	Binary	T2 lesion along one or both optic nerve(s), anywhere between orbit and optic chiasm
35	Lesion Enhancement	Binary	Gadolinium-enhancing lesion, correlating with a T2 lesion
36-39	Optic Nerve Enhancement	Binary	Contrast enhancement of any of : 'Optic Nerve(s)', 'Optic Nerve Sheath', 'Extra-optic Fat', or 'Extra-optic Muscle'
40-42	Other Enhancement	Binary	'Dural', 'Ependymal', or 'Perineural' (excluding optic nerves) enhancement
43-45	Leptomeningeal Enhancement	Binary	Contrast enhancement of the arachnoid and pia mater; if present, leptomeningeal enhancement is also scored as 'Linear' or 'Nodular'
46-47	Compartment of Enhancement	Binary	Lesional contrast enhancement present in 'Supratentorial' or 'Infratentorial' compartments
48	Proportion of Discrete Lesions	Categorical	Proportion of T2 lesions having well-defined lesion boarders in all planes: 0-25%, 26-50%, 51-75%, 76-100%

DWI, diffusion-weighted imaging; ADC, apparent diffusion coefficient

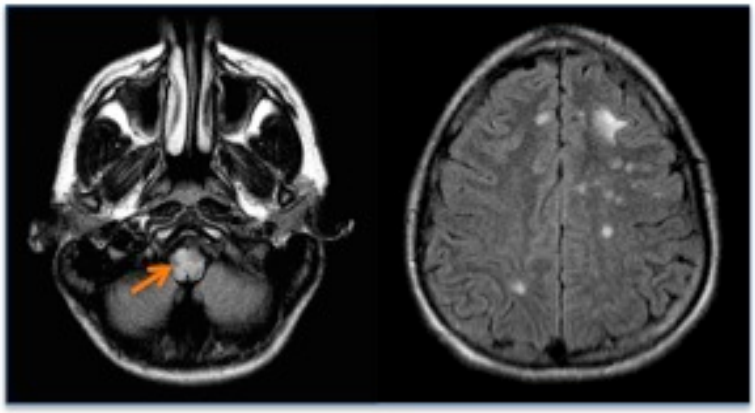
Appendix 2: MRI scoring tool manual and atlas

(Chapters 5, 6 and 10)

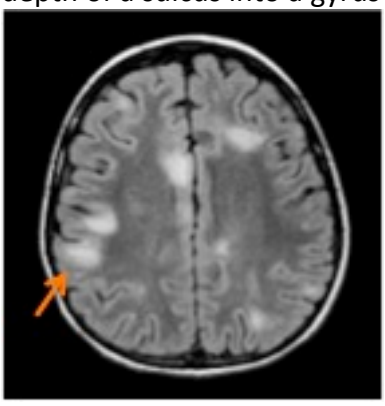
All parameters defined below are dichotomous (i.e. 'present'/'absent'), with the exception of #1.

1. Lesion count: total number of T2 lesions within the brain; lesion counts greater than 15 are binned as '>15'

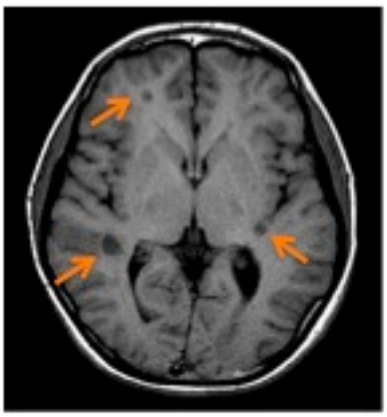
2. Bilateral lesion distribution: scan contains T2 lesions on either side of, or spanning across, the midline in either the supratentorial or infratentorial region, or both



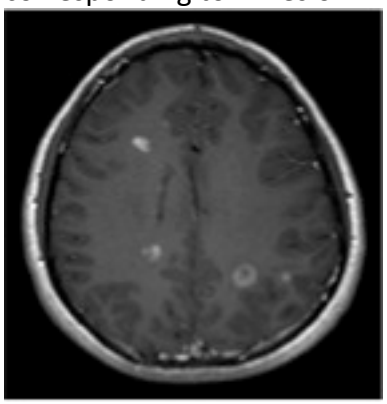
3. Gyral projections: a T2 lesion continuously projecting from subcortical white matter at the depth of a sulcus into a gyrus (or gyri) and abutting the cortical ribbon at the gyral apex



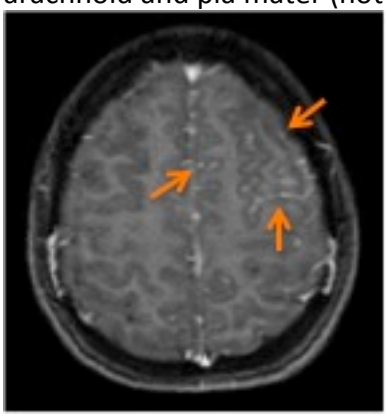
4. T1-hypointensity: Abnormal region of white matter with all or a portion of the lesion being *hypointense to cortical grey matter* on T1-weighted imaging; should be non-enhancing on post-contrast T1-weighted images and hyperintense on T2-weighted or FLAIR images; cross-referencing of T1-hypointense lesions with FLAIR imaging is recommended to exclude perivascular spaces from being scored as T1-hypointense



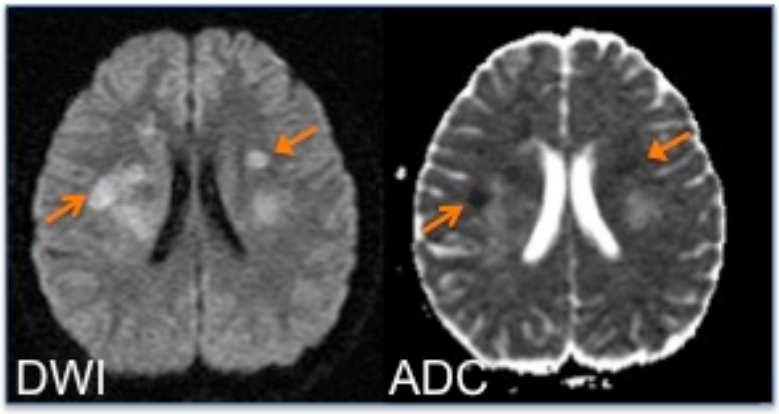
5. Lesion contrast-enhancement: nodular or ring-like hyperintense signal on T1-weighted contrast-enhanced imaging (not hyperintense on T1-weighted pre-contrast imaging) corresponding to T2 lesion



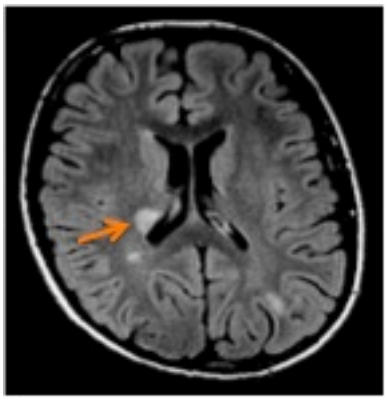
6. Leptomeningeal contrast-enhancement: linear or nodular hyperintensity (minimum 3mm length or diameter) on T1-weighted contrast-enhanced imaging corresponding anatomically to arachnoid and pia mater (not hyperintense on T1-weighted pre-contrast imaging)



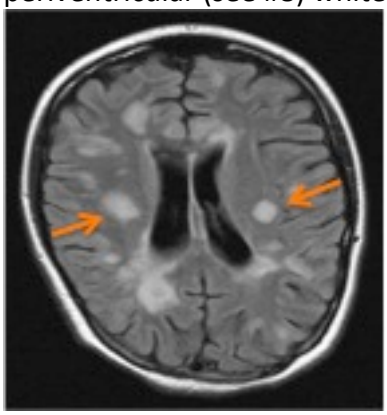
7. Diffusion restriction: hyperintensity on diffusion weighted imaging corresponding to a T2 lesion and correlated with hypointensity on apparent diffusion coefficient map



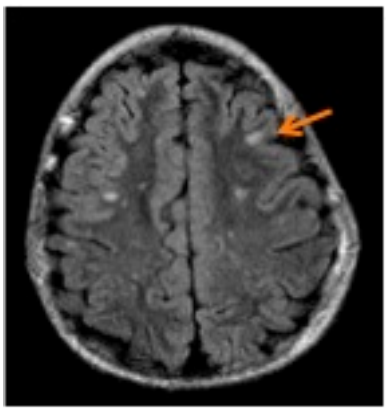
8. Periventricular lesion: white matter T2 lesion abutting any portion of the lateral ventricles only (excludes third and fourth ventricles); lesions involving the corpus callosal white matter are included; lesions within the thalami or basal ganglia (see #12-13) abutting lateral ventricles are excluded



9. Cerebral white matter lesion: supratentorial non-juxtacortical (see #10) and non-periventricular (see #8) white matter T2 lesion; excludes intracallosal lesions (see #11)

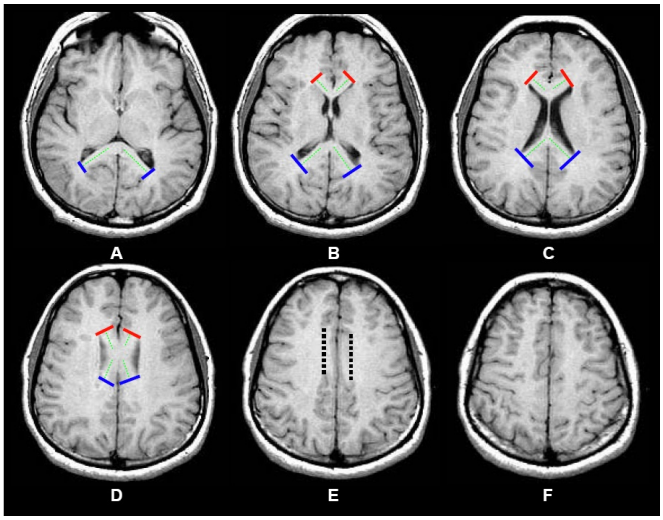


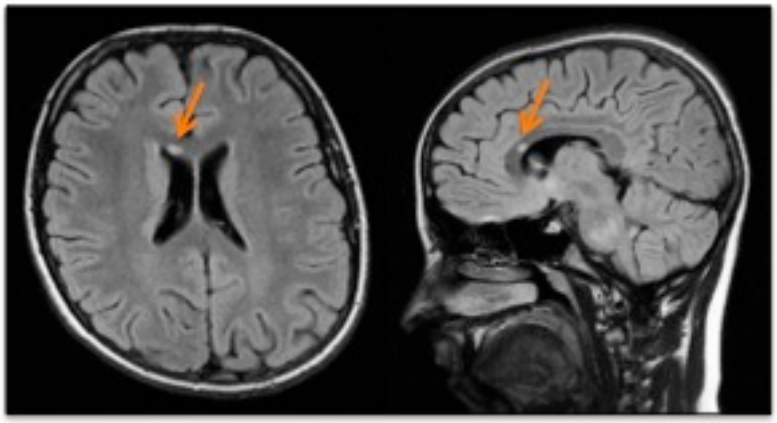
10. Juxtacortical lesion: supratentorial white matter T2 lesion contiguous with the cortical ribbon, i.e. involves subcortical U-fibers[95,96]



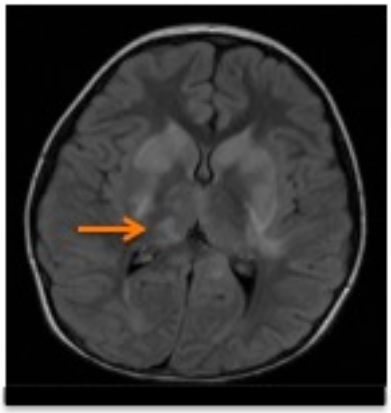
11. Intracallosal lesion: T2 lesion contained entirely within the margins of the corpus callosum (defined in image below adapted from[92]); 1mm of normal appearing white matter surrounding lesion to be confirmed as intracallosal rather than periventricular (see #8)

A-D: Line (red or blue) drawn from anterior (or posterior) tip of lateral ventricle perpendicular to the long axis of the callosal fibers (green lines) and then extending to the cortical ribbon of the midline; **E:** lateral margin of the lateral ventricle to be utilized when lateral ventricles are no longer visible; medial margin of the lateral ventricle is extrapolated from its location on the most superior axial image showing the lateral ventricles: 1) on last axial image showing lateral ventricles, note location of most medial portion of lateral ventricle closest to midline, 2) on each more superior axial slice, draw line parallel to interhemispheric fissure that touches this most medial point (extrapolate by viewing previous slice), 3) anterior and posterior limits of this line are where the line touches cortical ribbon of midline

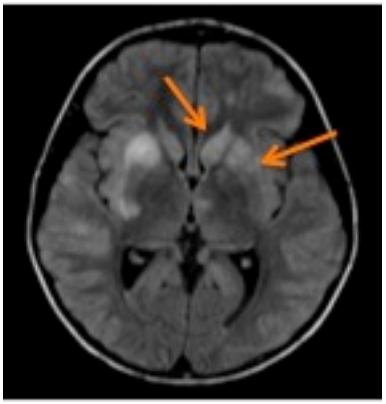




12. Thalamic lesion: T2 lesion either entirely or partially contained within the thalamus; bi-thalamic lesions counted as discrete lesions



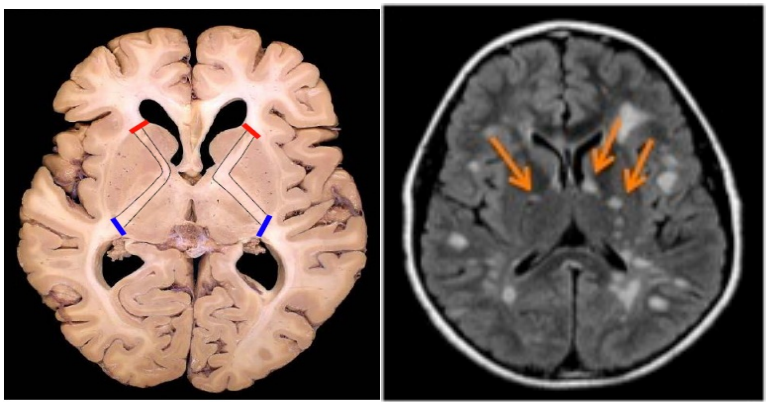
13. Basal ganglia lesion: T2 lesion either entirely or partially contained within the caudate (includes head and tail), putamen, or globus pallidus (includes interna and externa)



14. Internal capsule lesion: T2 lesion centered in the anterior or posterior limb of the internal capsule, defined as the supratentorial white matter bounded laterally by the lentiform nuclei and medially by the caudate and thalami (image below, adapted from[92])

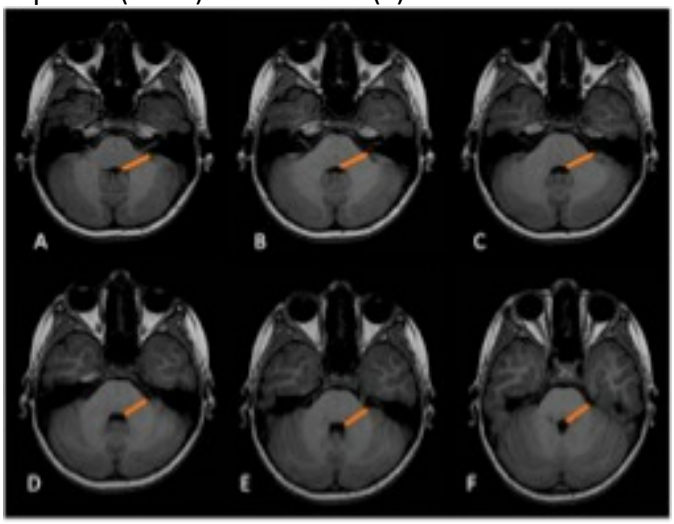
Anterior margin of anterior limb: line drawn from lateral margin of lateral ventricle to the anterolateral aspect of lentiform nucleus. Posterior margin of posterior limb: line drawn from

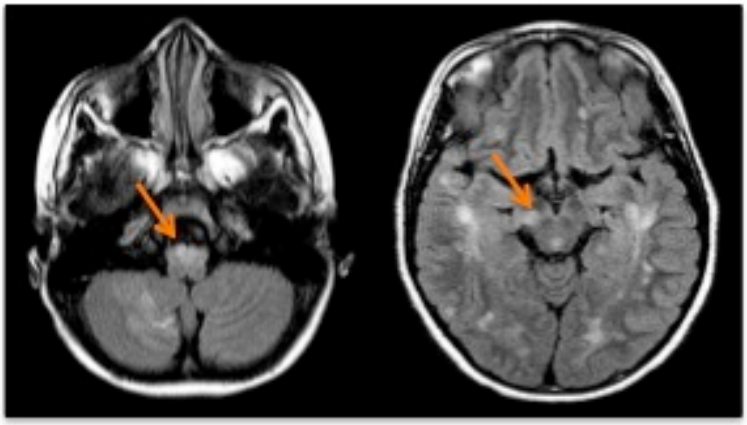
medial margin of lateral ventricle to posterolateral aspect of lentiform nucleus, extrapolated from its location of the most superior axial image in which lateral ventricles are visualized



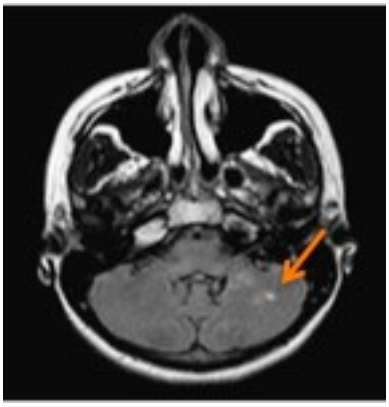
15. Brainstem lesion: T2 lesion within the brainstem which extends from the most inferior aspect of the medulla oblongata (at the level of the decussation of the pyramids) to the most superior portion of the midbrain (at the level of the red nuclei); posterior limits of the brainstem defined below.

Limit between brainstem and cerebellum defined by a line drawn from the lateral groove of the facial colliculus to the deepest (most posteriorly extending) aspect of the cerebellopontine angle, where cranial nerves 7 and 8 emerge from the pons (D & E). This line is extrapolated to superior (A – C) and inferior (F) axial slices.





16. Cerebellar lesion: T2 lesion involving the white matter or grey matter of any of the following: cerebellar white matter and cortices, dentate nuclei, vermis, flocculus or nodulus; anterior limits of the cerebellum defined in image above (#15)



Appendix 3: Frequency of excluded MRI parameters as a factor of diagnosis (Chapter 5)

	ADEM (n=16)		MS (n=27)		SV-cPACNS (n=12)		p-value	
	Rater 1	**Rater 2**	**Rater 1**	**Rater 2**	**Rater 1**	**Rater 2**	**Rater 1**	**Rater 2**
Frontal Lobar Location	12 (75)	14 (88)	25 (92)	21 (78)	7 (58)	7 (58)	0.038	0.340
Temporal Lobar Location	9 (56)	11 (69)	13 (48)	14 (52)	3 (25)	3 (25)	0.246	0.151
Parietal Lobar Location	12 (75)	13 (81)	18 (67)	21 (78)	3 (25)	3 (25)	0.020	0.003
Occipital Lobar Location	6 (38)	9 (56)	10 (37)	11 (41)	1 (8)	1 (8)	0.191	0.060
ACA Vascular Territory	9 (56)	10 (63)	14 (52)	13 (48)	4 (33)	6 (50)	0.5	0.930
MCA Vascular Territory	15 (94)	13 (81)	25 (93)	23 (85)	8 (67)	8 (67)	0.06	0.263
PCA Vascular Territory	11 (69)	10 (63)	17 (63)	19 (70)	3 (25)	2 (17)	0.05	0.005
Vertebrobasilar Vascular Territory	12 (75)	11 (69)	16 (59)	15 (56)	4 (33)	3 (25)	0.09	0.128
Cerebellar Peduncle	7 (44)	7 (44)	9 (33)	10 (37)	1 (8)	0	0.124	0.028
Target Lesion	0	1 (6)	10 (38)	12 (46)	0	1 (8)	0.002*	0.016
Nodular Leptomeningeal Enhancement§	0	0	0	0	0	0	--	--
Linear Leptomeningeal Enhancement§	1 (100)	2 (100)	2 (100)	1 (100)	2 (100)	1 (100)	--	--
Dural Enhancement¶	0	1 (9)	0	0	0	0	--	0.508
Supratentorial Lesion Enhancement¶	3 (27)	5 (45)	12 (55)	12 (55)	4 (40)	4 (40)	0.370	0.841
Infratentorial Lesion Enhancement¶	1 (9)	2 (18)	7 (32)	8 (36)	0	1 (10)	0.080	0.532

ACA, anterior cerebral artery; MCA, middle cerebral artery; PCA, posterior cerebral artery.

Numbers represent n (%). Frequencies of each parameter are compared within each rater separately, as a factor of diagnosis. Adjusting for multiple comparisons, significance was defined as $p<0.003$. §Leptomeningeal enhancement was present in 5 children (1 ADEM, 2 MS, 2 SV-cPACNS) according to Rater 1 and 4 children (2 ADEM, 1 MS, 1 SV-cPACNS) according to Rater 2. ¶Gadolinium was administered in 11 children with ADEM, 22 with MS and 10 with SV-cPACNS. *Although significant after correcting for multiple comparisons, the panel agreed to exclude 'Target Lesions' due to lack of agreement on its definition.

Appendix 4: Empirical rationale for parameters of the MRI scoring tool (Chapter 5)

1. **Lesion count**: T2 lesion count is a necessary component in evaluating dissemination in space criteria (DIS) for MS diagnosis. At least nine T2 lesions are required to meet dissemination in space according to the 2001 and 2005 McDonald criteria.[89,101] This requirement was recently decreased to one or more (clinically-silent) lesions in two of the following regions: periventricular, juxtacortical, infratentorial and spinal cord.[90] MRI criteria for DIS specific for pediatric-onset MS require at least five T2 lesions.[92]
2. **Bilateral lesion distribution:** Unilateral MRI abnormalities are less common in demyelination and may be more common in vascular occlusive disease or malignancy.
3. **Gyral projection**: Gyral Projection may be a less subjective term to describe the large, confluent lesions seen in children with ADEM[106] or very young children presenting with a first attack of MS.[86]
4. **T1 Hypointensity**: T1 hypointense non-enhancing lesions, termed “black holes”, have been associated with focal areas of chronic tissue damage on histopathology.[85]. The presence of T1 hypointense lesions predict MS in children with acute demyelination,[50] and distinguish MS from ADEM in children.[93]
5. **Lesion contrast enhancement**: The presence of asymptomatic contrast-enhancing lesions, when present simultaneously with non-enhancing T2 lesions, fulfills current McDonald criteria for dissemination in time.[90]

6. **Leptomeningeal contrast enhancement**: Leptomeningeal enhancement may be present in small-vessel primary angiitis of the CNS,[265] in CNS infections[279,280] and in neoplasm,[281-283] but is not a feature of CNS demyelination.
7. **Diffusion restriction**: The presence of decreased diffusion supports the diagnosis of an arterial ischemic event,[284-286] where the clinical presentation may mimic that of acute CNS demyelination.
8. **Periventricular lesion**: Presence of periventricular lesions is an important aspect in pediatric MS diagnostic criteria[92] and the McDonald criteria for dissemination in space.[89,90,101] Children with acute CNS demyelination who have one or more periventricular lesions on MRI are at high risk for MS diagnosis.[50]
9. **Cerebral white matter lesion**: The presence of cerebral white matter lesions is included as a parameter to capture those lesions that are non-juxtacortical and non-periventricular; the panel deemed it useful in evaluating the overall T2 lesion burden.
10. **Juxtacortical lesion**: Presence of juxtacortical lesions is component of the McDonald criteria for DIS.[90](909}[101]
11. **Intracallosal lesion**: The presence of intracallosal lesions is highly specific for MS, but is less sensitive due to its low prevalence in children with MS, especially on MRI scans acquired at onset. This parameter may be thought of a similar to a lesion perpendicular to the long axis of the corpus callosum, a feature proposed by the French group as specific but not sensitive for pediatric-onset MS.[91]
12. **Thalamic lesion**: Thalamic lesions are frequently reported in children with ADEM.[106,343]

13. **Basal ganglia lesion**: Basal ganglia lesions are less typically seen in children with MS, and when present may suggest another diagnosis, such as ADEM,[106,343] or metabolic or mitochondrial disorders.[344-350]
14. **Internal capsule lesion**: Lesions involving the internal capsule were described on MRI scans of children with established MS,[92] but may be less prevalent in children with MS at the time of onset.
15. **Brainstem lesion**: Brainstem lesions are more frequently observed in children with MS compared to adult patients,[109] This parameter is an important component of the pediatric MS criteria for DIS[92] and McDonald criteria for DIS[89,90,101]
16. **Cerebellar lesion**: Cerebellar lesions occur in both pediatric- and adult-onset MS patients, however, at a greater frequency in children compared to adults.[109]

Appendix 5: Sequence parameters of standardized MRI protocol (Chapter 6)

	T1w pre- & post-contrast¶	**PD**	**T2w**	**FLAIR**
Sequence	3D RF-SPGR	TSE	TSE	2D multi-slice turbo-IR
Orientation	axial	axial	axial	axial†
Matrix (frequency x phase)	256 x 192	256 x 256	256 x 256	256 x 192
FOV: AP x LR (mm)	250 x 187.5	250 x 250	250 x 250	250 x 187.5
Slice Thickness (mm)	3	3	3	3
TR (ms)	30	2200-2700*	4000-5420*	9000
TE (ms)	8-11	10-15	80-85	66
TI (ms)				2500
ETL		3	7-8	9
Flip Angle (^{o})	30	90	90	

SPGR, spoiled gradient-recalled echo; PD, proton density; T2w, T2-weighted; FLAIR, fluid attenuated inversion recovery; TSE, turbo spin-echo; IR, inversion recovery; FOV, field of view; TR, repetition time; TE, echo time; TI, inversion time; ETL, echo train length. ¶Magnevist [Bayer], Gadopentetate Dimeglumine, 0.2ml/kg [0.1mmol/kg] to a maximum dose of 10 ml; time between contrast injection and sequence acquisition: 10 minutes. *Manufacturer-dependent. †Sagittal FLAIR also acquired with matrix: 256 x 256, FOV: inferior-superior 250mm x anterior-posterior 250mm.

Appendix 6: Summary of studies on MRI features and predictors of MS in children (Chapter 6)

Systematic Review A literature search was conducted in PubMed, and limited to articles published in English between January 1, 1980 and September 1, 2011. Search terms were "multiple sclerosis" OR "MS", "pediatrics", "magnetic resonance imaging" OR "MRI", "predictors", "diagnostic criteria", and "sensitivity and specificity". Manuscripts describing MRI features of children with established MS, in addition to those with incident CNS demyelination, were included provided that the data included analysis of conventional MRI. References in the original articles and published reviews were also assessed. Only original manuscripts were included; case reports were excluded. For each article included, we summarize the study design, outcome of interest, and key findings in the subsequent table.

Interpretation The published research on MRI diagnostic criteria for MS or MRI features that predict subsequent MS diagnosis or severity is summarized in the table below, along with a brief commentary on the relation between such studies and the current report. Several cross-sectional and case-control analyses explored performance of published MRI criteria in cohorts of children with acute demyelination or established MS. Published MRI criteria have also been evaluated as correlates of early relapse and MS severity. Case-control studies, performed by our group on a pediatric cohort distinct from the present study population, have identified MRI features that aid in the differentiation of MS from non-demyelinating disease and acute disseminated encephalomyelitis. We report a national incident study of acute demyelination in the pediatric population in which we have optimized a consistent MRI acquisition protocol at all

participating centers and developed a standardized MRI scoring tool to evaluate parameters predictive of a future MS diagnosis – two important unique components of our work. The MRI predictors of MS diagnosis proposed in our study can be easily adjudicated by clinicians. We provide new information on the predictive utility of T1-hypointense lesions for MS diagnosis, a finding that may be of interest not only to those caring for pediatric patients but also potentially for prediction of adult-onset MS.

Author (Year)	No. of patients No. of controls	Design	Population	Outcome	Key Findings
Kurne[351] (2010)	30 patients	Cross-sectional	MS	Application of published MRI criteria to pediatric patients with MS	McDonald DIS[89] 25/30 (83%) McDonald DIT[89] 30/30 (100%) KIDMUS[91] 24/30 (80%)
Neuteboom[262] (2010)	28 patients	Retrospective cohort	MS	Clinical variables and published MRI criteria as predictors of early MS relapse	≥3 McDonald DIS criteria,[89] ≥3 periventricular lesions or lesion(s) perpendicular to the corpus callosum[91] associated with early relapse
Ketelslegers[103] (2010)	28 MS patients 21 ADEM controls	Case-control	MS ADEM	Application of published MRI criteria to distinguish MS from ADEM	Pediatric MS-ADEM criteria[93] best at differentiating 1st attack of MS from ADEM; Se 75%, Sp 95%, PPV 96%, NPV 74%
Callen[93] (2009)	28 MS patients 20 ADEM controls	Case-control	MS ADEM	Develop MRI criteria to distinguish MS from ADEM	2 of: absence of diffuse bilateral pattern, ≥2 periventricular lesions, black hole(s); Se 81%, Sp 95%, PPV 95%, NPV 79%
Callen[92] (2009)	38 MS patients 45 controls	Case-control	MS Migraine SLE	Develop MRI criteria to distinguish MS from OND	2 of: ≥5 T2 lesions, ≥2 periventricular lesion, ≥1 brainstem lesion(s); Se 90%, Sp 98%, PPV 97%, NPV 90%

Neuteboom[102] (2008)	117 patients	Retrospective cohort	MS ADS	Associations between MS outcome and prognostic factors at ADS	Children meeting MRI criteria more likely to be diagnosed with MS¶ McDonald DIS[89]: Se 60%, Sp 92%, PPV 81%, NPV 83% KIDMUS[91]: Se 49%, Sp 96%, PPV 83%, NPV 81%
Mikaeloff[302] (2006)	197 patients	Prospective cohort	MS	Clinical, CSF, and MRI predictors of MS severity	Severity risk higher in females, and in patients with time between 1st and 2nd attack ≤1 year, KIDMUS criteria[91] (HR 1.89, 95% CI 1.26-2.85), no change in mental status, or progressive course.
Dale[352] (2007)	40 patients	Prospective cohort	ADS	MRI and OCB predictors of MS following ADS	53% of MS patients met KIDMUS criteria[91]¶
Mikaeloff[57] (2007)	132 patients	Prospective cohort	ADEM	MRI and clinical predictors of MS outcome in patients with ADEM	ON, family history of MS, McDonald DIS criteria[89] (HR 2.52, 95% CI 1.04-6.12), and no sequelae after first attack associated with MS
Mikaeloff[51] (2004)	296 patients	Prospective cohort	ADS	Clinical, CSF, and MRI features associated with MS diagnosis	Rate of MS higher in patients ≥10 years of age at onset, with initial MRI suggestive of MS* (HR 1.54, 95% CI 1.02-2.33), and in those with ON lesion
Mikaeloff[91] (2004)	116 patients	Prospective cohort	ADS	MRI predictors of MS and	Lesion(s) perpendicular to corpus callosum: HR 2.89, 95% CI 1.65-5.06; solely

				severe disability following ADS	well defined lesions: HR 1.71, 95% CI 1.29-2.27; Se 21%, Sp 100%, PPV 100%, NPV 61%; No MRI features predicted disability
Balassy[82] (2001)	4 patients	Retrospective cohort	MS	Characterization of MRI features	Mean lesion count was 12.25; 16-30% of all lesions were infratentorial
Sindern[353] (1992)	31 early-onset patients 72 adult-onset patients	Retrospective cohort	MS	Characterization of clinical, MRI and OCB features at onset	Similar frequency of MRI abnormalities in early-onset (23/28, 82%) and adult-onset (60/67, 89%) patients
Verhey[50] (2011)	284 patients	Prospective cohort	ADS	MRI predictors of MS following ADS	≥1 periventricular and ≥1 T1 hypointense lesion predict MS HR 34.27, 95% CI 16.69-70.38; Se 84%, Sp 93%, PPV 76%, NPV 96%

HR, hazard ratio; CI, confidence interval; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; SLE, systemic lupus erythematosus; OND, other non-demyelinating neurologic disease; CSF, cerebrospinal fluid. ¶The authors did not specify the number of children with normal brain MRI.*Authors defined “MS-suggestive MRI” as a scan acquired at incident demyelination showing well-limited multiple lesions in juxtacortical and/or subcortical locations, according to Paty (1988).[300]

Appendix 7: Demographic and clinical characteristics of excluded patients, n=28 (Chapter 7)

Patient Number	Group	Reason for Exclusion	Sex	ADS	Age at Onset, years	Diagnosis
1	Prospective	Failed QC	F	ADEM	3.9	Monophasic ADS
2	Prospective	No MRI scan	F	TM	3.3	Monophasic ADS
3	Prospective	Failed QC	F	Polyfocal	12.4	Monophasic ADS
4	Prospective	Failed QC	M	ON	10.3	Monophasic ADS
5	Prospective	No T1 scan	F	ADEM	8.9	Monophasic ADS
6	Prospective	Failed QC	F	ON	14.6	MS
7	Prospective	Failed QC	F	Polyfocal	16.8	MS
8	Prospective	Failed QC	M	ADEM	2.8	Monophasic ADS
9	Prospective	Failed QC	M	ADEM	3.5	Monophasic ADS
10	Prospective	Failed QC	F	ON	16.3	MS
11	Prospective	No T1 scan	F	Monofocal	13.7	Monophasic ADS
12	Prospective	No MRI scan	M	TM	12.5	MS
13	Archived	Failed QC	M	Polyfocal	7.2	Relapsing ADS*
14	Archived	No MRI scan	F	Monofocal	14.8	Monophasic ADS
15	Archived	Failed QC	M	ADEM	4.3	Monophasic ADS
16	Archived	No MRI scan	M	TM	8.1	Monophasic ADS
17	Archived	Failed QC	M	TM	3.6	Monophasic ADS
18	Archived	Failed QC	M	Polyfocal	5.9	Monophasic ADS
19	Archived	Failed QC	M	ADEM	7.6	Monophasic ADS
20	Archived	Failed QC	M	TM	6.8	MS
21	Archived	Failed QC	F	ADEM	4.2	Monophasic ADS
22	Archived	Failed QC	F	Polyfocal	12.4	MS
23	Archived	No MRI scan	F	TM	15.4	Monophasic ADS
24	Archived	Failed QC	M	Monofocal	8.1	Monophasic ADS
25	Archived	Failed QC	M	ADEM	4.3	Monophasic ADS
26	Archived	Failed QC	M	Monofocal	13.0	Monophasic ADS
27	Archived	Failed QC	M	Polyfocal	5.7	Monophasic ADS
28	Archived	Failed QC	M	Polyfocal	10.9	Monophasic ADS
Summary Statistics			**F: 12 (43%)**	**Non-ADEM: 21 (75%)**	**Mean (SD): 9.0 (4.5)**	**MS: 6 (21%)**

QC, quality control; F, female; M, male. *This child presented with an “ADEM-like” incident demyelinating episode, and subsequently has had multiple episodes of optic neuritis; brain MRI does not meet criteria for multiple sclerosis. The child has been treated with intravenous

immunoglobulins, and has not experienced further clinical or MRI evidence of relapse. Until present, the child does not meet criteria for neuromyelitis optica.

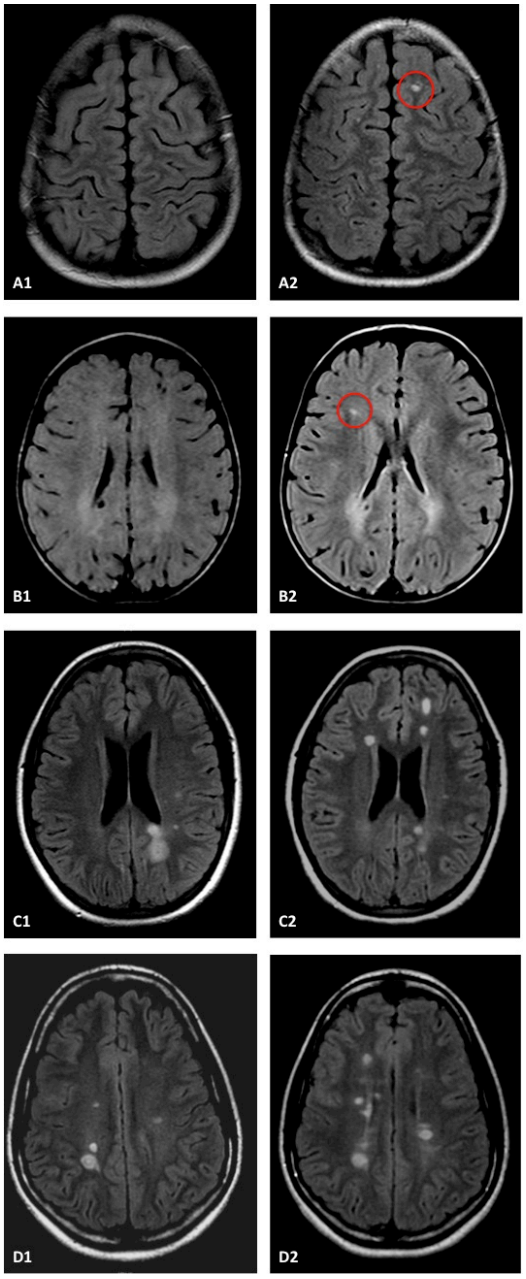
Appendix 8: Clinical and MRI features of children meeting 2010 McDonald criteria but with no second attack (Chapter 8)

Patient	Age at ADS (yrs)	Sex	ADS	Final Dx	Length of follow-up (yrs)	# of scans	Clinical DIS at baseline*	2010 DIS at baseline	2010 DIS on serial scans	2010 DIT at baseline	2010 DIT on serial scans	2005 +ve
1	5.3	F	ADEM	SV-pACNS	1.23	2	N/A	No	Yes	No	Yes	Yes
2	3.3	M	ADEM	Relapsing non-MS	4.12	3	N/A	Yes	Yes	Yes	Yes	No
3	15.8	M	ADEM	Mono-ADS	4.10	4	N/A	Yes	Yes	Yes	No	No
4	2.9	M	ADEM	Mono-ADS	3.14	4	N/A	No	Yes	No	Yes	No
5	8.2	M	ADEM	Mono-ADS	4.33	4	N/A	Yes	Yes	N/A¶	Yes	Yes
6	1.1	F	ADEM	Mono-ADS	3.25	3	N/A	Yes	Yes	No	Yes	Yes
7	3	M	ADEM	Mono-ADS	5.91	2	N/A	Yes	Yes	Yes	No	N/A†
8	2.8	M	ADEM	Mono-ADS	4.32	4	N/A	Yes	Yes	Yes	No	No
9	12.5	F	ADEM	Mono-ADS	4.05	5	N/A	Yes	Yes	No	Yes	No
10	2.2	F	ADEM	Mono-ADS	3.38	2	N/A	Yes	Yes	Yes	No	N/A†
11	13	F	ON	Mono-ADS	5.83	5	No	No	Yes	N/A§	Yes	Yes
12	15	M	ON	Mono-ADS	3.53	5	No	Yes	Yes	Yes	Yes	No
13	8.6	F	TM	Mono-ADS	3.34	2	No	Yes	Yes	Yes	No	N/A†
14¶	9	M	ON	Relapsing non-MS	4.64	8	No	Yes	Yes	No	No¶	Yes
15	11.4	F	ON	Mono-ADS	3.97	6	No	Yes	Yes	Yes	Yes	No
16	10.6	F	ON	Recurrent ON	5.36	8	No	No	Yes	No	Yes	No
17	12.2	F	ON	NMO	3.65	10	No	No	Yes	No	Yes	Yes
18	14.9	M	Polyfocal	Mono-ADS	6.17	3	Yes	No	Yes	Yes	Yes	Yes

19	13	M	Polyfocal	Mono-ADS	3.72	6	Yes	Yes	Yes	No	Yes	Yes
20	14.6	F	Polyfocal	Mono-ADS	2.66	5	Yes	Yes	Yes	No	Yes	Yes
21	11.9	F	Polyfocal	Mono-ADS	3.92	6	Yes	No	Yes	No	Yes	Yes
22	6.9	F	Polyfocal	Mono-ADS	5.58	3	Yes	Yes	Yes	Yes	Yes	No
23	9.6	M	Polyfocal	Mono-ADS	4.32	6	Yes	No	Yes	Yes	No	No
24	15.2	F	Polyfocal	SV-pACNS	4.67	13	Yes	Yes	Yes	No	Yes	Yes

F, female; M, male; Dx, diagnosis; SV-pACNS, small vessel primary angiitis of the CNS; Mono-ADS, monophasic ADS; NMO, neuromyelitis optica. *Clinical DIS was not adjudicated at baseline in children with ADEM owing to the inherent requirement for polyfocal deficits in the definition of ADEM. ¶Scoring for MRI DIT on baseline scan was not possible as gadolinium was not administered. §This patient did not met MRI DIT criteria, however did meet clinical criteria for DIT because of the occurrence of a second episode of ON. †Only two MRI scans appropriate for analysis, precluded evaluation of the 2005 criteria.

Appendix 9: New lesion accrual in children with monophasic ADEM compared to that of children with MS (Chapter 8)



The figure contrasts the MRI appearance of new lesions detected on serial scans (Time point 1=Column 1; Time point 2=Column 2) in two children with ADEM at onset (A and B), with the appearance of new lesions in two children diagnosed with clinically defined MS (C and D).

Appendix 10: Clinical features of children diagnosed with non-demyelinating diseases or neuromyelitis optica (Chapter 8)

Patient	ADS	Final Dx	Enrolled prior to 2009	# scans	Clinical DIS at baseline	2010 DIS at baseline	2010 DIS on serial scans	2010 DIT at baseline	2010 DIT on serial scans	2005 DIS	2005 DIT	2010 +ve	2005 +ve
1	ON	NMO	Yes	11	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes
2	ADEM	NMO	No	1	N/A*	Yes	N/A¶	Yes	N/A¶	No	N/A§	Yes	N/A§
3	Polyfocal	SV-pACNS	Yes	14	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
4	ADEM	SV-pACNS	Yes	2	N/A*	Yes	Yes	No	Yes	Yes	N/A§	Yes	Yes
5	ADEM	Viral enceph-alitis	No	1	N/A*	Yes	N/A¶	Yes	NA	Yes	N/A§	Yes	N/A§
6	ADEM	SV-pACNS	Yes	6	N/A*	No	No	No	No	No	No	No	No
7	ADEM	CMV	No	3	N/A*	Yes	Yes	No	No	Yes	No	No	No
8	ADEM	Neoplasm	Yes	1	N/A*	No	N/A¶	No	N/A¶	No	N/A§	No	N/A§
9	ADEM	Sarcoid-osis	Yes	3	N/A*	No	No	No	No	No	No	No	No
10	ADEM	Neoplasm	Yes	9	N/A*	No	No	No	No	No	No	No	No
11	ADEM	Stroke	Yes	3	N/A*	No	No	No	No	No	No	No	No
12	ADEM	Mitochon-drial	Yes	1	N/A*	No	N/A¶	No	N/A¶	No	N/A§	No	N/A§
13	Polyfocal	SV-pACNS	No	1	Yes	No	Yes	No	No	Yes	No	No	N/A§
14	TM	SLE	Yes	2	No	No	No	No	No	No	No	No	N/A§
15	TM	Lyme	Yes	8	No	No	No	No	No	No	No	No	No
16	ON	White dot syndrome	Yes	1	No	No	No	No	No	No	No	No	N/A§

17	ON	Mitofusin	Yes	1	No	No	N/A¶	No	N/A¶	No	N/A§	No	N/A§
18	Monofocal-other	Cerebel-litis	Yes	2	No	No	No	No	No	No	No	No	N/A§
19	Monofocal-other	Neoplasm	Yes	2	No	Yes	Yes	No	No	Yes	No	No	N/A§
20	Monofocal-other	Cerebel-litis	Yes	3	No	No	No	No	No	No	No	No	No

Dx, diagnosis; SV-pACNS, small vessel primary angiitis of the CNS; NMO, neuromyelitis optica; CMV, cytomegalovirus; SLE, system lupus erythematosus.*Clinical DIS was not adjudicated at baseline in children with ADEM owing to the inherent requirement for polyfocal deficits in the definition of ADEM. ¶Serial scans not obtained, and in one patient the baseline scan did not meet quality requirements. §The number of available images was not sufficient for evaluation of the 2005 or 2010 criteria.

Appendix 11: Comparison of published performance of McDonald DIS criteria in pediatric MS populations and in the present cohort (Chapter 8)

	Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**
Mikaeloff*[91]	52	63	53	38
Hahn¶[104]	53	--	--	--
Callen§[92]	76	100	100	85
Ketelslegers†[103]	61	91	60	63
Present cohort: 2005 McDonald criteria for DIS (first scan, ADEM excluded)	59	88	59	88
Present cohort: 2005 McDonald criteria for DIS‡ (first scan, ADEM excluded	78	81	54	93

*Cohort of 116 children with acute CNS demyelination. MRI at onset was analyzed using the DIS component of the 2001 McDonald criteria.[101] ¶Analysis of MRI scans from 20 children with CDMS. MRI scans acquired at onset were analyzed using the DIS component of the 2001 McDonald criteria;[101] only sensitivity evaluated. §Analysis of MRI scans from 38 children with CDMS and 45 children with non-demyelinating relapsing CNS disorders. MRI scans acquired at the second MS-defining clinical event were evaluated using the DIS component of the 2005 McDonald criteria.[89] †Analysis of 29 children with MS and 21 with non-relapsing ADEM. MRI acquired at onset was analyzed using the DIS component of the 2005 McDonald criteria.[89] ‡Defined as either 2005 MRI DIS criteria, or as 2 lesions plus presence of cerebrospinal fluid oligoclonal bands.

Appendix 12: Spinal cord MRI scoring tool (Chapter 9)

1. Lesion location: Assessed in the sagittal plane using the spinal vertebral bodies as landmark**;** lesion(s) scored as within one or more of the following regions: cervical, thoracic, lumbar.

2. Number of segments: Number of spinal vertebral body segments spanned by the lesion.

3. Lesion focality: Focal lesion(s) span <3 spinal vertebral body segments in length**;** longitudinally-extensive lesion(s) span ≥ 3 spinal vertebral body segments in length.

4. Intramedullary extent: Assessed in the axial plane**;** lesion(s) scored as involving one or more of the following intramedullary regions: anterior, central, posterior, complete, central h-shaped grey matter, anterior horn cells.

5. Presence of gadolinium-enhancement: Presence of gadolinium-enhancement corresponding to a lesion when cross-referenced with T1 pre-gadolinium and T2-weighted scans.

6. Presence of brain involvement: Presence of brain lesions on the brain MRI acquired at the time of spinal cord MRI scan.

Appendix 13: Publications arising from dissertation

Verhey LH, Branson HM, Laughlin S, Shroff MM, Benseler SM, Feldman BM, Streiner DL, Sled JG, Banwell B. Development of a standardized MRI scoring tool for CNS demyelination in children. *Am J Neuroradiol*, in press.

Verhey LH, van Pelt-Gravesteijn ED, Ketelslegers IA, Neuteboom RF, Catsman-Berrevoets CE, Feldman BM, Streiner DL, Sled JG, Hintzen RQ, Banwell B. Validation of MRI predictors of multiple sclerosis diagnosis in children with acute CNS demyelination. *Multiple Sclerosis and Related Disorders*, submitted.

Verhey LH, Shroff MM, Banwell B. Pediatric-onset multiple sclerosis. *Neuroimag Clin N Am*, in press.

Verhey LH, Sled JG. Advanced MRI in pediatric-onset multiple sclerosis. *Neuroimag Clin N Am*, in press.

Verhey LH, Narayanan S, Banwell B. Standardized MRI acquisition and reporting in pediatric-onset multiple sclerosis. *Neuroimag Clin N Am*, in press.

Sadaka Y, **Verhey LH**, Shroff MM, Branson HM, Arnold DL, Narayanan S, Sled JG, Bar-Or A, Sadovnick AD, McGowan M, Marrie RA, Banwell B, and the Canadian Pediatric Demyelinating Disease Network. 2010 McDonald criteria for diagnosing pediatric multiple sclerosis. *Ann Neurol* epub ahead of print, March 4, 2012 DOI: 10.1002/ana.23575.

Verhey LH, Branson HM, Shroff MM, Callen DJA, Sled JG, Narayanan S, Sadovnick AD, Bar-Or A, Arnold DL, Marrie RA, Banwell B, and the Canadian Pediatric Demyelinating Disease Network. MRI parameters for prediction of multiple sclerosis diagnosis in children with acute CNS demyelination: a prospective national cohort study. *Lancet Neurol* 2011;10:1065-1073.

Thomas T, Branson HM, **Verhey LH**, Shroff M, Stephens D, Magalhaes S, Banwell B. The demographic, clinical, and magnetic resonance imaging (MRI) features of transverse myelitis in children. *J Child Neurol* 2012;27:11-21.

Verhey LH, Branson HM, Makhija M, Magalhaes S, Shroff M, Banwell B. Magnetic resonance imaging features of the spinal cord in pediatric multiple sclerosis: a preliminary study. *Neuroradiology* 2010;52:1153-62.

Verhey LH, Banwell B. Inflammatory, vascular and infectious myelopathies in children. In: *Handbook of Neuropediatrics*. Editors: Dulac O, Sarnat H, Lassonde M. Elsevier, in press.