Acquired Demyelinating Syndromes

Focus on Neuromyelitis Optica and childhood-onset Multiple Sclerosis

Daniëlle van Pelt

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Acquired Demyelinating Syndromes:
Focus on Neuromyelitis Optica and childhood-onset Multiple Sclerosis

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focus op neuromyelitis optica en multiple sclerose op de kinderleeftijd

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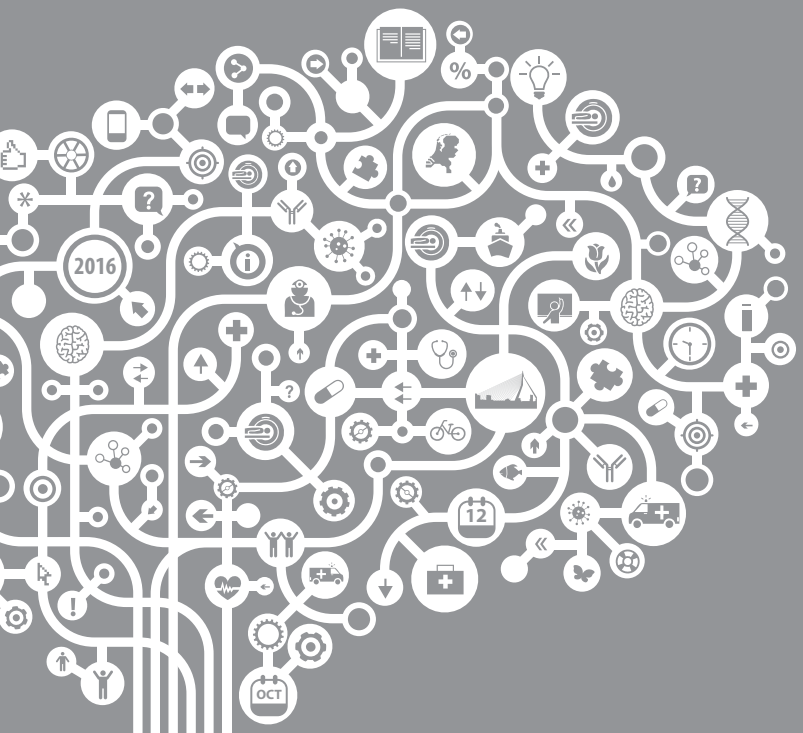
TABLE OF CONTENTS



Chapter 1

General introduction

ACQUIRED DEMYELINATING SYNDROMES OF THE CENTRAL NERVOUS SYSTEM

Acquired demyelinating syndromes (ADS) of the central nervous system (CNS) cover a broad spectrum of neurological symptoms caused by inflammation and damage to the myelin sheet.[1, 2] Multiple sclerosis (MS) is the most familiar diagnosis within this spectrum and most commonly affects young adults.[3] Previously, CNS demyelination was thought to be either one event (i.e. monophasic) or included an ongoing chronic relapsing disease course. The latter patients were subsequently diagnosed with MS.[4] In the past years it became clear that not all patients with relapsing CNS demyelination have MS, which has important therapeutic implications. Nowadays, the spectrum of ADS includes monophasic demyelinating events like: isolated optic neuritis (ON), transverse myelitis (TM), other clinically isolated syndromes (CIS), monophasic neuromyelitis optica spectrum disorders (NMOSD), and acute disseminated encephalomyelitis (ADEM).[1, 2, 5] In addition the spectrum includes more chronic and relapsing types of demyelination like: relapsing remitting MS[3], secondary progressive MS, primary progressive MS, multiphasic ADEM[6], ADEM followed by recurrent optic neuritis (ADEM-ON)[7] and relapsing NMOSD[5]. These syndromes overlap and therefore can be difficult to recognize and distinguish. In case of a first demyelinating event a careful patient history, physical examination and adequate diagnostics are of high importance in order to make the accurate diagnosis. First other causes should be excluded, like for example infectious diseases, metabolic disorders, and vascular diseases.[3, 8] The diagnostic process is complicated by the aspect of time, since it is difficult to identify the patients who will have a chronic and relapsing disease course at the first event. For example, optic neuritis can stay a single event (i.e.

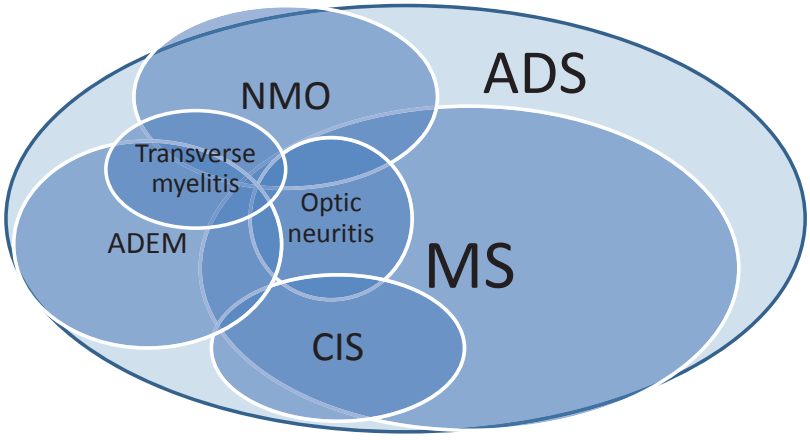


Figure 1.1 The spectrum of acquired demyelinating syndromes (ADS) of the central nervous system (CNS). *ADS = acquired demyelinating syndromes, ADEM = acute disseminated encephalomyelitis, CIS = clinically isolated syndrome, MS = multiple sclerosis, NMO = neuromyelitis optica.*

idiopathic ON) but also can occur in MS, NMOSD, chronic relapsing inflammatory optic neuropathy (CRION)[9], or in other autoimmune diseases with CNS involvement.[10] In other words, the group of ADS patients is heterogeneous and their clinical characteristics are dynamic. It can take several years, and in rare cases even decades, before a second and NMOSD or MS diagnostic event occurs.[5, 11] However, accurate and early diagnoses are of importance for the patient for establishing their prognosis and for the correct treatment initiation. Figure 1.1 illustrates the spectrum of ADS and its overlap.

The first part of this thesis on ADS focuses on NMOSD and describes the incidence and clinical features of NMOSD in the Netherlands. The second part, and main focus of this thesis, is on ADS in children. The goal of our studies is to reveal the clinical spectrum of ADS and to find diagnostic and prognostic markers, which allow for an early and safe diagnosis.

NEUROMYELITIS OPTICA SPECTRUM DISORDERS

Neuromyelitis optica (NMO), previously known as Devic's disease, is a rare variant of MS, characterized by optic neuritis and transverse myelitis.[5, 12] Classic NMO, as described by doctor Eugene Devic in 1894, was a monophasic illness including coincident bilateral optic neuritis and transverse myelitis.[13] However, in current literature there are clues that earlier cases of NMO have been described.[14] In the past years the spectrum of NMO has broadened and the nomenclature of Devic's disease became insufficient and outdated.[15] At present the unifying term neuromyelitis optica spectrum disorders (NMOSD) is used.

In 2004 an important milestone in the field of NMO was reached by the discovery of specific antibodies directed against aquaporin-4 (AQP4-IgG), which led to the distinction of MS.[16, 17] The identification of this specific antibody has led to the understanding of NMO pathophysiology as a B-cell mediated astrocytopathy, whereas the inflammatory response causes demyelination as collateral damage.[18] The timeline and growth in knowledge of NMOSD are illustrated in Figure 1.2. In 2006 the antibody was incorporated in the diagnostic criteria for NMO.[19] Since then diagnostic AQP4-IgG assays have been improved.[20] In the Netherlands the highly sensitive diagnostic AQP4-IgG cell-based assay (CBA) is performed at one centralized NMO expert center at Sanquin Diagnostic Services in Amsterdam.[21] Aquaporin-4 antibodies are present in the majority of NMOSD patients (±77%).[15] Serum AQP4 antibodies are highly specific for the disease.[22] The presence of AQP4-IgG allowed for a broadening of the clinical NMO spectrum including limited forms such as isolated or recurrent optic neuritis, transverse myelitis, brainstem syndromes (including area postrema syndrome with intractable nausea, vomiting and hiccups) and cerebral syndromes (including narcolepsy and acute diencephalic clinical syndrome).[5, 15] NMOSD diagnosis is based on clinical characteristics supported by AQP4-IgG status and MRI findings.[15] Typical MRI findings in NMO are a longitudinally extensive

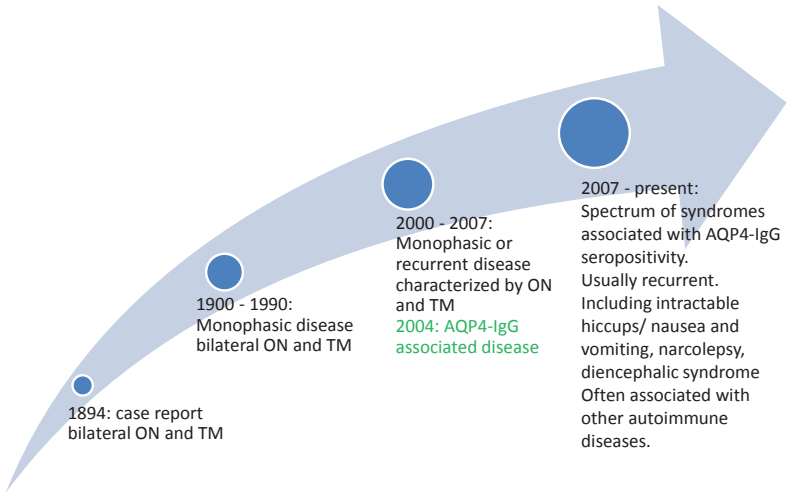


Figure 1.2 Timeline and growth in knowledge of NMOSD, from the case report of doctor Eugene Devic in 1894 until present.This figure is adapted from the teaching course 'Diagnostic criteria for NMO 2014: update' presented by BG Weinshenker at the 30th Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), 2014 Boston.

transverse myelitis (LETM) exceeding three or more contiguous vertebra.[19, 23] However short, sometimes even asymptomatic, spinal cord lesions can occur in a minority of NMOSD patients and might delay NMOSD diagnosis and treatment initiation.[24, 25] MRI brain lesions occur in up to 70% of the NMOSD patients typically, but not solely, at sites with high aquaporin-4 expression.[26] Current diagnostic criteria for NMOSD are presented in Table 1.1.[15] Prior to NMOSD diagnosis other causes for the symptoms including MS, infectious diseases, or systemic autoimmune disease like sarcoidosis, vasculitis or systemic lupus erythematosus (SLE), should be excluded. In patients with NMOSD a remarkable high rate of other autoimmune diseases has been observed, including thyroid diseases, SLE or myasthenia gravis[27], which can cause a diagnostic dilemma.[28, 29] Interestingly, AQP4-IgG can be present years before the first NMOSD attack, as described in patients with SLE.[30] However, clinical characteristics of NMOSD patients with and without other autoimmune diseases seem similar.[31]

NMOSD represent a group of rare autoimmune diseases: worldwide reported NMO incidence rates range from 0.05-0.4 per 100,000 people.[32] Exact incidence figures of NMOSD in the Netherlands were previously unknown. In most cases the disease has a relapsing course.[5] NMO is not a genetic disease, although the high rate of autoimmune comorbidity in NMO patients and their families reflects a genetic predilection for autoimmunity and amplified immune response.[12] NMO frequently has been misdiagnosed as MS.[5] In Table 1.2 definitions and characteristics, which can help to differentiate NMO from MS, are presented.[5, 12, 15] The distinction of NMO from MS is of utmost importance for

Table 1.1 Neuromyelitis optica spectrum disorders (NMOSD) diagnostic criteria (as defined by Wingerchuk et al. 2015).[15]

NMOSD with AQP4-IgG	1. At least 1 core clinical characteristic 2. Positive test for AQP4-IgG 3. Exclusion of alternative diagnoses
NMOSD without AQP4-IgG **or** **NMOSD with unknown AQP4-IgG status**	1. At least 2 core clinical characteristics occurring as a result of one or more clinical attacks and meeting all of the following requirements: a. At least 1 core clinical characteristic must be optic neuritis, or acute myelitis with LETM, or area postrema syndrome b. Dissemination in space (2 or more different core clinical characteristics) c. Fulfillment of additional MRI requirements, as applicable 2. Negative tests for AQP4-IgG, or testing unavailable 3. Exclusion of alternative diagnoses
Core clinical characteristics	1. Optic neuritis (ON) 2. Acute myelitis 3. Area postrema syndrome: episode of otherwise unexplained hiccups or nausea and vomiting 4. Acute brainstem syndrome 5. Symptomatic narcolepsy or acute diencephalic clinical syndrome with NMOSD-typical diencephalic MRI lesions 6. Symptomatic cerebral syndrome with NMOSD-typical brain lesions
Additional MRI requirements for NMOSD without AQP4-IgG and NMOSD with unknown AQP4-IgG status	1. Acute ON: requires brain MRI showing (a) normal findings or only nonspecific white matter lesions, or (b) optic nerve MRI with T2-hyperintense lesion or T1-weighted gadolinium enhancing lesions extending over > ½ optic nerve length or involving the chiasm. 2. Acute myelitis: requires associated intramedullary MRI lesion extending over ≥3 contiguous segments (LETM), or ≥3 contiguous segments of focal spinal cord atrophy in patients with history compatible with acute myelitis 3. Area postrema syndrome: requires associated dorsal medulla/area postrema lesions 4. Acute brainstem syndrome: requires associated periependymal brainstem lesions

establishing the prognosis and initiating the correct treatment. The treatment of attacks and chronic treatment differ significantly between MS and NMOSD. In NMOSD relapses are more severe than in MS and cause deterioration and progression of disability.[23, 33, 34] Acute NMOSD relapses should be urgently treated with high dose intravenous methylprednisolone in order to minimise disability.[35-37] If corticosteroid therapy is insufficient patients can benefit from plasmapheresis. Escalation of the acute therapy improved the outcome of NMOSD patients in a large German cohort and decreased the proportion of non-responders.[38] Preferably relapses and thus further deterioration are prevented. Therefore immunosuppressive treatment is indicated in relapsing NMOSD patients.[35-37] AQP4-IgG seropositive patients are at high risk for future relapses and therefore chronic treatment is advised in these patients after the first event. MS therapeutics can be potentially harmful for NMOSD patients and should be avoided.[39-42] Immunosuppressive therapy with azathioprine or mycophenolate mofetil is advised in NMOSD patients.[35-37, 43]

Table 1.2 Definitions and characteristics of MS and NMOSD (based on: Wingerchuk et al. 2007[5], Pittock et al. 2015[12], Wingerchuk et al. 2015.[15]

	MS	NMOSD
Definition	CNS symptoms and signs that indicate the involvement of the white matter tracts Evidence of dissemination in space and time based on clinical or MRI findings No other explanation	Core clinical characteristics (i.e. ON, TM, area postrema syndrome, acute brainstem syndrome, narcolepsy, diencephalic syndrome, other cerebral syndrome) Supported by typical MRI findings and/ or AQP4-IgG seropositivity No other explanation
Clinical course	85% relapsing remitting 15% primary progressive Not monophasic	80-90% relapsing course 10-20% monophasic course
Median age of onset (years)	29	39
Sex (F:M)	2:1	9:1
Secondary progressive course	Common	Rare
MRI brain	Periventricular white matter lesions	Usually normal or nonspecific white matter lesions; 10% unique hypothalamic, callosal, splenial, periventricular, periaqueductal, medullary, brainstem
MRI spinal cord	Short segmented peripheral lesions	Longitudinally extensive (≥3 vertebral segments) central lesions
CSF white blood cell number and differential count	Mild pleocytosis Mononuclear cells	Occasional prominent pleocytosis Polymorphonuclear cells and mononuclear cells
CSF oligoclonal bands	85%	15-30%
Coexisting autoimmune disease	Rare	Common: SLE, SS, MG, thyroid, APL
Attack prevention therapies	Interferon-β, glatiramer acetate, fingolimod, natalizumab	Azathioprine, mycophenolate mofetil, rituximab, mitoxantrone

APL = antiphospholipid syndrome, AQP4 = aquaporin-4, CNS = central nervous system, CSF = cerebrospinal fluid, MG = myasthenia gravis, MRI = magnetic resonance imaging, MS = multiple sclerosis, NMOSD = neuromyelitis optica spectrum disorders, ON = optic neuritis, SLE = systemic lupus erythematosus, SS = Sjogren's syndrome, TM = transverse myelitis.

In case of failure of the first-line treatments, rituximab should be considered. Future immunotherapeutic targets include complement proteins, the IL-6 receptor, neutrophils, eosinophils, CD19 and AQP4.[12, 36, 44]

At the Dutch NMO expert center, which includes Sanquin Diagnostic Services in Amsterdam and the NMO expert clinic at Erasmus MC in Rotterdam, we focus on the accurate diagnosis and treatment of NMOSD patients. The expert center is a referral center for clinicians in the Netherlands for the confirmation of NMOSD diagnosis and treatment advice.

ACQUIRED DEMYELINATING SYNDROMES IN CHILDHOOD

ADS also occur in children and, although rare, can evolve into childhood-onset MS.[8, 11] Diagnosing childhood-onset ADS can be challenging due to unfamiliarity of clinicians with these rare diseases. Furthermore, children can be too young to report their symptoms to their caregivers. Symptoms can be mild and usually are self-limiting and therefore a delay can occur before a clinician is consulted. Up to 10% of all MS patients have their first attack during childhood, prior to their 18th birthday.[8] In the Netherlands a nationwide prospective study was started in 2007 investigating ADS in children.[45] The main goal of the PROUDkids study (PRedicting the OUtcome of a Demyelinating event in children) is to identify prognostic factors, which predict a future MS diagnosis at the first event of ADS. Children with a first event of ADS and their families face their lives with uncertainty about their future.[46] For this reason, adequate counselling, early diagnosis and treatment initiation are of utmost importance. Reported incidence rates of pediatric ADS range from 0.66 per 100,000 in the Netherlands to 1.66 per 100,000

Table 1.3 IPMSSG consensus definitions for acquired demyelinating syndromes in children (as defined by Krupp et al. 2013).[51]

CIS Clinically Isolated Syndrome	• A first monofocal or multifocal CNS demyelinating event; encephalopathy is absent, unless due to fever
ADEM Acute Disseminated Encephalomyelitis	• A first polyfocal clinical CNS event with presumed inflammatory cause • Encephalopathy that cannot be explained by fever is present • MRI typically shows diffuse, poorly demarcated, large, >1–2 cm lesions involving predominantly the cerebral white matter; T1 hypointense white matter lesions are rare; Deep gray matter lesions (e.g. thalamus or basal ganglia) can be present • No new symptoms, signs or MRI findings after three months of the incident ADEM
Multiphasic ADEM	New event of ADEM three months or more after the initial event that can be associated with new or re-emergence of prior clinical and MRI findings. Timing in relation to steroids is no longer pertinent.
MS Multiple Sclerosis	Any of the following: • Two or more nonencephalopathic CNS clinical events separated by more than 30 days, involving more than one area of the CNS • Single clinical event and MRI features rely on 2010 Revised McDonald criteria[52] for DIS and DIT (but criteria relative for DIT for a single attack and single MRI only apply to children ≥12 years and only apply to cases without an ADEM onset) • ADEM followed three months later by a nonencephalopathic clinical event with new lesions on brain MRI consistent with MS
NMO Neuromyelitis Optica	All are required: • Optic neuritis • Acute myelitis At least two of three supportive criteria • Contiguous spinal cord MRI lesion ≥3 vertebral segments • Brain MRI not meeting diagnostic criteria for MS • AQP4-IgG seropositive status

DIS = dissemination in space, DIT = dissemination in time.

children per year in other cohorts.[1, 45, 47-49] Approximately 5 – 10 children are diagnosed with MS per year in the Netherlands. In 2007 the International Pediatric MS Study Group (IPMSSG) first developed diagnostic criteria for childhood-onset ADS.[50] In 2012 these criteria were revised based on the tremendous growth in research and gain in knowledge of childhood-onset ADS.[51] The current IPMSSG definitions of the various ADS subtypes are presented in Table 1.3.

A first demyelinating event in children can present with symptoms caused by a single lesion (monofocal) or by multiple lesions (polyfocal).[52] In the Dutch ADS cohort 22% of the children presented with ON, 24% with ADEM and 30% with polyfocal CIS without encephalopathy.[45] Any type of ADS can be the first presentation of MS in children. Based on previous cohort studies, it is estimated that 21-32% of children who presented with a first event of ADS will have a future diagnosis of MS.[53, 54] MS percentages differ between the various reported pediatric ADS cohorts.[53-56] This is partly explained by the introduction and revision of diagnostic criteria, differences in study design and referral bias.[52]

ADEM is a relatively common subtype of ADS, especially in young children.[6] The acute event of ADEM is often preceded by viral infections and characterized by polyfocal neurological deficits and encephalopathy. Encephalopathy, defined as behavioural changes and or alterations in consciousness not explained by fever[51], is not a typical feature of MS.[53] Children with ADEM usually have a good prognosis.[57, 58] A minority of children with ADEM have a severe deteriorating disease course, which can lead to ICU admission and sometimes death.[58, 59] MRI typically shows large poorly demarcated lesions in the white and gray matter (basal ganglia).[60, 61] The symptoms and MRI findings of ADEM can fluctuate within three months after the first onset of symptoms.[51, 62] Fluctuations within this time period are considered as part of one event.[51] ADEM usually is monophasic, but in rare cases a second event of ADEM can occur (i.e. multiphasic ADEM).[57, 63] A minority of children with ADEM are diagnosed with MS during follow-up.[51, 60] In the Dutch cohort 5 out of 92 children with ADEM (6%) converted to MS during follow-up.[58] However, one event of ADEM followed by a second non-ADEM event might still reflect a transient demyelinating disease. Therefore, children with ADEM have to fulfill strict criteria prior to MS diagnosis: i.e. ADEM should be followed by two non-encephalopathic events, or one new event with the appearance of new MS-specific MRI lesions fulfilling dissemination in time and space.[51] In addition, a disease course with isolated relapsing optic neuritis can occur after the first event of ADEM (ADEM-ON).[7]

NMO occurs approximately in 3% of all children with ADS.[45, 64] Children with NMO can have a very diverse clinical presentation with diffuse ADEM-like inflammatory lesions on first brain MRI.[64-67] In one study of 88 children with AQP4-IgG seropositive NMOSD, 45% had episodic cerebral manifestations including encephalopathy.[68] Since the clinical syndromes of ADEM and NMO can overlap in children, AQP4-IgG testing should be considered in children with an ADEM-like event including ON and LETM.[51, 64, 65] A recent

American study in 38 children with NMO demonstrated the NMOSD 2015 criteria apply well in the childhood setting (sensitivity 97%) and could diminish treatment delay in children with NMOSD.[69]

MS diagnosis in childhood

Prognostic factors for a future MS diagnosis in children are: female gender, age at onset ≥ 10 years old, onset of symptoms without encephalopathy, elevated IgG index and/or positive oligoclonal bands in cerebrospinal fluid (CSF), presence of MS-like lesions on MRI.[53-55] As in adults, children who had a clinical event of CNS demyelination supported by clinical and/or radiological evidence of dissemination in time and space can be diagnosed with MS.[51, 70] Alternative diagnoses should be excluded. The differential diagnosis of MS in children is much more complex than in adults and includes a long list of other diagnoses like CNS infectious diseases, neoplasms, leukodystrophy, and inflammatory diseases.[8, 71] Additional laboratory results and MRI can aid in the diagnostic workup and differentiation of ADS from other diseases. MRI is an important diagnostic tool in childhood-onset ADS by confirming demyelinating whiter matter lesions. In the past years several MRI criteria have been developed for early MS diagnosis and distinction from other ADS subtypes and alternative diagnoses in children.[61, 72, 73] MRI in MS patients typically shows T2 lesions in locations characteristic for MS: periventricular, juxtacortical, infratentorial and spinal cord.[70] Unique for the current 2010 McDonald diagnostic MRI criteria for adults is the opportunity to confirm MS diagnosis at the incident event. Several studies have confirmed that the 2010 McDonald MRI criteria, designed for adults,

Table 1.4 MRI criteria childhood-onset MS.

	2010 McDonald MRI criteria[51, 52]	Verhey criteria[61]
Original criteria	*Dissemination in space:* ≥ 1 lesion in 2 of 4 characteristic areas: - periventricular - juxtacortical - infratentorial - spinal cord *Dissemination in time:* New T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI or Simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions*	≥1 T1 hypointense lesion and ≥1 periventricular lesion
Purpose	Prognostic and diagnostic for MS in adults	Prognostic for MS in a childhood-onset ADS population
Sensitivity	100%[77]	84%[61]
Specificity	86%[77]	93%[61]

*at the first event this criterion applies to children ≥12 years old according to IPMSSG 2012 consensus definitions.[51]

apply well in pediatric populations and led to an earlier MS diagnosis.[56, 74-77] In a large Canadian cohort of 212 children with ADS the sensitivity of the 2010 criteria applied at baseline was 100%, the specificity 86%.[77] Compared with adults, children with MS have a higher number of total T2 lesions at disease onset.[78, 79] In Table 1.4 the most recent MRI criteria for childhood-onset MS are presented.

CSF analysis can be supportive for the MS diagnosis and for the exclusion of other diagnoses.[80] CSF oligoclonal bands (OCB) are detected in 92% of pediatric MS patients. In contrast, CSF OCB are uncommon in children with ADEM.[57] In a large German cohort including 357 children with isolated optic neuritis the presence of CSF OCB at onset was an independent predictor of MS diagnosis.[81]

Course and prognosis of childhood-onset MS

Children with MS have more frequent relapses and more severe attacks than adults with MS.[82-84] A primary progressive course (PP-MS) is extremely rare in children with MS.[83, 85] In case PP-MS is suspected in children, clinicians should carefully consider alternative diagnoses.[8, 11] Despite a relatively higher relapse rate, disease progression is slower in children than in adults with MS.[85, 86] A possible explanation for this could be that the developing CNS of childhood-onset MS patients has more plasticity to recover.[85] However, children with MS reach states of irreversible disability at a younger age, approximately 10 years earlier than adults with MS.[86, 87] Indicating that there is an endpoint in the plasticity of the CNS. Clinical symptoms of children with MS can be comparable with adults, but cognitive dysfunction and fatigue occur probably more frequently in children than in adults.[88-93] Cognitive impairment is reported in nearly one-third of the children with MS and their cognitive outcome is heterogeneous.[90]

Risk factors in childhood-onset MS

MS is a complex multifactorial disease in which environmental and genetic factors interact.[3, 94] However, the exact cause of MS is unknown. First degree relatives of a patient with MS have an increased MS risk of 2-5%.[94] MS risk in monozygotic twins is approximately 25% and not 100% as would be expected if MS solely was a genetic disease.[94, 95] The presence of the major *HLA-DRB1*15* MS risk allele increases MS risk in children.[53, 96, 97] It was shown in a large Canadian pediatric ADS cohort that children harbouring one or two *HLA-DRB1*15* alleles were more likely to be diagnosed with MS than children with ADS lacking *HLA-DRB1*15* alleles.[97] Large international MS genome-wide association studies (GWAS) have been performed in search of novel genetic variants in adults.[98, 99] Up to now 200 MS risk single nucleotide polymorphisms (SNPs) have been identified.[100] Although there are strong genetic determinants of MS, epidemiological studies have shown environmental factors that are involved in the etiology of MS as well.[101] Children with ADS are an interesting group to study these environmental risk factors for MS, since

children are close to the onset of the disease and are not exposed to as many different environmental factors as adults. Studying children with MS might reveal novel risk factors and insights in the disease mechanism of MS. Epidemiological studies have shown a latitude in MS prevalence, whereas MS is more prevalent in countries remote from the equator.[101-103] This had led to the hypothesis that vitamin D deficiency is a potential risk factor for MS, since people who live further from the equator are less exposed to sunlight and have lower levels of vitamin D. In both adults and children with MS, lower serum vitamin D levels are associated with a higher MS risk and higher relapse rates.[53, 104-106]

Moreover, migration studies have shown that, children who were born in high MS prevalence countries and move to countries with low MS prevalence during childhood, adapt to the low risk of the country where they live.[101, 107, 108] Suggesting exposure to specific environmental factors early in life contributes to MS risk. Previous viral infections have been studied as a potential risk factor for MS. Several studies have confirmed the significant increased frequency of Epstein-Barr virus (EBV) seropositivity in adults and children with MS as compared with healthy controls and patients with a monophasic demyelinating event.[53, 109-114] There are concerns vaccinations might increase the risk of CNS demyelination, but in a large case-control study including 780 MS cases and 3885 controls no associations were found between hepatitis B vaccination, human papilloma virus (HPV) vaccination, or vaccinations of any type and CNS demyelination.[115] In childhood-onset MS both boys and girls are equally affected, but after puberty females are more often affected.[11, 86] This indicates that gender and puberty enhance MS risk.[116] Smoking increases risk of adult-onset MS[117, 118]. A French cohort study reported the risk of MS is increased two-fold in children who were exposed to second hand smoking.[119] Early age obesity is associated with an increased MS risk.[120, 121] An association that can be confounded by vitamin D status, since vitamin D levels are lower in obese people. However, in a large Swedish cohort an interaction was observed in obesity and *HLA-DRB1*15*.[122] When adjusted for vitamin D status, obesity caused an increased MS risk. Furthermore, higher dietary salt intake might contribute to MS severity and relapse rate.[123] A recent American study did not find an association between higher sodium intake and MS risk in children.[124]

Treatment of childhood-onset ADS

The care of children with ADS should be managed in multidisciplinary teams.[11, 125] The Erasmus MC Sophia Children's hospital has a national expert center for childhood-onset MS and variants within the spectrum of ADS. The team includes pediatric and adult MS neurologists, nurses, physiotherapists, occupational therapists, neuropsychologists, ophthalmologists, urologists and ambulatory teachers. A specialized pediatric MS nurse has a central role in coordinating the care and counselling of children with ADS. The center has expertise in the acute and chronic treatment of children with ADS. In

addition, it has an advisory role for pediatricians and neurologists in the Netherlands, who are less frequently consulted with children with ADS. The center has an important role in counselling, increasing the awareness of childhood-onset ADS and educating the school system. For example, via a special website (http://www.kindermscentrum.nl/) and the organisation of an annually informative day, where children with MS and their families can meet and share their experiences. At the specialized outpatient clinic children are guided in the transition from pediatric to adult care.

The acute treatment of children with ADS is with intravenous corticosteroid therapy.[11, 126, 127] The common regimen is 10-30 mg/kg/dose of methylprednisolone intravenous for 3 – 5 days. In case children do not respond to the first pulse of corticosteroids, a second pulse could be considered. Intravenous immunoglobulins or plasmapheresis could be considered in the acute phase when children not tolerate or not respond to corticosteroids.

The effect of chronic immunomodulatory treatment on MS is well established in adults[128], but is less proven in children.[129] The small numbers of patients and more strict ethical concerns challenge therapeutic trials in children with MS.[130] First-line treatment with interferon-β or glatiramer acetate have not been formally evaluated in children with MS.[129] However, several case series report a reduction in relapse rate and an overall high safety-profile of those first-line therapies in children with MS.[129, 131] Most notable side effect of interferon-β is a transient increase of liver transaminases.[52, 131] Side effects are less severe in case the dose is titrated. Currently DMT is initiated at 25% of the full dose and titrated gradually. Long- term effects on growth, puberty and adverse effects of immunomodulatory therapies for MS in children have yet to be established.[127] MS is a chronic and ongoing disease and despite the initiation of DMT relapses can still occur. Switching to more aggressive second-line therapy with more severe side effects requires a careful consideration of the potential risks and benefits per individual patient. The IPMSSG provided a definition for inadequate treatment response and recommends switching treatments in case children are compliant on full-dose therapy for at least 6 months and have an increase or no reduction in relapse rate, or new MRI lesions as compared with the pre-treatment period, or in case they had 2 or more relapses within a 12-month period or less.[129] Options for switching are, change between first-line therapies, or switch to second-line therapy with natalizumab. An Italian study reported a cohort of 101 children of whom the majority tolerated natalizumab well.[132] In this cohort strong MS suppression was observed with only 9 relapses during a mean follow-up of 34.2± 18.3 months. Long-term effects of natalizumab in children are unknown and there are concerns for the risk of progressive multifocal leukoencephalopathy (PML).[132, 133] Patients at high risk of PML can be identified by serum anti-JC virus antibodies.[134] Children at high risk of PML might switch to oral second-line therapy with fingolimod in the future. A Brazilian study reported no serious adverse events of fingolimod in 17 children with MS.[135] The effect

and safety of fingolimod is investigated in the ongoing PARADIGMS trial: a double-blind randomized controlled trial which compares the safety and effect of fingolimod versus intramuscular interferon-β-1a.[136] Recently, new oral first-line therapies came available for adults with MS: dimethyl fumarate and teriflunomide.[137, 138] First-line treatment with dimethyl fumarate was safe and well tolerated in a small cohort of 13 American children with MS.[139] Novel exciting MS therapeutics are currently underway.[140]

SEARCH FOR NEW BIOMARKERS: MYELIN OLIGODENDROCYTE GLYCOPROTEIN ANTIBODIES

Current additional investigations, including laboratory findings in blood (AQP4-IgG) and CSF (oligoclonal bands) and MRI techniques, lack sensitivity to accurately diagnose patients with the subtype of ADS at the first event. This underscores the urgent need for new biomarkers, which can help to distinct monophasic ADS from chronic relapsing ADS including NMOSD and MS.[141, 142] Interesting markers are antibodies directed to myelin oligodendrocyte glycoprotein (MOG-IgG) or other myelin peptides.[143] Myelin oligodendrocyte glycoprotein is a protein expressed exclusively in the CNS on the surface of the myelin sheath and oligodendrocytes (Figure 1.3).[143, 144] The protein is a minor component of myelin (0.05%) and is located at the outermost lamella of the myelin sheet. Antibodies directed to MOG (MOG-IgG) have been shown to induce or contribute to demyelination in various animal models. Optimization of antibody detection has enabled the reliable identification and association of MOG-IgG with a spectrum of CNS demyelinating disorders, including ADEM, bilateral and recurrent ON, as well as LETM in both children and adults.[145]

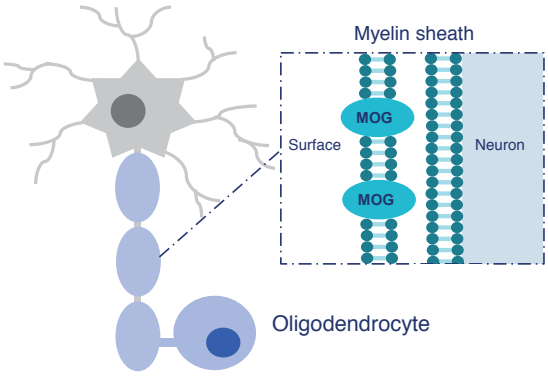


Figure 1.3 Schematic view of oligodendrocytes and proteins of the myelin sheath adapted from Hemmer et al.[144]

SCOPE OF THIS THESIS

This thesis focuses on two important topics within the spectrum of acquired demyelinating syndromes (ADS): neuromyelitis optica spectrum disorders (NMOSD) and childhood-onset ADS including MS. Both NMOSD and childhood-onset ADS, are rare variants within the spectrum of demyelination and can be difficult to recognize. In general, early and correct diagnoses are of importance for both patient groups for accurate counselling and for early treatment possibilities. Here we aimed to reveal the spectrum of NMOSD in adults and of ADS in children, and to improve the diagnostic process. In addition, we searched for prognostic and diagnostic biomarkers in ADS.

The first part of this thesis focuses on NMOSD. In **chapter 2** the nationwide incidence of AQP4-IgG seropositive NMOSD in the Netherlands was assessed. In **chapter 3** we investigated whether antibodies directed to MOG (MOG-IgG) are present in AQP4-IgG seronegative NMOSD patients, and compared the clinical features of MOG-IgG seropositive patients versus AQP4-IgG seropositive and seronegative NMOSD patients.

The second part of this thesis describes the spectrum of ADS in children and our search for prognostic markers. In **chapter 4** we investigated the utility of the 2012 revised International Pediatric Multiple Sclerosis Study Group (IPMSSG) diagnostic definitions for childhood-onset ADS. Furthermore, MRI predictors for MS diagnosis in children with a first event of ADS were validated in **chapter 5**. In **chapter 6** we studied the disease course after the onset of CIS in children and adults, and compared the time to MS diagnosis and relapse rates. We investigated whether genetic risk loci identified in adults with MS are associated with a risk for childhood-onset MS, and if these genes can predict MS diagnosis in children presenting with ADS in **chapter 7**. In **chapter 8** we studied if serum MOG-IgG could distinguish the different subtypes of childhood-onset ADS and if MOG-IgG could predict the disease course after the first event. In addition, we searched for prognostic biomarkers for MS in cerebrospinal fluid in **chapter 9**.

The main findings of this thesis and interpretation of our results are discussed in **chapter 10**. At the end suggestions for future research are described.

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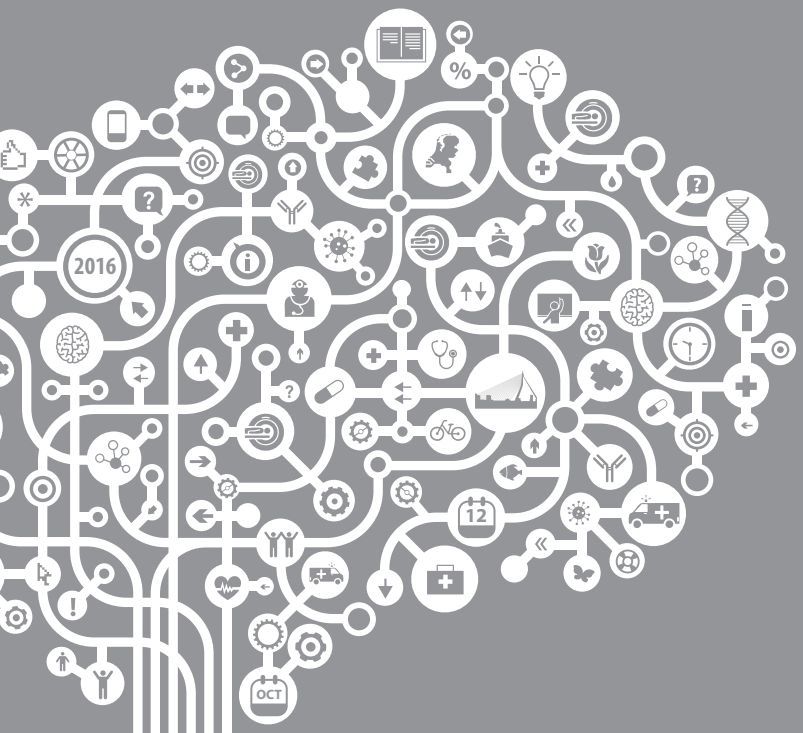
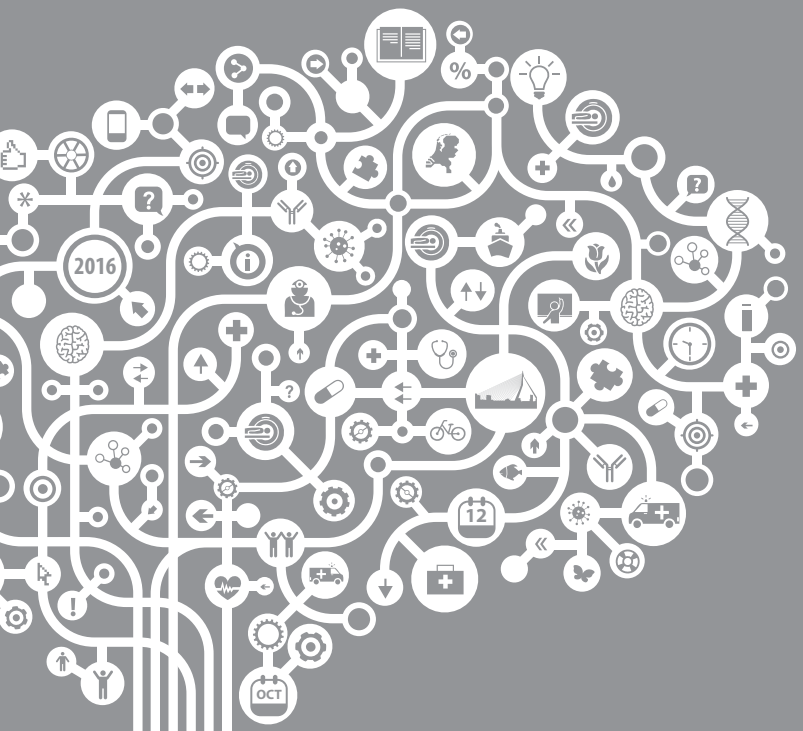


Part one

Neuromyelitis optica spectrum disorders



Chapter 2

Incidence of AQP4-IgG seropositive neuromyelitis optica spectrum disorders in the Netherlands: about one in a million

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ABSTRACT

Neuromyelitis optica (NMO) is a rare autoimmune disease affecting the optic nerves and spinal cord. In the majority of NMO patients anti-aquaporin-4 antibodies (AQP4-IgG) are detected. Here we assessed a nationwide incidence of AQP4-IgG seropositive NMO spectrum disorders (NMOSD) in the Netherlands based on results of one central laboratory. Data were collected since the introduction of the highly sensitive cell based assay for six consecutive years. Samples of 2,795 individual patients have been received, of them 94 (3.4%) were seropositive. Based on the Dutch population with 16,6 million inhabitants the mean incidence of AQP4-IgG seropositive NMOSD was calculated at 0.09 per 100,000 people.

INTRODUCTION

Neuromyelitis optica (NMO) is a rare autoimmune disease classically affecting the optic nerves and spinal cord.[1] Exact incidence figures of NMO in the Netherlands are currently unknown. The clinical spectrum of NMO has broadened in the past years and besides Devic's syndrome it includes limited forms such as isolated or recurrent optic neuritis, transverse myelitis, brainstem syndromes and other cerebral presentations.[2, 3] In approximately 77% of the patients with NMO spectrum disorders (NMOSD) specific antibodies against aquaporin-4 (AQP4-IgG) are detected.[2] In the Netherlands diagnostic testing of these antibodies is performed in one centralised NMO expert centre. This provides a unique chance to get insight in a nationwide incidence of AQP4-IgG seropositive NMOSD. Epidemiological figures of NMOSD are of interest for patient care and counseling and for the estimation of the socioeconomic burden of the disease. The purpose of this study is to estimate a nationwide incidence of NMOSD in the Netherlands.

METHODS

Patients

This study was conducted at the Dutch national NMO expert centre which includes Sanquin Diagnostic Services in Amsterdam and the NMO expert clinic at the Erasmus university Medical Centre (Erasmus MC) in Rotterdam. We collected demographic data (age and gender) from serum samples sent for routine AQP4-IgG diagnostics. Data were collected since the introduction of the highly sensitive cell based assay (CBA) for AQP4-IgG detection in May 2009 for six consecutive years. Samples sent in from abroad, mainly Belgium and the Dutch Caribbean, were excluded from this study (*n*=139 patients). Of these foreign patients 8 were AQP4-IgG seropositive. Incidence rates were calculated as the number of AQP4-IgG seropositive patients per year divided by the number of Dutch inhabitants per 100,000 people. Population figures were extracted from Statistics Netherlands.[4] From the patients known at the Erasmus MC in Rotterdam clinical data were collected. Magnetic resonance images (MRIs) were evaluated for the presence of lesions, longitudinally extensive transverse myelitis (LETM)[3] and cerebral NMO-like lesions.[5] In five patients known at Erasmus MC the diagnosis of NMOSD was made prior to the time of the AQP4-IgG assay in 2009 based on their clinical characteristics and therefore they were not included in the incidence calculations. This study was approved by the Medical Ethical Committee of the Erasmus MC in Rotterdam. All patients from the Erasmus MC provided informed consent.

AQP4-IgG cell based assay

We used a CBA for AQP4-IgG detection as has previously been described.[6] In short, patient serum was incubated with HEK293 cells transiently transfected with AQP4-M23 (final serum dilution 1:20). After washing, cells were subsequently incubated with goat anti-human IgG Allophycocyanin (APC) conjugated secondary antibody and analysed after washing using fluorescence-activated cell sorter (FACS). The cut-off was determined in every assay as average deltaMFI + 10 standard deviations of 8 individual negative control sera.

Statistical analysis

Statistical analysis was performed using SPSS 21.0. The Chi-Square test and Mann-Whitney *U* test were used to compare categorical and continuous data respectively.

RESULTS

During six consecutive years, from May 2009 until May 2015, 3,207 samples of 2,795 individual Dutch patients have been received for AQP4-IgG testing. Samples were sent from 85 different hospitals including all 8 university hospitals. Of all included patients 94 (3.4%) were seropositive. Two hundred and forty children and adolescents less than 18 years old were included, of them 8 (3.3%) were AQP4-IgG seropositive. The mean age of AQP4-IgG seropositive patients was 47.6 years ± 18.2 compared with 41.0 years ± 16.1 in the seronegative group ($p<0.01$). Seventy-eight (83%) of the seropositive patients were female in contrast to 1,698 (63%) female patients in the seronegative group ($p<0.01$). The incidence rates of 6 consecutive years are presented in Table 2.1.

Table 2.1 Incidence rates of 6 consecutive years of AQP4-IgG seropositive NMOSD in the Netherlands. Population figures were extracted from Statistics Netherlands.[4]

Year	*Number of AQP4-IgG seropositive NMOSD patients*	*Number of Dutch inhabitants*	*Incidence per 100.000 people*
1: May 2009 – April 2010	15	16,486,000	0.09
2: May 2010 – April 2011	15	16,575,000	0.09
3: May 2011 – April 2012	12	16,656,000	0.07
4: May 2012 – April 2013	16	16,730,000	0.10
5: May 2013 – April 2014	18	16,778,000	0.11
6: May 2014 – April 2015	13	16,829,000	0.08
Mean/year	**15**[a]	**16,676,000**	**0.09**

[a]Results rounded to the nearest integer.

AQP4-IgG = aquaporin-4 immunoglobulin G, NMOSD = neuromyelitis optica spectrum disorders.

The mean incidence of NMOSD during the past six years in the Netherlands was calculated at 0.09 per 100,000 people. Considering that approximately 77% of NMOSD patients has antibodies directed to AQP4[2], the estimated incidence of NMOSD in general (including AQP4-IgG seropositive and seronegative cases) is 0.12 per 100,000 people. Thirty-six of the 94 AQP4-IgG seropositive NMOSD patients (38%) are known at the Erasmus MC and their clinical data are presented in Table 2.2. Seventy-eight percent of

Table 2.2 Clinical characteristics of 36 AQP4-IgG seropositive NMOSD patients known at Erasmus MC.

	AQP4-IgG seropositive NMOSD patients, *n*=36
Age at onset, mean years (SD)	41.6 (18.9)
Females, *n* (%)	28 (78%)
Caucasians, *n* (%)	27 (75%)
AID comorbidity, *n* (%)	8 (22%)
Time from first onset of symptoms to AQP4-IgG assay, median months (range)	7.9 (0.3 – 248.8[a])
Type of onset, *n* (%)	
ON	12 (33%)
TM	18 (50%)
NMO	4 (11%)
Brainstem and or cerebral syndromes	2 (6%)
CSF elevated IgG index >0.68 and/ or positive OCB, *n* (%)	11/31 (35%)
MRI cerebral lesions, *n* (%)[b]	17/34 (50%)
NMO like[5]	4 (12%)
Aspecific	13 (41%)
MRI spinal cord lesions, *n* (%)[b]	30/33 (91%)
LETM	24 (73%)
Relapse, *n* (%)	24 (67%)
Chronic treatment, *n* (%)	30 (83%)
Follow-up, mean years (SD)	5.4 (5.4)
Type at last Follow-up, *n* (%)	
ON	3 (8%)
TM	11 (31%)
NMO	21 (58%)
Brainstem and or cerebral syndromes	1 (3%)

[a]The extreme of 248.8 months from onset to sampling was caused by a patient with recurrent optic neuritis in 1988, 2004 and later. In this particular case NMOSD diagnosis could not have been made prior to the AQP4-IgG testing. [b]MRIs performed at onset and/or follow-up.

AID = autoimmune disease, AQP4-IgG = aquaporin-4 immunoglobulin G, CSF = cerebrospinal fluid, IgG = immunoglobulin G, LETM = longitudinally extensive transverse myelitis, NMO(SD) = neuromyelitis optica (spectrum disorders), OCB = oligoclonal bands, ON = optic neuritis, TM = transverse myelitis, MRI = magnetic resonance imaging.

them were females. Twenty-four patients had LETM at some point during their disease course. Eventually at last follow-up 21 patients (58%) fulfilled classic NMO criteria with optic neuritis and transverse myelitis.[3]

DISCUSSION

Here we report the incidence of AQP4-IgG seropositive NMOSD in the Netherlands, derived from data of the Dutch national NMO expert centre, is nearly one in a million: 0.09 per 100,000 people. Unique for this study is that we have a nationwide coverage given that the CBA is performed in one central laboratory. Our incidence figure is within the range of previous described incidence rates which range from 0.05 – 0.4 per 100,000 people.[7] It has to be considered that epidemiological studies on NMOSD are difficult to compare since they are based on different selection and inclusion criteria. For example different clinical definitions and AQP4-IgG assays were used. Also the ethnicities of included patients and the geographic coverage differed. Two studies performed in comparable geographic areas in Denmark and the United Kingdom differed essentially from our study, as both studies also included AQP4-IgG seronegative NMOSD patients and did not have nationwide coverage.[8, 9]

In a comparable Austrian study an incidence of 0.05 was calculated.[10] The main difference with our study is that the patients they identified were all Caucasian. However there are indications that some ethnic groups are overrepresented in NMOSD.[11] In the Netherlands we estimated the incidence of NMOSD is more than twice as high in non-Caucasians. Based on 25 percent of the patients known at Erasmus MC were non-Caucasian and 11.9 percent of the Dutch inhabitants are non-Caucasian[4] we estimated a mean annual incidence rate of NMOSD for non-Caucasians of 0.19 per 100,000 people and for Caucasians of 0.08 per 100,000 people.

We think our findings reflect the real incidence of AQP4-IgG seropositive NMOSD in the Netherlands. However, we cannot exclude that mild cases and forme fruste types of the disease[2] have been missed. Fifty-eight percent of the NMOSD patients at the Erasmus MC fulfilled classic NMO criteria.[3] Unfortunately we did not have access to the clinical data of all patients and therefore we could not present this figure for all NMOSD patients in the Netherlands. Only the clinical data of patients known at the Erasmus MC are presented, however covering over one third of the study population.

More awareness and better recognition of NMOSD might increase the incidence in the future. Further demographic studies and international collaboration in the NMO field would add to a better understanding of NMOSD.

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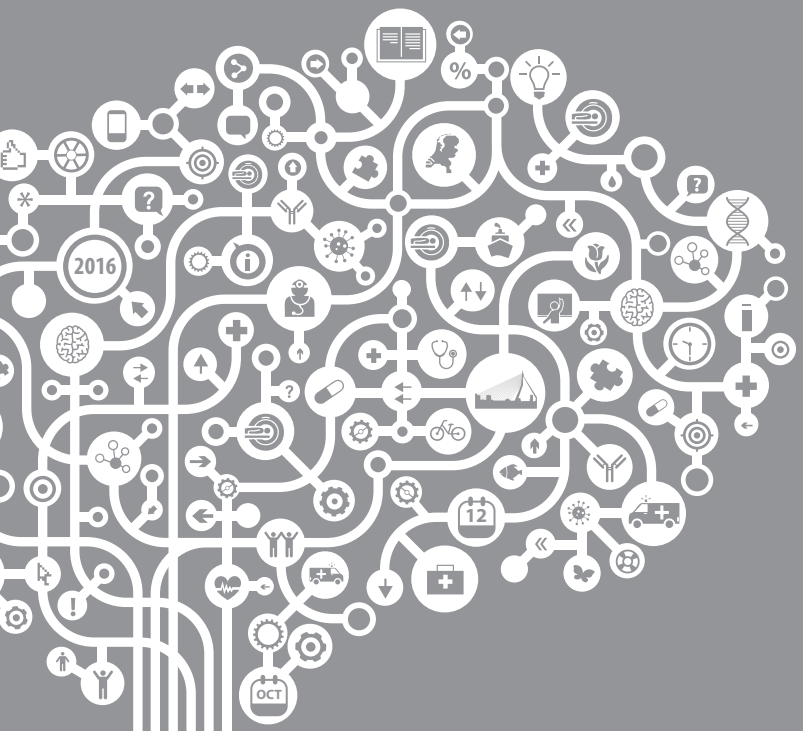

Chapter 3

Neuromyelitis optica spectrum disorders: comparison of clinical and MRI characteristics of AQP4-IgG versus MOG-IgG seropositive cases in the Netherlands

E.D. van Pelt*, Y.Y.M. Wong*, I.A. Ketelslegers, D. Hamann, R.Q. Hintzen.

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ABSTRACT

Background and purpose Neuromyelitis optica spectrum disorders (NMOSD) are a group of rare inflammatory demyelinating disorders of the CNS. The identification of specific antibodies directed to aquaporin 4 (AQP4-IgG) led to the distinction from multiple sclerosis (MS). However, up to 25% of the clinically diagnosed NMO patients are seronegative for AQP4-IgG. A subgroup of these patients might be identified by antibodies directed to myelin oligodendrocyte glycoprotein (MOG-IgG). Our objective was to investigate whether the clinical characteristics of these patients differ.

Methods Using a cell based assay, samples of 61 AQP4-IgG seronegative patients and 41 AQP4-IgG seropositive patients with clinically NMOSD were analysed for the presence of MOG-IgG. Clinical characteristics of the AQP4-IgG, MOG-IgG seropositive and double seronegative NMOSD patients were compared.

Results Twenty of the 61 AQP4-IgG seronegative patients tested MOG-IgG seropositive (33%). MOG-IgG seropositive patients were more frequently males in contrast to AQP4-IgG seropositive patients (55% versus 15%, $p<0.01$) and Caucasians (90% versus 63%, $p=0.03$). They more frequently presented with coincident optic neuritis (ON) and transverse myelitis (TM) (40% versus 12%, $p=0.02$) and have a monophasic disease course (70% vs 29%, $p<0.01$). AQP4-IgG seropositive patients were 2.4 times more likely to suffer from relapses as compared with MOG-IgG seropositive patients (relative risk 2.4, 95% CI 1.2 – 4.7). AQP4-IgG seropositive patients had higher EDSS levels at last follow-up ($p<0.01$).

Conclusion Antibodies directed to MOG identify a subgroup of AQP4-IgG seronegative NMO patients with generally a favourable monophasic disease course.

INTRODUCTION

Neuromyelitis optica spectrum disorders (NMOSD) are a group of rare inflammatory demyelinating disorders of the central nervous system (CNS), characterised by severe episodes of optic neuritis (ON) and/or longitudinally extensive transverse myelitis (LETM).[1, 2] Antibodies directed to aquaporin 4 (AQP4-IgG) are specific for neuromyelitis optica (NMO) and distinguish NMO from multiple sclerosis (MS).[3] Despite the development of highly sensitive cell based assays (CBA)[4] 10-25% of the patients clinically diagnosed with NMO are AQP4-IgG seronegative.[1] In the Netherlands AQP4-IgG was found in 74% of the recurrent NMO cases.[5] The presence of antibodies directed to myelin oligodendrocyte glycoprotein (MOG-IgG) has been reported in a subgroup of patients with NMO and NMOSD[6-13] and ON.[14-16] These MOG antibodies are associated with CNS demyelinating syndromes, particularly with children with an acute-disseminated-encephalomyelitis-like disease onset.[17, 18] In case of NMOSD MOG antibodies seem to be associated with a male predominance and relative mild disease course.[7, 9, 10] Double seropositive (AQP4-IgG and MOG-IgG) NMOSD patients seem to be rare and only a few cases have been described.[6, 10, 16] In this study we investigated the presence of MOG-IgG in NMOSD patients referred to our clinic. Clinical characteristics of MOG-IgG seropositive patients were compared with AQP4-IgG seropositive patients, as well as MOG-IgG seropositive patients with double seronegative NMOSD patients.

METHODS

Patients

This study was conducted at the Dutch national NMO expert centre which includes Sanquin Diagnostic Services in Amsterdam and the NMO expert clinic at the Erasmus MC in Rotterdam. We included patients with NMOSD referred to the Dutch NMO expert centre at Erasmus MC between 2000 and 2015 retrospectively. Patients either presented primarily at Erasmus MC, or were referred by ophthalmologists and neurologists in (non-) academic hospitals in the Netherlands. All patients fulfilled the following inclusion criteria: (i) age at first presentation at Erasmus MC ≥ 18 years, (ii) diagnosis of NMOSD according to current diagnostic criteria for NMO[19], except for AQP4-IgG seropositive status, or limited forms of NMO defined as LETM ≥ 3 vertebral segments or bilateral ON or recurrent unilateral ON.[1] Patients were not included in case they were diagnosed with MS, or a non-demyelinating inflammatory cause like other systemic autoimmune diseases (e.g. systemic lupus erythematous, sarcoidosis) or ophthalmologic diseases (e.g. Leber hereditary optic neuropathy, acute ischemic optic neuropathy). Additionally 41 AQP4-IgG seropositive NMOSD patients from the Dutch NMO expert centre of whom

serum samples were available were included. Clinical characteristics were compared between MOG-IgG seropositive NMOSD patients and AQP4-IgG seropositive and double negative patients respectively. Data on auto-immune comorbidity, defined as coexisting clinically diagnosed auto-immune disease(s), were collected. Relapses were defined as new neurological symptoms lasting for at least 24 hours with objective findings at neurological examination. Patients without relapses were defined as monophasic during the current observation period, irrespective of its duration. In order to compare the disease severity for patients with ON, the nadir visual acuity was retrieved from medical records. For all patients the expanded disability status scale (EDSS) was assessed.[20] Cerebral and spinal cord magnetic resonance images (MRIs) were evaluated for the presence of specific NMO-like lesions.[21] This study was approved by the Medical Ethical Committee of Erasmus MC in Rotterdam. All patients provided informed consent.

Cell culture and cell based assays

All samples were tested blindly at Sanquin, Amsterdam. We used cell based assays (CBA) for MOG-IgG and AQP4-IgG detection as has previously been described.[5,17] Briefly, patient serum was incubated with HEK293 cells transiently transfected with AQP4-M23 (final serum dilution 1:20) or LN18 cells stably transfected with full length MOG (final serum dilution 1:200). After washing, cells were subsequently incubated with goat anti-human IgG Allophycocyanin (APC) conjugated secondary antibody (Jackson ImmunoResearch Laboratories, Brunschwig Chemie B.V., Amsterdam, The Netherlands (specific for human IgG)) and analysed after washing using fluorescence-activated cell sorter (FACS). The cut-off was determined in every assay as the average mean fluorescence intensity + 10 standard deviations of eight individual negative control sera. Our assay has an anti-IgG specific detection antibody and thus no IgM anti-MOG or IgM anti-AQP4 is detected.

Statistical analysis

Patients were divided in three groups: AQP4-IgG seropositive, MOG-IgG seropositive and double seronegative NMOSD. Statistical analyses were performed using SPSS 21.0. (SPSS, Chicago, IL, USA). The chi-square test and Fisher exact test were used in order to compare categorical data. Mann-Whitney *U* test and Student's *t* test were used for continuous data when appropriate. In case p-values were < 0.05 results were considered significant.

RESULTS

In all, 102 NMOSD patients were included, 61 of them were AQP4-IgG seronegative and 41 were AQP4-IgG seropositive. Twenty of the 61 AQP4-IgG seronegative patients tested

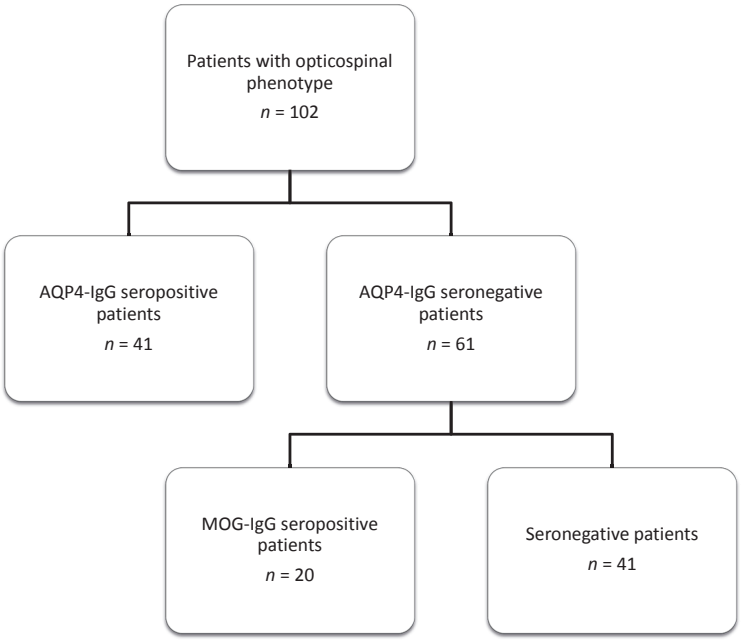

Figure 3.1 Overview of included patients.

MOG-IgG seropositive (33%). In none of the AQP4-IgG seropositive patients MOG-IgG was detected. An overview of the included patients is presented in Figure 3.1. The median time to sampling was 10.7 months (0 – 401.5). Thirteen patients (12.7%) received chronic treatment whilst the sample was collected.

In Table 3.1 clinical characteristics are presented for MOG-IgG seropositive (*n*=20), AQP4-IgG seropositive (*n*=41) and double seronegative (*n*=41) NMOSD patients. Compared with AQP4-IgG seropositive patients, MOG-IgG seropositive patients were more frequently males (55% versus 15%, $p<0.01$) and Caucasians (90% versus 63%,

Table 3.1 Clinical characteristics of MOG-IgG seropositive (*n*=20) and AQP4-IgG seropositive (*n*=41) and seronegative (*n*=41) patients with clinically NMOSD.

	MOG-IgG+ (*n*=20)	**AQP4-IgG+ (*n*=41)**	**Seronegative (*n*=41)**	***p*-value[a]**	***p*-value[b]**
Female, *n* (%)	9 (45)	35 (85)	25 (61)	**0.00**	0.24
Mean age at onset, years (SD)	36.2 (14.2)	42.0 (16.1)	40.4 (13.0)	0.23	0.26
Autoimmune comorbidity, *n* (%)	1 (5)	8 (20)	6 (15)	0.25	0.41
Familial autoimmune disease in first and/or second degree relatives, *n* (%)	5/18 (28)	19/38 (50)	22/38 (58)	0.12	**0.04**
Caucasian, *n* (%)	18 (90)	26 (63)	34 (83)	**0.03**	0.70

Table 3.1 (continued)

	MOG-IgG+ (*n*=20)	**AQP4-IgG+ (*n*=41)**	**Seronegative (*n*=41)**	***p*-value[a]**	***p*-value[b]**
Follow-up, median, months (range)	23.9 (2.7-463.8)	61.5 (4.3-312.9)	23.9 (5.8-267.6)	0.09	0.49
Monophasic disease course	8.0 (2.7 – 463.8)	11.9 (4.3 – 61.5)	17.8 (5.8 – 202.4)	0.43	0.06
Relapsing disease course	106.4 (30.5 – 234.3)	115.8 (14.3 – 312.9)	34.6 (17.7 – 267.6)	0.81	0.07
Monophasic disease, *n* (%)	14 (70)	12 (29)	25 (61)	**0.00**	0.49
Phenotype at onset, *n* (%)					
ON	8 (40)	16 (39)	15 (37)	0.94	0.80
Bilateral ON	6/8 (75)	6/16 (38)	11/15 (73)	0.19	1.00
LETM	4 (20)	20 (49)	15 (37)	**0.03**	0.19
ON and TM	8 (40)	5 (12)	11 (27)	**0.02**	0.30
Bilateral ON	7/8 (88)	4/5 (80)	8/11 (73)	1.00	0.60
Phenotype at last follow-up, *n* (%)					
ON	6 (30)	4 (10)	10 (24)	0.07	0.64
LETM	3 (15)	11 (27)	12 (29)	0.35	0.34
ON and TM	11 (55)	26 (63)	19 (46)	0.53	0.53
CSF elevated protein >0.60, *n* (%)	4/14 (29)	12/32 (38)	9/32 (28)	0.74	1.00
CSF oligoclonal bands, *n* (%)	3/16 (19)	10/28 (36)	7/35 (20)	0.31	1.00
CSF IgG index >0.68, *n* (%)	4/13 (31)	13/27 (48)	15/34 (44)	0.30	0.40
EDSS at onset, median (range)	4.0 (2.0-8.0)	4.0 (1.5-9.0)	3.0 (1-9.5)	0.40	0.16
EDSS at best recovery, median (range)	1.0 (0-8.0)	2.0 (0-8.0)	3.0 (1.0-8.0)	0.06	**0.01**
EDSS at last FU, median (range)	1.0 (0-10)	3.0 (1.0-10.0)	3.0 (1.0-8.0)	**0.01**	**0.02**
Nadir visual acuity, median (range)	0.05 (0.003-0.7)	0.003 (0-1.0)	0.05 (0.002-1.0)	0.16	0.83
Chronic treatment, *n* (%)[c]	4 (20)	34 (83)	17 (43)	**0.00**	0.10
Duration, months (range)	40.3 (2.8 -79.1)	30.4 (1.3 – 152.4)	32.3 (3.5 – 213.3)	0.98	1.00

[a]Comparison between MOG-IgG and AQP4-IgG seropositive patients; [b]comparison between seropositive MOG-IgG and seronegative patients; [c]55 patients received chronic therapy: four MOG-IgG seropositive patients (one azathioprine, one mycophenolate, one immunomodulatory treatment, one mycophenolate and rituximab), 34 AQP4-IgG seropositive patients (27 azathioprine, five mycophenolate, two rituximab), 17 seronegative patients (11 azathioprine, four mycophenolate, one low dose oral prednisone, one mitoxantrone). Significant results are reported in bold.

AQP4-IgG = aquaporin-4 immunoglobulin G, CSF = cerebrospinal fluid, EDSS = expanded disability status scale, LETM = longitudinally extensive transverse myelitis, MOG-IgG = myelin oligodendrocyte glycoprotein immunoglobulin G, NMOSD = neuromyelitis optica spectrum disorder, ON = optic neuritis, TM = transverse myelitis.

Table 3.2 MRI features of MOG-IgG and AQP4-IgG seropositive patients with NMOSD.

	MOG-IgG+ (*n*=20)	**AQP4-IgG+ (*n*=41)**	***p*-value**
Brain			
NMO specific brain lesions, *n* (%)	0	12/38 (32)[a]	**0.01**
Aspecific brain lesions, *n* (%)	4 (20)	12/37 (32)	0.32
Spinal cord			
Affected spinal cord segments			
Cervical	9/12 (75)	31/36 (86)	0.66
Thoracic	10/12 (83)	30/36 (83)	1.00
Lumbar	3/12 (25)	5/36 (14)	0.66
Whole spinal cord	3/12(25)	5/36 (14)	0.66
Central grey matter	10/12 (83)	30/36 (83)	1.00

[a]Magnetic resonance imaging was reviewed for the presence of NMO specific brain lesions as recently described.[21] Five patients with diencephalic lesions surrounding the third ventricle and cerebral aquaduct, two patients with dorsal brainstem lesions, two patients with both diencephalic and dorsal brainstem lesions, one patient with a dorsal brainstem, diencephalic and periependymal lesion, one patient with a specific lesion of the internal capsule and one patient with an extensive confluent hemispheric lesion were detected.

AQP4-IgG = aquaporin-4 immunoglobulin G, MOG-IgG = myelin oligodendrocyte glycoprotein immunoglobulin G, MRI = magnetic resonance imaging, NMO = neuromyelitis optica, NMSOD = neuromyelitis optica spectrum disorder.

p=0.03). They more frequently presented with coincident ON and transverse myelitis (TM) (40% versus 12%, *p*=0.02). AQP4-IgG seropositive patient more often presented with longitudinally extensive transverse myelitis (LETM) (49% versus 20%, *p*=0.03). In Table 3.2 MRI features are presented. The cerebral MRI features as recently described in NMOSD patients[21] were only found in het AQP4-IgG seropositive patients and not in the MOG-IgG seropositive patients (32% versus 0%, *p*<0.01). An example of typical NMO dorsal brainstem (a) and midbrain (b) lesions of one of our patients is presented in Figure 3.2. Spinal cord MRI features and cerebrospinal fluid (CSF) findings were similar in the AQP4-IgG versus MOG-IgG seropositive groups. MOG-IgG seropositive patients more frequently had a monophasic disease course (Table 3.1) (70% vs 29%, *p*<0.01). AQP4-IgG seropositive patients were 2.4 times more likely to suffer from relapses as compared with MOG-IgG seropositive patients (RR 2.4, 95% CI 1.2 – 4.7). EDSS at last follow-up was higher for AQP4-IgG seropositive patients (*p*<0.01). Presenting phenotype was not predictive for a relapsing disease course. Table 3.3 shows the clinical characteristics of the relapsing patients. TM relapses were more frequently seen in the AQP4-IgG group compared with the MOG-IgG seropositive patients (76% versus 17%, *p*=0.01).

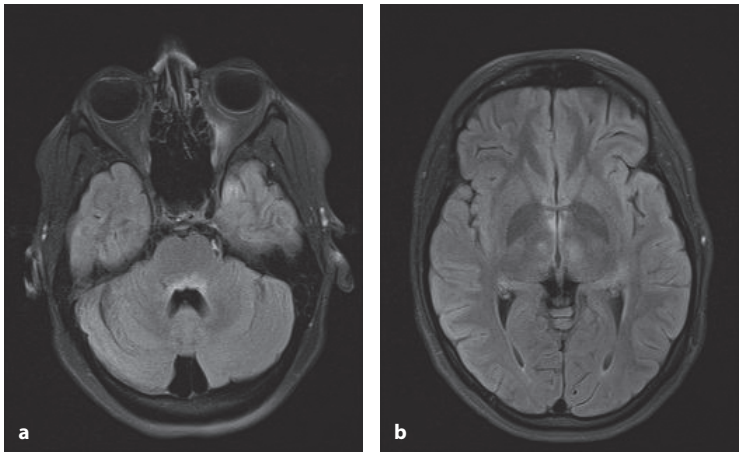

Figure 3.2 Transversal MRI fluid-attenuated inversion recovery images presenting typical NMO dorsal brainstem (a) and midbrain (b) lesions of one of our NMO patients with AQP4-IgG seropositivity.

Table 3.3 Clinical characteristics of patients with relapsing NMOSD.

	MOG-IgG+ (*n*=6)	**AQP4-IgG + (*n*=29)**	**Seronegative patients (*n*=16)**	***p*-value[a]**	***p*-value[b]**
Phenotype at onset, *n* (%)					
ON	4 (67)	13 (45)	9 (56)	0.40	1.00
LETM	0 (0)	13 (45)	3 (19)	0.06	0.53
NMO	2 (33)	3 (10)	4 (25)	0.20	1.00
Annualised relapse rate, median (range)	0.46 (0.05-1.13)	0.41 (0.07-1.92)	0.67 (0.09-2.00)	0.74	0.17
Time from onset to first relapse median, months (range)	28 (8-219)	18 (1-192)	13 (3-96)	0.20	**0.04**
Relapse ON, *n* %	4 (67)	16 (55)	10 (63)	0.68	1.00
Relapse bilateral ON, *n* %	2 (33)	3 (10)	5 (31)	0.20	1.00
Relapse transverse myelitis, *n* %	1 (17)	22 (76)	7 (44)	**0.01**	0.35
Relapse simultaneous ON and TM, *n* (%)	1 (17)	4 (14)	1 (6)	1.00	1.00
Chronic therapy, *n* (%)[c]	3 (50)	26 (90)	8 (50)	**0.05**	1.00
Duration, months (range)	57.4 (2.8 – 79.1)	42.0 (1.8 – 152.4)	32.8 (8.7 – 213.3)	0.92	0.92

[a]Comparison between MOG-IgG and AQP4-IgG seropositive patients; [b]comparison between seropositive MOG-IgG and seronegative patients; [c]37 patients received chronic therapy: three MOG-IgG seropositive patients (one azathioprine, one mycophenolate, one mycophenolate and rituximab); 26 AQP4-IgG seropositive patients (23 azathioprine, one mycophenolate, two rituximab) and eight seronegative patients (four azathioprine, two mycophenolate, one low dose oral prednisone, one mitoxantrone).

AQP4-IgG = aquaporin-4 immunoglobulin G, LETM = longitudinally extensive transverse myelitis, MOG-IgG = myelin oligodendrocyte glycoprotein immunoglobulin G, NMO = neuromyelitis optica, NMSOD = neuromyelitis optica spectrum disorder, ON = optic neuritis, TM = transverse myelitis.

DISCUSSION

Antibodies directed to MOG can be detected in a subgroup of children with acquired demyelinating syndromes.[17, 18] Recently it has been reported that MOG-IgG also can be found in adults with clinical NMOSD phenotypes and ON.[6-16] In this study we confirm that MOG-IgG is present in approximately one third of the AQP4-IgG seronegative NMOSD cases. An overview of previous studies using a CBA for MOG-IgG testing in NMO(SD) and this study is presented in Table 3.4. In line with previous studies we found a male and Caucasian predominance, frequent coincident ON and TM, more often a monophasic disease course and lower EDSS levels at follow-up in MOG-IgG seropositive cases.[7, 9, 10] Six out of 8 (75%) MOG-IgG seropositive patients with coincident ON and TM had a monophasic disease course as originally described as Devic's syndrome.[1] Follow-up periods between the MOG-IgG and AQP-IgG seropositive patients differed slightly, but not significantly. It cannot be excluded that upon further follow up some of the monophasic MOG-IgG seropositive patients will have relapses. In our study we observed a relatively high rate of a monophasic disease course in the AQP4-IgG seropositive patients, which is a result of our protocol to start immunosuppressive treatment in this group as early as possible, often already after the first attack.

In addition to previous studies, cerebral MRIs from MOG-IgG and AQP4-IgG seropositive patients were assessed for presence of NMO specific brain lesions.[21] These lesions were not present in MOG-IgG seropositive NMOSD patients, which might be explained by different underlying disease mechanisms.

AQP4-IgG, MOG-IgG seropositive NMOSD and seronegative NMOSD seem to be similar in their clinical opticospinal phenotype, although there might be pathophysiological differences between these NMOSD subgroups.[22, 23] Further neuropathological studies are needed in order to improve criteria for clinical NMOSD phenotype, since MOG-IgG seropositivity might reflect a separate demyelinating syndrome.

The presence of MOG-IgG further expands the spectrum of NMOSD. Although the presence of MOG-IgG is rare, the detection of it seems beneficial in clinical practice to differentiate patients with NMOSD from MS[13] and may identify a subgroup of NMOSD patients with favourable outcome with lower EDSS levels at follow-up and less relapses. However it is still important to realise that some MOG IgG seropositive patients experience frequent relapses and have prominent residual neurological deficits.

MOG-IgG was found in 33% of the clinical NMOSD AQP4-IgG seronegative cases. This is higher than has been described in some of the previous studies (Table 3.4).[8-13, 15, 16] However, it is difficult to compare these percentages considering the different study protocols and inclusion criteria.

Our relative high percentage of MOG-IgG seropositivity is probably caused by the retrospective character of our study and selection bias of patients who have been

Table 3.4 Overview of Cell Based Assays (CBA) on MOG-IgG in NMO and NMOSD.

Study	Patients (age at first presentation)	NMO inclusion criteria	Assay	Number of MOG-IgG seropositive cases[a]	MOG-IgG seropositivity as percentage of the AQP-IgG seronegative cases[a]	MOG-IgG seropositivity as percentage of the total included group[a]	Number of double seropositive (AQP4-IgG and MOG-IgG) patients
Mader et al.[6]	Children and adults (range 2 – 84 yrs)	Definite NMO *n*=45, High Risk-NMO ((recurrent) LETM, or recurrent ON) *n*=53	CBA – HEK 293 A cells	9	39%	9%	1
Kitley et al.[8]	(young) adults (range 16 – 34 yrs MOG-IgG seropositives)	AQP4-IgG seronegative NMO/ NMO-SD *n*=27 Control group 44 AQP4-IgG seropositive NMO patients	CBA – HEK 293 cells	4	15%	6%	0
Kitley et al.[7]	(young) adults (mean age 32.2 ± 17.1 yrs MOG-IgG seropositives, 44.9 ± 14.8 yrs AQP4-IgG seropositives).	46 patients with a first CNS demyelinating event with AQP4-IgG *n*=20, Or MOG-IgG *n*=9	CBA – HEK 293 cells	9	35%	20%	0
Sato et al.[9]	Children and adults (range 3 – 78 yrs)	Definite NMO *n*=101, and NMO-SD *n*=114	CBA – HEK 293 cells	16	21%	7%	0
Tanaka et al.[11]	(young) adults (range 15 -78 yrs)	AQP4-IgG seronegative patients with TM or ON *n*=48 Control group 14 AQP4_IgG seropositive NMO patients	CBA – HEK 293 cells	4	8%	6%	0
Höftberger et al.[10]	Adults (range 18 – 77 yrs)	Definite NMO *n*=48 LETM *n*=84, ON *n*=39, ADEM with LETM *n*=3	CBA – HEK 293 cells	17	13%	10%	2

Study	Patients (age at first presentation)	NMO inclusion criteria	Assay	Number of MOG-IgG seropositive cases[a]	MOG-IgG seropositivity as percentage of the AQP-IgG seronegative cases[a]	MOG-IgG seropositivity as percentage of the total included group[a]	Number of double seropositive (AQP4-IgG and MOG-IgG) patients
Ramanathan et al.[14]	(young) adults (range 17 – 59 yrs)	AQP4-IgG seronegative NMO/ NMO-SD *n*=23	CBA – HEK 293 cells	9	39%	39%	n/a[b]
Martinez-Fernandez et al.[16]	Children and adults (range 5 – 65 yrs)	Idiopathic ON *n*=51 Definite NMO *n*=48	CBA – HEK 293 cells	14	23%	14%	2
Probstel et al.[12]	(young) adults (range 15 – 60 yrs)	NMO and NMO-SD *n*=48	CBA – TE 671 cells	4	24%	8%	0
Waters et al.[13]	Children and adults (range 1.3 – 70 yrs MOG-IgG seropositives)	Consecutive serum samples sent for routine AQP4-IgG testing *n*=1,109	CBA – HEK 293 T cells	65	6%	6%	0
Nakajima et al.[15]	(young) adults (16-84 yrs)	Idiopathic ON *n*=29	CBA – HEK 293 cells	8	29%	28%	0
Van Pelt and Wong et al.	(young) adults (mean age 40.2 ± 14.8 yrs)	NMO and NMO-SD defined as LETM, bilateral ON and/or recurrent ON *n*=102	CBA – LN18	20	33%	20%	0
Total	**Children and young adults**	**Definite NMO, NMOSD, ON**	**CBA**	**179**	**11%**	**9%**	**5**

[a]Patients seropositive for both AQP4-IgG and MOG-IgG were excluded; bnot applicable, only AQP4-IgG seronegative patients were included.

ADEM = acute disseminated encephalomyelitis, AQP4-IgG = aquaporin-4 immunoglobulin G, CNS = central nervous system, LETM = longitudinally extensive transverse myelitis, MOG-IgG = myelin oligodendrocyte glycoprotein immunoglobulin G, NMO = neuromyelitis optica, NMSOD = neuromyelitis optica spectrum disorder, ON = optic neuritis, TM = transverse myelitis.

referred to our NMO expert centre at Erasmus MC Rotterdam. The scope of the current study was to compare clinical characteristics of MOG-IgG versus AQP4-IgG seropositive NMOSD patients, rather than to determine the prevalence of MOG-IgG seropositive CNS demyelination. Further studies are needed in order to present epidemiological figures of AQP4-IgG and MOG-IgG seropositivity in the Netherlands.

This study confirms MOG-IgG seropositivity in a subgroup of patients with clinically NMOSD in the Netherlands. Limitation of our study is a relative small sample size and therefore we could not make statistical corrections. Also we have not collected sequential samples.

Even though AQP4-IgG and MOG-IgG represent a considerable amount of patients with the clinical profile of NMOSD, there is still a group of patients without antibodies. Future studies are needed to gain more insight in this group of seronegative NMOSD patients in order to possibly detect new autoantibodies, to better customise treatment for individual patients and to predict their prognosis.

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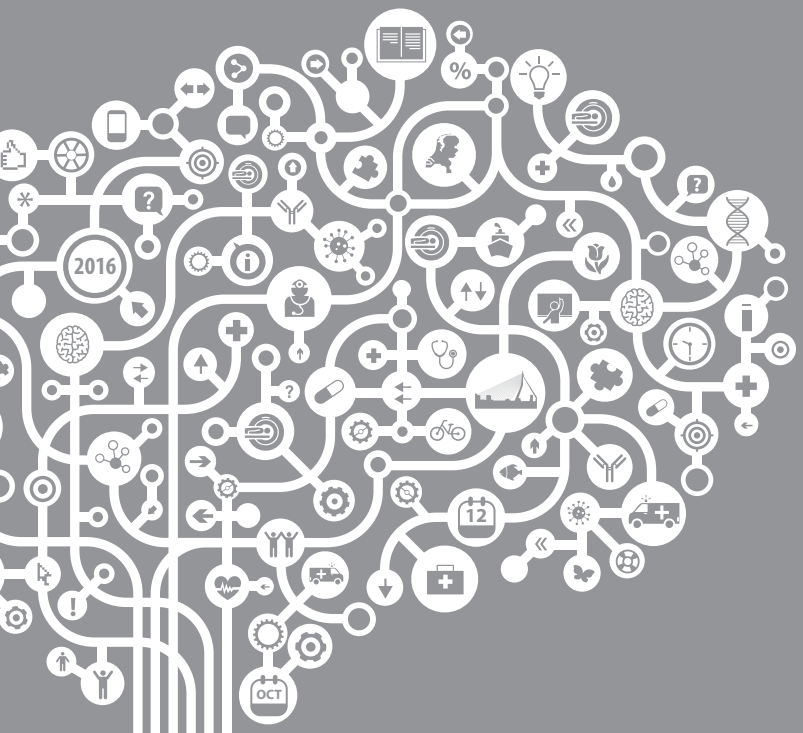
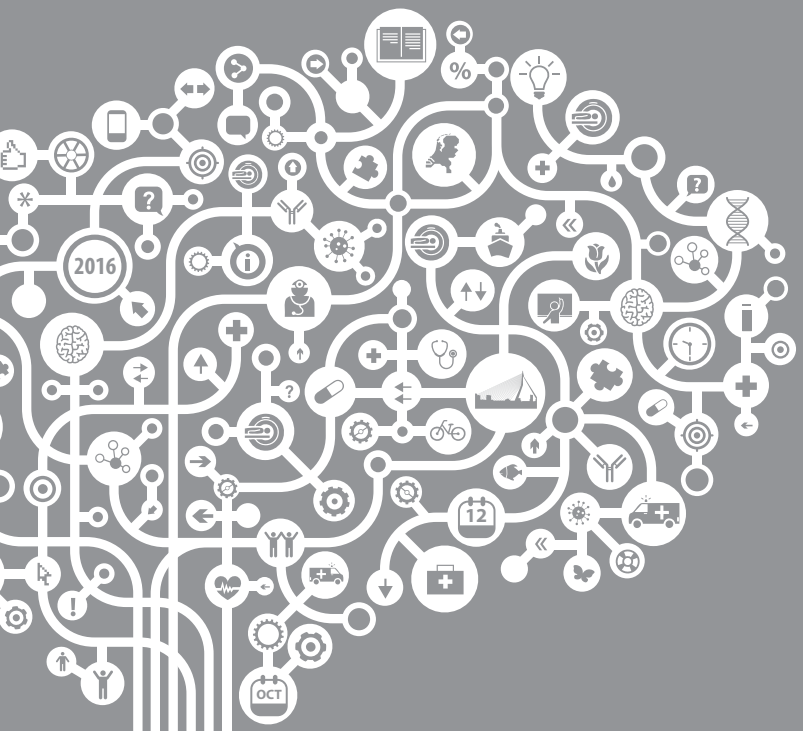


Part two

Acquired demyelinating syndromes in children



Chapter 4

Application of the 2012 revised diagnostic definitions for paediatric multiple sclerosis and immune-mediated central nervous system demyelination disorders

E.D. van Pelt, R.F. Neuteboom, I.A. Ketelslegers, M. Boon, C.E. Catsman-Berrevoets, R.Q. Hintzen. On behalf of the Dutch Study Group for Paediatric MS.

Journal of Neurology Neurosurgery and Psychiatry, 2014

ABSTRACT

Background Recently, the International Paediatric MS Study Group (IPMSSG) definitions for the diagnosis of immune-mediated acquired demyelinating syndromes (ADS) of the central nervous system including paediatric multiple sclerosis (MS) have been revised.

Objective To evaluate the 2012 revised IPMSSG consensus definitions in a cohort of children with ADS prospectively followed from January 2007.

Methods Children with ADS who had an MRI scan obtained within 90 days after first disease onset were included. The sensitivity and specificity of the 2007 and 2012 IPMSSG consensus definitions were assessed. The time to MS diagnosis applying the 2007 and 2012 definitions was compared using survival analysis and log-rank test.

Results Eighty-two children with ADS were included. Thirty-five children were diagnosed with paediatric MS, of whom 30 experienced a second clinical event. The final diagnosis applying either the 2007 or 2012 IPMSSG definitions corresponded. The revised 2012 definitions had sufficient sensitivity (80%) and high specificity (100%). MS diagnosis was made 3.4 months earlier (X^2=8.24, p=0.004) applying the new definitions. In 14 children MS diagnosis was made at first MRI.

Conclusions MS diagnosis can be made reliable and early using the 2012 IPMSSG consensus definitions. This is beneficial for adequate counseling of children and their families and for early treatment possibilities.

INTRODUCTION

Recently, the diagnostic criteria for the diagnosis of immune-mediated acquired demyelinating syndromes (ADS) of the central nervous system (CNS) including paediatric multiple sclerosis (MS) have been revised by the International Paediatric Multiple Sclerosis Study Group (IPMSSG).[1] The new definitions aim to improve consistency in terminology in the heterogeneous group of paediatric demyelinating disorders. They incorporate the in 2010 revised McDonald criteria for MS.[2] Diagnosing MS earlier in the disease course is favourable because disease modifying treatment is also beneficial in children with MS.[3] Previous studies already showed that the 2010 McDonald magnetic resonance imaging (MRI) criteria, which allow diagnosis at disease onset, are useful for early MS diagnosis in children.[4-8] But they recommended to be cautious when applying these MRI criteria to young children with acute disseminated encephalomyelitis (ADEM).[4, 6] Therefore the IPMSSG included in the revised 2012 definitions that the 2010 McDonald MRI criteria for dissemination in time (DIT) and space (DIS) cannot be applied at first event in children with ADEM and children younger than 12 years old.[1] In the new IPMSSG consensus definitions, diagnostic criteria for ADEM and neuromyelitis optica (NMO) have been sharpened and the term recurrent ADEM is excluded. Our aim was to evaluate the new 2012 IPMSSG consensus definitions in our clinical cohort of prospectively included children with ADS.

METHODS

Patients and definitions

Children younger than 18 years, who presented with a first episode of acquired demyelination of the CNS between January 2007 and April 2013, were prospectively included. All children were identified by the Dutch Study Group for Paediatric MS, which consists of paediatric neurologists in 13 major paediatric neurology centres in the Netherlands, or the children were identified by Dutch paediatricians who participate in the NSCK (Netherlands Paediatric Surveillance Unit) as has been described elsewhere.[9] Children were eligible for this study when a cerebral MRI scan had been obtained within 90 days after first onset of symptoms. All patients had a minimum follow-up time of 2 years, unless they were diagnosed with definite MS. MS diagnosis could be made either on evidence of a second clinical attack at least 30 days after the initial attack, or on MRI evidence of DIT within 2 years. Patients were classified as MS, ADEM, clinically isolated syndrome (CIS), NMO, or other relapsing demyelinating disorders according to the 2007[10] and new 2012[1] IPMSSG consensus definitions.

Standard protocol approvals, registrations and patient consents

This study was approved by the Medical Ethical Committees of the Erasmus MC in Rotterdam and of the other participating centres. Written informed consent was obtained from all children and/or their parents.

MRI analysis

MRI scans were performed on 1.5-Tesla MRI scanners with slice thicknesses of 3-5 mm. Scans were archived as electronic images. The presence of lesions was determined on T2-weighted and fluid attenuated inversion recovery (FLAIR) images. All scans were scored blinded to clinical data by 2 experienced raters (E.D.v.P. and R.F.N.). MRI scans were classified as meeting the 2007 IPMSSG definitions for MS[10] (based on the 2001 McDonald MRI criteria[11]) or the 2012 IPMSSG definitions for MS[1] (based on the 2010 McDonald MRI criteria[2]). It should be noted that according to the 2007 IPMSSG consensus definitions, DIS can also be met by the combination of abnormal cerebrospinal fluid (CSF) and two lesions on MRI of which one must be in the brain. The 2012 IPMSSG definitions did not incorporate CSF findings anymore. According to the 2007 IPMSSG consensus definitions, MRI criteria for DIT can only be applied on a MRI scan made 3 months after disease onset in children ≥ 10 years old.[10] In contrast, using the 2012 IPMSSG consensus definitions MS can be diagnosed after a single event in children ≥ 12 years old, not meeting ADEM criteria and meeting the 2010 McDonald MRI criteria for DIS and DIT on the baseline MRI scan.[1]

Statistical analysis

Analyses were performed using SPSS version 20.0. Patient characteristics were compared using Chi-Square and student's *t*-test. Test characteristics of the 2007 and 2012 diagnostic criteria (i.e. sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy) were calculated using clinical definite MS (CDMS) as endpoint. Children with MS diagnosis based on MRI criteria who started disease modifying treatment (DMT) before CDMS diagnosis (*n*=2) and those with a follow-up of <1 years (*n*=3) were excluded from the analysis of test characteristics. Kaplan-Meier survival analyses were used to analyse time to diagnosis according to the 2007 and 2012 IPMSSG consensus definitions. The time to diagnosis was compared using a log-rank test. Survival analysis included all patients with a first MRI with gadolinium and either the presence of a follow-up scan or a diagnosis of CDMS according to the 2010 McDonald MRI criteria.

RESULTS

Ninety-two children presented with a first demyelinating event between January 2007 and April 2013 and were eligible for this study. Eight patients were excluded because cerebral MRI was not acquired at onset (*n*=6 not at onset, *n*=2 only spinal cord MRI). One patient was excluded because the MRI was not of sufficient quality. One patient with monophasic ADS was excluded because of loss to follow-up within 2 years after onset. Eighty-two children were analysed of whom 41 were younger than 12 years at onset.

In Table 4.1, patient characteristics including sex, age at first onset of symptoms, type of onset, follow-up time in months and final diagnosis are presented. CSF data are also presented in Table 4.1, since the 2007 IPMSSG definitions incorporate CSF in the criteria for DIS, whereas CSF positivity is defined by either the presence of oligoclonal bands or an elevated IgG index.[10]

There were no discrepancies in final diagnosis according to the 2007 or 2012 IPMSSG criteria for paediatric MS and other CNS demyelinating disorders. Thirty-five children

Table 4.1 Patient characteristics.

	Multiple sclerosis (*n*=35)		**Other ADS (*n*=47)**		***p*-value**
Female/male	20/15		30/17		NS
Age at onset					
Mean, SD	14.7 ± 2.8		7.5 ± 4.8		*p*<0.001
Range	5.3 – 17.7		1.0 – 16.7		
Median	15.5		6.2		
Type of onset (%)					*p*<0.001
Optic Neuritis	6 (17.1%)		10 (21.3%)		
Transverse Myelitis	1 (2.9%)		2 (4.3%)		
Other monofocal CIS	12 (34.3%)		2 (4.3%)		
Polyfocal CIS	16 (45.7%)		13 (27.7%)		
ADEM	-		20 (42.6%)		
CSF analysis performed (%)	31 (88.6%)		45 (95.7%)		NS
Positive CSF (valid %)*	26 (83.9%)		8 (17.8%)		*p*<0.001
Follow-up time months (mean, SD)	32 ± 17		38 ± 12		NS
Final diagnosis according to the 2007 and 2012 consensus definitions	CDMS	30	CIS	22	
	Definite MS based on MRI evidence	5	ADEM	19	
			NMO	4	
			Other relapsing ADS	2	

*CSF positivity is defined by either the presence of oligoclonal bands or an elevated IgG index.[10]
ADEM = acute disseminated encephalomyelitis, ADS = acquired demyelinating syndromes, CDMS = clinical definite multiple sclerosis, CIS = clinically isolated syndrome, CSF = cerebrospinal fluid, MRI = magnetic resonance imaging, MS = multiple sclerosis, NMO = neuromyelitis optica, NS = non-significant.

are diagnosed with MS according to both 2007 and 2012 IPMSSG definitions. Five of them did not have a second clinical event confirming CDMS during current follow-up. Two of these patients have started DMT, which may have suppressed a second clinical event. Children with MS were older (F=18.35, $p<0.001$) and have a different type of onset ($X^2=27.6$, $p<0.001$) as compared to children with monophasic or other relapsing demyelinating disorders. None of the children currently diagnosed with MS presented with ADEM, as defined by a polyfocal event with encephalopathy.[1]

Four patients were diagnosed with NMO according to both 2007 and 2012 consensus definitions.[1, 10] Three of them suffered from a relapsing disease course. Anti-aquaporin-4 antibodies were tested in 39 patients (48%) as part of their diagnostic follow-up using a cell based assay and fluorescence activated cell sorter (FACS).[12] Two patients had anti-aquaporin-4 IgG seropositivity, one patient with recurrent NMO and one patient who presented with a brainstem syndrome and was diagnosed with NMO related spectrum disorder.[13] Two patients had a final diagnosis of other relapsing demyelinating disorders: one patient suffered from a relapsing optic neuritis (ON) in absence of MRI lesions fulfilling DIT for MS diagnosis and aquaporin-4 antibodies for NMO spectrum diagnosis. The other patient suffered from a first event of ADEM followed by one event of ON without presence of new MRI lesions during follow-up.[14] None of the children with ADEM-onset had a subsequent event of ADEM (multiphasic ADEM).

In Table 4.2 sensitivity and specificity of the 2007 and 2012 IPMSSG consensus definitions are presented with CDMS as endpoint using only the baseline characteristics and using also follow-up MRI. The 2012 IPMSSG consensus definitions had better sensitivity (79 vs 68%), NPV (88 vs 71%) and accuracy (92 vs 82%) compared to the 2007 definitions. The test characteristics differed not significantly. For the calculations of DIS and DIT 2010 McDonald MRI criteria at onset, only children who had a first MRI with gadolinium were included. Two patients who presented with ADEM fulfilled the 2010 McDonald MRI criteria at the first MRI and one of them also fulfilled DIS and DIT 2001 and 2010 McDonald MRI criteria at second MRI (>3 months after onset). Both these patients had negative CSF at onset and did not have a second event. Follow-up time of these patients was 53 and 58 months respectively. However, according to the 2012 IPMSSG definitions the 2010 McDonald MRI criteria do not apply for children with an ADEM-onset and both children were younger than 12 years old. Therefore according to both the 2007 as 2012 IPMSSG consensus definitions these children cannot be diagnosed as MS. In contrast, 2 other patients presenting with ADS and younger than 12 years fulfilled the 2010 McDonald MRI criteria for DIS and DIT at baseline, but because of their age they were not diagnosed with MS at first MRI according to 2012 IPMSSG consensus definitions. Both these patients eventually showed clinical progression and thus were diagnosed with CDMS and turned out to have a false-negative test result at baseline. Applying the 2010 DIS and DIT McDonald MRI criteria at disease onset, thus irrespective of the age limit of 12

Table 4.2 Test characteristics of the MRI criteria include the 2007 and 2012 IPMSSG consensus definitions when applied to distinguish MS from other acquired demyelinating syndromes (ADS) of the CNS.

	First MRI	**First MRI**	**First MRI**	**First MRI**	**First MRI**	**First and second MRI**	**First and second MRI**
	2007 DIS McDonald 2001 criteria (at least 3 out of 4); - ≥9 esions on T2-weighted images or 1 gadolinium-enhancing lesion - ≥3 Periventricular lesions - ≥1 Juxtacortical lesion - ≥1 Infrantentorial lesion	2007 DIS - abnormal CSF - 2 lesions on the MRI of which 1 must be in the brain*	2012 DIS McDonald 2010 criteria (at least 2 out of 4); - ≥1 Spinal cord - ≥1 Periventricular - ≥1 Juxtacortical lesion - ≥1 Infrantentorial lesion	2012 DIT McDonald 2010 criteria; - Simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions. - Excluding children ≤12 years old†	2012 DIS+DIT McDonald 2010 for DIS and DIT at baseline. - Excluding children ≤12 years old†	2007 DIS+DIT McDonald 2001 DIT; - New T2- or gadolinium enhancing lesion(s) more than 3 months after onset on follow-up MRI. - Excluding children ≤10 years old‡	2012 DIS+DIT McDonald 2010 DIT; - New T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, with reference to a baseline scan irrespective of the timing of the baseline scan - Simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions at any time§
Sensitivity (%)	70	75	93	63	58	68	79
Specificity (%)	96	89	77	100	100	100	100
PPV (%)	91	81	71	100	100	100	100
NPV (%)	83	85	95	82	80	71	88
Accuracy (%)	86	84	83	86	85	82	92

In this table test characteristics are presented with CDMS as endpoint using first and second MRI.

* Calculated for patients when CSF analysis was performed (*n*=73).

† Calculated for children who had a first MRI with gadolinium (*n*=65).

‡ Calculated for children who had a second MRI following 3 months after onset (*n*=39).

§ Calculated for children who had their first MRI with gadolinium and/or had second MRI, excluding children ≤12 years old for DIT 2010 at baseline (*n*=73).

ADS = acquired demyelinating syndromes, CNS = central nervous system, CSF = cerebrospinal fluid, DIS = dissemination in space, DIT = dissemination in time, IPMSSG = International Paediatric MS Study Group, MRI = magnetic resonance imaging, MS = multiple sclerosis, NPV = negative predictive value, PPV = positive predictive value.

years, resulted in similar test characteristics (sensitivity 67%, specificity 100%, PPV 100%, NPV 83%) as when applied only in children older than 12 years (Table 4.2, column 5). In a subgroup of 41 children younger than 12 years at onset, including 4 patients with MS, we found a lower sensitivity (50%) but higher NPV (95%) applying the 2010 McDonald MRI criteria, irrespective of the age limit at first MRI, but a high sensitivity (100%) when a follow-up MRI is included.

Sixty-five children, including 24 children with MS, had a first MRI with gadolinium administration. MS was diagnosed in 14 (47%) children at first MRI (excluding 2 children younger than 12 years) using the 2012 IPMSSG definitions. Six (20%) of these patients had a spinal cord lesion contributing to DIS McDonald MRI criteria.

Twenty-seven children were eligible for the survival analysis. Kaplan Meier survival curves (Figure 4.1) show the time to MS diagnosis using the 2007 versus 2012 IPMSSG consensus definitions. MS diagnosis in children can be made significantly earlier (X^2=8.24, p=0.004) using the revised 2012 consensus definitions based on the 2010 McDonald MRI criteria, similar as in adults.[15] The mean time to diagnosis was 8.5 ± 7.4 months and 5.1 ± 7.2 months applying the 2007 and 2012 IPMSSG definitions respectively (t=6.87, p<0.001). The average time to MS diagnosis is 3.4 months earlier using the 2012 IPMSSG definitions. The mean time to a second event defining CDMS was 10.1 ± 8.7 months.

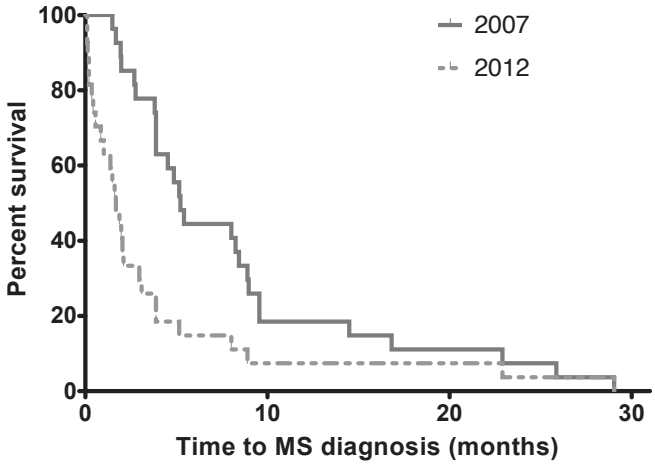


Figure 4.1 Kaplan-Meier survival curves showing the time to MS diagnosis using the 2007 versus 2012 International Pediatric Multiple Sclerosis Study Group (IPMSSG) consensus definitions. The 2012 IPMSSG definitions allow for an earlier diagnosis (χ^2=8.24, p=0.004).

DISCUSSION

In this study the revised 2012 IPMSSG consensus definitions for paediatric MS and immune-mediated CNS demyelinating disorders were evaluated in a prospective nationwide cohort of children aged 1.0 to 17.7 years old with the full spectrum of immune-mediated CNS demyelination disorders. The 2010 revised McDonald MRI criteria have already been studied and found useful in children with CNS demyelination.[4-8] In contrast to these previous studies, not only the revised 2010 McDonald MRI criteria were considered in the present study, but also other clinical characteristics as proposed in the 2012 IPMSSG definitions. This means that the 2010 McDonald MRI criteria for DIT on the first MRI can only be applied to children of 12 years and older and having a first clinical event not meeting criteria for ADEM. This study showed that the 2012 IPMSSG consensus definitions allow for an equally reliable, but earlier MS diagnosis in all children, also those younger than 12 years. Fourteen (47%) MS patients could be diagnosed at first MRI using the 2012 IPMSSG definitions. Six of these patients (20%) fulfilled DIS McDonald criteria because of the presence of spinal cord lesion(s), confirming the importance of a spinal cord MRI at onset.[7]

An important finding in our study is that the final diagnoses corresponded applying either the 2007 or 2012 IPMSSG consensus definitions. However, using the 2012 definitions, a diagnosis of MS can be made earlier, which is beneficial for adequate counseling of the children and their families, as prognosis can be uncertain after ADS, and children with MS may benefit from early treatment.

Test characteristics were better when applying the 2012 IPMSSG definitions compared to the 2007 definitions. When including follow-up MRI scans for the application of the 2007 and 2012 IPMSSG definitions, both have a high specificity and PPV, indicating the absence of false positive test results. This is because the McDonald MRI criteria do not apply for children aged ≥ 10 years (2007 IPMSSG definitions) and ≥ 12 years at baseline (2012 IPMSSG definitions) and for children with an onset of ADEM (2012 IPMSSG definitions). Test characteristics for DIT based on MRI criteria according to 2007 IPMSSG definitions were calculated for 39/77 patients with a follow up scan ≥ 3 months after the onset of clinical symptoms. This may have introduced some bias as the decision to make a second MRI might not be fully independent of the initial observed disease activity.

When applying the 2010 McDonald MRI criteria to all children (regardless of age) test characteristics are comparable although sensitivity would be higher, due to the finding that 2 patients ≤ 12 years old with paediatric MS tested false negative. In a subgroup of children aged younger than 12 years old we found lower sensitivity and NPV, indicating more false negative results. A second MRI increased the sensitivity. When the DIS and DIT 2010 criteria are not applied in children with ADEM at onset but regardless of age,

high specificity and PPV was found, which might warrant exclusion of the age limit. Further investigation of the age limit is needed.

In contrast to the study of Sadaka et al.[4] we did not find 100% sensitivity and NPV for the 2010 McDonald MRI criteria. This is probably explained by the fact we did not use a standardised MRI protocol including systematic frequent follow-up MRIs. Despite the lack of a systematic MRI protocol, we did find earlier MS diagnosis in the Dutch cohort which resembles general clinical practice.

We only included patients with a minimum follow-up of 2 years (unless they were diagnosed with MS based on MRI criteria within 2 years (*n*=3)) because the time interval between first and second attack in paediatric MS is typically less than 12 months.[16, 17] Despite a relatively long follow-up it still might be that patients currently defined as monophasic will experience relapses in the future.

A first onset of ADEM is an important clinical parameter when using the 2012 consensus definitions. In our prospective cohort none of the patients with a first onset of ADEM developed MS during follow-up, confirming its role as a negative predictor for MS. Using the 2012 consensus definitions, children with ADEM can be diagnosed with MS earlier if they experience a second non-ADEM event with new MRI lesions at least 3 months after onset. We did not have such patients in our current cohort. Relapse after ADEM typically occurs within 2 years after onset, but in a small subgroup can occur many years later.[1]

Using the new 2012 consensus definitions for the diagnosis of paediatric MS, CSF analysis is not strictly needed. Although in clinical practice CSF analysis in children presenting with neurological symptoms is still of importance in order to exclude other diagnoses as infections and malignancies.[18] It should be further investigated if CSF analysis is of use in doubtful cases were MRI criteria have not been completely fulfilled.

The results of our prospective multicentre cohort show that the 2012 IPMSSG consensus definitions apply well for a reliable and early diagnosis of paediatric MS and ADS.

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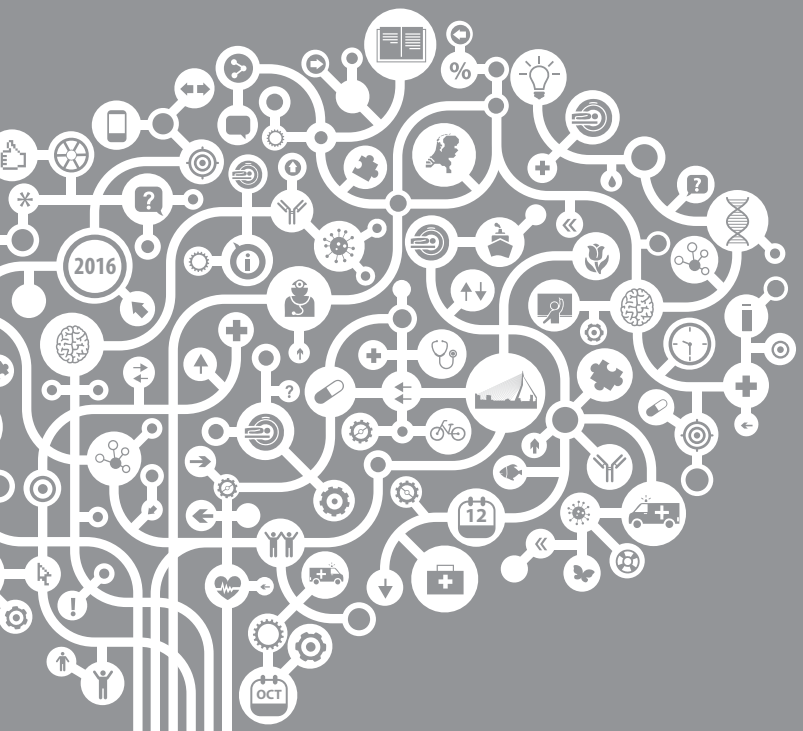

Chapter 5

Validation of MRI predictors of multiple sclerosis diagnosis in children with acute CNS demyelination

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ABSTRACT

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 115 children identified with ADS between December 1993 and 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.

INTRODUCTION

The ability of magnetic resonance imaging (MRI) to aid in identifying children at risk for multiple sclerosis (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 acute demyelinating syndromes (ADS) of the central nervous system (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%).[1] 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.

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 13 pediatric healthcare centers in the Netherlands.

Archived group

Children less than 17 years of age who presented with an ADS, based on clinical features, between 1990 and 2007 inclusive were identified from hospital records by pediatric neurologists at 11 participating centers. These centers are located in nine large cities with complete geographic coverage of the Netherlands. 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 being consecutively enrolled into a nationwide prospective study at each of the 13 participating centers (8 academic pediatric hospitals and 5 regional hospitals). To confirm nationwide inclusion of chil-

dren with ADS, email surveys were sent out to pediatricians through the Netherlands Pediatric Surveillance Unit (response rate: 85%) in which they reported suspected cases of ADS. Uniform definitions for ADS[2] 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,[2] 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 incident demyelinating attack. Each participant had at least two years of clinical observation following acute demyelination. Children with neuromyelitis optica were excluded.[3] 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,[4] 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.[5] 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.[2] 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 at-

tenuated 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.[1] 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.[1] Two parameters were scored on each baseline scan as described in our original work:[1] i) *T1-hypointense lesions* were defined as lesions hypointense relative to cortical grey matter and nonenhancing 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).[6] 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,[6] 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).[1, 7-9] 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[4] or MRI evidence of DIT alone[5]). Statistical analyses were conducted using Stata version 11 (College Station, Texas, USA).

RESULTS

Of 115 children and adolescents presenting with an incident ADS between December 1993 and December 2009 who were eligible, 87 were analyzed (Figure 5.1). 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 Supplementary Table 5.1, the 28 children excluded from the present analysis did not differ from the 87 participants retained in the study with respect to sex (*p*=0.2307), ADEM versus non-ADEM presentations (*p*=0.4833), mean age at onset (*p*=0.7691), or proportion of patients diagnosed with MS (*p*=0.1951).

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 5.1). 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

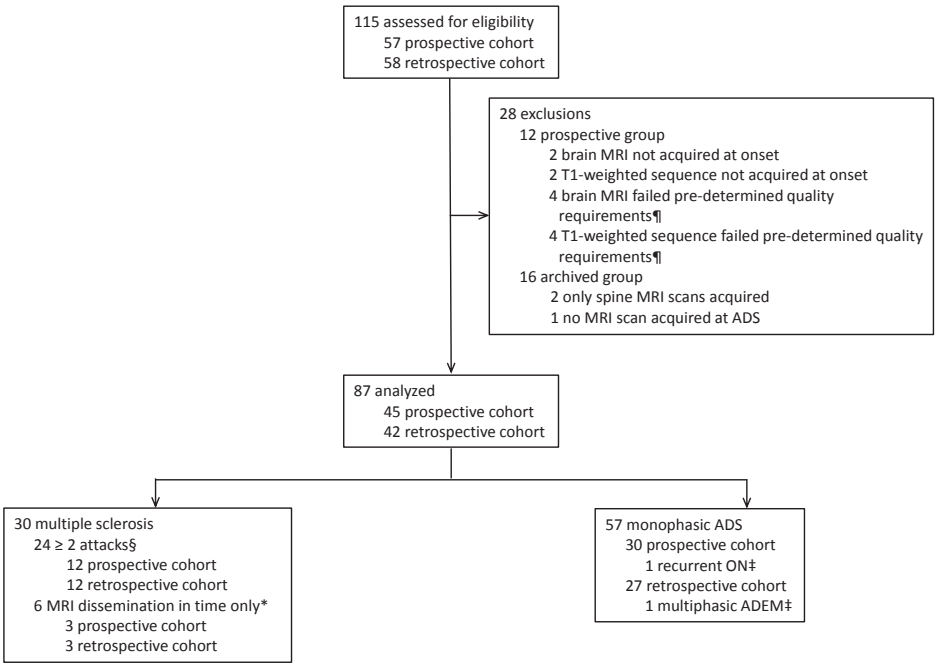

Figure 5.1 Description of cohort:
¶ MRI scans do not pass quality control if they are degraded by motion or dental hardware artifact, and if images have poor grey matter-white matter contrast or in adequate coverage of the brain.
§ Attacks separated by at least 28 days and localize to distinct regions of the central nervous system.[4]
*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.[5]
‡ 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.[2]
MRI = magnetic resonance imaging, ADS = acute demyelinating syndrome, ON optic neuritis.

Table 5.1 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)	0.734
Age at onset							
<10 years	19 (45%)	4 (27%)	15 (56%)	26 (58%)	1 (7%)	25 (83%)	0.286
≥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%)	0.521
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)	--	0.121§
Time to MS diagnosis (years)	--	1.2 (0.5-1.7)	--	--	0.3 (0.2-0.7)	--	0.019§
2nd clinical attack	--	12 (80%)	--	--	12 (80%)	--	1.0§
ADS phenotype							
ADEM	13 (31%)	1 (7%)	12 (44%)	15 (33%)	0 (0%)	15 (50%)	0.605
Transverse myelitis	5 (12%)	1 (7%)	4 (15%)	2 (4%)	1 (7%)	1 (3%)	
Optic neuritis	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%)	0.779

*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 demyelination4, 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.[5]

Data are mean (standard deviation, SD), median (interquartile range, IQR) or number (%).

MS = multiple sclerosis, ADS = acute demyelinating syndrome, ADEM = acute disseminated encephalomyelitis.

(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 acquired.

Of 87 children, 30 (34%) children were diagnosed with MS, and 57 (66%) have experienced monophasic ADS to date (Table 5.1). Of the 30 children diagnosed with MS, 24 (80%) experienced two or more clinical attacks,[4] whereas the remaining 6 (20%) patients were diagnosed with MS based on MRI evidence of DIT[5] alone. The median time from incident demyelination to MS diagnosis 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=0.019). 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.[2] Of the 27 children with clinically monophasic ADEM, 17 had serial MRI scans and none had new lesions.

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 5.2 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=0.0686), specificity (86.7% vs. 85.2%; p=0.8706), PPV (77.8% vs. 71.4%; p=0.6783), and NPV (96.3% vs. 82.1%; p=0.0915) 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.

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 5.2, when MS diagnosis was defined on the basis of clinical relapses only[4], 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

Table 5.2 Performance of MRI parameters for predicting MS diagnosis.

	MS diagnosis according to occurrence of 2nd attack[4] *or* 2005 McDonald DIT[5]			**MS diagnosis according to occurrence of 2nd attack[4] alone**		
	Archived Group (*N*=42)	Prospective Group (*N*=45)	*p*-value	Archived Group (*N*=42)	Prospective Group (*N*=45)	*p*-value
True Positives, *n*	10	14		8	11	
True Negatives, *n*	23	26		24	26	
False Negatives, *n*	5	1		4	1	
False Positives, *n*	4	4		6	7	
Sensitivity, % (95% CI)	66.7 (38.4-88.2)	93.3 (68.1-99.8)	0.0686	66.7 (34.9-90.1)	91.7 (61.5-99.8)	0.1314
Specificity, % (95% CI)	85.2 (66.3-95.8)	86.7 (69.3-96.2)	0.8706	80.0 (61.4-92.3)	78.8 (61.1-91.0)	0.8988
Positive Predictive Value, % (95% CI)	71.4 (41.9-91.6)	77.8 (52.4-93.6)	0.6783	57.1 (28.9-82.3)	61.1 (35.7-82.7)	0.8192
Negative Predictive Value, % (95% CI)	82.1 (63.1-93.9)	96.3 (81.0-99.9)	0.0915	85.7 (67.3-96.0)	96.3 (81.0-99.9)	0.1717
Positive Likelihood Ratio (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)	
Negative Likelihood Ratio (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)	

DIT = dissemination in time, CI = confidence interval.

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.

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, supporting our initial work.[1] 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[7] from which the MRI parameters were originally developed.[1] Specifically, both studies utilized the International Pediatric MS Study Group consensus definitions[2] 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 cohort 93%, Canadian study 84%) and specificity (Dutch cohort 87%, Canadian study 93%) of the MRI parameters.

We then applied the MRI parameters to 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 estimate of sensitivity (67%), although not statistically significantly different, is lower than that from the prospective cohort (93%). Although all MRI scans analyzed were considered to be of sufficient quality for scoring, the increased number of false negatives in the archived cohort may be due to image quality and variable sequence acquisition in the archived dataset compared with the prospective cohort. Specifically in the archived set, 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[10] includes axial FLAIR and T2 sequences with contiguous three millimeter slices, we anticipate that these sequences will enhance sensitivity of our MRI parameters.

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[11-13], MRI parameters that effectively stratify unselected groups of children with ADS, including those with ADEM, into high and low likelihood for MS diagnosis 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[14, 15], 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.

Our study has several limitations. In the archived collection of MRI scans, acquisition orientation, slice thickness, interslice gap and image contrast were not consistent, and images were acquired on 1.0 T and 1.5 T scanners. The children enrolled in the prospective Dutch program were imaged with more consistent protocols, and were all imaged at 1.5 T. Our primary goal was to devise a predictive tool suited to application in the clinical context, and across variable imaging protocols. While the results of our predictive model performed better when applied to more rigorously acquired MRI scans, our parameters remained valid even within the limits of variable sequence parameters or quality. We also acknowledge the limitation of including both a retrospective and prospective patient sample in our present analysis. For the retrospective pediatric MS patients, MS diagnosis was made largely on the basis of confirmed relapsing clinical disease, given that consistent serial MRI studies were not available for earlier diagnostic confirmation of MS. In contrast, for the prospectively enrolled children, MRI studies were more frequently and consistently obtained, and thus new lesions on MRI were more likely to be detected, as reflected by the shorter time from first attack to MS diagnosis in this group. For our purposes, however, the outcome was not time to MS diagnosis; rather, we focused only on whether our MRI parameters correctly distinguished children with MS from those with monophasic disease. Finally, as is true for all predictive models, it is imperative to acknowledge that some children currently considered to have monophasic disease, may ultimately experience new disease activity leading to a diagnosis of MS.

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 (suggestive of established pathology) are robust predictors of a future diagnosis of MS in children with ADS.

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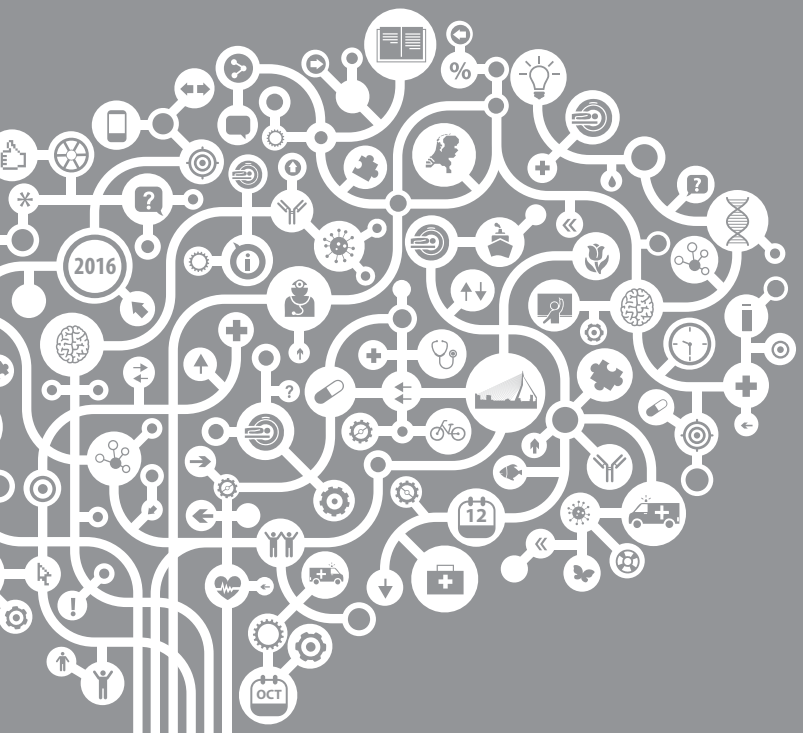
SUPPLEMENTAL MATERIAL

Supplementary Table 5.1 Demographic and clinical characteristics of excluded patients (*n*=28).

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%)**

*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.

QC = quality control, MRI = magnetic resonance imaging, F = female, M = male, ADS = acute demyelinating syndrome, ADEM = acute disseminated encephalomyelitis, TM = transverse myelitis, ON = optic neuritis, MS = multiple sclerosis



Chapter 6

Disease course after CIS in children versus adults: a prospective cohort study

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Submitted

ABSTRACT

Background Clinically isolated syndrome (CIS) is a first demyelinating event of the central nervous system (CNS) and can be a single event. After CIS a chronic disease course with ongoing inflammation and relapses might occur, resulting in a diagnosis of multiple sclerosis (MS). Whether children and adults with CIS have the same disease course has not been prospectively explored yet.

Methods Patients with CIS, whose age ranged from 1-50 years, were prospectively followed. We divided the patients in four different age groups: patients 1-10, 11-16, 17-29, and 30-50 years old. Among these groups demographic data, disease course, time to MS diagnosis and annualized relapse rates (ARR) were compared.

Results We included 383 CIS patients, of whom 218 (56.9%) were diagnosed with MS. 11-16 year-old children had the highest rate of MS conversion (85% versus 51% in the other age groups together, $p<0.01$) and the shortest time to MS diagnosis (median time 2.6 months (IQR: 0.62-5.94) versus 7.8 months (IQR: 2.0-25.5) in the other age groups together, $p<0.01$). Highest ARR were found in 1-10 year-old children with MS (ARR: 0.79), followed by 11-16 year-old children with MS (ARR: 0.62). In 30-50 year-old adults with MS the ARR was 0.41.

Conclusion Children with CIS tend to have a more inflammatory disease course appearing from the high relapse rate in all children, and the highest rate of MS conversion in 11-16 year-old children. This supports early initiation of first-line disease modifying therapy in children with CIS who are at high risk for a future MS diagnosis.

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) which can cause a broad spectrum of neurological deficits.[1] Worldwide over 2.5 million people suffer from MS.[1] MS mostly affects young women in their early twenties or thirties, and although rare, MS can also occur in children.[2] MS can be diagnosed when a first event of CNS demyelination, a so-called clinically isolated syndrome (CIS), is followed by a new clinical event.[3-5] In addition, MS can be diagnosed after CIS when new lesions, which fulfill MS diagnostic criteria for dissemination in time and space, are detected on Magnetic Resonance Imaging (MRI).[4, 5] However CIS can be a single event. Whether childhood-onset versus adulthood-onset CIS and MS reflect the same disease is unknown.[6-8] Previous studies focused on MS and did not compare the disease course between children and adults after the onset of CIS.[9-15] Despite existing parallels in childhood-onset and adulthood-onset MS, there are fundamental differences in presentation and disease course. Several studies have reported a higher relapse rate and MRI lesion load in children than in adults with MS.[10, 12, 13, 16, 17] On the other hand, the progression of disability is slower in children with MS.[14, 15] It should be noted, however, that some of the previous studies included retrospective cohorts[11, 14, 15, 17], and some were conducted before the immunomodulatory treatment era in children.[9-11, 14, 15] Current recommendation and use of disease modifying therapies (DMT) in children with MS likely influenced their disease course and relapse rate.[18] Several studies have shown initiation of first-line DMT (interferon or glatiramer acetate) in adults with CIS delays MS diagnosis and disability accumulation.[19-21] In children with CIS it is not common practice yet to offer first-line DMT prior to MS diagnosis. Here, we performed a clinical prospective follow-up study of 383 patients with CIS, whose age ranged from 1 - 50 years, in order to report a comparison of childhood-onset versus adulthood-onset CIS and MS.

METHODS

Patients and definitions

Patients with CIS were included between April 2006 and August 2015, either in our prospective cohort of adults with CIS (PRedicting the Outcome of a Demyelinating event, PROUD study)[22], or in our prospective cohort study of children with a first episode of acquired demyelination of the CNS (PROUDkids).[23] Both studies are ongoing multicenter observational studies conducted at Erasmus MC in Rotterdam, the Netherlands, and study protocols have been described previously.[22, 23] Patients younger than 51 years old were included within 6 months after the first onset of their clinical symptoms. At baseline, a MRI scan was performed and routine laboratory tests were done. Patients

with alternative diagnoses other than CIS were excluded. After baseline patients were reassessed at least annually.

We used a prediction rule for defining children with CIS at high risk of MS when they fulfilled two out of three criteria at onset: more than nine T2 white matter lesions, and/or at least one gadolinium-enhancing lesion on MRI, and/or positive oligoclonal bands (OCB) in cerebrospinal fluid.[24, 25] MS was diagnosed in adults according to the 2010 McDonald criteria.[5] Children were diagnosed with CIS and MS according to the diagnostic criteria from the International Pediatric Multiple Sclerosis Study Group (IPMSSG).[4] Clinically definite multiple sclerosis (CDMS) was defined as two attacks with (para)clinical evidence of two separate lesions as described by Poser[26] and should include two non-encephalopathic events.[4] The expanded disability status scale (EDSS scale) was used to define disability.[27] EDSS scores were obtained at least 2 months after a relapse occurred. Secondary progressive MS is defined as a history of gradual worsening after an initial relapsing-remitting disease course, with or without exacerbations during the progressive course.[28] This study was approved by the medical ethical committees of Erasmus MC in Rotterdam and of the other participating centers. Written informed consent was provided for all patients.

Data analysis

Statistical analysis was performed using SPSS v20 (SPSS Inc., Chicago, Illinois, USA) and GraphPad Prism 5 (GraphPad., San Diego, USA). We divided the patients in four different age groups of clinical interest, based on evidence that puberty enhances CNS autoimmunity in females[29]: i.e. pre-puberty, puberty, young adults and adults (patients 1-10, 11-16, 17-29, and 30-50 years old). Among these groups we compared demographic data, disease course, time to MS diagnosis and annualized relapse rate (ARR). We used the Kolmogorov-Smirnov test to assess normal distribution of the data. For the comparison of continuous data between two groups we applied a two-tailed *t*-test, or when the data were not normally distributed a Mann-Whitney *U* test. For the analysis of multiple groups, we used the one-way-ANOVA for normally distributed data, and Kruskal-Wallis test for not normally distributed data. Categorical data were analyzed using the Chi-square or Fisher's exact test. Kaplan-Meier survival analyses were used to analyse the time to MS diagnosis. Univariate and multivariable Cox proportional hazard regression models were used to obtain hazard ratios for MS diagnosis and EDSS 4.0 respectively. Patients who were not diagnosed with MS, or did not reach EDSS 4.0 during follow-up respectively, were considered as censored observations in those analyses. Negative binomial regression, with the natural logarithm of follow-up years after MS diagnosis as offset, was used to analyse ARR after CIS. This offset was used to correct for the different follow-up times between patients. We used a generalized estimating equations (GEE) model to compare ARR with and without disease-modifying treatment. This GEE model

includes the effect and interaction of treatment and age group and accounts for the correlation within patients.

RESULTS

Characteristics of patients with CIS

We included 383 patients who met our inclusion criteria. The youngest included patient had CIS at the age of 1 year, and the oldest included patient was 50 years at the time of CIS. 218 patients (56.9%) were diagnosed with MS during follow-up. Patient characteristics for the four different age groups are shown in Table 6.1.

CIS patients in the age group 11-16 had the highest rate of MS conversion (85% versus 51% in the other age groups together $p<0.01$) and the shortest time to MS diagnosis (median time 2.6 months (IQR: 0.62-5.94) versus 7.8 months (IQR: 2.0-25.5) in the other age groups together, $p<0.01$).The time to MS diagnosis is presented in Figure 6.1. Hazard ratios for future MS diagnosis for the different age groups are shown in Table 6.2.

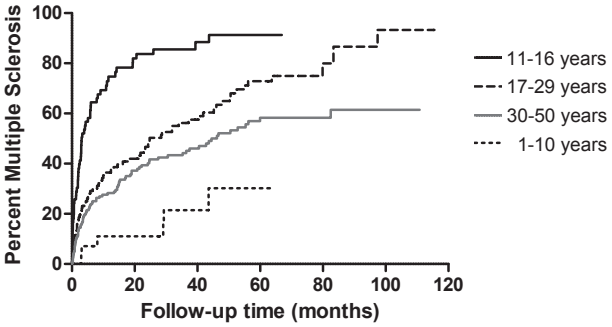

Figure 6.1 Kaplan-Meier curves for time to MS diagnosis for patients in different age categories. (Log Rank test $p<0.01$).

Disease course after MS diagnosis

ARR corrected for follow-up were higher in children than in adults with MS (mean ARR 0.66 vs 0.43, $p<0.01$). ARR in MS patients per age group, and ARR with and without DMT are presented in Table 6.3.

The time to reach EDSS 4.0 in patients who were diagnosed with MS was shorter in 30-50 year-old adults than in the other groups, hazard ratio 3.0 ($p=0.04$). Using a multivariable COX regression model including MRI and CSF features ($n=119$), in which we corrected for optic neuritis at CIS, the presence of gadolinium enhancing lesions and positive OCB in CSF, the hazard ratio was 4.5 ($p=0.03$). Kaplan-Meier curves for time to EDSS 4.0 in MS patients are demonstrated in Figure 6.2. Eight patients in the age group

Table 6.1 Patient characteristics.

Age groups	1-10 years (*n*=28)	11-16 years (*n*=66)	17-29 years (*n*=115)	30-50 years (*n*=174)	Total (*n*=383)	*p*-value
Female sex, n(%)	16 (57.1%)	39 (59.1%)	85 (73,9%)	128 (73.6%)	286 (70.0%)	*p*=0.05
Mean age, years (SD)	6.6 (3.05)	14.8 (1.51)	24.7 (3.8)	38.9 (3.8)	28.1 (11.8)	
Caucasian ethnicity, n(%)	19 (70.4%)	33 (50.8%)	79 (70.5%)	139 (80.8%)	270 (70.5%)	*p*<0.01
Type of clinical onset						
ON, n(%)	10 (35.7%)	20 (30.3%)	49 (42.6%)	64 (36.8%)	143 (37.3%)	NS
Spinal cord, n(%)	2 (7.1%)	9 (13.6%)	28 (24.3%)	52 (29.9%)	91 (23.8%)	*p*<0.01
Other monofocal symptoms, n(%)	6 (21.4%)	16 (24.2%)	22 (19.1%)	39 (22.4%)	83 (21.7%)	NS
Other polyfocal symptoms, n(%)	10 (35.7%)	21 (31.8%)	16 (13.9%)	19 (10.9%)	66 (17.2%)	*p*<0.01
Features first MRI						
≥9 lesions on T2-weighted images, n(%)	6 (22.2%)	38 (57.6%)	48 (42.1%)	59 (34.1%)	151 (39.7%)	*p*<0.01
Dissemination in space[a], n(%)	8 (28.6%)	49 (74.2%)	56 (49.1%)	78 (45.3%)	191 (50.3%)	*p*<0.01
Gadolinium-enhancing lesions, n(%) (*n*=284)	4 (17.4%)	29 (55.8%)	36 (42.4%)	53 (43.4%)	122 (43.3%)	*p*=0.02
CSF findings						
WBC count (·10^6/L), median (IQR) (*n*=216)	5.0 (2.0-16.0)	8.0 (4.3-18.0)	10.0 (6.5-19.5)	4.0 (3.0-10.0)	7.0 (3.0-17.8)	*p*<0.01
Positive OCB, n(%) (*n*=252)	6 (27.3%)	43 (82.7%)	57 (79.2%)	74 (69.8%)	180 (71.4%)	*p*<0.01
IgG index, median (IQR) (*n*=242)	0.64 (0.49-0.79)	0.95 (0.68-1.42)	0.86 (0.59-1.35)	0.74 (0.56-1.20)	0.81 (0.57-1.30)	*p*<0.01
Follow-up						
CDMS, n(%)	6 (21.4%)	45 (68.2%)	49 (42.6%)	62 (35.6%)	162 (42.3%)	*p*<0.01
MS[b], n(%)	6 (21.4%)	56 (84.8%)	72 (62.6%)	84 (48.3%)	218 (56.9%)	*p*<0.01
Time CIS to CDMS, months (median, IQR)	16.5 (4.00-32.4)	8.9 (3.00-17.3)	18.5 (8.30-42.78)	18.8 (7.78-38.9)	15.2 (6.46-33.8)	*p*<0.01
Time CIS to MS[b], months (median, IQR)	16.0 (3.06-32.36)	2.6 (0.62-5.94)	8.8 (1.61-31.1)	6.5 (1.97-22.06)	4.8 (1.57-21.1)	*p*<0.01
Follow-up, months (mean, SD)	38.3 (16.5)	37.5 (19.8)	45.4(29.9)	50.1 (29.6)	45.6 (27.8)	*p*<0.01
Initiation of DMT before MS diagnosis, n(%)	1 (3.6%)	1 (1.5%)	16 (13.9%)	20 (11.3%)	38 (9.9%)	*p*=0.03

[a]DIS according to McDonald 2010 criteria[5] [b]MS diagnosis according to McDonald 2010 criteria[5] *p*-values describe the comparison between all age groups.

CIS = clinically isolated syndrome, CDMS = clinically definite multiple sclerosis, DMT = disease modifying therapies, IgG = immunoglobulin G, MS multiple sclerosis, NS = non-significant, ON = optic neuritis, OCB = oligoclonal bands, WBC = white blood cell count.

30-50 were diagnosed with secondary progressive MS (SPMS), and none of the younger patients were diagnosed with SPMS during follow-up ($p<0.01$).

Using a prediction rule at onset (methods), 42 out of 94 (45%) 1-16 year-old children with CIS were defined at high risk for a future MS diagnosis. Of them 25 ≥12 year-old children were diagnosed with MS at the first event.[4] The other 17 children were diagnosed with MS during follow-up based on new relapses or on MRI-criteria. None of the children with CIS who remained monophasic during follow-up were defined as having a high risk for MS by using this prediction rule (sensitivity 68%, specificity 100%).

Table 6.2 Hazard ratios for MS diagnosis, univariate cox regression analysis.

Age groups (years)	Number of patients	Number of events	Hazard ratio (95% CI)	*p*-value
1-10	28	6	0.4 (0.2-0.9)	0.03
11-16	66	56	3.6 (2.6-5.1)	<0.01
17-29	115	72	1.5 (1.1-2.1)	0.01
30-50	174	84	1(ref)	

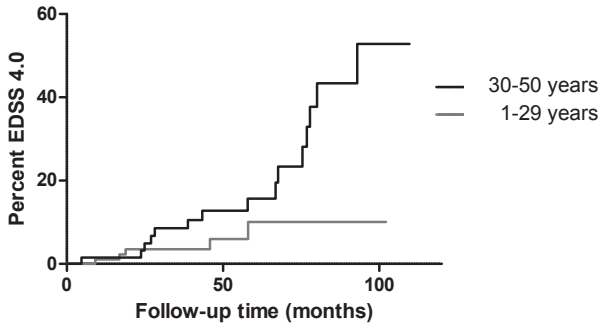

Figure 6.2 Kaplan-Meier curves for time to EDSS 4.0 for MS patients in age groups 1-29 and 30-50. Legend: We merged the younger age groups (1-10, 11-16, 17-29) since the patient numbers were small and their KM-curves overlapped.

Table 6.3 ARR corrected for follow-up in MS patients per age group.

Age group	Total years at risk after MS diagnosis	Total number of relapses	Overall ARR (95% CI)	ARR with-outDMT	ARR with DMT	*p*-value[a]	Interaction coefficient of DMT	*p*-value[b]
0-10 (*n*=6)	15.4	12	0.79 (0.42-1.48)	5.22	0.43	<0.01	5.64	0.033
11-16 (*n*=56)	154.3	94	0.62 (0.49-0.77)	2.13	0.47	<0.01	2.08	0.040
17-30 (*n*=72)	200.6	104	0.52 (0.42-0.65)	1.16	0.45	<0.01	1.19	ns
30-50 (*n*=84)	283.6	114	0.41 (0.34-0.51)	0.81	0.37	<0.01	Ref.	Ref.

[a] Presented p-values for the comparison of ARR with and without DMT within the different age groups.
[b] Presented p-values for the interaction coefficient of DMT for the different age groups.
ARR = annualized relapse rate, DMT = disease modifying therapies, CI = confidence interval

DISCUSSION

The purpose of our study was to report a comparison of childhood-onset versus adulthood-onset CIS and MS in a large prospective cohort during the current immunomodulatory treatment era. The results of our study suggest a more inflammatory disease course, rather than a neurodegenerative disease course, in 11-16 year-old children with CIS, as this group had the highest rate of MS conversion and the shortest time to MS diagnosis, higher ARR, higher MRI lesion load and a more inflammatory CSF profile. A secondary progressive MS disease course, which reflects a more neurodegenerative instead of an inflammatory phase of MS, was found in eight 30-50 year-old adult patients. However, secondary progression in MS is age dependant and probably therefore has not been observed yet in our younger patients with the current follow-up.[30] In addition, we found higher EDSS progression rates in adults than in children. Previous studies on childhood-onset MS versus adulthood-onset MS, have already reported a higher relapse rate in children than in adults.[10, 12, 13] In our unique prospective study we followed children and adults after the first attack. Since we collected a large cohort, we could compare subgroups and draw conclusions for different age groups of clinical interest. Overall, we found a female predominance both in patients with CIS and MS, except in young children who were diagnosed with MS before puberty, of whom four out of six MS patients were boys. The difference in sex distribution in children diagnosed with MS before and after puberty and the higher rates of MS diagnoses after puberty suggests that sex hormones contribute to the onset of MS.[2, 23, 29] A remarkable high rate of non-Caucasian ethnicities was observed in 11-16 year-old children with CIS. Higher rates of non-Caucasian ethnicities have been reported in childhood-onset MS and might reflect a higher vulnerability of developing MS at a younger age.[23, 31, 32] A possible explanation for this might be that non-Caucasians miss certain protective genes since their ancestors were born in countries with a low prevalence of MS.[23] In the lowest age group of 1-10 years old, 28.6% of the children with CIS were non-Caucasians, while 83.3% of the children with MS were non-Caucasians.

The shorter time to MS diagnosis in 11-16 year-old children is partly explained by the current diagnostic MRI criteria which allow for an early MS diagnosis in a subgroup of the patients at first MRI, however not in children younger than 12 years of age.[4, 5] Still, we observed a high rate of CDMS and short time to CDMS diagnosis in 11-16 year-old children.

Early initiation of DMT in adults with CIS might have influenced their disease course. However, a relatively small proportion of adults (12.5%) started with DMT before MS diagnosis and in a subanalysis (data not shown) we found similar results when we excluded these patients. Relapse rates decrease after the initiation of DMT in both children and in adults with MS. It is unlikely our observed differences in ARR are only caused by

early initiation of DMT, since a small proportion of CIS patients started with DMT prior to the MS diagnosis and pre-treatment ARR differed significantly. First-line DMT is already offered to adults with CIS who are at high risk for a future MS diagnosis, but not to children with CIS. However, DMT especially reduce MS relapse rates and have been shown beneficial in children with MS.[18] In addition, DMT might prevent disability progression. Therefore early initiation of first-line DMT in children with CIS who are at high risk for MS seems logical. By using a prediction rule at onset, we found that 42 out of 94 (45%) 1-16 year-old children with CIS were defined as at high risk for MS, and could have started with first-line DMT at onset, since all 42 children were diagnosed with MS during follow-up. The utility of this prediction rule should be validated in other prospective cohorts for its usefulness and safety. A limitation of our study is that the follow-up is limited and relatively shorter in children with CIS since our prospective study in adults started prior to our prospective study in children. With the current follow-up, however, we could already demonstrate important differences in rates of MS diagnosis and relapses. It would be interesting to follow our cohort in order to compare the clinical outcome and disease progression over several decades. Another limitation of our study is a selection bias since we only included patients with CIS, while in children it is known that MS can present with a spectrum of acquired demyelinating events, including ADEM.[4] However, it is not expected that this influences our results much, since it is uncommon that children are diagnosed with MS after ADEM, using the current diagnostics criteria.[4] In addition, there could have been a selection in patients who were reported for our prospective studies by physicians from other hospitals. Furthermore, we do not have a standard MRI protocol, gadolinium was not administered to all patients at the first event, nor was a lumbar puncture performed in all patients. Nevertheless, our data resemble clinical practice. In summary, we found a more inflammatory disease course of CIS and MS in children. This could argue for early initiation of first-line disease modifying therapy in children with CIS who are at high risk for a future MS diagnosis.

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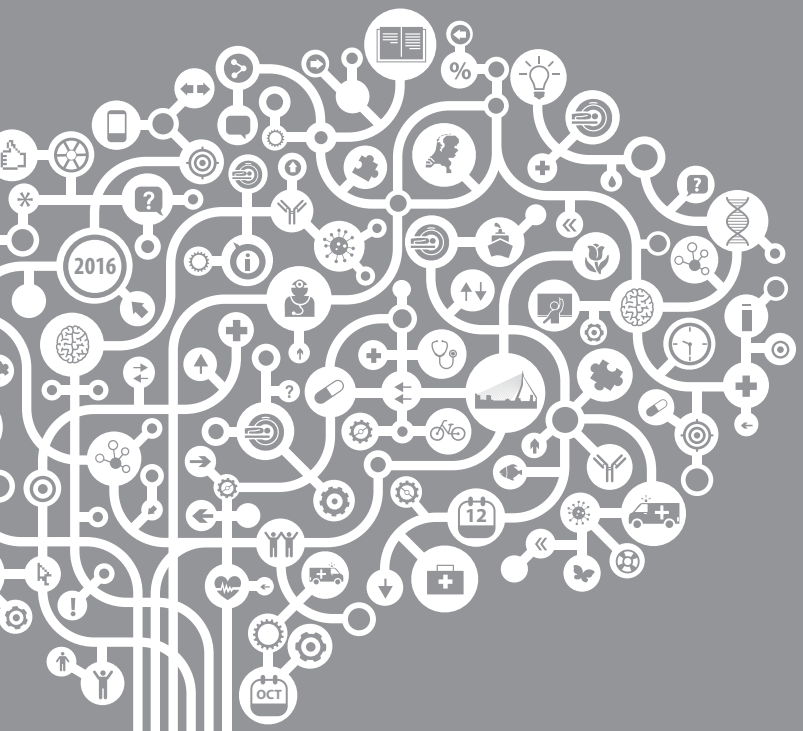

Chapter 7

Risk genes associated with pediatric-onset MS but not with monophasic acquired CNS demyelination

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ABSTRACT

Objective To investigate whether 57 genetic risk loci recently identified in a large-scale genome-wide association study (GWAS) in adult MS patients are also associated with a risk for pediatric-onset MS and whether they can predict MS diagnosis in children presenting with acquired demyelinating syndromes (ADS).

Methods We included 188 children with ADS, of which 53 were diagnosed with MS, 466 adult-onset MS patients and 2046 adult controls in our cohort study. Weighted genetic risk scores (wGRS) were calculated to evaluate genetic effects.

Results Mean wGRS was significantly higher for pediatric-onset MS patients (7.32 ± 0.53) as compared with monophasic ADS patients (7.10 ± 0.47, p=0.01) and controls (7.11 ± 0.53, p<0.01). We found no difference in mean wGRS of participants with monophasic ADS (7.10 ± 0.47) and controls (7.11 ± 0.53). The ability of the wGRS for the 57 SNPs to discriminate between children with MS and those with monophasic ADS was moderate (AUC =0.64), but improved with the addition of sex and *HLA-DRB1*15* (AUC =0.70). The combined effect of 57 SNPs exceeded the effect of *HLA-DRB1*15* alone in our risk models for pediatric- and adult-onset MS.

Conclusion The previously reported 57 SNPs for adult-onset MS also confer increased susceptibility to pediatric-onset MS, but not to monophasic ADS.

INTRODUCTION

Multiple sclerosis (MS) is being increasingly diagnosed in childhood.[1] In children, an initial attack of central nervous system (CNS) demyelination (acquired demyelinating syndrome or ADS) frequently remains monophasic. Approximately 21-32% of the children with ADS will display further MRI or clinical evidence of inflammatory CNS demyelination meeting diagnostic criteria for MS.[2, 3] This is in contrast with adults, where the majority of patients are diagnosed with MS after an initial event of acute CNS demyelination.[4] Pediatric-onset MS has been proposed as a unique time window for the study of early MS disease mechanisms. However, it is not known whether pediatric- and adult-onset MS share the same genetic risk factors. A recent Canadian study of a large ADS cohort reported that children harboring one or more *HLA-DRB1*15* alleles were more likely to be confirmed to have MS, compared to ADS children lacking *HLA-DRB1*15* alleles.[5] While HLA alleles thus contribute to the risk of both pediatric- and adult-onset MS[6], large-scale genome-wide association studies (GWAS) recently identified 57 non-HLA genetic risk loci in adult MS patients compared to controls.[7] Whether these 57 single nucleotide polymorphisms (SNPs) also contribute to the risk of either MS in children, or childhood-onset ADS more generally is not known. The objective of this study was to investigate whether the 57 SNPs identified in adult-onset MS are associated with increased risk of pediatric-onset MS, and whether such SNPs distinguish children with MS from children with monophasic ADS. We utilized a previously published approach to generate compound-weighted genetic risk scores[8], and compared the predictive value of the 57 SNPs with the predictive value of *HLA-DRB1*15* alone, in distinguishing children with MS from children with monophasic ADS and controls from the general population.

METHODS

Patients and definitions

Children younger than 16 years 0 days (Canadian cohort) and 17 years 0 days (Dutch cohort) of age who presented with ADS between 2001 and 2009 were enrolled in either the prospective Canadian Pediatric Demyelinating Disease Study or the Dutch Study group for Pediatric MS study. The Canadian National Pediatric Demyelinating Disease Study Group consists of 23 participating pediatric health care centers across Canada. The Dutch Study Group for Pediatric MS consists of 13 participating pediatric healthcare centers in the Netherlands. Initial phenotypes were characterized by clinical history and physical examination as clinically monofocal optic neuritis (ON), clinically monofocal transverse myelitis (TM), other clinically monofocal disease, clinically polyfocal disease, or acute disseminated encephalomyelitis (ADEM[9]). Children were diagnosed with MS

based either on evidence of a second clinical attack after at least 30 days or MRI evidence of dissemination in time.[9, 10] The recent 2010 iteration of the McDonald criteria for MS diagnosis was not available at the time of clinical classification, and was not used for this work. All participants had a minimum follow-up of 2 years from initial ADS. A total of 466 adult-onset (> 18 years) MS patients with either whole blood or saliva available to extract DNA were identified through the Rotterdam MS center. DNA from a control group of 2046 unrelated European adults from the general population was obtained from individuals enrolled in the longitudinal Rotterdam Study.[11] In order to control the effect of genetic variation due to ancestry, only participants with self-reported European ancestry were included in the analyses presented in the paper. Potential stratification was corrected for by genomic control and principal component analysis.

Standard protocol approvals, Registration and Patient Consents

Institutional ethical approval by an ethical standard committee on human experimentation was obtained at all 23 sites participating in the Canadian National Pediatric Demyelinating Disease Study and all 13 sites participating in the Dutch national study and for the Rotterdam study. Written informed consent for genetic analysis was obtained from all participants and/ or their families.

SNP Selection and Genotyping

DNA isolation and purification from saliva (Oragene DNA Purification kit, DNA Genotek ®) or whole blood samples[12] was performed. Genotyping was performed using the Illumina Human610-Quad Bead array and the 57 risk SNPs of interest were extracted.[7] An overview of these 57 risk SNPs and their odds ratios (ORs) obtained from a recent GWAS[7] are presented in Supplementary Table 7.1. A tagging SNP (rs9271366) for the *HLA-DRB1*15* locus was used. This SNP is in linkage disequilibrium (LD) (r^2 =0.957)[13] with the most often described *HLA-DRB1*15* tagging SNP (rs3135388)[14] in MS and is strongly correlated with the presence of *HLA-DRB1*15* itself.[15] We confirmed this linkage with *HLA-DRB1*15* in our pediatric patients with an overlap of 97.4% for *HLA-DRB1*15* allele typed using PCR amplification[5] and the tagging SNP rs9271366 (r^2 =0.95, p<0.01). All genotyping was carried out blinded to clinical data.

Genetic risk score computation

Unweighted genetic risk scores (uwGRS) were calculated by adding the total number of risk alleles for the 57 non-HLA SNPs carried by each individual. Weighted genetic risk scores (wGRS) were calculated by multiplying the number of risk alleles for each SNP with the effect size (log odd ratios) obtained from the literature[7, 15] (Supplementary Table 7. 1) and then taking the sum across all 57 risk SNPs.[8, 16] To assess the additional effect of *HLA-DRB1*15* status (as determined by presence of the rs9271366 tagging risk SNP), we

also calculated the wGRS for the 57 risk SNPs with and without including *HLA-DRB1*15* status. To determine how well our genetic risk scores discriminated between children with MS and monophasic ADS we constructed receiver operating characteristic (ROC) curves by plotting the sensitivity of the continuous wGRS scores against '1 -specificity' and calculated the area under the ROC curve (AUC). AUC is a measure of how well the model is able to distinguish between patients and non-patients and varies between 0.5 (no discrimination) and 1 (perfect discrimination). Sex, a known risk factor in MS[1, 17, 18], was also included in the final adjusted models. For comparative purposes, we tested the performance of the wGRS using a risk model for our adult-onset MS patients and controls. This study was reported based on the guideline for the Reporting of Genetic Risk Prediction Studies (GRIPS).[19]

Statistical analysis

Statistical analyses were performed using R software.[20] The Welch two-sample *t*-test was used to compare the means of the GRS. PredictABEL package[21] was used to compute univariate ORs. For the construction of the ROC curves and computation and comparison of the AUC values, we used PredictABEL[21] and ROCR[22] packages for the R software. SPSS Statistical Software (IBM Company, version 20) was used to analyze categorical and continuous variables (ex: sex and mean age) using Chi-Square and one-way-ANOVA tests and to calculate Spearman's Rank Correlation Coefficient for *HLA-DRB1*15* allele and tagging SNP rs9271366.

RESULTS

We identified 209 children with European background and with ADS. We excluded 8 patients with relapsing diseases that were not MS (multiphasic / recurrent ADEM *n*=2, optic neuritis after ADEM *n*=1, and recurrent ON *n*=5). Two patients with neuromyelitis optica (NMO) and 5 patients with other alternative diagnoses (vasculitis *n*=1, cerebellitis *n*=1, CNS infection *n*=2 and one patient with progressive unexplained visual loss) were excluded. Three patients withdrew from the Canadian prospective study within 6 months of ADS and were excluded. Two patients were excluded because poor DNA quality did not permit accurate genotyping and one patient was excluded because data for one risk SNP was missing. A total of 188 children with ADS were therefore included in this study. Of these 188 pediatric ADS patients, 53 children were diagnosed with MS during a mean overall follow-up period of 8.1 months (range 1.1 – 37.0 months, median 5.4 months, 90th percentile 17.6 months). The mean age at ADS (13.1 years ± 3.08) of children subsequently diagnosed with MS was higher than the mean age (9.0 years ± 4.57) of those who remained monophasic ($F = 21.82$, $p<0.01$). There was a greater

proportion of females in the MS group as compared to the monophasic ADS group (χ^2 =7.880, p<0.01). Of the 135 children who had monophasic ADS, 52 had ADEM as their ADS phenotype. Patient and control characteristics are presented in detail in Table 7.1. We did not find any clinical differences between the children from the Canadian and the Dutch cohort (appendix 1).

Table 7.1 Characteristics of patients and controls.

	Pediatric-onset MS	Pediatric-onset Monophasic ADS	Controls	Adult-onset MS
Total, n	53	135	2,046	466
Sex, % female	68	45	56	72
Age at onset, y, mean ± SD	13.1 ± 3.1	9.0 ± 4.6	NA	NA
Type of onset				
Optic Neuritis	10 (18.9%)	26 (19.3%)	NA	NA
Transverse Myelitis	4 (7.5%)	28 (20.7%)		
Clinically monofocal disease	17 (32.1%)	11 (8.1%)		
Clinically polyfocal disease	19 (35.8%)	18 (13.3%)		
ADEM	3 (5.7%)	52 (38.5%)		
Follow-up time, months (range)	63.5 (25-115)	66.0 (24-160)	NA	NA

ADEM = acute disseminated encephalomyelitis, ADS = acquired demyelinating syndrome, MS = multiple sclerosis, NA = not available or not applicable.

There was modest population stratification (inflation factor 1.07). However, exclusion of outliers regarding genomic kinship had no influence on the results. Univariate ORs of the 57 risk SNPs for our patients with pediatric-onset and adult-onset MS are presented in Supplementary Table 7.1. We calculated the wGRS to investigate whether there is implication of the 57 risk SNPs in pediatric-onset MS risk, and if so, whether it is similarly implicated as in adults. The mean wGRS was similar between pediatric-onset MS and adult-onset MS (7.32 ± 0.53 vs. 7.40 ± 0.52, p=0.29). The mean wGRS differed significantly between both MS groups and general population controls (p<0.01). We found a significantly higher mean wGRS in pediatric-onset MS patients as compared to children with monophasic ADS (7.32 ± 0.53 vs. 7.10 ± 0.47, p=0.01) and as compared to controls (7.32 ± 0.53 vs. 7.11 ± 0.53, p<0.01). In contrast, there was no difference in mean wGRS of participants with monophasic ADS (7.10 ± 0.47) and controls (7.11 ± 0.53).We did not find differences in mean GRS between children from the Canadian and the Dutch cohort (appendix 1). As an exploratory analysis, we were also interested in whether mean wGRS differed between children with an ADEM (n=52) presentation at ADS and controls or children with pediatric-onset MS. Since ADEM reflects a specific ADS presentation with encephalopathy and typical MRI features and often is post-infectious.[9] No difference was found in mean wGRS between children with ADEM (7.06 ± 0.45) and controls, while

the mean wGRS was higher in children with MS as compared to the children with ADEM (7.32 ± 0.53 vs. 7.06 ± 0.45, $p<0.01$). Similar results were found for all comparisons using the unweighted GRS. In Figure 7.1A the distribution of the number of risk alleles in pediatric-onset MS patients and children with monophasic ADS is presented. Figure 7.1B displays the distribution of wGRS values as plot boxes for children with MS, children with monophasic ADS, adults with MS and controls from the general population.

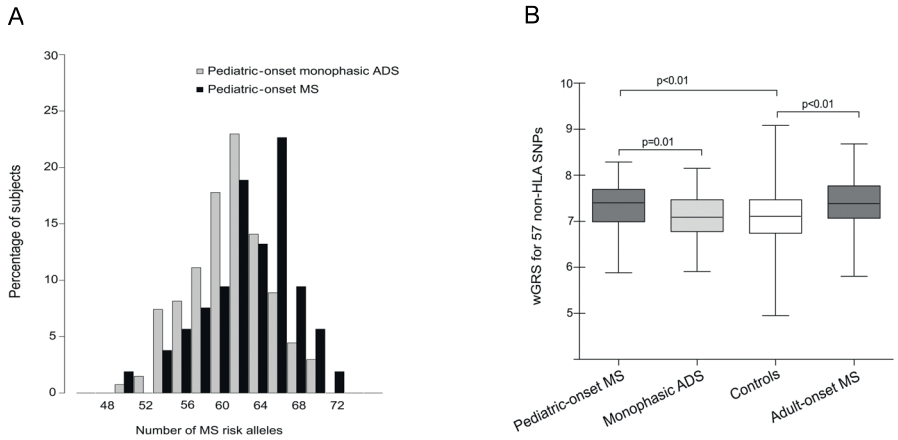

Figure 7.1 Distribution of genetic parameters in patients and controls.
(A) Distribution of the number of risk alleles in children with multiple sclerosis (MS) (black) and monophasic acquired demyelinating syndrome (ADS) (green). It should be noted that patients can harbor 0, 1, or 2 alleles for each risk single nucleotide polymorphism (SNP). (B) Box plots present the distribution of weighted genetic risk score (wGRS) values in patients and controls. The distribution of wGRS is presented for children with MS, children with monophasic ADS, adults with MS, and controls from the general population using box plots. We found a significantly higher mean wGRS in patients with pediatric onset MS as compared to children with monophasic ADS (7.32 ± 0.53 vs 7.10 ± 0.47, p=0.01) and as compared to controls (7.32 ± 0.53 vs 7.11 ± 0.53, p=0.01). The mean wGRS in patients with adult-onset MS was significantly higher compared with controls (7.40 ± 0.52 vs 7.11 ± 0.53, p=0.01). The mean wGRS was similar between pediatric-onset MS and adult-onset MS (7.32 ± 0.53 vs 7.40 ± 0.52, p=0.29).
HLA = human leukocyte antigen.

We next examined how well the wGRS values were able to discriminate between children with MS and monophasic ADS or controls, as well as between adults with MS and controls. To do this, we created ROC-curves and calculated AUC-values, including the effects of *HLA-DRB1*15* and sex. AUC values and their confidence intervals are presented in Table 7.2. The ability of the wGRS for the 57 non-HLA risk SNPs to discriminate between children with MS and those with monophasic ADS was moderate (AUC =0.64), but improved with the addition of sex and *HLA-DRB1*15* (AUC =0.70). Comparable AUCs of the wGRS for the 57 risk SNPs (AUC =0.66) and for the 57 risk SNPs combined with sex and *HLA-DRB1*15* (AUC =0.73) were found in our adult-onset MS patients when

Table 7.2 AUC values for risk models for pediatric-onset MS versus monophasic ADS and adult-onset MS versus controls from the general population.

	Pediatric-onset MS		Adult-onset MS	
	AUC	95% CI	AUC	95% CI
*HLA-DRB1*15*	0.60	0.52 - 0.67	0.63	0.60 - 0.65
*HLA-DRB1*15* and sex	0.66	0.58 - 0.74	0.67	0.64 - 0.69
57 non-HLA SNPs	0.64	0.54 - 0.73	0.66	0.63 - 0.68
57 non-HLA SNPs and *HLA-DRB1*15*	0.66	0.58 - 0.75	0.71	0.69 - 0.74
57 non-HLA SNPs, *HLA-DRB1*15* and sex	0.70	0.62 - 0.79	0.73	0.71 - 0.76

AUC = area under the receiver operating characteristic curve, CI = confidence interval, HLA = human leukocyte antigen, MS = multiple sclerosis, SNP = single nucleotide polymorphism.

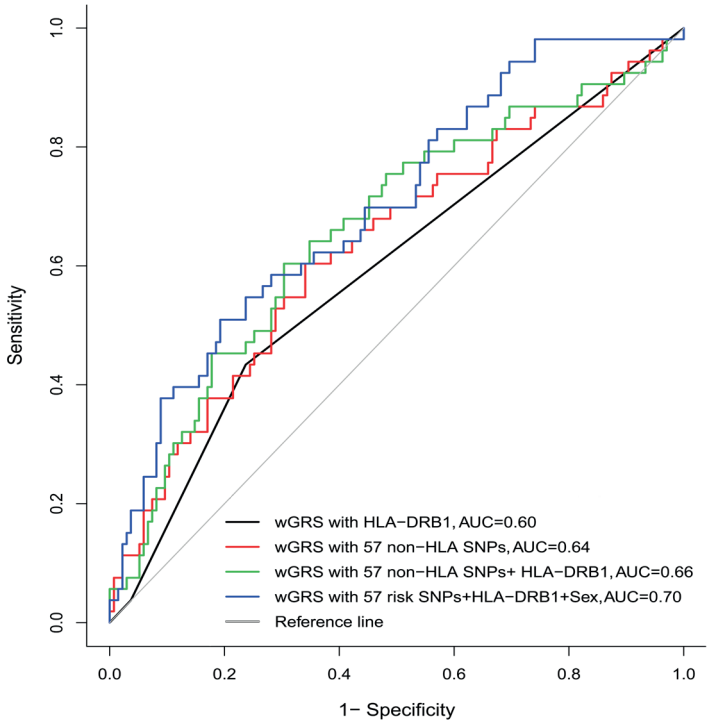

Figure 7.2 Receiver operating characteristic curve for models identifying diagnosis of pediatric-onset multiple sclerosis among children with acquired demyelinating syndrome.
The results for 4 separate models to identify pediatric-onset multiple sclerosis (MS) cases among children with acquired demyelinating syndrome (ADS): weighted genetic risk score (wGRS) with *HLA-DRB1*15* (black); wGRS with 57 non-HLA risk loci (red); wGRS with *HLA-DRB1*15* and 57 non-HLA risk loci (green); and wGRS with *HLA-DRB1*15*, including 57 non-HLA risk loci and sex (blue).
AUC = area under the curve, HLA = human leukocyte antigen, SNP = single nucleotide polymorphism.

compared with controls. The combined effects of the 57 risk SNPs exceeded the effect of *HLA-DRB1*15* alone in both models. In Figure 7.2, we present the ROC-curves for our model predicting pediatric-onset MS in children with ADS. In contrast, the same model using wGRS of the 57 non-HLA SNPs had no ability to discriminate between individuals with monophasic ADS and controls (AUC =0.50).

DISCUSSION

We report a unique analysis of the 57 non-HLA SNPs recently found to confer risk for adult-onset MS, in a large prospective pediatric ADS cohort including children ascertained to have MS and children with monophasic ADS. Using a compound weighted genetic risk score of the 57 SNPs, we found that mean wGRS significantly differs between pediatric-onset MS patients and controls and between children with MS and those with monophasic ADS. Our results indicate that the 57 non-HLA risk SNPs implicated in adult-onset MS, also contribute to risk of MS in children. These SNPs do not appear to confer a general risk of CNS inflammation in children since wGRS of children with monophasic ADS and controls did not differ.

Disease onset during childhood may represent a heightened genetic susceptibility (a greater "genetic load") or a particularly powerful interaction between genetic factors and childhood environmental risk exposures. We found no significant differences between the GRS of the 57 non-HLA risk SNPs in children and adults diagnosed with MS, suggesting a similar cumulative genetic contribution to disease risk in both pediatric-onset and adult-onset disease. However, whether the very same loci make the same contributions to the pathophysiology of pediatric- and adult-onset MS remains to be fully elucidated, and would require large sample sizes to distinguish individual SNP contributions.

While mean wGRS was higher in the pediatric-onset MS group as compared to both the monophasic ADS and control group, our AUC modeling indicated only a modest ability to discriminate between children with MS and monophasic ADS (AUC =0.70 for final adjusted model). The same model applied to adult-onset disease was comparable in its ability to distinguish between MS patients and controls (AUC =0.73 for final adjusted model) and its discriminatory ability was similar to other published models using a compound genetic risk score in adult-onset MS.[8,16] For comparison, the AUC of LDL-cholesterol as a risk predictor of coronary heart disease was 0.74 in men and 0.77 in woman in a large prospective study.[23]

As we expected, presence of *HLA-DRB1*15* alone had a high contribution to the overall predictive ability of the model (AUC =0.60). However, a surprising finding of our study was that the predictive value as reflected in AUCs of the 57 non-HLA risk SNPs together

(AUC =0.64) was larger than the predictive value of the major MS risk allele *HLA-DRB1*15* (AUC =0.60) alone. We found a similar result in our adult MS patients.

There are several limitations in our study. While our pediatric MS cohort is relatively large given the rarity of this condition, our overall numbers still limit comparisons between groups. Future studies would be aided by large-scale multinational collaborations to facilitate the inclusion of more patients. Despite a mean duration of follow-up of 66.0 months (range 24 – 160), it remains possible that some of the children currently classified as having monophasic disease will be diagnosed with MS in the future. We do not expect that this number will be very high, since pediatric MS studies have demonstrated a high early relapse rate[24] and given that the time interval between incident attack and second event is typically less than 12 months.[2, 25] In order to study a genetically homogenous group as possible, our study focused on individuals of European ancestry. Replication studies including individuals of mixed ethnicities will be valuable, though the field is currently hampered by differences in the distribution and linkage disequilibrium of the genetic variants. Other areas of future study include the generation of more complex prediction models that incorporate not only genetic susceptibility but also known environmental factors such as serum 25-hydroxyvitamin D levels and viral exposures.[26] As has been seen in models for adult-onset MS and other autoimmune diseases, it is likely that the incorporation of non-genetic risk factors to the current genetic risk model will lead to improved predictive ability for pediatric-onset MS.[27]

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Supplementary Table 7.1 Overview of the 57 GWAS-implicated risk SNPs and their odds ratios (ORs), and the univariate ORs for patients with adult-onset and pediatric-onset MS.

Chr	dbSNP rs-number	Position	Gene	Allele	OR *	95%CI	OR	95%CI	OR	95%CI
1	rs4648356	2699024	*MMEL1(TNFRSF14)*	C	1·14	1·12-1·16	1·26	1·08-1·48	1·55	0·98-2·45
1	rs11810217	92920965	*EVI5*	A	1·15	1·13-1·16	0·99	0·84-1·16	1·68	1·12-2·51
1	rs11581062	101180107	*VCAM1*	G	1·12	1·1-1·13	1·08	0·93-1·27	1·13	0·74-1·72
1	rs1335532	116902480	*CD58*	A	1·22	1·19-1·24	1·12	0·89-1·41	0·62	0·37-1·02
1	rs1323292	190807644	*RGS1*	A	1·12	1·1-1·14	1·13	0·94-1·36	1·50	0·86-2·6
1	rs7522462	199148218	*C1orf106(KIF21B)*	G	1·11	1·1-1·13	1·25	1·07-1·47	2·04	1·23-3·41
2	rs12466022	43212565	*No gene*	C	1·11	1·1-1·13	1·24	1·05-1·46	1·06	0·68-1·64
2	rs7595037	68500599	*PLEK*	A	1·11	1·1-1·12	1·17	1·02-1·35	1·59	1·06-2·38
2	rs17174870	112381672	*MERTK*	G	1·11	1·09-1·13	1	0·85-1·19	0·94	0·6-1·47
2	rs10201872	230814968	*SP140*	A	1·14	1·12-1·16	1·23	1·03-1·47	0·97	0·58-1·62
3	rs11129295	27763784	*EOMES*	A	1·11	1·09-1·12	1·12	0·97-1·29	1·18	0·8-1·74
3	rs669607	28046448	*No gene*	C	1·13	1·12-1·15	1·17	1·02-1·35	1·30	0·88-1·91
3	rs2028597	107041527	*CBLB*	G	1·13	1·06-1·21	1·14	0·85-1·53	0·80	0·4-1·6
3	rs2293370	120702624	*TMEM39A(CD80)*	G	1·13	1·11-1·15	1·17	0·97-1·42	1·13	0·68-1·9
3	rs9282641	123279458	*CD86*	G	1·21	1·18-1·24	0·96	0·74-1·25	1·47	0·64-3·37
3	rs2243123	161192345	*IL-12A*	G	1·08	1·06-1·1	1·03	0·88-1·2	1·18	0·79-1·78
4	rs228614	103797685	*NFKB1(MANBA)*	G	1·09	1·07-1·1	1·08	0·94-1·24	1·24	0·84-1·84
5	rs6897932	35910332	*IL7R*	G	1·11	1·09-1·13	1·22	1·03-1·43	1·53	0·95-2·46
5	rs4613763	40428485	*PTGER4*	G	1·2	1·8-1·22	1·18	0·97-1·44	1·10	0·64-1·88
5	rs2546890	158692478	*IL12B*	A	1·11	1·1-1·13	1·02	0·89-1·18	0·90	0·61-1·32
6	rs12212193	91053490	*BACH2*	G	1·09	1·08-1·1	1·01	0·87-1·16	1·38	0·94-2·03
6	rs802734	128320491	*THEMIS*	A	1·1	1·09-1·12	1·16	0·99-1·37	0·91	0·6-1·38
6	rs11154801	135781048	*MYB(AHI1)*	A	1·13	1·11-1·15	1·11	0·96-1·28	1·07	0·72-1·58
6	rs17066096	137494601	*IL22RA2*	G	1·14	1·12-1·15	1·23	1·04-1·44	1·42	0·93-2·17
6	rs13192841	138008907	*No gene*	A	1·1	1·09-1·12	1·24	1·06-1·45	1·06	0·69-1·64
6	rs1738074	159385965	*TAGAP*	G	1·13	1·12-1·15	1·09	0·94-1·26	0·97	0·65-1·44
7	rs354033	148920397	*ZNF746*	G	1·11	1·1-1·13	1·05	0·89-1·24	0·89	0·57-1·38
8	rs1520333	79563593	*IL7*	G	1·1	1·08-1·11	1·11	0·94-1·3	1·15	0·75-1·76

8	rs4410871	128884211	*MYC*	G	1·11	1·09-1·12	1·15	0·97-1·35	1·01	0·65-1·56
8	rs2019960	129261453	*PVT1*	G	1·12	1·1-1·13	1·05	0·89-1·25	0·95	0·59-1·53
10	rs3118470	6141719	*IL2RA*	G	1·12	1·1-1·13	1·19	1·03-1·38	0·82	0·54-1·26
10	rs1250550	80730323	*ZMIZ1*	A	1·1	1·09-1·12	1·12	0·97-1·3	1·03	0·69-1·55
10	rs7923837	94471897	*HHEX*	G	1·1	1·08-1·11	1·11	0·95-1·28	1·00	0·67-1·5
11	rs650258	60588858	*CD6*	G	1·12	1·1-1·13	1·16	1-1·35	0·84	0·56-1·24
11	rs630923	118259563	*CXCR5*	C	1·12	1·1-1·14	1·28	1·05-1·58	1·57	0·86-2·88
12	rs1800693	6310270	*TNFRSF1A*	G	1·12	1·11-1·14	1·14	0·98-1·31	1·06	0·72-1·56
12	rs10466829	9767358	*CLECL1*	A	1·09	1·08-1·11	1·13	0·98-1·3	1·14	0·78-1·68
12	rs12368653	56419523	*CYP27B1*	A	1·1	1·09-1·12	1·05	0·91-1·21	0·97	0·66-1·43
12	rs949143	122161116	*ARL6IP4*	G	1·08	1·04-1·12	1·18	1·02-1·38	1·06	0·7-1·61
14	rs4902647	68323944	*ZFP36L1*	G	1·11	1·1-1·13	1·12	0·97-1·29	0·95	0·64-1·39
14	rs2300603	75075310	*BATF*	A	1·11	1·09-1·12	0·97	0·82-1·14	0·86	0·56-1·31
14	rs2119704	87557442	*GALC(GPR65)*	C	1·22	1·19-1·25	1·01	0·77-1·32	2·12	0·78-5·79
16	rs2744148	1013553	*SOX8*	G	1·12	1·1-1·14	1·12	0·92-1·36	0·88	0·5-1·55
16	rs7200786	11085302	*CLEC16A(CIITA)*	A	1·15	1·13-1·16	1·19	1·04-1·38	1·09	0·74-1·6
16	rs13333054	84568534	*IRF8*	A	1·11	1·1-1·13	0·98	0·83-1·16	0·94	0·59-1·49
17	rs9891119	37761506	*STAT3*	C	1·11	1·09-1·12	1·23	1·06-1·42	0·94	0·63-1·4
17	rs180515	55379057	*RPS6KB1*	G	1·09	1·08-1·11	1·04	0·9-1·21	1·13	0·77-1·68
18	rs7238078	54535172	*MALT1*	A	1·12	1·1-1·14	0·99	0·84-1·17	0·78	0·51-1·19
19	rs1077667	6619972	*TNFSF14*	G	1·16	1·14-1·18	1·35	1·12-1·63	1·27	0·77-2·1
19	rs8112449	10381064	*TYK2(ICAM3)*	G	1·08	1·07-1·1	1·15	0·98-1·35	0·91	0·6-1·37
19	rs874628	18165700	*MPV17L2(IL12RB1)*	A	1·11	1·09-1·12	1·16	0·99-1·37	1·43	0·9-2·28
19	rs2303759	54560863	*DKKL1(CD37)*	C	1·11	1·09-1·13	1·3	1·11-1·52	1·18	0·77-1·8
20	rs2425752	44135527	*CD40*	A	1·11	1·1-1·13	1·17	1-1·37	0·76	0·48-1·22
20	rs2248359	52224925	*CYP24A1*	G	1·12	1·1-1·13	1·11	0·95-1·28	0·76	0·52-1·13
20	rs6062314	61880157	*TNFRSF6B*	A	1·16	1·14-1·19	1·23	0·92-1·66	0·55	0·31-1
22	rs2283792	20461125	*MAPK1*	C	1·1	1·08-1·11	1·11	0·97-1·29	1·41	0·95-2·09
22	rs140522	49318132	*SCO2*	A	1·1	1·09-1·12	1·08	0·93-1·25	1·45	0·98-2·16

*Weighted genetic risk score (wGRS) was calculated by multiplying the number of risk alleles for each SNP with the effect size (log OR) obtained from the GWAS.

Appendix 1. supplementary statistical analysis

Canadian and Dutch cohorts

There were no overt clinical differences between the children from the Canadian and the Dutch cohort (mean age at onset, sex, type of onset, mean time of follow-up).

Mean age at onset: one-way ANOVA F = 5.17, df = 35, p = 0.68

Sex: Chi Square χ^2 = 2.7, df = 1, p = 0.10

Type of onset: Chi Square χ^2 = 9.1, df = 4, p = 0.06

Mean time of follow-up: one-way ANOVA F= 27.3, df = 30, p = 0.83

We also did not find differences in the Genetic Risk Scores between children from the Canadian and the Dutch cohort.

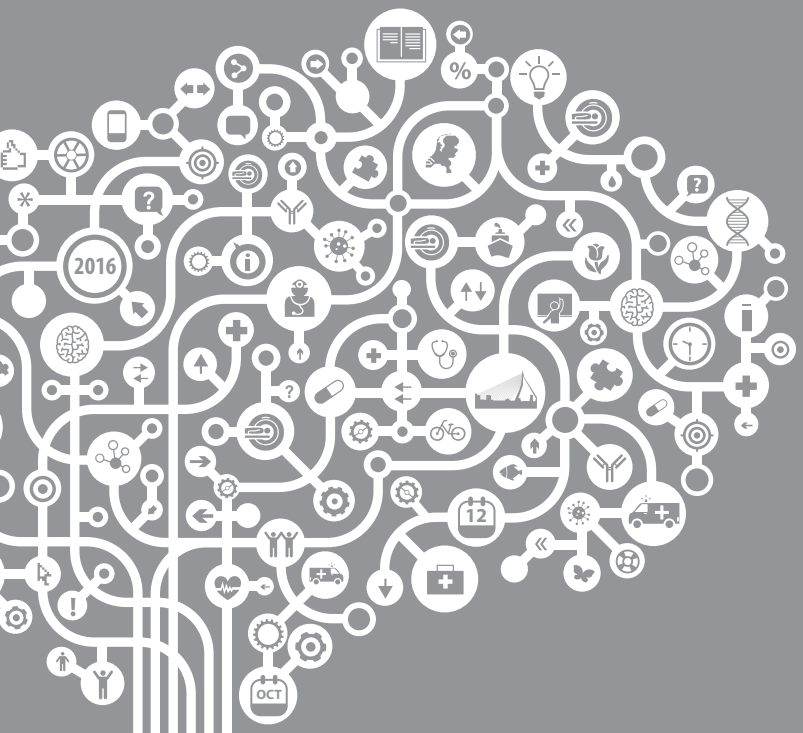
uwGRS 57 SNPs: Welch two-sample t-test p = 0.49

wGRS 57 SNPs: Welch two-sample t-test p = 0.55

wGRS 57 SNPs + HLA: Welch two-sample t-test p = 0.14

Controlling for possible population stratification

In order to control the effect of genetic variation due to ancestry, only participants with self-reported European ancestry were included in the analyses presented in the paper. Potential stratification was corrected by genomic control and principal component (PC) analysis. There was modest population stratification (inflation factor 1.07). However, exclusion of outliers regarding genomic kinship had no influence on the results.



Chapter 8

Anti-MOG antibodies plead against MS diagnosis in an ADS cohort

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ABSTRACT

Background Acquired demyelinating syndromes (ADS) in children are a group of distinct first immune-mediated demyelinating events of the central nervous system (CNS). Predictive biomarkers for future diagnosis are lacking. A putative target antigen is myelin oligodendrocyte glycoprotein (MOG). We analysed the presence of MOG-antibodies in a cohort of ADS patients.

Methods 117 children with ADS form a nationwide cohort were analysed with a cell-based assay, divided in 5 groups: optic neuritis (ON; *n*=20), transverse myelitis (TM; *n*=7), other monofocal ADS (*n*=22), polyfocal ADS without encephalopathy (*n*=44) and polyfocal ADS with encephalopathy (*n*=24). Additionally, 13 children with other neurological diseases (OND), 31 healthy children and 29 adult ADEM patients were tested.

Results Twenty-one of the 117 children with ADS tested anti-MOG seropositive (18%). The group of patients with polyfocal ADS plus encephalopathy (ADEM) had the highest prevalence of anti-MOG seropositivity (42% versus 18% in non-encephalopathic polyfocal ADS patients). Forty-seven ADS children had a final diagnosis of multiple sclerosis (MS). In only one of them MOG-antibodies were detected (2%), with only borderline positivity. Only 1 out of 29 adult ADEM patients tested anti-MOG seropositive.

Conclusions MOG-antibodies are strongly skewed towards ADS children that present with an ADEM-like disease-onset. The presence of such antibodies pleads against a future diagnosis of MS.

INTRODUCTION

Acquired demyelinating syndromes (ADS) in children are first immune-mediated demyelinating events of the central nervous system (CNS).[1, 2] The clinical spectrum is very heterogenic, including optic neuritis (ON), transverse myelitis (TM), other clinically isolated syndromes, acute disseminated encephalomyelitis (ADEM) and neuromyelitis optica (NMO). These distinct disease entities may be challenging to diagnose accurately at the first event. Disease course and prognosis are also variable and all these different subtypes of ADS can represent a first episode of multiple sclerosis (MS). It is essential to distinguish monophasic disease forms from a chronic relapsing disease like MS early in the disease course, because prompt initiation of disease-modifying treatment has been recommended for children with MS.[3]

Clinical evidence suggests that ADS includes several distinct disorders with different underlying pathophysiology. Preferably, certain subsets of ADS patients characterized by humoral autoimmunity might be identified through the use of disease-specific autoantibodies. For example, NMO is now considered to be an antibody-mediated disease that is distinct from MS, on account of the discovery of the disease-specific autoantibody against aquaporin-4 (AQP4).[4, 5]

In the search for disease-specific autoantibodies in ADS, myelin oligodendrocyte glycoprotein (MOG) is a putative target antigen. This protein is expressed on the surface of myelin sheaths and oligodendrocytes, and thus specific to the CNS. Previous studies already showed that MOG-antibodies can cause demyelination in vitro and can induce experimental autoimmune encephalomyelitis (EAE).[6, 7] Based on the current knowledge, antibodies to MOG have been detected in a subgroup of patients presenting with CNS demyelinating diseases[8, 9] and are especially present in patients with ADEM[10, 11], in children with very early-onset MS[12], and with higher titers in the youngest children and children with ADEM.[11, 13-15] But the sensitivity of MOG-antibody assays to discriminate patients with CNS demyelinating disease varies and is reported to be only as high as 46%.[16] This is in part due to the patient population that is included, since the antibodies are more prevalent is the ADEM subgroup of ADS[10-15] and might not be a sensitive marker for ADS in general. Previous studies focused mainly on specific subgroups of ADS patients based on diagnosis, like paediatric onset MS[10-12, 14, 16], ADEM[10, 11, 13, 14, 17] and CIS.[11, 13, 14] Recently MOG-antibodies were also detected in patients with anti-AQP4 negative NMO or NMO spectrum disorders such as bilateral or recurrent ON and longitudinally transverse myelitis.[18-26]

To date, it is unsure which subgroup the children with MOG-antibodies represent within the spectrum of ADS.[16] In the current study we investigated the presence of MOG-antibodies in a cohort comprised of all ADS subtypes and compared their presence between the different ADS subtypes based on clinical presentation. We hypothesized

that the MOG-antibodies are prevalent in the younger ADS children who are more likely to have a polyfocal onset with encephalopathy, which is the strict definition for ADEM.[27]

MATERIALS AND METHODS

Patients and controls

Children with a first demyelinating event of the CNS (ADS), younger than 18 years, enrolled in the nationwide cohort of the Dutch pediatric MS study[2, 28], were consecutively included in this study. At first event, patients were divided into five groups, based on clinical presentation: ON, TM, other monofocal disease-onset (mono ADS), polyfocal disease-onset without encephalopathy (poly ADS –) and polyfocal disease-onset with encephalopathy (poly ADS +). Encephalopathy was defined as altered consciousness or change in behaviour, which cannot be explained by fever, systemic illness or postictal symptoms.[2] A diagnosis of MS could be made when a second demyelinating attack occurred, with clinical and/or MRI evidence of dissemination in time and space at least one month after onset.[29] After a first attack with encephalopathy, a second event without encephalopathy at least three months after onset and a third event with clinical or MRI evidence of dissemination in time are needed for a diagnosis of MS.[27] Follow-up information was provided by the clinical physician and by telephone interview of the parents. As control groups we included healthy children and children with other neurological diseases (OND). Furthermore we tested a group of adult patients with a clinical diagnosis of ADEM. This study was approved by the Medical Ethical Committees of the Erasmus University Medical Centre in Rotterdam and of the other participating centres.

Cell culture

Human MOG-transfected and untransfected LN18 cells were cultured in IMDM medium (Bio Whittaker, Verviers, Belgium) containing 10% fetal calf serum (Bodinco, Alkmaar, The Netherlands), penicillin 100 U/ml / streptomycin 100 ug/ml (Gibco, Merelbeke, Belgium), and 50 µM 2-mercaptoethanol (Sigma-Aldrich, Steinheim, Germany).

Cell-based assay

In order to detect antibodies to native intact MOG we used a glial LN18 cell line (a kind gift of Prof. B. Hemmer, Technical University of Munich, Germany) that stably expressed full-length MOG tagged with eGFP (GeneCopeia, Inc., OmicsLink Clone, Cat.# EX-M0097-M03). For the detection and quantification of antibodies binding to MOG expressed on the cell surface a fluorescence activated cell sorter (FACS, LSRII and FCS 3.0 software, Becton Dickinson) was used. MOG-LN18 and untransfected LN18 cells were harvested by trypsinization and washing into ice-cold PBAE buffer (PBS/0.5% BSA (vol/vol, Celliance,

Kanakee, Illinois, USA) /0.1% azide /1 mM EDTA (all from Sigma)). Equal numbers of MOG-transfected LN18 cells and untransfected LN18 cells were mixed to a final concentration of 100000 cells per well and incubated with patient or control samples (1:50 in PBAE) in 96 wells round-bottom microtiter plates (Greiner Bio-One, Alphen a/d Rijn, The Netherlands) for 30 min on ice. After three times washing with PBAE cells were incubated with goat anti-human IgG- Allophycocyanin (APC) conjugated secondary antibody (Jackson ImmunoResearch Laboratories, BrunschwigChemie B.V., Amsterdam, The Netherlands) at 1:500 in PBAE containing containing 10% (vol/vol) normal goat serum (NGS, Sanquin, Amsterdam, The Netherlands) for 25 min on ice. Cells were than washed 4 times in PBAE and analyzed immediately by FACS. Binding of human IgG was determined by measuring APC fluorescence after setting an acquisition gate of 10,000 events on GFP-positive cells. In each assay we tested 8 individual negative control sera (apparently healthy lab workers), one strongly positive and one low positive control serum. Quantitative levels of antibody titers were expressed as difference in median fluorescence intensity (ΔMFI) between MOG transfected and untransfected LN18. The assay cut-off was based on the average ΔMFI+3xSD of healthy and diseased control patients.

Statistical analysis

Statistical analysis was performed using SPSS 20.0. Chi-square test was used to compare categorical data and Kruskal-Wallis and Mann-Whitney *U* tests to compare continuous data. Differences in continuous data between two groups were compared using Student's *t*-test. Results were considered significant if *p*-values were <0.05. Bonferroni corrections were made when appropriate.

RESULTS

We included 117 patients with ADS, 13 children with other neurological disorders (OND) and 31 healthy children. The OND group consisted of patients with epilepsy (*n*=4), viral encephalitis (*n*=4), other autoimmune diseases (*n*=2: opsoclonus myoclonus syndrome and cerebral vasculitis), migraine (*n*=1), trauma (*n*=1) and hypertensive encephalopathy (*n*=1). In addition, 29 adult ADEM patients were tested. Demographic characteristics are shown in Table 8.1. MOG-antibodies were present in 21 of the 117 children with ADS (18%) and in none of the healthy or OND control children (Chi-Square=9.082, *p*=0.003). Eighteen anti-MOG seropositive patients had a polyfocal disease-onset. Two children had isolated ON as the initial event, one had right-sided hemiparesis, whereas all other 46 patients with a monofocal onset were seronegative.

Figure 8.1 shows the anti-MOG titers in the 5 separate clinical subgroups, healthy controls, children with other neurological diseases and adults with ADEM. The group of

Table 8.1 Demographics of patients and controls.

	ADS children	OND children	Healthy control children	Adult ADEM patients
Number	117	13	31	29
Female, *n* (%)	61 (52)	6 (46)	12 (39)	17 (59)
Mean age, years (range)	10.7 (0.5 - 17.5)	9.8 (1 - 16)	8.7 (2 – 16)	40 (18 – 82)
Mean time disease onset – sampling, years (range)	1.2 (0 – 13.5)	2.24 (0 – 11)		1.3 (0 – 14.1)
Sampling < 3 months, *n* (%)	73 (62)	5 (62)		19 (66)

ADEM = acute disseminated encephalomyelitis, ADS = acquired demyelinating syndromes, OND = other neurological diseases.

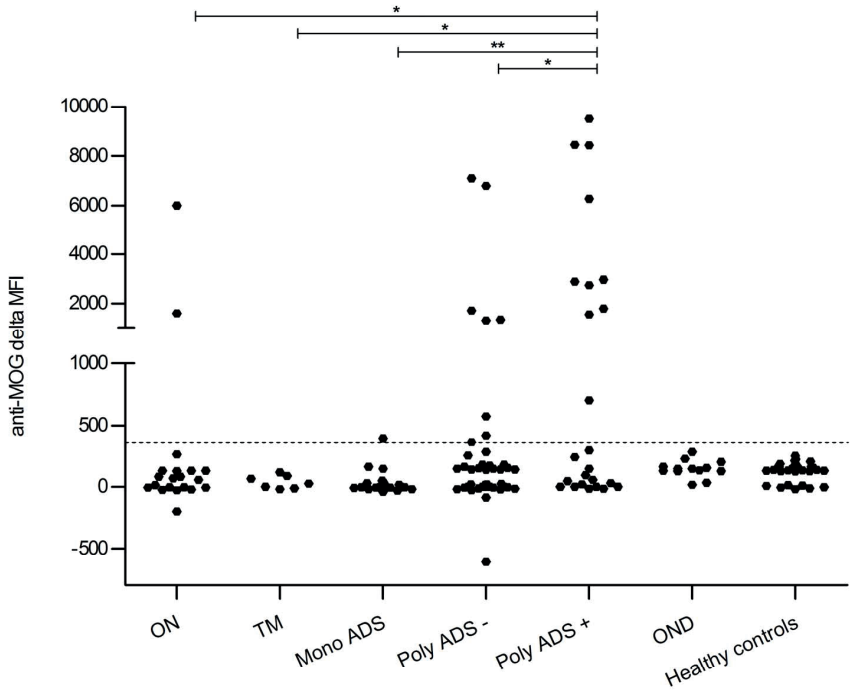

Figure 8.1 MOG-antibody titers in ADS patients (divided by clinical presentation) and control groups.
* $p<0.05$ ** $p<0.01$, dotted line represents the assay cut-off (ΔMFI 360)
ADS = acquired demyelinating syndrome, ΔMFI = median fluorescence intensity (delta is the change in this), MOG = myelin oligodendrocyte glycoprotein, mono ADS = monofocal ADS, ON: = optic neuritis, OND = other neurologic disorders, poly ADS - = polyfocal ADS without encephalopathy (negative), poly ADS + = polyfocal ADS with encephalopathy (positive), TM = transverse myelitis.

children with a polyfocal disease-onset plus encephalopathy had the highest frequency of anti-MOG seropositivity (42%). Also 18% of children presenting with polyfocal ADS without encephalopathy had MOG-antibodies. Most of them had an ADEM-like onset according to the International Paediatric MS Study Group (IPMSSG) criteria defined as

a first polyfocal clinical CNS event with encephalopathy and a presumed inflammatory cause and usually diffuse poorly demarcated lesions on cerebral MRI, although they presented without encephalopathy, and did not qualify as suffering from MS.[27] The clinical presentation and disease course of individual MOG-antibody positive children are outlined in Table 8.2. The characteristics of the MOG-antibody positive and negative paediatric patient groups are shown in Table 8.3. The MOG-antibody positive patients were 4.4 years younger on average than the MOG-antibody negative patients (p<0.001).

In the ADS cohort, 47 children had a final diagnosis of MS (mean follow-up time of 4.7 years) and except for one they were all seronegative. In contrast, of the 70 patients without MS diagnosis (mean follow-up time of 5 years) 29% was seropositive (p<0.001). Eleven of these 70 children developed a relapsing disease without fulfilling diagnostic criteria for MS. In eight of these 11 children MOG-antibodies could be detected (Table 8.2). Figure 8.2 shows the disease course of the 8 seropositive children with clinical relapsing disease without MS diagnosis. There were no significant differences between the monophasic and relapsing MOG-antibody seropositive patients in length of follow-up (4.02 vs 6.75 years) and in treatment (75% vs 67% were treated with Methylprednisolone after disease presentation).

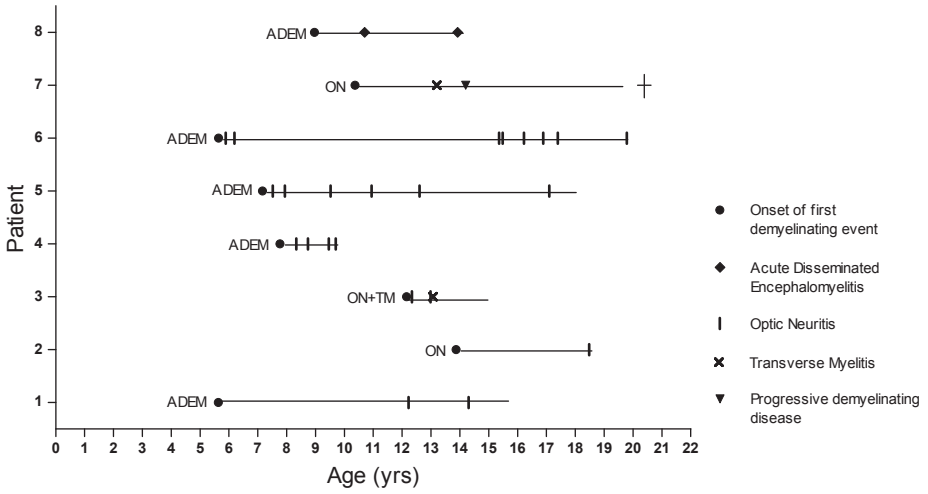

Figure 8.2 Disease courses of the 8 MOG-antibody positive children with relapsing disease.
ADEM = acute disseminated encephalomyelitis, BG = basal ganglia, CC = corpus callosum, MOG = myelin oligodendrocyte glycoprotein, MRI = magnetic resonance imaging, ON = optic neuritis, TM = transverse myelitis.

Clinical recovery of the MOG-antibody positive patients at last follow-up was complete in 12 patients. Eight patients had only mild residual symptoms, like fatigue, attention or behavioural deficits or mild persisting visual loss. One patient who initially presented

with optic neuritis had a subsequently progressive disease course and she died of complications (aspiration pneumonia) 9 years after disease onset (Table 8.2).

Table 8.2 Clinical presentation and disease course of MOG-antibody positive children.

Patient	Sex	Disease onset	Clinical diagnosis	Relapse(s)	Recovery	Remarks
1	F	Poly ADS -	ADEM	ON	Incomplete	Not meeting MS diagnostic criteria
2	M	ON	Bilateral ON	ON	Complete	Not meeting MS diagnostic criteria
3	F	Poly ADS -	NMO	ON+TM	Incomplete	AQP4-antibody negative; Not meeting MS diagnostic criteria
4	M	Poly ADS +	ADEM	ON	Incomplete	
5	M	Poly ADS +	ADEM	ON	Complete	
6	F	Poly ADS -	ADEM	ON	Incomplete	Not meeting MS diagnostic criteria
7	F	ON	ON	TM	Died 9 years after onset	Subsequently progressive disease course
8	M	Poly ADS -	ADEM	ADEM	Complete	
9	M	Mono ADS	CIS	MS	Complete	
10	F	Poly ADS -	Bilateral ON and TM	Basal ganglia lesions on follow-up brain MRI	Complete	AQP4-antibody negative; Not meeting NMO or MS diagnostic criteria
11	F	Poly ADS +	ADEM		Incomplete	
12	M	Poly ADS +	ADEM		Complete	
13	F	Poly ADS +	ADEM		Complete	
14	F	Poly ADS -	ADEM		Complete	
15	M	Poly ADS +	ADEM		Complete	
16	M	Poly ADS +	ADEM		Complete	
17	M	Poly ADS -	ADEM		Complete	
18	F	Poly ADS -	ADEM		Incomplete	
19	M	Poly ADS +	ADEM and LETM		Incomplete	AQP4-antibody positive
20	M	Poly ADS +	ADEM		Complete	
21	M	Poly ADS +	ADEM		Incomplete	

ADEM = acute disseminated encephalomyelitis, ADS = acquired demyelinating syndrome, AQP4-antibody = aquaporin-4 antibody, F = female, LETM = longitudinally extensive transverse myelitis, M = male, MOG = myelin oligodendrocyte glycoprotein, MRI = magnetic resonance imaging, MS = multiple sclerosis, NMO = neuromyelitis optica, ON = optic neuritis, Poly ADS - = polyfocal disease-onset without encephalopathy, Poly ADS+ = polyfocal disease-onset with encephalopathy, TM = transverse myelitis.

In only one adult patient with ADEM we were able to detect MOG-antibodies (3% versus 42% of the children with poly ADS +; Pearson Chi-Square, p=0.002). This man was 37 years old at disease-onset. The sample was obtained within 1 month after onset and he had a monophasic disease.

In 11 children follow-up samples were tested, 9 patients were anti-MOG seronegative at onset and remained negative (including 6 children with MS, one with mono ADS, one with ON, one with poly ADS +). A second sample was obtained in 2 anti-MOG seropositive children (patients 4 and 7 in Table 8.2). In both patients MOG-antibodies remained detectable, respectively 3 months and 7 months after last relapse.

Table 8.3 Characteristics of MOG-antibody positive and negative paediatric patients.

	MOG Ab + patients (n=21)	MOG Ab – patients (n=96)	p-value
Female, *n* (%)	9 (43)	52 (54)	0.48
Mean age, years ± SD	7.1 ± 4.5	11.5± 4.6	<0.001
Mean time disease onset – sampling, years ± SD	2.7± 4.2	0.9 ± 2.1	0.06
Sampling < 3 months, *n* (%)	13 (62)	60 (63)	0.95
Mean follow-up time, years ± SD	5.2 ± 4.3	4.8 ± 4.5	0.72

MOG Ab = myelin oligodendrocyte glycoprotein antibody

DISCUSSION

It is now widely accepted that antibodies to MOG are specific for demyelinating CNS diseases in children[10-14, 17] and also in adults with NMO and NMO-spectrum disorders.[19-26] However, this biomarker appears to lack sensitivity to discriminate (subgroups of) children with ADS from healthy children or children with other neurological diseases. In this study we investigated MOG seropositivity amongst the complete spectrum of acquired demyelinating syndromes. We used a cell-based assay in a glial cell-line to maintain optimal natural conformation and glycosylation of the MOG molecule, as such assays discriminate best between clinical sub-groups.[16]

We here observed that antibodies were almost exclusively detected in children with a polyfocal disease-onset. MOG-antibodies were more frequently present in children with a polyfocal disease-onset plus encephalopathy (42% positivity in this group fulfilling ADEM criteria according to the IPMSSG definitions)[27] when compared to all other patient groups. Also a significant part of the children with a polyfocal onset but without encephalopathy tested positive (18%). This group fulfilled the IPMSSG criteria for ADEM, except for the lack of encephalopathy at onset.

Of the total set of 21 anti-MOG seropositive patients, only 5 did not have a typical ADEM presentation. One of them had recurrent ON. This is in line with a previous study show-

ing that MOG-antibodies in paediatric patients with ON are predominantly detected in children with recurrent disease.[18] Three anti-MOG positive girls had a NMO (spectrum) disorder, but without detectable AQP4-antibodies, confirming previous studies showing that a subgroup of AQP4-antibody negative NMO patients do have MOG-antibodies.[19-26]

An interesting observation is that the four children in this cohort with a clear ADEM onset followed by multiple episodes of ON only, all tested anti-MOG seropositive. As this phenomenon has already been described by Huppke et al, we suggest that this may represent a newly identified disease entity.[30]

Only one of the children with MS in our cohort was anti-MOG seropositive, which is in contrast to former studies.[9-12, 14, 17, 19, 21] It has been discussed that if these antibodies can be measured in MS patients, the titers are lower in comparison to ADEM patients.[11, 13, 14] This is in line with our observation in this child, as he had a very low antibody titer (ΔMFI 395) just above the cut-off value of our assay (ΔMFI 360). Another explanation may be a different application of the diagnostic criteria for paediatric MS. In the current study children only with 2 non-ADEM episodes were diagnosed with MS, whereas in another study only a second non-ADEM attack or clinically silent new lesions on MRI were already sufficient for MS diagnosis.[14]

It is still unclear whether MOG-antibodies have demyelinating activity or whether they represent an epiphenomenon of myelin destruction. However, it is unlikely that these antibodies merely reflect fulminant white matter damage, as MOG-antibodies were virtually absent in adult ADEM patients. Similarly, we never encountered an anti-MOG positive adult MS patient (data not shown).

MOG polymorphisms vary among patients[31] and genetic factors affect conformation, glycosylation or expression of the MOG protein. The cell-based assay used here has the highest chance of finding naturally occurring, relevant auto-antibodies. It will be of interest to investigate in the future if some polymorphisms are associated with the rise of anti-MOG antibodies or even protect against the recognition of certain epitopes.[16, 32]

Some studies showed that these antibodies may remain detectable during the disease course[10, 12, 18], whereas two longitudinal studies showed that MOG-antibodies in some ADEM patients disappear over time.[11, 14] We did not obtain sequential samples routinely. In two of the anti-MOG positive children a second sample was tested and the antibodies remained detectable long after disease activity. Furthermore in 6 of the 21 anti-MOG positive patients, their sample was obtained during remission instead of during the active stage of the disease. Despite the small numbers in our study, this may contradict the observation that antibodies are only present as a kick-off of the disease or reflect the presence of a chronic active disease.[8, 14]

The main limitation of the study is that in 38% of ADS patients the samples were obtained during remission of this first attack instead of close to attack onset. Still, time of sampling does not seem to be of pivotal importance, otherwise it would be more likely

for the MS patients to be anti-MOG positive, as they have a chronic recurrent disease. The study is further limited by the lack of serial sampling, although the few samples we tested showed no difference in seropositivity in the follow-up sample.

This study is the first to describe the presence of MOG-antibodies in an unbiased cohort encompassing all clinical ADS subtypes in children and with relatively long follow up (over 4 years). ADS are a heterogeneous group of clinical phenotypes and diagnosis can be inaccurate, partly because substantial clinical overlap between the subgroups can exist. Here we zoomed in on the clinical features at disease-onset of MOG-antibody positive patients. Most of these seropositive patients had an ADEM-like disease and only one was diagnosed with MS. In our study the presence of MOG-antibodies in children with a first attack of CNS demyelination strongly pleads against future diagnosis of MS. We expect that the future value of testing for MOG-antibodies in a clinical setting will depend on international collaboration, on assay standardization and on consensus about the proper cut-off values. Such studies are underway.

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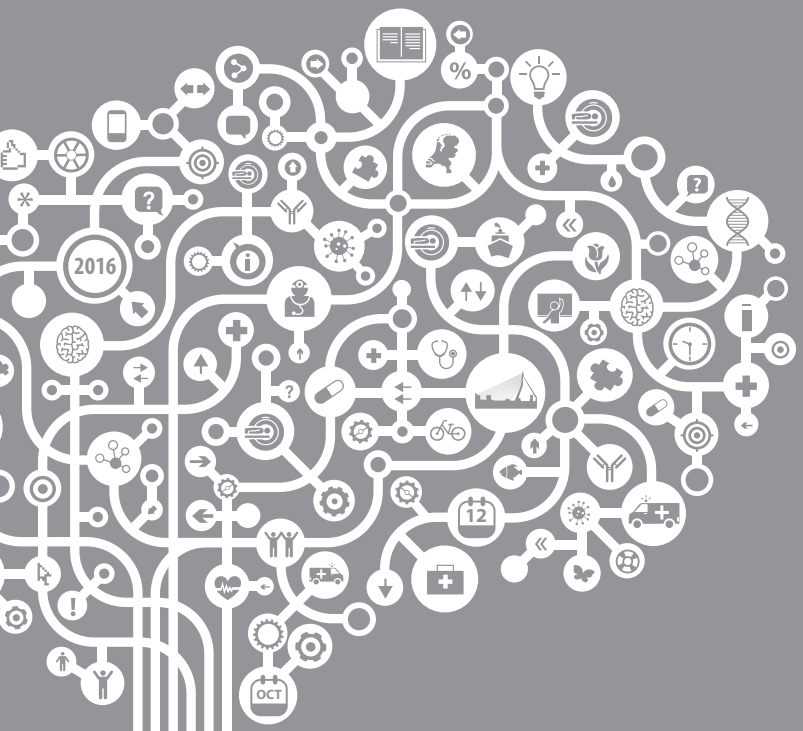

Chapter 9

Gray matter related proteins are associated with childhood-onset Multiple Sclerosis

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ABSTRACT

Objective To identify CSF biomarkers for multiple sclerosis (MS) in children with an initial acquired CNS demyelinating syndromes (ADS).

Methods CSF was collected from a cohort of 39 children with initial ADS, of them 18 were diagnosed with MS and 21 had a monophasic disease course. Proteomic analysis of trypsinised CSF (20 µl) was performed by nano liquid chromatography Orbitrap mass spectrometry. Univariate statistical analysis was used to identify differentially abundant proteins between childhood-onset MS and monophasic ADS.

Results A total of 2260 peptides corresponding with 318 proteins were identified in the total set of samples. Of these 2260 peptides, 88 were identified as most distinctive between MS and ADS. 53 peptides, corresponding to 14 proteins, had higher abundance in children with MS as compared to monophasic ADS. Twelve out of these 14 proteins were linked to neuronal functions and structures such as synapses, axons and CNS proteases (example: neurofascin, carboxypeptidase E, brevican core protein, and contactin-2). The other two were functionally related to immune function. The 35 peptides identified with decreased abundance in children with MS corresponded to seven proteins. Six of them linked to innate immune function (example: haptoglobin, haptoglobin-related protein, c4b-binding protein alpha chain and monocyte differentiation antigen CD14) and one to cellular adhesion (protein diaphanous homolog 1).

Conclusion At first onset of ADS, CSF of children diagnosed with MS showed increased abundance of CNS gray matter related proteins, whereas CSF of children with a monophasic disease course showed increased abundance of innate immunity related proteins.

INTRODUCTION

A few percent of all MS patients experience their first event in childhood.[1] In children, such a first event can present with a spectrum of clinical features of acquired demyelinating syndromes (ADS) including optic neuritis (ON), transverse myelitis (TM), acute disseminated encephalomyelitis (ADEM), neuromyelitis optica (NMO), and other clinically monofocal or polyfocal symptoms.[2] In most children with ADS, the disease course remains monophasic. However approximately 21% to 32% of these children will subsequently be diagnosed with MS.[3, 4] Current MS diagnosis is based on a combination of clinical features, CSF findings and MRI-criteria for dissemination in time and space.[1, 3, 5] These factors are insufficient to predict the disease course at first event. The availability of a biomarker that helps to differentiate between children with monophasic ADS, and those subsequently diagnosed with MS is needed. Moreover, identification of CSF proteins that are associated with childhood-onset MS can provide further insight into the disease pathophysiology. So far, one study was published that compared CSF of children with MS and monophasic ADS which suggested disturbed axoglial biology during early MS events.[6] In the present study, we investigated CSF, a body fluid that reflects ongoing CNS pathology[7] in a fully unbiased manner. Samples were analyzed by high resolution, and sensitive nano-liquid chromatography Orbitrap mass spectrometry (LC-MS).[8] Our aim was to find CSF protein markers expressed during first event of CNS demyelination that can help to distinguish children with monophasic ADS (*n*=21) from children with MS (*n*=18).

METHODS

Patients

Children younger than 18 years old who presented with a first acquired demyelinating event were identified by the Dutch Study Group for Pediatric MS, which includes 13 major pediatric neurology centers in the Netherlands as described earlier.[9] Children were diagnosed with MS in case they had a second demyelinating attack of the CNS and/or MRI evidence of a new lesion at least one month after onset.[1] Clinical features and physical examination defined initial clinical phenotypes of the children. This study included 41 children with ADS of them 22 had a monophasic disease course and 19 were diagnosed with MS. The CSF samples were collected at first clinical presentation. Children were symptomatic at time of sampling. Children were not on immunomodulatory treatment at time of sampling. CSF samples were collected, processed and stored following previously described protocols.[8, 10]

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the Clinical Research Ethics Board of the Erasmus University. Written informed consent was obtained from all patients and/or their families.

Sample preparation and LC-MS measurements

CSF samples (20 µL) were digested using an in-solution trypsin digestion protocol followed as previously described.[11] Prepared samples were analyzed by LC-MS/MS using an Ultimate 3000 nano RSLC system (Thermo Fischer Scientific, Germering, Germany) online coupled to a hybrid linear ion trap/Orbitrap mass spectrometer (LTQ Orbitrap XL; Thermo Fisher Scientific, Bremen, Germany) using data - dependent acquisition method. LC-MS data were analyzed using the Progenesis LC-MS software package (version 3.6, Nonlinear Dynamics Ltd, Newcastle - upon - Tyne, United Kingdom). Detailed LC-MS measurements, protein identification and quantification, are available in the e-methods at Neurology.org/nn.

Statistical analysis

The Wilcoxon test (unpaired, two tailed) was used to analyze the differences in the abundance of peptides between children with MS, and monophasic ADS. *p*-values <0.01 were considered statistically significant. Relative quantitative differences of peptide abundances between samples of children with MS and monophasic ADS samples were calculated as $\log_2$ ratio between median abundances of both groups. A set of significantly distinct peptides and proteins was determined by applying following stringent criteria: (a) peptides that had at least 1.5 fold difference in expression at a p-value <0.01; (b) protein identified by at least 2 peptides, and (c) at least 40% differentially abundant peptides ($p<0.01$) per protein, whereby peptides of a given protein were required to have representation in the same direction (increased or decreased abundance in MS). In the above dataset we also excluded those proteins that had only one peptide differentially abundant out of a total two. To verify these findings and to determine the statistical background level, we performed a permutation analysis on the entire dataset (2260 peptides) between samples of children with MS ($n=18$) and monophasic ADS ($n=21$) sample groups included with the above mentioned criteria. Whereby, we determined the statistical background level on a set of significantly distinct peptides. The random permutation test, on the dataset with randomized sample group assignment, was repeated 1000 times through which, the resulting thresholds were saved. For all calculations and graphics, we used the R software package (R version 3.0.2, http://www.R-project.org, accessed on February 4th, 2014).[12] For other calculations SPSS 15.0 (SPSS, Chicago, IL, USA) and Microsoft Excel 2010 were used.

RESULTS

Patient characteristics

We have analyzed CSF samples of 18 children with MS and 21 with monophasic ADS patients. One MS and one monophasic ADS patients were excluded because of <200 required alignment vectors (weak alignment) found during Progenesis LC-MS analysis. From 18 children diagnosed with MS, five had ON, two had TM, three had clinical monofocal, and eight had clinical polyfocal symptoms as their presenting symptoms of onset. From the 21 children with monophasic ADS, two had experienced ON, two had TM, eight had clinical polyfocal symptoms and nine had ADEM. No significant differences were observed between the two groups in terms of gender, CSF levels of total protein, albumin, albumin CSF/serum quotient, leukocyte and IgG concentration. The mean age at onset of children diagnosed with MS (14.17 ± 1.5) was found to be significantly higher in comparison to children with monophasic ADS (6.89 ± 4.9), reflecting the epidemiology of these phenotypes. In addition, CSF elevated IgG index and positive OCB were more frequently present in children with MS ($p \leq 0.01$). Patients characteristics are shown in Table 9.1.

Identification of proteins that discriminates MS from monophasic ADS

We have detected 50,119 peptide precursors from Progenesis label-free analysis LC-MS experiment from all trypsin digested protein in CSF samples. A Mascot database search in the human subset of the Uniprot database resulted into 2260 unique peptides that corresponded with 318 proteins (listed as a supplementary Table e-1). The total protein concentrations of digested peptide samples quantified (integrated UV area at 214 nm) during LC–MS measurements did not show any significant difference (p=0.54) between CSF samples of children with MS and monophasic ADS group. To check technical variability, at regular interval we measured 12 reference samples (pooled CSF samples from all patients). Here, also the total protein concentrations of digested peptide samples quantified (integrated UV area at 214 nm) during LC – MS measurements between Reference group 1 (n=6) and 2 (n=6) did not show any difference (Reference group 1 (n=6) =382.94 ± 153.28; versus Reference group 2 (n=6), 429.34± 661.90, p=0.3). In addition, a number of MS/MS fragmentation spectra also did not show any significant difference between samples of children with MS and monophasic ADS. In particular, the measured MS/MS fragmentation spectra for MS, and monophasic ADS samples were 16796 ± SD 1795 and 16971 ± 1153 (p=0.72) respectively. Moreover, the database identified MS/MS spectra for MS and monophasic ADS samples were 1607 ± 280 and 1626 ± 256 (p=0.83) respectively.

Comparing abundance of identified peptides (n=2260) from CSF samples of children with MS and monophasic ADS patients and using the stringent criteria as described in

Table 9.1 Clinical characteristics of children with multiple sclerosis and monophasic acquired demyelinating syndrome.

	Monophasic ADS (*n*=21)			**MS (*n*=18)**			***p* Value[a]**
	Mean ± SD	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**	**Monophasic ADS *vs* MS**
Age at onset, years	6.89 ± 4.91	5.81	1.14 - 17.11	14.17 ± 1.50	14.29	11.14 - 16.21	$p<0.01$
Sex, % females	71.4	N/A	N/A	61.1	N/A	N/A	NS
Protein, g/L	0.36 ± 0.21 (*n*=21)	0.3	0.18-1.15	0.33 ± 0.14 (*n*=17)	0.32	0.19-0.75	NS
Albumin, g/L	0.23 ± 0.20 (*n*=11)	0.16	0.11-0.79	0.18 ± 0.07 (*n*=14)	0.17	0.08-0.36	NS
Leukocytes x10^6	37.12 ± 34.91 (*n*=20)	28.5	1-118	20.98 ± 25.68 (*n*=17)	13	1.0-87	NS
IgG, g/L	0.05 ± 0.07 (*n*=11)	0.03	0.01-0.27	0.05 ± 0.03 (*n*=13)	0.05	0.03-0.12	NS
IgG index	0.61 ± 0.10 (*n*=12)	0.58	0.5-0.79	1.36 ± 0.72 (*n*=17)	1	0.67-3	$p<0.01$
Elevated IgG index[b], n (%)	2 (16.7)	N/A	N/A	16 (94.1)	N/A	N/A	$p<0.01$
Positive OCB, n (%)	2 (12.5) (*n*=16)	N/A	N/A	14 (87.5) (*n*=16)	N/A	N/A	$p<0.01$
Relapsing disease, n (%)	0 (0)	N/A	N/A	18 (100)	N/A	N/A	$p<0.01$

This table shows clinical characteristics and routine CSF findings of children with MS (at disease onset) and children with monophasic ADS. Data are presented as the mean ± SD or median and range. In case of missing data, the number of patients with available data is indicated in parentheses.

[a] Calculated by Mann-Whitney test and $p < 0.01$ was considered significant.

[b] IgG index considered elevated 0.68 or higher.[4]

ADS = acquired demyelinating syndrome; MS = multiple sclerosis; NA = not applicable; NS = not significant; OCB = oligoclonal bands.

Table 9.2 Identification of proteins differentially abundant in CSF samples of children with MS (*n*=18) and samples of children with monophasic ADS (*n*=21).

Trend in MS[a]	Description	Sign./ total[b]	Fold change mean (Min-Max)[c]	*p* Value[d] mean (Min-Max)
Increased abundance in MS (*n*=14)	Amyloid-like protein 2	2/2	3.8 (2-5.5)	0.006 (0.003-0.008)
	Neurofascin	3/3	2.5 (2.1-3)	0.002 (0.001-0.002)
	Carboxypeptidase E	2/3	2.4 (2.1-2.6)	0.0004 (0.0002-0.0005)
	Neuronal growth regulator 1	2/3	2.3 (2.1-2.5)	0.0008 (0.0008-0.0009)
	Contactin-2	4/9	2.3 (1.5-3.2)	0.002 (0.0005-0.003)
	Amyloid beta A4 protein	6/11	2.2 (1.8-2.6)	0.002 (0.00003-0.008)
	Brevican core protein	5/7	2.1 (1.7-2.6)	0.002 (0.0001-0.005)
	Disintegrin and metalloproteinase domain-containing protein 22	2/2	1.9 (1.7-2.1)	0.0004 (0.0001-0.0006)
	Tyrosine-protein phosphatase non-receptor type substrate 1	3/4	1.9 (1.5-2.1)	0.003 (0.001-0.006)
	Dickkopf-related protein 3	6/11	1.8 (1.6-2)	0.002 (0.00004-0.006)
	Neuronal cell adhesion molecule	9/18	1.8 (1.6-2.1)	0.004 (0.001-0.009)
	Ig kappa chain V-III region POM	2/2	1.8 (1.7-1.8)	0.002 (0.0009-0.003)
	Ig gamma-1 chain C region	5/11	1.7 (1.5-1.8)	0.0016 (0.0002-0.005)
	Kallikrein-6	4/7	1.7 (1.5-1.9)	0.001 (0.0006-0.004)
Decreased abundance in MS (*n*=7)	Apolipoprotein B-100	7/17	661 (3.5-3930)	0.0026 (0.00002-0.01)
	C4b-binding protein alpha chain	2/4	8.1 (2.9-13.4)	0.008 (0.007-0.008)
	Haptoglobin	18/29	3.7 (2-12.1)	0.002 (0.00004-0.01)
	Haptoglobin-related protein	2/4	3.3 (3.1-3.5)	0.0002 (0.00001-0.0005)
	Leucine-rich alpha-2-glycoprotein	2/3	2.6 (2.3-3)	0.008 (0.007-0.009)
	Monocyte differentiation antigen CD14	3/3	1.9 (1.8-2)	0.006 (0.004-0.009)
	Protein diaphanous homolog 1	2/2	1.8 (1.7-1.9)	0.005 (0.001-0.009)

The table shows 21 proteins, of which 14 were identified with increased abundance in CSF samples of children with MS and 7 were identified with decreased abundance in CSF samples of children with MS. The table includes the direction of difference (trend) in MS, name of the protein (description), fold expression difference, and *p* value. All proteins given in the table were identified with at least 2 unique peptides; differentially abundant peptides ($p < 0.01$) had at least 1.5-fold difference in expression (median) between groups; and for the same protein, 40% of identified peptides were differentially abundant with the expression in the same direction (i.e., either higher or lower in MS) of the same protein. Details of each peptide of the indicated proteins are listed in Tables e-2 and e-3.

[a] Protein abundance is either significantly increased or decreased in children with MS compared to children with monophasic ADS.

[b] Number of differentially identified peptides $p < 0.01$/total number of identified peptides for the same protein.

[c] Fold expression difference calculated based on median abundance from 18 patients with MS and 21 patients with ADS. Shown is the average of fold difference for all peptides of the same protein. Minimum and maximum range for the same is indicated.

[d] Calculated by Wilcoxon test. Given in the table is the mean p value and range for all differentially abundant peptides of the same protein.

ADS = acquired demyelinating syndrome; MS = multiple sclerosis.

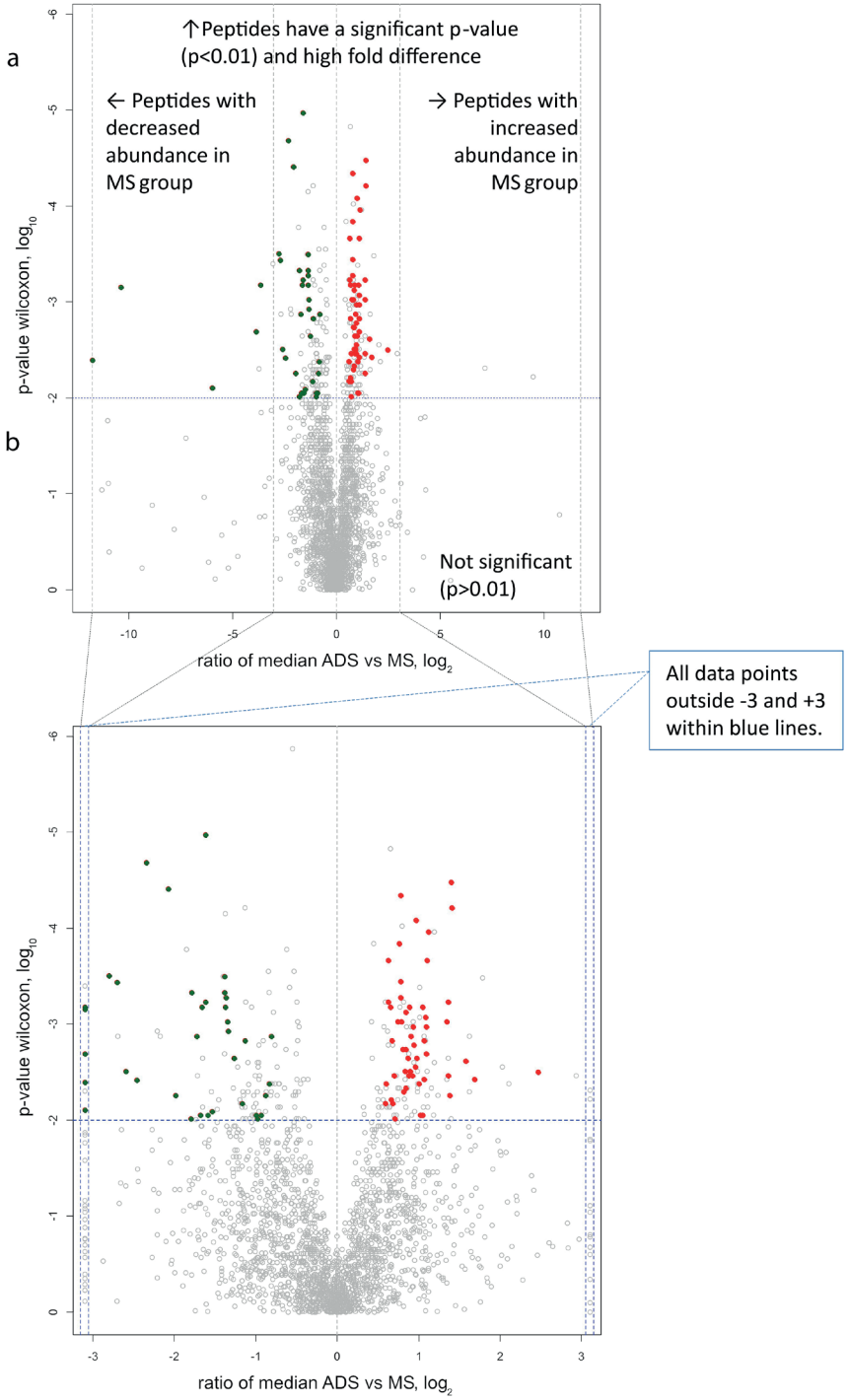

Figure 9.1 Volcano plot.

← **Figure 9.1** Volcano plot.
Peptides (*n*=2,260) showing distribution of fold change and statistical significance. In this plot each point represents a peptide, and shows the ratio between CSF samples of children with MS (*n*=18) and monophasic ADS (*n*=21) plotted against the level of statistical significance. Y-axis shows p-values, obtained (plotted at $\log_{10}$) from a Wilcoxon tests performed between abundances of peptides. X-axis shows the ratio of the median between MS and monophasic ADS samples (plotted on $\log_2$). (a) Above the dashed horizontal line, red points (*n*=53 peptides) were found with increased in abundance (right side of the vertical line), green points (*n*=35 peptides) decreased in abundance (at the left side of the vertical dashed line) in the MS group (compared to monophasic ADS). (b) Peptides shown in gray color point below the dashed horizontal line did not pass stringent statistical criteria for identification of a candidate peptide.

statistical analysis, we found a total of 88 differentially abundant peptides (supplementary Table e-2 and e-3). Of these 88 peptides, 53 were significantly increased (Table 9.2 and supplementary Table e-2) and 35 decreased in CSF samples of children with MS (Table 9.2 and supplementary Table e-3) as compared with monophasic ADS. Peptides with increased abundance (*n*=53) in the MS group corresponded with 14 proteins and peptides with decreased abundance (*n*=35) corresponded with 7 proteins. An inventory of these 21 proteins is given in Table 9.2.

Moreover, fold expression difference between MS and monophasic ADS groups groups and statistical significance are plotted simultaneously for our entire data set (*n*=2260 peptides) as a Volcano plot (Figure 9.1). This permutation analysis resulted in 20 ± 41 (median 9) false positive peptide markers, which indicated that our observations are not due to chance alone, because more than 90% of the permutations yielded 0-4 significant hits i.e. in 900. Only 4 times out of 1000 more than 88 hits with low p-value were detected (FDR: 0.4%). Whereas, contrasting to this mere background chance, from the actual data set (true hits) 88 peptides were identified. Therefore, comparison of permutated data with the real data indicated that the occurrence of differentially abundant peptides related to MS or monophasic ADS was highly significant ($p<0.001$). The outcome of the permutation test is shown as a histogram (supplementary Figure e-1).

Identified proteins with increased abundance (*n*=14) in MS (Table 9.2 and supplementary Table e-2) were: amyloid-like protein 2 (2/2, 2 significant peptides for a total of 2 peptides), neurofascin (3/3), carboxypeptidase E (2/3), neuronal growth regulator 1 (2/3), contactin-2 (4/9), amyloid beta A4 (6/11), brevican core protein (5/7), disintegrin and metalloproteinase domain-containing protein 22 (2/2), tyrosine-protein phosphatase non-receptor type substrate 1 (3/4), dickkopf-related protein 3 (6/11), neuronal cell adhesion molecule (9/18), Ig kappa chain V-III region POM (2/2), Ig gamma-1 chain C region (5/11) and kallikrein-6 (4/7). Proteins identified with decreased abundance (*n*=7) in MS (Table 9.2 and supplementary Table e-3) were: apolipoprotein B-100 (7/17), c4b-binding protein alpha chain (2/4), haptoglobin (18/29), haptoglobin-related protein (2/4), leucine-rich alpha-2-glycoprotein (2/3), monocyte differentiation antigen CD14

Table 9.3 Function of proteins identified with increased abundance in CSF samples of children with MS compared with samples of children with monophasic ADS.

Proteins (Accession no.)	Functions and expressions	Dhaunchak et al.[6]	Schutzer et al.[13]
Amyloid-like protein 2 (Q06481)	Amyloid precursor protein family, memory processes, concentrated in synapses[18], regulates neuronal stem cell differentiation during cortical development	×	** Variant
Neurofascin (O94856)	Synapse formation, located at CNS paranodal domain and expressed by oligodendrocytes[20], target for autoantibody-mediated axonal injury in MS[21]	NS	×
Contactin-2 (Q02246)	Axon connection, majorly expressed on CNS juxtaparanodal domain[19, 20]	NS	***
Amyloid beta A4 protein (P05067)	Component of amyloid plaques (Alzheimer brains)[35], neuronal adhesion	×	×
Brevican core protein (Q96GW7)	Major proteoglycan in perisynaptic extracellular matrix of brain, inhibits neurite outgrowth from cerebellar granule neurons[23]	NS	Decreased expression in first attack CIS-MS relative to established RR-MS and controls
Carboxypeptidase E (P16870)	Found in brain and throughout neuroendocrine system, involved in synthesis of most neuropeptide[36]	Same trend (4 sign./21 total, p=0.004)	×
Neuronal growth regulator 1 (Q7Z3B1)	Promotes outgrowth and expressed on reactive astrocytes after entorhinal cortex lesion (in mice)[37]	NS	×
Tyrosine-protein phosphatase non-receptor type substrate 1 (P78324)	Supports adhesion of cerebellar neurons, neurite outgrowth and glial cell attachment[28]	×	×
Neuronal cell adhesion molecule (Q92823)	Localized at the node of Ranvier and in unmyelinated axons, Paranodal region of CNS, axo-glial contact[19]	NS	**
Disintegrin and metalloproteinase domain-containing protein 22 (Q9P0K1)	Expressed in the juxtaparanodal complex[19]	Not same trend (2 sign./10; p=0.01,0.02)	×
Dickkopf-related protein 3 (Q9UBP4)	Expressed in the brain and spinal cord, synapse formation[13]	×	**
Kallikrein-6 (Q92876)	Elevated in active MS[26], regulates early CNS demyelination in a viral (mouse) model of MS[26]	×	**

Table 9.3 (continued)

Proteins (Accession no.)	Functions and expressions	Dhaunchak et al.[6]	Schutzer et al.[13]
Ig gamma-1 chain C region (P01857)	Elevated in adult MS and CIS as compared to non-inflammatory neurological diseases[38]	×	×
Ig kappa chain V-III region POM (P01624)	Elevated in adult MS as compared to non-inflammatory and inflammatory neurological diseases[38]	×	×

Nodes, paranodes, juxtaparanodes (begins at the innermost axoglia junction of paranode), and internodes are structural parts of a myelinated axon.
Summary of the function of differentially abundant increased protein markers in MS and their overlap with previous studies. Most of the proteins were assigned as either neuronal or immune-related molecules based on previous reports and database searches. The first column shows the name of the protein and Uniprot accession number. The second column shows the function of the proteins. The third column shows comparison with a previous study on children with MS (Dhaunchak et al.) using ADS-MS (*n*=8, mean age 12 years) vs monophasic ADS (*n*=11, mean age 10 years). The fourth column shows overlap with the work of Schutzer et al., who used CIS-MS (*n*=9, age 18–42 years) and established MS and controls (*n*=6, age 31–54 years).
× = protein was not identified; ** variant = variant of same protein was found with increased expression in first-attack CIS-MS group compared to established RRMS and controls; ** = increased expression in first-attack CIS-MS group compared to established RRMS and controls; *** = protein was found with increased expression in first-attack CIS-MS relative to RRMS but with decreased expression in first-attack CIS-MS vs controls; NS = protein was detected in pediatric CSF samples but no statistical difference in their abundance was reported between MS and monophasic ADS group comparison; same trend (trend is the direction of difference in MS) = when abundance of the protein was compared between 2 groups (MS vs monophasic ADS), expression of the identified protein showed overlap with our study; not same trend 5 when abundance of protein was compared, expression of the identified protein did not show overlap with our study.
ADS = acquired demyelinating syndrome; CIS = clinically isolated syndrome; MS = multiple sclerosis; NS = not significant; RRMS = relapsing remitting MS.

(3/3), and protein diaphanous homolog 1 (2/2). The function of these 14 proteins and overlap with previous studies[6, 13] are summarized in Table 9.3.

Among the 14 proteins with increased abundance in the MS, 12 were associated with CNS structure and functions (86%), especially to the gray matter (Table 9.3), compared to 17% of the total identified proteins related to CNS structure and functions. Seven proteins identified with decreased abundance in the MS (relative to monophasic ADS group) were components of the innate immune system and inflammation (Table 9.4).

For each peptide that passed (53 increased and 35 decreased in MS) all stringent criteria (as described in statistical analysis): the associated protein, database search identification details, p-value, fold expression difference, total number of peptides per protein, number of peptides identified below 0.01, median abundance, frequency/occurrence of identifications by MS/MS is shown in the supplementary Table e-2 and e-3. Proteins identified in the current study did not exhibit any myelin related proteins, for instance; myelin oligodendrocyte glycoprotein and myelin basic protein.

Table 9.4 Function of proteins identified with decreased abundance in CSF samples of children with MS compared to samples of children with monophasic ADS.

Differential proteins (Accession number)	Functions	Dhaunchak et al.[6]	Schutzer et al.[13]
Apolipoprotein B-100 (P04114)	Innate immune related, not produced in CNS[39]	×	×
C4b-binding protein alpha chain (P04003)	Innate immune defense, involved in complement activity[31]	×	×
Haptoglobin (P00738)	Innate immune defense[29]	×	×
Haptoglobin-related protein (P00739)	Innate immune defense[40]	×	×
Leucine-rich alpha-2-glycoprotein (P02750)	Induced by inflammation and involved in cell adhesion, high expression in the deep cerebral cortex[25], novel marker of granulocytic differentiation[29]	×	×
Monocyte differentiation antigen CD14 (P08571)	Main modulator of innate immune system[32]	×	×
Protein diaphanous homolog 1 (O60610)	Coordinates cellular dynamics by regulating microfilament and microtubule function, role in cell–matrix adhesions, variant related to innate immune function[34]	×	×

Summary of the function of differentially abundant decreased protein markers in MS and their overlap with previous studies. Most of the proteins were assigned as either neuronal or immune-related molecules based on previous reports and database searches. The first column shows the name of the protein and Uniprot accession number. The second column shows the function of the proteins. The third column shows comparison with a previous study on children with MS (Dhaunchak et al.) using ADS-MS (*n*=8, mean age 12 years) vs monophasic ADS (*n*=11, mean age 10 years). The fourth column shows overlap with the work of Schutzer et al., who used CIS-MS (*n*=9, age 18–42 years) and established MS and controls (*n*=6, age 31–54 years).

ADS = acquired demyelinating syndrome; CIS = clinically isolated syndrome; MS = multiple sclerosis. × = Protein was not identified.

We examined for the influence of age of onset on the 88 candidate peptides abundances for children with MS and monophasic ADS by correlation analysis. We found a mean coefficient of determination (± SD) for MS 0.04 ± 0.06, and for monophasic ADS 0.05 ± 0.05. Thus, by correlation analysis no significant correlation was found between age and peptide abundance for children with MS and monophasic ADS for all 88 peptides.

DISCUSSION

In the current study, we have used LC-MS proteomic approach to search for differences in CSF proteome between children with MS and monophasic ADS in children. Benefit of this Orbitrap technique is the possibility to identify relatively vast amounts of different peptides, and assess their abundances, in a small sample volume. We observed a striking difference between the two groups (children with monophasic ADS versus MS), using stringent statistical criteria. We searched for the known functions of the 88 peptides corresponding to 21 distinctive proteins (14 increased and 7 decreased in abundance in MS), using biological databases (www.geneontology.org and www.nextprot.org) and literature. Recently, two research groups[6, 13] demonstrated the identification of axoglial and gray matter proteins using mass spectrometry in CSF of MS patients . Similar to our study, Dhaunchak and co-workers[6] compared CSF of 8 children with MS with 11 children with monophasic ADS. The overlap of some proteins in the MS group is noticeable (e.g. carboxypeptidase E), despite clear differences in sample handling such as depletion of abundant proteins with possible carrier function for other proteins, and exclusion of proteins with less than 5 kDa weight (Table 9.3).

Our results show overlap with molecules identified in the CSF of adult acute onset MS cases by Schutzer et al.[13], who performed the mass spectrometry analysis in CSF on CIS cases versus established relapsing remitting MS (RR-MS) and controls (Table 9.3). They showed proteins that distinguished these CIS patients from both established RR-MS and controls. For example, they showed a significant increase of kallikrein-6, dickkopf-related protein 3 at first clinical onset in comparison to established RR-MS and controls (Table 9.3). They also showed a significant increase in contactin-2 (neuronal membrane protein) in first-attack MS patients relative to established RR-MS (Table 9.3).[13]

Our findings illustrate that the neurodegenerative arm of MS neuropathology is already active at the earliest stage of clinical disease, also in children. Consistent with the findings of others and our own previous studies[6, 14-16], we again observed a striking lack of myelin proteins in these clear-cut cases of acute demyelination. This may not directly imply absence of such free proteins in this type of pathology, but rather it may reflect the specific physicochemical properties of the hydrophobic myelin components,

and perhaps different pathways of elimination from the CSF (e.g. via draining macrophages).[17] In any case, we must be cautious in using the dominant presence of CNS gray matter over white matter proteins as proof that neurodegeneration is a primary event in MS and would precede demyelination. The presence of CNS gray matter may simply represent damage by inflammation, and the molecules identified may lead to a better understanding of this presumed inflammation induced neurodegeneration. It should be stressed that not all differentially abundant proteins were overrepresented in MS; some were underrepresented, pointing at more complex mechanisms, such as a perturbation in the physiology of the axoglial apparatus (Table 2 and Tables e-2 and e-3).[6] A confounding factor in our study could be the fact that, due to the skewed occurrence of monophasic disease at younger age, both groups were not matched according to age. We doubt, however whether this has influenced the results, as we did not see an age effect in our groups on the abundance of the 21 identified proteins.

Of 14 proteins with increased abundance in MS (Table 9.2 and 9.3), two were associated with the amyloid beta A4 protein family. Amyloid-like protein 2 is mainly concentrated at neuronal synapses[18] and has a role in memory processes. While, amyloid beta A4 protein is associated with neurite growth, neuronal adhesion and axonogenesis (Table 9.3). Six (of 14) proteins: contactin-2[19], neurofascin[20], neuronal growth regulator 1, brevican core protein[6], neuronal cell adhesion molecule[19] and disintegrin and metalloproteinase domain-containing protein 22[19] are shown to be located at the paranodal and, juxtaparanodal region of the CNS of myelinated axons (Table 9.3). Contactin-2 is axonal glycoprotein which is shown at juxtaparanodal domain of myelinated axons.[13] Neurofascin plays a role in the assembly of nodes of Ranvier in the CNS.[21] Two isoforms of neurofascin are shown to interact with contactin-associated protein and contactin-1 to form paranodal junction, that attaches the myelin loop to the axon; and helps to separate voltage gated sodium channels at node and potassium channels at juxtparanodal region.[20] Disruption of neurofascin localization shows early changes preceding demyelination and remyelination in MS.[22] Interestingly, contactin-2 and neurofascin are previously reported as autoimmune targets in MS.[20] Neuronal growth regulator 1 is shown to be located at paranodal region of CNS and play a role in axo-glia contact at the node of Ranvier. Identification of contactin-2 and neurofascin in CSF of children with MS is consistent with a previous study[6] however, they did not found significant difference in CSF of children with MS and monophasic ADS (Table 9.2 and 9.3). Brevican core protein is known as CNS specific proteoglycan[19] at the surface of neuroglial sheaths, where it is enriched in perisynaptic extracellular matrix.[23]

Among the proteins with increased abundance in MS (of 14), the proteases/peptidases protein named as carboxypeptidase E, plays a role in synthesis of most neuropeptides.[24] Next, disintegrin and metalloproteinase domain-containing protein 22 are highly expressed in brain and localized at juxtaparanode.[19] This molecule presumed to be work

as a major neuronal receptor.[25] Another brain related protease was kallikrein-6, which is a secreted serine protease[13], and it is described to regulate early CNS demyelination in a viral model (expression in the brain and spinal cord of mice) of MS.[26] Additionally CSF kallikrein-6 elevated level is reported in MS (adults) as compared to neurological controls (Table 9.3).[27]

Among other proteins with increased abundance in MS (of 14), tyrosine-protein phosphatase non-receptor type substrate 1, is implicated in the neurite outgrowth, glia-cell attachment and also shown to support adhesions of cerebellar neurons.[28] Next the highest expression of dickkopf-related protein 3 is reported in the brain and spinal cord (Table 9.3).[13] Thus the majority of proteins with increased abundance in our MS group (compared to monophasic ADS) were neuronal related with the exception of two immune function related proteins Ig gamma-1 chain C region and Ig kappa chain V-III region POM (Table 9.3).

Our study reports seven proteins with decreased abundance in pediatric-onset MS as compared to the monophasic ADS (Table 9.2 and supplementary Table e-3) group. Six of these seven proteins have previously been reported as a specific component of innate immune functions (Table 9.4). Haptoglobin, an acute phase protein[29] was identified as the most distinctive one (18/29, 18 significant peptides for a total of 29 peptides) from those which were elevated in monophasic ADS children (compared to children with MS). Another study in adults showed increased haptoglobin concentration in NMO comparison to adult MS patients.[30] C4b-binding protein alpha chain which is a crucial component of complement cascade and inhibits function of complement component.[31] Interestingly, we also found monocyte differentiation antigen CD14, which is shown to be mainly expressed on cells of monocytic lineage (macrophages and monocytes).[32] Higher CD14 levels might be linked with increased levels of cytokines triggering inflammatory processes in monophasic ADS children. Monocytic cells secrets soluble sCD14 (activation product of activated monocytes), so this may affect the enormous macrophage activation during acute monophasic ADS.[33] Among seven identified proteins, protein diaphanous homolog 1, has a role in cell adhesion, it is also expressed in brain and, its variant are shown to be required for innate immune response to gram-negative bacterial infection.[34]

Overall in the MS group we found a significant over-representation of proteins associated with changes in CNS gray matter, axons, synaptic regulation, node of Ranvier and brain proteases (Table 9.3). Several of these proteins are part of the axoglial apparatus and may relate to disturbances in axo-glia interaction[6] (Table 9.3). Two of them (contactin-2 and neurofascin) have been identified as possible axo-glial auto-antigens in MS.[20] The overlap of proteins observed in previous studies[6, 13] (Table 9.3) as part of the axo-glia apparatus and gray matter provides validation for these proteins. Further insight into the role of these proteins in early-onset MS can be useful for disease process

understanding and might be useful as a future tool to differentiate MS from monophasic ADS in children.

These pathologically relevant proteins (mostly CNS gray matter related), elevated in CSF of early-onset MS in children might be involved in early disease mechanisms. Further insight into the role of these proteins can be useful for disease process understanding. Moreover, such proteins might be useful as a future tool to differentiate children MS from children with monophasic ADS. In addition, knowing the start of MS could immediate an earlier treatment with disease modifying therapies. However, the current research is designated as discovery phase study, which serves as a base for the follow-up on verification and validation phase studies which can provide in clinically valuable biomarkers. In future, it would be interesting to further validate our findings with an independent technology more importantly in an independent sample group.

The data presented in this study indicate that monophasic ADS can be differentiated from MS in children primarily by CNS gray matter proteins and immune-related proteins. Our findings point to perturbed axoglial physiology as a hallmark of the earliest events of MS pathogenesis.

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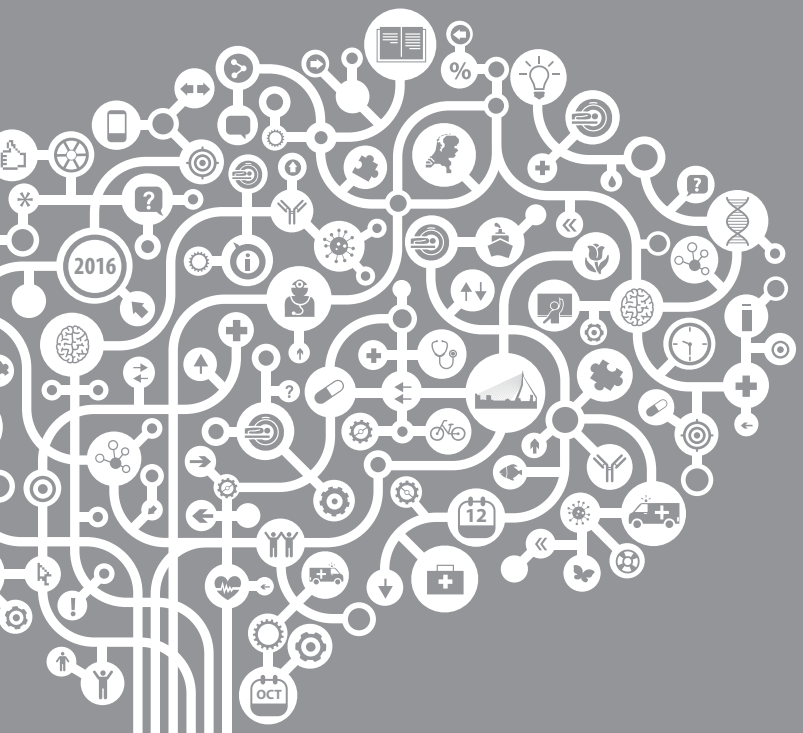
SUPPLEMENTAL MATERIAL

Supplemental data at Neurology.org/nn http://nn.neurology.org/content/2/5/e155
Supplemental Methods
Supplementary Figure e-1. Permutation test.
Supplementary Table e-1: All identified peptides 2260 (corresponded to 318 proteins).
Supplementary Table e-2: Peptides (*n*=53) identified with increased abundance in MS as compared to monophasic ADS group and passed all the criteria for candidate peptide.
Supplementary Table e-3: Peptides (*n*=35) identified with decreased abundance in MS as compared to monophasic ADS group and passed all the criteria for candidate peptide.



Chapter 10

General discussion

Acquired demyelinating syndromes (ADS) cover a broad spectrum of inflammatory demyelinating syndromes of the CNS, of which multiple sclerosis (MS) is the most common subtype. This thesis focuses on two relatively rare clinical subtypes of ADS: neuromyelitis optica spectrum disorders (NMOSD) and childhood-onset ADS including MS. Awareness and recognition of uncommon ADS subtypes are of importance for clinicians, since those require a distinct diagnostic and therapeutic approach. In this thesis we aimed to reveal the spectrum of ADS by describing the clinical features of NMOSD and childhood-onset ADS, in order to improve the diagnostic process. Here, the most important findings from our studies are outlined and discussed in relation to other studies. Future directions and steps for further research are discussed.

PART ONE: NEUROMYELITIS OPTICA SPECTRUM DISORDERS

Demographic features of NMOSD

NMOSD is a rare variant of CNS inflammation, previously often misdiagnosed as MS.[1, 2] However, treatment regimens and prognosis differ significantly between NMOSD and MS and therefore it is important to distinguish these diseases. Prior to our study, incidence numbers of NMOSD in the Netherlands were unknown. Based on results of a national centralized NMO expert center, we could asses the mean nationwide incidence of AQP4-IgG seropositive NMOSD in the Netherlands is 0.09 per 100,000 people per year (**chapter 2**). The Dutch incidence is within the range of previous reported incidence rates which range from 0.05 – 0.4 per 100,000 people.[3] It should be noted that these are minimum incidence figures, since mild cases and forme fruste types of the disease could have been missed. The incidence of NMOSD in the Netherlands is estimated more than twice as high in non-Caucasians. Africans seem to be more predisposed to NMOSD than whites.[3-5] This is supported by a recently reported high incidence of AQP4-IgG seropositive NMOSD patients of 0.65 per 100,000 people in a population with 90% people of Afro-Caribbean origin.[4] Moreover, Afro-Caribbean NMOSD patients are younger at disease onset, have more multifocal attacks and have a higher likelihood of visual disability than Caucasian patients.[6] Differences in clinical features and outcomes between ethnic groups indicate the need for a future tailored treatment approach for individual groups.

The recently revised diagnostic criteria for NMOSD broaden the clinical spectrum of NMO.[7] Most importantly, the new diagnostic criteria allow for an earlier NMOSD diagnosis[8, 9] even under the assumption of an unknown AQP4-IgG status.[8] Previously, patients had to have optic neuritis (ON) and transverse myelitis (TM) in order to fulfill definite NMO criteria.[10] Now, AQP4-IgG seropositive patients can be diagnosed with NMOSD after one event of isolated ON, TM or area postrema syndrome.[7] In patients without AQP4-IgG, dissemination in space with two different core clinical characteristics (Table 1.1)

and exclusion of alternative diagnoses are required for NMOSD diagnosis. Recognition of NMOSD patients can be a diagnostic challenge since not all NMOSD patients present with classical bilateral ON and longitudinally extensive TM (LETM). Spinal cord lesions can be short in a minority of the NMOSD cases[11], and sometimes even asymptomatic.[12] Clues to a NMOSD diagnosis are a non-Caucasian ethnicity, ON with severe vision loss and minimum response to steroids, preceding ON or brainstem syndrome, personal or serological evidence of autoimmunity and a brain MRI without MS lesions.[12] More awareness and better recognition of the broadening clinical spectrum of NMOSD might increase the incidence of NMOSD in the future.

The search for new prognostic markers in NMOSD

Despite the high specificity of AQP4-IgG for NMOSD, the antibody is present in approximately 77% of the NMO cases and lacks sensitivity to diagnose all patients with NMOSD.[7, 13-15] Therefore, researchers focused on identifying new diagnostic and prognostic biomarkers for the AQP4-IgG seronegative NMOSD patients.[16, 17] In the past years, MOG autoantibodies were evaluated as a potential novel marker for NMOSD.[18, 19] In the Dutch cohort we have confirmed the presence of MOG-IgG in 33% of the AQP4-IgG seronegative patients with a clinical NMOSD phenotype[7], including limited forms as recurrent ON, bilateral ON and LETM[1] (**chapter 3**). In addition to previous studies, we evaluated MRIs for NMO-specific cerebral lesions, which typically occur at sites with high aquaporin-4 expression.[20, 21] NMO-specific cerebral lesions were absent in the MOG-IgG seropositive patients which is likely explained by the different underlying disease mechanisms.[16, 22] In general, MOG-IgG seropositive patients with a clinical NMOSD phenotype have a relative benign disease course, which is often monophasic, and if relapses occur those are less severe.[23-25] However, MOG-IgG seropositive cases with a worse disease course including ongoing relapses and disability progression have been observed.[24-27] At the NMO expert center at Erasmus MC, we follow a few MOG-IgG seropositive patients who suffered from frequent disabling relapses and required chronic immunosuppressant therapies. This demonstrates the importance of an individualized treatment approach and careful consideration of chronic treatment initiation in a subgroup of MOG-IgG seropositive patients.

Reported MOG-IgG seropositivity rates as percentage of the AQP4-IgG seronegative NMOSD cases, including limited forms of NMO like recurrent ON or LETM, range between 7-39%.[23-35] Double seropositive cases (both AQP4-IgG and MOG-IgG) are extremely rare, limited to only a few cases.[25, 28, 31, 35] Since study protocols and inclusion criteria differ between various cohorts, it is not easy to compare MOG-IgG seropositivity rates. At the NMO expert center at Erasmus MC we were also interested in limited forms of NMOSD and included patients with bilateral ON, recurrent ON and LETM. This and referral bias to expert clinics might contribute to differences in reported MOG-IgG seropositivity rates.

PART ONE: NEUROMYELITIS OPTICA SPECTRUM DISORDERS

Main findings

- The incidence of AQP4-IgG seropositive NMOSD in the Netherlands is 0.09 per 100,000 people per year.
- In approximately one-third of the Dutch AQP4-IgG seronegative patients with a clinical NMOSD phenotype MOG-IgG is detected.
- MOG-IgG seropositive patients with a clinical NMOSD phenotype generally have a more favorable monophasic disease course than AQP4-IgG seropositive NMOSD patients.

Clinical implications

- MOG-IgG should be tested in AQP4-IgG seronegative patients with a clinical NMOSD phenotype as part of their standard diagnostic work up.

Future studies should collect serial serum samples to study if MOG-IgG persists in patients with a clinical NMOSD phenotype. Furthermore, MOG-IgG seropositive patients should be followed in order to investigate if they truly have a more favorable disease course than AQP4-IgG seropositive NMOSD patients. Collaborative international studies in search of new additional biomarkers for NMOSD diagnosis and for establishing the prognosis and therapeutic management of individual patients are promising.[17, 36, 37] Recently, it has been questioned whether MOG-IgG seropositivity justifies a diagnosis of NMOSD.[22] Clinical phenotypes of MOG-IgG seropositive and AQP4-IgG seropositive patients overlap. However, there are important differences in their underlying disease mechanisms.[16, 22] Neuromyelitis optica refers to the classical syndrome as first described by Eugene Devic in 1894.[38] The old nomenclature became out-dated, since the spectrum of NMOSD is growing and includes limited forms of NMOSD as isolated ON, or isolated TM, or brainstem syndromes.[1, 2, 7] Elevated creatine kinase (CK) levels[39-41], and hypothalamic endocrinopathies, including symptoms as galactorrhoea[42], are examples of rare features of NMOSD. Some have proposed the novel umbrella term autoimmune aquaporin-4 channelopathy as this term better suits the broad clinical spectrum, which includes more than inflammation of the optic nerve and spinal cord.[2] In addition, this term clearly refers to the underlying disease mechanism. Major disadvantage of this term is that it excludes the AQP4-IgG seronegative NMOSD patients, who share clinical similarities with the AQP4-IgG seropositive NMOSD patients.[43]

PART TWO: ACQUIRED DEMYELINATING SYNDROMES IN CHILDREN

Diagnosing childhood-onset ADS

In children with ADS, the most relevant risk parameters for MS can be found amongst MRI parameters, which can help to identify the children in whom an incident event of ADS represent the first attack of MS. In the past years, several MRI criteria have been developed for the diagnosis of ADS, including MS, in children and for the distinction from other diseases.[44-46]

The current IPMSSG diagnostic definitions for childhood-onset MS, incorporate the 2010 revised McDonald MRI criteria for MS.[47, 48] Aims of the 2010 MRI criteria were to simplify the existing criteria[49, 50], and to allow for an earlier definite MS diagnosis. Unique for the 2010 McDonald criteria is that MS can be diagnosed in patients who fulfill criteria for dissemination in time and space, already after a single attack based on a single baseline MRI.[48] With the 2010 McDonald MRI criteria for relapsing-remitting MS, detection of CSF OCB is not obligatory to confirm MS diagnosis. However, in clinical practice CSF analysis is often performed in children presenting with CNS demyelination in order to exclude alternative diagnoses, most importantly infectious diseases, prior to the initiation of acute treatment with intravenous methylprednisolone. Previous studies reported the 2010 McDonald MRI criteria allow for an early MS diagnosis in children.[51-55] But the diagnostic accuracy is less when applied in young children and children with ADEM.[51, 53] Therefore the 2012 IPMSSG stated the McDonald 2010 MRI criteria cannot be applied in children less than 12 years old and in children with ADEM at the first event.[47] In **chapter 4** we evaluated the utility of the most recent MRI criteria, as per the 2012 IPMSSG consensus definitions in a Dutch prospective cohort including children with the full spectrum of ADS. Here, we demonstrated the 2012 IPMSSG consensus definitions allow for an equally reliable but earlier MS diagnosis in all children (including children ≤12) than with the 2007 IPMSSG definitions. This is beneficial for the adequate counselling of patients and parents, who face their lives with insecurity. In addition, an earlier MS diagnosis allows for earlier initiation of disease modifying therapy (DMT), which might be of long-term benefit.[56-58]

The IPMSSG 2012 definitions also include a revised definition of MS diagnosis after ADEM. However, using the IPMSSG definitions, a definite MS diagnosis is observed less often than previously reported[59] in children initially presenting with ADEM (<10%).[47, 60-62] We endorse the IPMSSG panel's advice not to apply the 2010 McDonald MRI criteria in children with a first event of ADEM. Children with ADEM usually have large and enhancing lesions[47, 59, 60, 63], which could lead to false-positive MS diagnoses. Other MRI criteria are more useful to discriminate children with ADEM from those with MS.[46] In the Dutch cohort it was confirmed that the Callen MS-ADEM criteria (i.e. fulfilling at least 2 out of 3: a) absence of a diffuse bilateral lesion pattern, b) presence of black holes and c) ≥2 periventricular lesions) were the most useful criteria to differentiate MS from ADEM in children.[64]

The Canadian study group proposed new MRI parameters for the prediction of MS diagnosis in children with ADS.[60] In their large-scale prospective cohort study including 284 children with ADS, 14 MRI parameters were assessed for MS risk prediction using a multivariable prediction model. Here, 57 children were diagnosed with MS during follow-up. Two MRI parameters were associated with an increased likelihood of MS diagnosis: T1-hypointense lesions and periventricular lesions (Figure 10.1). MS risk was highest when both parameters were present. In collaboration with our Canadian col-

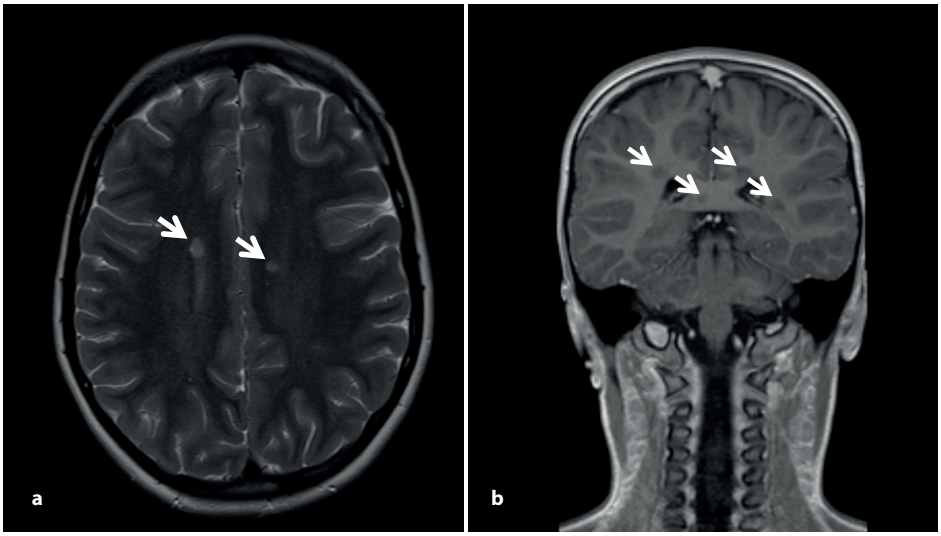


Figure 10.1 Example of periventricular lesions (arrows) (a) and T1-hypointense lesions (arrows)(b).

leagues we validated these parameters in a large independent Dutch cohort of children with ADS (**chapter 5**).

Verhey MRI parameters[60] were evaluated in both the Dutch retrospective[65] and prospective[66] ADS cohorts. Overall, we found that the presence of ≥1 T2-periventricular and ≥1 T1-hypointense lesions reliably identify children with MS. We found a higher sensitivity of 93.3% and specificity 86.7% in the prospective group than in the retrospective group (66.7% and 85.2% respectively). The higher number of false negatives in the retrospective group might be explained by poorer quality of the images. In addition, we reported lower specificity in the prospective cohort, than in the original study (87% versus 93%).[60] In our report we speculated this was caused by the fact that serial imaging was not standardly performed in the Dutch cohort and thus fulfillment of dissemination in time based on MRI for MS diagnosis might be underappreciated. Currently, one of the four children initially classified as false positive is diagnosed with MS during follow-up, increasing the specificity of the Verhey criteria to 90% in the prospective cohort.

Nowadays, serial imaging in children with ADS is performed on a more regular basis since its importance for early MS diagnosis, monitoring the effect of DMT and surveillance for severe therapeutic side-effects including opportunistic infections such as progressive multifocal leukoencephalopathy (PML).[67] In our opinion, serial imaging is only required in children in whom it might be of clinical relevance, since MRI scans are invasive for children. Moreover, an intravenous administration route is required, and in younger children there is the additional risk of narcosis. For example, children with isolated ON or TM with a normal cerebral MRI at onset have a very low risk for a future MS diagnosis[61] and in these children continuing serial MRI scans seem not strictly neces-

sary. Clinical follow-up and surveillance for new symptoms are sufficient in this specific category of children who are at low risk for a future MS diagnosis.

This year novel MRI criteria for the diagnosis of MS were reported.[68] The 2016 MS diagnostic MRI criteria include two new CNS sites which can contribute to dissemination in space: optic nerve lesions and cortical lesions. These criteria need to be validated in pediatric and adult cohorts of CNS demyelination. Furthermore, non-conventional imaging techniques as diffusion tensor imaging (DTI) should be further studied, as these images could be used as a prognostic marker in children presenting with ADS.[69] Functional MRI (fMRI) can provide insight into the adaptive and compensatory mechanisms in childhood-onset MS.[70, 71] Combined with neuropsychological assessments, fMRI might provide relevant implications for counselling and rehabilitation strategies.

Children versus adults: outcome and management

There are important differences in childhood-onset versus adulthood-onset MS. Children with MS can present with a broad spectrum of demyelinating syndromes, including ADEM.[72] In addition, the differential diagnosis in children is far more complex.[73-75] Children with MS have more frequent and more severe attacks.[76-78] Still, children with MS take longer to reach stages of progressive disability, but do so at a younger age.[79-81] However, these observations are based on studies conducted prior to the immunomodulatory treatment era in children.[76, 79, 80] Current recommendation and use of DMT in children with MS, likely influences their disease course.[82] In **chapter 6** we compared the disease course in 383 children and adults presenting with CIS. Over 50 percent of the included patients were diagnosed with MS during follow-up. 11-16 year-old children had the highest rate of MS conversion and the shortest time to MS diagnosis. Overall we found a female predominance, but as in line with previous studies not in young children who were diagnosed with MS before puberty.[83, 84] This suggests sex hormones might contribute to the onset of MS.[85] Moreover, we found that children with MS more frequently had a non-Caucasian ethnicity than adults with MS. Previous studies reported a higher vulnerability for ADS and a future MS diagnosis in non-Caucasian children.[66, 86-89] The exact underlying mechanism is unclear, but it might be that those children lack certain protective genes since their ancestors were born in countries with a low prevalence of MS.[66, 90] In addition, a higher load of certain environmental risk factors, as for example lower vitamin D levels in Afro-American children, might contribute to a higher MS vulnerability in non-Caucasian children. Moreover, non-Caucasian children with MS have a higher relapse rate and more severe relapses.[91, 92] This indicates the need for future tailored treatment protocols for individual groups based on their disease course. In **chapter 6** we also validated a prediction rule for a future definite MS diagnosis and early first-line treatment initiation in 11-16 year-old children. In adult CIS patients with a high risk for a future MS diagnosis, it is already common practice to start with DMT at onset.[93]

In children long term effects of DMT are less well known and DMT is only prescribed to children with a definite MS diagnosis.[82, 94] However, all children who fulfilled our prediction rule had a definite MS diagnosis during follow-up. From an empirical point of view, it seems logical to start DMT in an as early as possible stage. However, the effect of DMT initiation in children with CIS who are at a high risk of MS needs to be established.

Prognostic markers in childhood-onset ADS

Prospective follow-up studies of children with ADS are of great interest to study the genetic and environmental risk factors that contribute to MS, since any child presenting with ADS can be diagnosed with MS in the future. Studying children with ADS could identify new MS risk factors and underlying disease mechanisms. As in adults, genetic risk factors contribute to MS risk in children.[95] The presence of one or both *HLA-DRB1*15* alleles confer to MS susceptibility in children.[61, 95, 96] In a Canadian cohort of 266 children with ADS, including 64 children with MS, the increased risk of MS in children harbouring *HLA-DRB1*15* alleles was larger in children who reported a European ancestry (OR 3.3, p=0.001 versus OR 2.0, p=0.15 in children with a non-European ancestry).[95] Besides the major *HLA-DRB1*15* risk allele for MS, 200 SNPs with a relatively small effect on MS risk have been identified.[97] In **chapter 7** we studied the effect of 57 MS risk SNPs[98] (at that time known from a large-scale international GWAS) in children with ADS in collaboration with the Canadian study group for childhood-onset ADS. Using a compound weighted genetic risk score, we found higher risk scores in children diagnosed with MS than in children with monophasic ADS. The genetic risk score of children with monophasic ADS was equal to the risk score of healthy controls. From this study we learned, genetic risk loci known from adults with MS also confer to increased MS susceptibility in childhood. The ability of the genetic risk score to discriminate between children with MS and monophasic ADS was moderate and comparable with the model for adult MS and controls. This risk model is of interest for studying genetic risk effects in groups of patients, but is not useful for prediction of the disease course in individual cases. Interestingly, the combined effect of 57 minor risk genes exceeded the effect of the major MS risk allele *HLA-DRB1*15* alone. Whether the very same risk loci are involved in childhood-onset and adult-onset MS, and among various ethnicities, remains to be elucidated. The identification of novel genetic risk factors in childhood-onset ADS might reveal new pathways for MS disease mechanisms and future therapeutic intervention strategies.[99] However, future genetic studies are complicated by the requirement of large sample sizes. It would be interesting to study the effect of the currently 200 risk SNPs[97] in a more complex prediction model for childhood-onset MS, including *HLA-DRB1*15*, EBV serology and vitamin D levels. In a recent American prospective cohort study, no association was found between the compound genetic risk score of 110 MS risk SNPs and relapse rate.[100] However, only children with CIS and MS were included in this study, and not the full spectrum of childhood-onset ADS.

Besides genetics, several environmental factors contribute to MS risk in childhood, including vitamin D level. In a Canadian cohort of 302 children with ADS every 10 nmol/L decrease in 25-hydroxyvitamin D levels led to an increased MS risk (HR 1.11, CI 1.0-1.25).[61] Furthermore, an increase in vitamin D levels was associated with decrease in relapse rate in a retrospective study of 110 children with CIS and MS.[101] Recently is has been reported, this association is linked to children carrying at least one *HLA-DRB1*15* allele.[100] Whether vitamin D suppletion has a therapeutic effect on CNS demyelination is unknown.[102] In the Netherlands vitamin D suppletion is recommended in healthy toddlers by the Dutch Health Council. At the outpatient clinic in Erasmus MC Sophia, vitamin D suppletion is started in children with 25-hydroxyvitamin D levels below the normal threshold (≤ 50 nmol/L)[103] for the purpose of their general health and normal bone growth. Remote EBV infection contributes to MS risk in adults and children.[61, 104-107] This is recently confirmed in a prospective Canadian study including 247 children with ADS, of whom 58 were diagnosed with MS.[108] Eighty-five percent of the children with MS, compared with 44% of the children with monophasic ADS, were seropositive for remote EBV infection (HR 3.6, CI 1.6 – 7.9). Increased rates of EBV viral reactivation, as measured by monthly oral swabs, were found in children with MS (average detection rate 50.6% in patients with MS and 20.4% in controls, p=0.01).[109] Suggesting a selective impairment in the immunological control of EBV in children with MS. On the other hand, MS risk is lower in children who had a previous CMV infection.[107, 108] One American cohort study in 189 children with MS and 38 controls reported higher levels of antibody response to EBNA-1 in *HLA-DRB1*15* positive children.[110] Their results demonstrate a possible gene-environment interaction in the disease mechanism of MS, in which genotype influences the humoral response to EBV. In adults, gene-environment interactions have been described between environmental factors as smoking and obesity and *HLA-DRB1*15*.[102]

MOG-IgG in childhood-onset ADS

MOG-IgG further elucidates the spectrum of childhood-onset ADS and is mainly detected in young children with an ADEM-like disease onset, including polyfocal symptoms and often encephalopathy.[111-115] In the Dutch cohort we identified MOG-IgG in 21 out of 117 (18%) children with ADS (**chapter 8**). Most of the MOG-IgG seropositive children had a polyfocal disease onset (86%), of whom 10 children had encephalopathy, fulfilling 2012 IPMSSG criteria for ADEM.[47] In contrast, MOG-IgG was detected in only one of the adults with ADEM (3% versus 42% in children with ADEM), confirming that MOG-IgG is especially detected in young patients. In this study we reported 4 children with a relatively new ADS phenotype of ADEM followed by recurrent optic neuritis (ADEM-ON).[116] Interestingly, all of the 4 children with ADEM-ON were MOG-IgG seropositive. Currently, we have included 7 children with ADEM-ON in the prospective ProudKids study. In one child MOG-IgG was not detected, nor was AQP4-IgG, despite sequential sampling and

sampling during relapse. In our experience, children with ADEM-ON can suffer from severe ON with fast progression of visual loss and frequent relapses. However, they seem to recover well with acute treatment with intravenous methylprednisolone. The chronic management of children with ADEM-ON is comparable with, and based on experience of, treatment of NMOSD.[117] Six children with ADEM-ON who had an oral prednisone taper, had relapses when the dose was reduced below 10 milligrams. Therefore we advise to decrease the prednisone taper very slowly, depending on the clinical features.

Further studies in international collaboration are needed, so that clinicians can share their experiences and study what are the best acute and chronic treatment strategies for MOG-IgG associated diseases, including ADEM-ON. Only one of the 21 MOG-IgG seropositive children was diagnosed with MS during follow-up. Low titers of MOG-IgG can be detected in young children with MS[112-114], as was the case in this specific patient with MS. The presence of MOG-IgG pleads against MS, as MOG-IgG seropositive patients much more likely develop one of the various non-MS ADS subtypes (i.e. CIS, monophasic ADEM, multiphasic ADEM[118], ADEM-ON or NMOSD). Similar results have been reported by the pediatric ADS study group from the United Kingdom.[119] Furthermore, MOG-IgG has not been detected in adult MS patients at the outpatient clinic of Erasmus MC (unpublished data).

As in adults, MOG-IgG is detected in children with a NMOSD phenotype in whom AQP4-IgG was not detected.[120] The optic nerve is a frequently involved location in MOG-IgG seropositive patients.[27, 31] MOG antibodies are specific for CNS demyelination and are not detected in other diseases.[19] The diagnostic assays improved in the past years and the current CBA detecting full-length MOG-IgG reliably identify patients within the spectrum of CNS demyelinating disorders. However, the exact role of these antibodies is not clear yet. They might induce demyelination, or represent a bystander effect. It is unlikely that the antibodies solely reflect fulminant demyelination, since MOG-IgG is not detected in all patients with ADEM, or in patients with MS.[119, 121] Loss of organisation of the cytoskeleton of oligodendrocytes incubated with purified MOG-IgG suggest a possible direct pathogenic effect of MOG-IgG.[122] Recently, it has been reported that MOG-IgG was detected in 7 out of 35 patients (20%) with a symptomatic primary EBV infection and in none of the controls.[123] This finding suggests EBV might be a possible trigger for MOG-IgG associated CNS demyelination. Further studies investigating the role of MOG-IgG and the pathogenesis of CNS inflammation are of interest, since those could shed light on new therapeutic interventions. However, MOG-IgG is already of relevance for clinicians, since the antibodies are specific for CNS demyelinating diseases and can aid in the diagnostic workup of patients by narrowing the differential diagnosis. Furthermore, detection of the antibody is of relevance for patient counselling, since the antibodies are present in a subgroup of ADS patients with generally a more favorable, non-MS, disease course.[119-121]

Search for novel biomarkers in childhood-onset ADS

Possible new biomarkers for CNS demyelination can be identified in the exciting field of proteomics. The large-scale study of proteins involved in ADS can provide further insight into the pathophysiology of MS. We studied the CSF proteome of 39 children with ADS, of whom 18 children were subsequently diagnosed with MS (**chapter 9**). In CSF collected at the first clinical event of CNS demyelination, we found peptides relating to 21 proteins to be differentially abundant in children with monophasic ADS and MS. Fourteen proteins had higher abundance in children with MS than in children with monophasic ADS. Of these proteins, 12 were linked to neuronal function and gray matter, illustrating neurodegeneration already occurs early in MS. This finding is supported by previous studies which reported an abundance of gray matter proteins in early MS.[124] Furthermore, some of the identified proteins overlap with a previous study, including CSF of 8 children with MS and 11 children with monophasic ADS, in which axoglial proteins were detected in the children with MS.[125] The presence of black holes on MRI at the first event[46] and limited age-expected brain growth and brain atrophy in children with MS[126] endorse neurodegeneration occurs at an early stage. Seven proteins were abundant in children with monophasic ADS, of which 6 proteins have a role in innate immunity. This demonstrates a more innate inflammatory disease mechanism in children with monophasic ADS, which might be explained by the children with an ADEM-like onset in this group, who often have a monophasic event preceded by infections. Our discovery study of the CSF proteome in children with ADS indicates different underlying disease mechanisms in monophasic ADS and MS. Future larger-sample size studies, preferably in international collaboration, are needed in order to investigate whether certain proteins relate to clinical symptoms and if these proteins can predict the outcome of MS diagnosis and relapse rate.

PART TWO: ACQUIRED DEMYELINATING SYNDROMES IN CHILDREN

Main findings

- In children with ADS, MOG-IgG identifies a subset of young children with an ADEM-like phenotype without a future MS diagnosis.
- 57 risk SNPs for adult-onset MS are associated with MS risk in children with ADS. The compound effect of 57 minor risk alleles exceeded the effect of the major MS risk allele *HLA-DRB1*15*.
- MRI is an important diagnostic tool in child-hood onset ADS. We found that the Verhey MRI criteria and IPMSSG 2012 consensus definitions reliably identify children with MS.
- Children with CIS have a more inflammatory disease course than adults with CIS, apparent from a higher rate of future MS diagnoses and more frequent relapses.

Clinical implications

- MOG-IgG should be tested in children presenting with CNS inflammatory syndromes as part of their standard diagnostic work up.
- The IPMSSG 2012 definitions for childhood-onset ADS, including MS, are validated.
- We offer a clinical prediction rule, which has to be validated, for early DMT initiation in 11-16 year-old children with CIS who are at high risk for a future MS diagnosis.

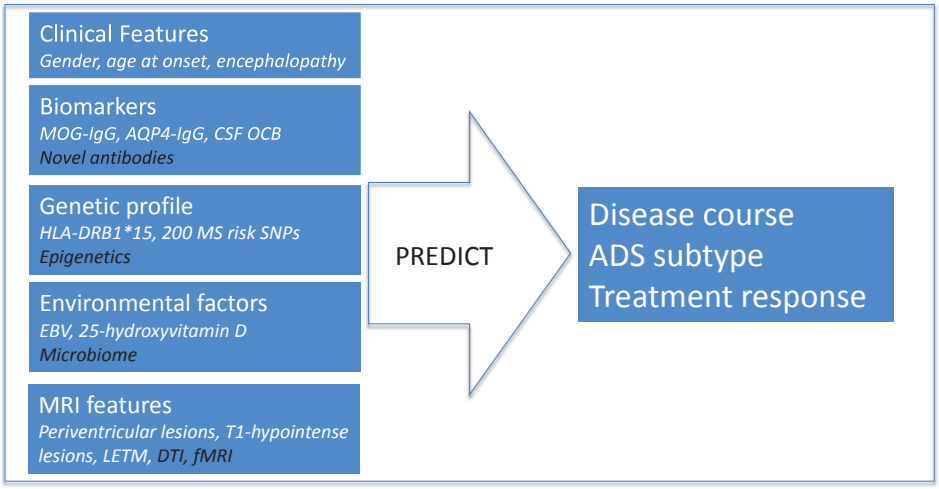


Figure 10.2 This figure presents the main goal of ADS research: the accurate and early prediction for individual patients of their ADS subtype and disease course based on their clinical features, prognostic markers and additional laboratory and MRI studies. In addition, possible novel prognostic and diagnostic markers are presented (black).

FUTURE RESEARCH

In the past decade, a tremendous growth occurred in the knowledge of NMOSD and childhood-onset ADS. However, still questions remain concerning exact disease mechanisms and early subtyping and prognosis of ADS. Currently, the cohorts of NMOSD and childhood-onset ADS are followed at Erasmus MC within the national NMO and pediatric ADS expert centers. As a result of the ongoing studies on NMOSD and childhood-onset ADS more epidemiological data in the Netherlands and long-term follow-up reports are expected. Specific recommendations for further studies regarding the results reported in the **chapter 2 – 9** are described in the previous paragraphs of the general discussion. The main goal of ADS research is summarized in Figure 10.2. In this model the ADS subtype and disease course are predicted for individual patients based on their clinical features, exposure to certain environmental factors, genetic profile, biomarkers and MRI features. The aim of future research is to identify novel prognostic and diagnostic markers which can predict the ADS subtype and determine the prognosis of individual patients more precisely.

Future international collaborative studies are very welcome to gain more insight into the disease mechanisms of NMOSD and childhood-onset ADS. Such studies are of importance to increase the number of included patients to achieve sufficient statistical power. International collaborative studies are of interest to investigate if the outcome of ADS differs in various world regions[127], and might reveal new environmental factors

involved in the pathogenesis of ADS. Ongoing prospective studies in NMOSD and childhood-onset ADS include the search for novel prognostic biomarkers and require large-scale standardized storage of blood and CSF samples (biobanking).[128] Novel biomarkers are needed to further specify the subtypes of ADS and determine the prognosis of individual patients. AQP4-IgG and MOG-IgG are useful markers in patients presenting with a first event of ADS and identify patients who are likely to have diseases distinct from MS.[7, 19] However, AQP4-IgG and MOG-IgG lack sensitivity to accurately subtype all patients with ADS. Future serum and CSF immunohistochemistal studies might reveal novel antibodies associated with ADS.

An exciting new focus in MS research is the gut microbiome. The gut microbiome has an important role in normal immune function.[129] The composition of the gut microbiota might represent a propagating factor for inflammatory signalling in MS.[130] A recent German study with a mouse model for human MS, presented at the 31st Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), reported that mice who received a poop transplant from a monozygotic twin with MS got worse disease than mice who received a poop transplant from the healthy twin.[131] These results suggest that the gut microbiome represent a propagating factor for MS rather than just a consequence of MS. American studies including relatively small sample sizes, already reported some microbiota stems are associated with pediatric MS and a higher relapse rate.[132, 133] Further studies in this novel field, including children with a European diet, are exciting as those studies might identify novel (supportive) therapeutic strategies. Likewise, such studies would be interesting in other autoimmune diseases, including NMOSD.

Furthermore, international collaboration would allow for larger sample sizes and genome-wide association studies in patients with various ethnicities. Such large-scale genetic studies might reveal new specific risk genes for childhood-onset ADS and for NMOSD. In addition, international collaboration would allow discovering the field of epigenetics, which is the study of heritable changes in genome function without underlying modifications in their nucleotide sequence.[134] Epigenetic modifications are the crossroad between environmental and genetic modifications and might reveal future diagnostic and therapeutic targets in ADS patients.

Future prospective studies including a broader spectrum of CNS inflammation with ADS and other autoimmune diseases (for example sarcoidosis, vasculitis or SLE) would be of great interest to study early prognostic and diagnostic markers in patients presenting with an incident event of CNS inflammation. Such an approach would resemble clinical practice, but is hampered by the rarity of these disorders.

Future research will focus on the improvement of treatment strategies. Novel therapeutic trials are underway both in the field of NMOSD and MS.[2, 135-137] As the number of chronic treatment possibilities is growing, a future individual treatment approach is

encouraged.[138] In this concept of personalized medicine the decision for chronic treatment initiation is based on an individual risk benefit analysis. Genetic profiling might contribute tothis personalized medicine approach, since certain single-nucleotide polymorphisms are associated with treatment response to interferon-β.[139] Moreover, *FCGR3A* genetic polymorphisms were associated with incomplete B-cell depletion and more than a 5-fold higher increased risk for relapses in NMOSD patients treated with rituximab.[140]

Future directions

- Ongoing prospective long-term follow-up studies of NMOSD and childhood-onset ADS at Erasmus MC in order to determine epidemiological data and search for new potential risk factors.
- Sequential serum sampling and follow-up of MOG-IgG seropositive patients in order to determine their long-term prognosis.
- International collaborative studies in NMOSD and childhood-onset ADS for novel prognostic factors and diagnostic biomarkers including:
  - serum and CSF immunohistochemistal studies in search of possible novel antibodies.
  - exploring the gut microbiome as a possible propagating factor for CNS inflammation.
  - (epi)genetic studies.
- Validation of the 2016 MS diagnostic MRI criteria in childhood-onset MS, plus studying non-conventional imaging techniques as potential diagnostic marker for ADS subtype and cognitive outcome.
- Initiation of prospective studies including a broader spectrum of CNS inflammation with ADS and other autoimmune diseases to study early prognostic and diagnostic markers in patients presenting with an incident event of CNS inflammation.
- International collaboration in order to optimize and study therapeutic treatment strategies in NMOSD and further evaluation of MS therapies in clinical trials in children.
- The search for parameters which support a personalized medicine approach for individual patients should be further elaborated, as there is an urgent need for such parameters in clinical practice.

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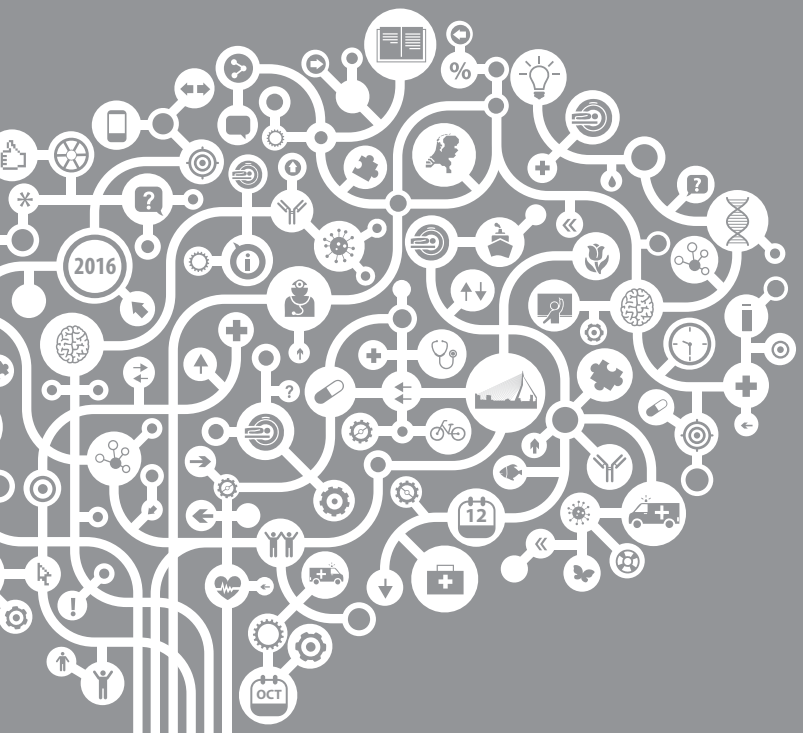


Chapter 11

Summary | Samenvatting

SUMMARY

Acquired demyelinating syndromes (ADS) are a group of immune mediated CNS inflammatory diseases, of which multiple sclerosis (MS) is the most common subtype. ADS cover a broad spectrum of clinical phenotypes with either a monophasic or relapsing disease course. These syndromes overlap and therefore can be difficult to recognize and distinguish. However, awareness and recognition of the various ADS subtypes are of importance for clinicians, since those require a distinct diagnostic and therapeutic approach. This thesis focuses on two relatively rare subtypes of ADS: neuromyelitis optica spectrum disorders (NMOSD) and childhood-onset MS.

Chapter 1 describes the heterogeneous spectrum of ADS, and the current knowledge of NMOSD and childhood-onset ADS respectively. NMOSD is a rare variant of MS, characterized by optic neuritis and transverse myelitis. In the majority of NMOSD patients serum aquaporin-4 antibodies (AQP4-IgG) are present. Next to this, the clinical spectrum of ADS in children, as well as the risk factors for childhood-onset MS and its disease course are described.

The first part of this thesis goes into NMOSD in the Netherlands. One of the goals of our research project was to gain epidemiological figures of NMOSD in the Netherlands. Here, AQP4-IgG is tested in one centralized Dutch NMO expert centre, which provides a unique change to assess a nationwide incidence figure. In **chapter 2** we report approximately 15 AQP4-IgG seropositive NMOSD patients are detected per year in the Netherlands. The mean incidence of AQP4-IgG seropositive NMOSD was calculated at 0.09 per 100,000 people, which is nearly one in a million. However, it is estimated that 77% of the NMOSD patients are AQP4-IgG seropositive, meaning nearly one quarter of the NMOSD patients are not identified by AQP4-IgG testing. Another goal of our study was to identify new possible biomarkers for AQP4-IgG seronegative NMOSD patients.

In **chapter 3** we studied if antibodies directed to myelin oligodendrocyte glycoprotein (MOG-IgG) are a possible diagnostic and prognostic marker for AQP4-IgG seronegative NMOSD patients. We found that 20 patients out of 61 AQP4-IgG seronegative patients were MOG-IgG seropositive. MOG-IgG seropositive NMOSD patients more frequently were males and had a Caucasian ethnicity compared with AQP4-IgG seropositive NMOSD patients. In addition, MOG-IgG seropositive NMOSD patients more frequently had coincident optic neuritis and transverse myelitis, and in general had a more favorable monophasic disease course than AQP4-IgG seropositive NMOSD patients.

The second part of this thesis describes the spectrum of ADS in children. The main goal of our studies on childhood-onset ADS is to find potential markers which can identify the children in whom an incident event of ADS represents the first attack of MS. In **chapters 4 and 5** the role of magnetic resonance imaging (MRI) was studied as a diagnostic and differentiating tool for children with ADS. We validated the 2012 revised diagnostic defi-

nitions for childhood-onset ADS (**chapter 4**) and found that the new diagnostic criteria allow for a safe and early MS diagnosis, which is beneficial for patient counselling and early chronic treatment initiation. In addition, the Verhey MRI criteria (i.e. presence of T1-hypointese and/ or periventricular lesions) for the prediction of MS were validated in our large independent Dutch cohort of children with ADS. These criteria reliably identify children with MS (**chapter 5**). In **chapter 6** the disease course after a first event of non-encephalopathic CNS demyelination (CIS) was compared between children and adults. Children with CIS have a more inflammatory disease course than adults, appearing from the high rate of MS conversion and the shortest time to MS diagnosis, higher annualized relapse rates, higher MRI lesion load and a more inflammatory CSF profile. This could argue for early initiation of first-line disease modifying therapy in children with CIS who are at high risk for a future MS diagnosis in line with current clinical practice in adults. Therefore we offer a clinical prediction rule which might be used for early treatment initiation in future 11-16 year-old children with CIS who are at high risk for MS.

Genetic risk loci identified in adults with MS were studied in children with ADS in **chapter 7**. Using a combined weighted genetic risk score we investigated if these genes are associated with a risk for childhood-onset MS, and if these genes can predict MS diagnosis. We found significant higher weighted genetic risk scores in children with MS than in children with monophasic ADS. The 57 genetic risk loci alter small risk effects, however when combined they exceeded the effect of the major risk gene in MS *HLA-DRB1*15*.

In **chapter 8** we studied the ability of MOG-IgG to differentiate between ADS subtypes and found that MOG-IgG was present in 18% of the children with ADS. MOG-IgG was especially detected in young children with ADEM and in some children presenting with a clinical NMOSD phenotype, who were seronegative for AQP4-IgG. Here, we identified four MOG-IgG seropositive children with a newly recognized clinical entity of ADEM followed by recurrent optic neuritis (ADEM-ON). Children with ADEM-ON have a relapsing disease course, however they do not fulfill diagnostic MS criteria and require a different chronic treatment strategy. MOG-IgG was detected in one child with MS who had his onset at a young age and had a very low MOG-IgG titer. In general, the presence of MOG-IgG is uncommon in MS and pleads against a future MS diagnosis.

We searched for new ADS biomarkers in cerebrospinal fluid (**chapter 9**) and found an increased abundance of CNS gray matter-related proteins in children with MS and an increased abundance of innate immunity-related proteins in children with monophasic ADS. The different identified proteins indicate that distinct underlying disease mechanisms play a role in childhood-onset monophasic ADS and MS. The abundance of CNS gray matter-related proteins in children with MS demonstrate neurodegeneration occurs already early in the disease course.

The main observations from our studies are summarized and discussed in **chapter 10**. Here recommendations for future research are described.

SAMENVATTING

Verworven demyeliniserende syndromen (Acquired demyelinating syndromes, ADS) vormen een groep immuungemedieerde inflammatoire ziekten van het centrale zenuwstelsel (CZS). Multipele sclerose (MS) is het meest bekende subtype en presenteert zich vaak op jong volwassen leeftijd. Daarnaast omvat ADS een breed spectrum aan klinische presentaties met een éénmalig of een recidiverend beloop. Het is van belang om de verschillende subtypes van ADS te kunnen herkennen, omdat dit consequenties heeft voor de behandeling. In dit proefschrift ligt het focus op twee relatief zeldzame subtypes van ADS: neuromyelitis optica (NMO) en MS op de kinderleeftijd.

In **hoofdstuk 1** wordt het heterogene spectrum van ADS beschreven. Tevens wordt de huidige kennis van NMO en MS op de kinderleeftijd samengevat. NMO is een zeldzame variant van MS en wordt gekenmerkt door vooral ontstekingen van de oogzenuw(en) en/of het ruggenmerg. Het merendeel van de NMO patiënten heeft bijzondere antistoffen in het bloed (anti-aquaporine-4, anti-AQP4). Daarnaast wordt in dit hoofdstuk het hele spectrum van ADS op de kinderleeftijd beschreven, evenals de bekende risicofactoren voor MS en het ziektebeloop van MS bij kinderen.

Het eerste deel van dit proefschrift gaat over NMO. Eén van de doelen van het onderzoek was het bepalen van de incidentie van NMO in Nederland. In Nederland wordt de antistoftest (anti-AQP4) bij NMO uitgevoerd in één landelijk gecentraliseerd laboratorium (Sanquin diagnostiek Amsterdam in samenwerking met het nationale NMO centrum in het Erasmus MC). Dit biedt de unieke mogelijkheid om een landelijke incidentie van NMO te bepalen. In **hoofdstuk 2** rapporteren we dat er ongeveer 15 anti-AQP4 positieve NMO patiënten per jaar worden gediagnostiseerd in heel Nederland. Daarmee hebben we de gemiddelde incidentie van anti-AQP4 positieve NMO berekend op 0.09 per 100,000 mensen, wat neerkomt op een kans van bijna 1 op de miljoen. Echter, naar schatting heeft ongeveer 77% van de NMO patiënten AQP4-antistoffen. Dit betekent dat nagenoeg een kwart van de NMO patiënten niet wordt geïdentificeerd door de anti-AQP4 test. Een ander doel van onze studie was om nieuwe biomarkers te onderzoeken voor anti-AQP4 negatieve NMO patiënten.

In **hoofdstuk 3** hebben we onderzocht of bijzondere antistoffen gericht tegen myeline oligodendrocyt glycoproteine (anti-MOG) kunnen fungeren als een diagnostische en prognostische marker in anti-AQP4 negatieve NMO patiënten. We hebben gevonden dat in 20 van de 61 anti-AQP4 negatieve NMO patiënten MOG-antistoffen aantoonbaar waren. Anti-MOG positieve NMO patiënten waren vaker man en hadden vaker een Kaukasische etniciteit in vergelijking met anti-AQP4 positieve NMO patiënten. Daarnaast hadden de anti-MOG positieve NMO patiënten vaker tegelijkertijd een oogzenuwontsteking en ruggenmergontsteking. In het algemeen hebben de anti-MOG positieve NMO patiënten vaker een gunstiger en éénmalig ziektebeloop.

Het tweede deel van dit proefschrift beschrijft het spectrum van ADS bij kinderen. Het hoofddoel van het onderzoek naar ADS op de kinderleeftijd is het vinden van potentiële markers die vroeg in het ziektebeloop de kinderen met MS kunnen identificeren, het liefst al ten tijde van de eerste aanval. In **hoofdstukken 4 en 5** wordt de rol van MRI bestudeerd om kinderen met verschillende vormen van ADS van elkaar te kunnen onderscheiden. In **hoofdstuk 4** onderzochten we de in 2012 gereviseerde internationale diagnostische criteria voor ADS bij kinderen. Het blijkt dat deze nieuwe criteria een betrouwbare en snellere MS diagnose geven. Dit biedt voordelen voor adequate voorlichting van patiënten en hun families en biedt de mogelijkheid om vroeg te starten met onderhoudsbehandeling voor MS. Daarnaast, zijn de Verhey MRI criteria gevalideerd in het Nederlandse ADS cohort. Deze Verhey criteria identificeren nauwkeurig de kinderen met MS (**hoofdstuk 5**). In **hoofdstuk 6** hebben we het ziektebeloop van kinderen met een eerste aanval van CZS demyelinisatie zonder encephalopathie (Clinically isolated syndrome, CIS) vergeleken met het beloop van volwassenen met CIS. Kinderen met CIS hebben een meer inflammatoir ziektebeloop dan volwassenen, wat blijkt uit het hoge aantal MS diagnoses, de korte tijd tot MS diagnose, hoge aanvalsfrequentie, de vele afwijkingen op de MRI en het ontstekingsbeeld in het hersenvocht bij kinderen. Dit zou een argument kunnen zijn om bij kinderen met CIS en een hoog risico op MS al vroeg te starten met ontstekingsremmende onderhoudsbehandeling, zoals momenteel al bij volwassen gebeurd. Daarom presenteren we een predictieregel die in de toekomst mogelijk gebruikt kan worden om de kinderen met CIS en een hoog risico op MS te identificeren.

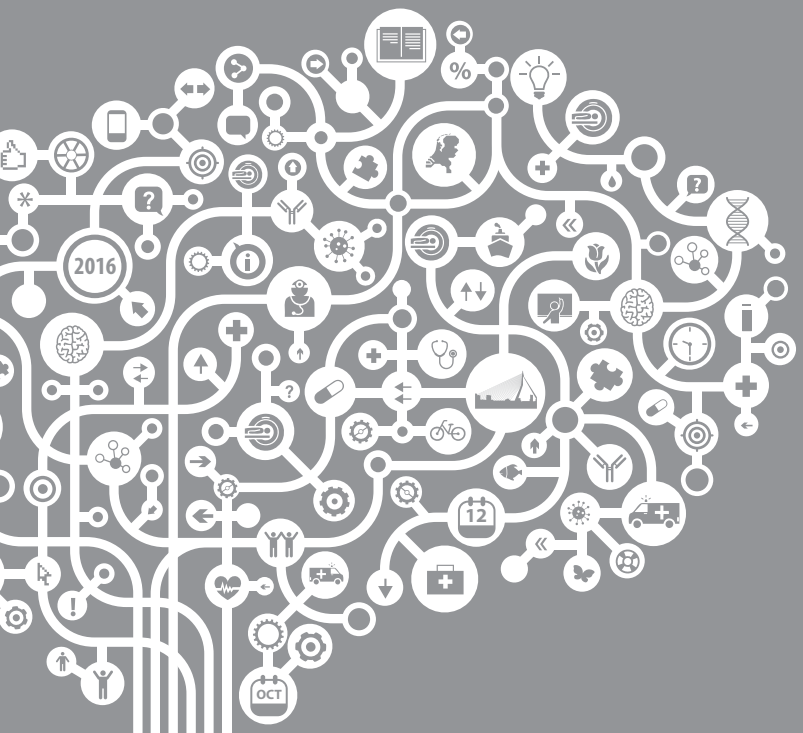
Risicogenen voor MS, bekend uit het onderzoek bij volwassenen, zijn bestudeerd in **hoofdstuk 7**. Met behulp van een zogenaamde gecombineerde genetische risicoscore, die rekening houdt met het effect van de afzonderlijke genen, hebben we onderzocht of deze genen zijn geassocieerd met een risico op MS op de kinderleeftijd. We vonden significant hogere genetische risicoscores in kinderen met MS in vergelijking met de kinderen met éénmalige ADS. De 57 genetische MS risicovarianten hebben afzonderlijk een relatief klein effect, maar het gecombineerde effect is groter dan het effect van het bekendste en grootste MS risicogen *HLA-DRB1*15*.

In **hoofdstuk 8** hebben we onderzocht of anti-MOG kan helpen de verschillende ADS types bij kinderen te onderscheiden en vonden dat anti-MOG aanwezig is bij 18% van de kinderen met ADS. Anti-MOG wordt met name gevonden bij jonge kinderen met ADEM en ook bij een paar kinderen met anti-AQP4 negatieve NMO. In deze studie, hebben we vier kinderen gevonden met een nieuwe klinische entiteit van ADEM gevolgd recidiverende oogzenuwontstekingen (ADEM-ON). Kinderen met ADEM-ON hebben een recidiverend ziektebeeld, maar voldoen niet aan de diagnostische criteria voor MS en hebben derhalve ook een andere behandeling nodig. Bij één kind met MS werd een laag positieve waarde van anti-MOG gevonden. Dit kind had het debuut van zijn ziekte

op zeer jonge leeftijd. In het algemeen, komt anti-MOG niet voor bij MS en pleit de aanwezigheid van anti-MOG tegen een toekomstige diagnose MS.

We hebben gezocht naar nieuwe biomarkers in het hersenvocht van kinderen met MS (**hoofdstuk 9**) en vonden een overschot van eiwitten gerelateerd aan de grijze stof van het CZS in het hersenvocht van kinderen met MS. In het hersenvocht van kinderen met een éénmalige vorm van ADS hebben we een overschot gevonden van eiwitten die gerelateerd zijn aan het aangeboren afweersysteem. Deze verschillende geïdentificeerde eiwitten suggereren dat verschillende ziektemechanismen een rol spelen bij éénmalige ADS en MS op de kinderleeftijd. Het overschot van eiwitten gerelateerd aan de CZS grijze stof in het hersenvocht laat zien dat neurodegeneratie al vroeg in het ziektebeloop optreedt.

De belangrijkste bevindingen uit onze studies worden samengevat en bediscussieerd in **hoofdstuk 10**. Hier worden ook aanbevelingen gedaan voor toekomstig onderzoek.



Chapter 12

Epilogue

Dankwoord

Authors and Affiliations

About the author

Publications

PhD portfolio

Abbreviations

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Daniëlle van Pelt was born on December 2nd 1985 in Gouda and raised in Stolwijk. She graduated in 2004 from the Sint Antonius College in Gouda, and proceeded to study medicine at the Erasmus University in Rotterdam. Between her general and final internships she worked on a research project at the pediatric neurology department of Erasmus MC Sophia (supervisor: Dr. C.E. Catsman-Berrevoets) where she studied the incidence of traumatic brain injury in children and adolescents in the catchment area of Erasmus MC in Rotterdam. After obtaining her medical degree in August 2010 she worked as a medical doctor at the department of neurology at the Groene Hart Hospital in Gouda. One year later she started her PhD research at the MS center of Erasmus MC under supervision of Prof. R.Q. Hintzen, and succeeded Dr. I.A. Ketelslegers as coordinator of the Dutch multicentre PROUDkids study (PRedicting the OUtcome of a Demyelinating event in children). The results of this research are described in this thesis. From April 2014 onwards she works as a resident in neurology at Erasmus MC in Rotterdam (Head: Prof. P.A.E. Sillevis Smitt). She is married to Thomas Gravesteijn, and they live in Gouda.

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JR Scheepe*, YYM Wong*, **ED van Pelt**, IA Ketelslegers, CE Catsman-Berrevoets, J van den Hoek, RQ Hintzen, RF Neuteboom. Neurogenic lower urinary tract dysfunction in the early disease phase of paediatric multiple sclerosis. *Multiple Sclerosis Journal* Published online 20 November 2015. doi:10.1177/1352458515618541

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OTHER PUBLICATIONS

ED van Pelt, A de Kloet, SR Hilberink, SAM Lambregts, E Peeters, ME Roebroeck, CE Catsman-Berrevoets. The incidence of traumatic brain injury in young people in the catchment area of the University Hospital Rotterdam, The Netherlands. *European Journal of Paediatric Neurology* 2011;15(6)519-526.

ED van Pelt, JC den Hollander, HJ de Vries, Y van Beek, DC Melles, WI van der Meijden, PJJ van Genderen. A tropical disease characterised by rapidly progressive skin lesions and haemolytic anaemia. *The Netherlands Journal of Medicine* 2011;69(9):400.

PHD PORTFOLIO

1. PhD training	Year	Workload (ECTS)
Courses		
Basic human genetics	2011	0.5
Basiscursus Regelgeving Klinisch Onderzoek (BROK)	2012	1.4
Introduction in GraphPad Prism, MolMed	2015	0.3
Biomedical English writing	2015	3.0
Seminars and workshops		
Basic immunology, Department of Immunology, Rotterdam	2012	0.5
Training in transition care, Hogeschool Rotterdam	2013	0.5
MS symposium NVN, Amsterdam	2016	0.5
Oral presentations		
Surveillance of acquired demyelinating syndromes of the CNS in children: NSCK research meeting, TNO Leiden	2012, 2013	1.0
Neuromyelitis optica, ErasMS Rotterdam	2012	0.5
Workshop rare variants of MS, Multidisciplinary MS Symposium, Ede	2012	1.0
Risk genes in pediatric onset ADS, Rotterdam	2013	0.5
Pediatric onset MS - Treatment, patient care and research in the Netherlands, Odense	2013	1.0
Application of the 2012 revised diagnostic definitions for pediatric MS and immune-mediated CNS demyelination disorders, Hasselt	2013	0.5
Workshop on childhood-onset ADS, Multidisciplinary MS Symposium, Ede	2013	1.0
MOG-IgG spectrum disorders: a new neuro-immunologic entity, NVN wetenschappelijke vergadering	2014	1.0
MOG-spectrum diseases, Rotterdam	2014	1.0
Multidisciplinary care for children with MS, samen beter congres, Zeist	2015	1.0
Poster presentations		
ECTRIMS (4 posters)	2013-2016	4.0
Annual MolMed Day, Rotterdam	2013	1.0
MS Research days	2012	1.0
International conferences		
Congress of the European Committees for Treatment and Research in Multiple Sclerosis (ECTRIMS); Amsterdam (2011), Lyon (2012), Copenhagen (2013), Boston (2014), Barcelona (2015), London (2016)	2011-2016	6.0
Meeting of the Dutch MS Research foundation; Oegstgeest (2011), Doorweth (2012), Hasselt (2013), Oegstgeest 2015	2011-2013, 2015	4.0
2. Teaching		
Lecturing		
'Acquired demyelinating syndromes in childhood', research master programme infection & immunity (MolMed), Rotterdam	2012-2014	3.0
Lecture 'treatment childhood-onset MS', minor students medicine, Rotterdam	2013	1.0

Supervising/guiding master thesis		
Aditi Broos February – July 2013 master student infection & immunity, Rotterdam	2013	2.5
Yu Yi M Wong June – August 2013 medical student Radboud MC, Nijmegen	2013	2.5
3. Other		
Guiding pre-university students in their final school year with their research paper on NMO (Laura Scholten, Eline Feitsma, Tessa Brouwer, Zaheda Sadoghy) and MS (Charlotte Hoffman).	2011, 2012	1.0
Video colleges pediatric MS, MS en wetenschap	2013	1.0
Co-investigator in clinical MS trials (Fingolimod, Natalizumab, Ocrelizumab, ParadigMS)	2011-2016	2.0

ABBREVIATIONS

ADEM	Acute disseminated encephalomyelitis
ADEM-ON	Acute disseminated encephalomyelitis followed by recurrent optic neuritis
ADS	Acquired demyelinating syndromes
AID	Autoimmune disease
AUC	Area under the curve
AQP4	Aquaporin-4
ARR	Annualized relapse rate
CBA	Cell-based assay
CD	Cluster of differentiation
CDMS	Clinically definite multiple sclerosis
CI	Confidence interval
CIS	Clinically isolated syndrome
CK	Creatine kinase
CNS	Central nervous system
CRION	Chronic relapsing inflammatory optic neuropathy
CSF	Cerebrospinal fluid
DIS	Dissemination in space
DIT	Dissemination in time
DMT	Disease modifying therapy
ΔMFI	Delta mean fluorescence intensity
DNA	Deoxyribonucleic acid
EAE	Experimental autoimmune encephalomyelitis
EDSS	Expanded disability severity scale
EBV	Epstein-Barr virus
FACS	Fluorescence activated cell sorting
FLAIR	Fluid-attenuated inversion recovery
GEE	Generalized estimating equations
GRIPS	Guideline for the reporting of genetic risk prediction studies
GRS	Genetic risk score
GWAS	Genomic-wide association study
HEK293	Human embryonic kidney cell line
HLA	Human leukocyte antigen
HPV	Human papilloma virus
ICU	Intensive care unit
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
IPMSSG	International Pediatric Multiple Sclerosis Study Group

IQR	Inter quartile range
LC-MS	Nano-liquid chromatography mass spectrometry
LD	Linkage disequilibrium
LETM	Longitudinally extensive transverse myelitis
LN18	Human malignant glioma cell line
LR	Likelihood ratio
MC	Medical center
MOG	Myelin oligodendrocyte glycoprotein
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
N	Number of patients
NA	Not applicable
NS	Not significant
NMO	Neuromyelitis optica
NMOSD	Neuromyelitis optica spectrum disorders
NPV	Negative predictive value
NSCK	Nederlands Signalerings Centrum Kindergeneeskunde (Netherlands Pediatric Surveillance Unit).
OCB	Oligoclonal bands
ON	Optic neuritis
OND	Other neurological diseases
OR	Odds ratio
PML	Progressive multifocal leukoencephalopathy
Poly ADS +	Polyfocal ADS with encephalopathy
Poly ADS -	Polyfocal ADS without encephalopathy
PP-MS	Primary progressive multiple sclerosis
PPV	Positive predictive value
PROUDkids	PRedicting the OUtcome of a Demyelinating event in children study
QC	Quality control
ROC	Receiver operating characteristic
RR	Relative risk
RR-MS	Relapsing-remitting multiple sclerosis
SD	Standard deviation
SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism
SP-MS	Secondary progressive multiple sclerosis
TM	Transverse myelitis
WBC	White blood cell count
wGRS	Weighted genetic risk score

Acquired Demyelinating Syndromes

Focus on Neuromyelitis Optica and childhood-onset Multiple Sclerosis

Acquired demyelinating syndromes (ADS) cover a broad spectrum of central nervous system (CNS) inflammatory demyelinating syndromes, of which multiple sclerosis (MS) is the most common subtype. This thesis focuses on two relatively rare clinical subtypes of ADS: neuromyelitis optica spectrum disorders (NMOSD) and childhood-onset MS. Awareness and recognition of uncommon ADS subtypes are of importance for clinicians, since those require a distinct diagnostic and therapeutic approach. Here we aimed to reveal the spectrum of ADS by describing the clinical features of NMOSD and childhood-onset ADS, in order to improve the diagnostic process. In addition, we searched for prognostic and diagnostic biomarkers in ADS.