MOVING FORWARD ON ACQUIRED DEMYELINATING SYNDROMES

DIAGNOSIS, DISEASE COURSE AND OUTCOME

Yu Yi M. Wong

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DIAGNOSIS, DISEASE COURSE AND OUTCOME

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Diagnose, ziektebeloop en uitkomst

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1.
General introduction

ACQUIRED DEMYELINATING SYNDROMES OF THE CENTRAL NERVOUS SYSTEM

Acquired demyelinating syndromes (ADS) of the central nervous system (CNS) include a broad spectrum of phenotypes and are mainly classified by the clinical localization of symptoms and signs. Neurological deficits are caused by inflammation and the subsequent damage to the myelin sheet [1,2] A first attack of ADS may occur as a transient illness or may represent the first attack of a chronic demyelinating disorder, such as multiple sclerosis (MS).[3] In earlier years, ADS patients were diagnosed with MS when they had a relapsing disease course during follow-up. However, due to the ongoing elucidation of the ADS spectrum, it became clear that not all patients with relapsing disease have MS. This clarification has important therapeutic implications.

The ADS spectrum includes patients with a monophasic disease course, such as clinically isolated syndromes (CIS) with monofocal (single) or polyfocal (multiple) localizations, monophasic neuromyelitis optica spectrum disorders (NMOSD) and acute disseminated encephalomyelitis (ADEM). [1,2,4] Yet, the ADS spectrum also includes relapsing (and chronic) variants of ADS, such as MS, multiphasic ADEM (MDEM), ADEM followed by recurrent optic neuritis (ADEM-ON) and relapsing NMOSD. [3–6]

The term 'ADS' has been introduced to cover the overlapping presenting phenotypes of these demyelinating syndromes. For example, optic neuritis (ON) can be a monophasic event (idiopathic ON) but can also be the first presentation of MS, NMOSD, recurrent ON, or even in context of other autoimmune diseases with CNS involvement.[7] One can envision that at incident presentation, distinction between monophasic and relapsing syndromes can be challenging despite a detailed patient history, neurological examination and additional relevant diagnostic tools. However, accurate and early distinction between ADS subtypes is of utmost importance for counselling and prognosis, and also for initiation of appropriate treatment in chronic demyelinating disorders. Moreover, accurate identification of monophasic patients may prevent overtreatment and therewith the potential side effects of immunomodulatory drugs.

ACQUIRED DEMYELINATING SYNDROMES IN CHILDHOOD

What are childhood-onset acquired demyelinating syndromes?

Acquired demyelinating syndromes in childhood are a rare group of disorders which can overlap in presenting phenotypes, as explained in the first paragraph of this chapter. At first presentation, the diagnostic process of these rare disorders can be challenging, as physicians are often dependent on the medical history by the caregivers because of the young age of the patient. A medical delay is not uncommon in this group. The National Pediatric MS center has

its outpatient clinic in the pediatric hospital of Erasmus MC-Sophia, where we try to assess patients as soon as possible after the first attack. This is made possible by the participation of pediatric neurologists and pediatricians in our national prospective study for children with ADS. The main goal of this study is to predict the outcome of a first demyelinating syndrome (PROUD-kids study).

The diagnostic work-up consists of a carefully taken history of the patient and caregivers, neurological examination, magnetic resonance imaging (MRI) scans of the brain (and spinal cord), cerebrospinal fluid analysis (CSF) and blood analysis. Due to the overlap in clinical and radiological phenotypes, additional biomarkers are needed to shorten the time to accurate diagnosis and therefore also shorten the time that parents and patients are being kept in uncertainty. Considering several subtypes of ADS have life-long therapeutic implications, adequate counselling and initiating treatment in the right group of patients is highly important. The first presentation of ADS in children encompasses a wider spectrum than in adults and includes a more extensive list of differential diagnoses, which makes the diagnostic process even more difficult.[8] The international pediatric MS study group proposed diagnostic criteria for ADS in children in 2007, and revised these in 2012, to aid the diagnostic process.[9]

The first attack of demyelination can be a monofocal or polyfocal presentation with and without encephalopathy. Every subtype can potentially be a first attack of an underlying chronic demyelinating syndrome such as MS. Up to one-third of ADS patients will eventually be diagnosed with MS during follow-up.[1,2,10] These estimates differ between studies because of different study designs. The same variation is found in incidence estimates of ADS, varying between 0.66 to 1.66 per 100.000 persons due to study designs.[1,2,10–12] Our previous work reported on the distribution of the clinical ADS phenotypes of Dutch patients, including polyfocal ADS without encephalopathy (ADEM, 30%), polyfocal ADS without encephalopathy (24%), ON (22%) and other monofocal ADS (19%).[10]

Clinically isolated syndromes (CIS) are a first episode of demyelination and can be monofocal or polyfocal without encephalopathy.[9] Examples of monofocal CIS are ON, transverse myelitis (TM), brainstem or cerebellar syndromes and CIS with hemispherical symptoms.

Acute disseminated encephalomyelitis (ADEM) is a relatively common subtype of ADS, where patients have a polyfocal presentation and encephalopathy.[13] Encephalopathy is defined as behavioral changes and altered consciousness which cannot be explained by fever, and is a feature not typically seen in MS.[1,9] ADEM is usually considered as a monophasic and benign illness that occurs mainly in young children with preceding infections.[5,13,14] However, the presentation of ADEM can be severe, leading to ICU admissions and sometimes death.[13] The MRI can show bilateral large hazy and poorly demarcated T2 hyperintense lesions in both the

white and the grey matter (cortical grey matter and deep gray matter like the basal ganglia and thalami).[15] The proportion of patients with relapsing disease after a first attack varies from 6-25%.[10 13,14,16,17] The relapsing disease can still be transient, like in multiphasic ADEM (MDEM), but can also be a chronic disorder such as MS, NMOSD or ADEM-ON.[6] Early distinction between patients who remain monophasic and who will relapse is important for counselling and follow-up.

Pediatric onset NMOSD accounts for 3-5% of all NMOSD cases with a mean age of 10 years old.[10,18] The proposed international criteria for adult NMOSD apply well in children with NMOSD and aid in quickly initiating immunosuppressive drugs with as little delay as possible.[4,19] The presentation can be diverse, including an ADEM-like presentation, and more often includes intracerebral lesions than in adult patients.[18–21] Therefore NMOSD antibody testing in patients with an ADEM-like presentation with (simultaneous) ON and spinal cord involvement should be considered. NMOSD will be discussed in more detail later in this chapter.

MS in childhood

Up to 10% of all MS patients have the first symptoms before age 18.[22] Pediatric onset MS has a relapsing remitting disease course in about 97% of the cases; an initial progressive disease course is extremely rare and should raise a red flag prompting for extensive assessment for other diagnosis.[23,24]

How is childhood-onset MS diagnosed?

Prognostic factors for MS diagnosis in childhood are age at onset >11 years, female gender, clinical presentation without encephalopathy, unique oligoclonal bands in cerebrospinal fluid (CSF), and MRI abnormalities.[15,25,26] In children, the presence of T1 hypointense lesions together with periventricular lesions on baseline MRI are highly predictive for future MS diagnosis in children.[15,27] Despite these predictive factors, physicians are at times unable to provide enough clarity on the diagnosis at time of first attack. Prediction of the subsequent course is even more difficult. Therefore the search for additional predictive biomarkers is necessary.

As in adults, children with a first event of CNS demyelination may be diagnosed with MS, if clinical or radiological evidence is present of dissemination in time (DIT) and space (DIS).[9,28] MS diagnosis can only be established 'per exclusionem', so alternative diagnoses need to be excluded first. The differential diagnosis of pediatric MS includes CNS infection, neoplasm, leukodystrophy, and systemic inflammatory diseases.[8] The McDonald 2010 criteria gave adult physicians the ability to establish MS diagnosis at time of a first attack of demyelination.[29] MRI abnormalities in MS patients typically show periventricular, juxtacortical, infratentorial, and spinal cord T2 weighted lesions.[29] Examples of the MS predilection sites are shown in *Figure 1.1.*

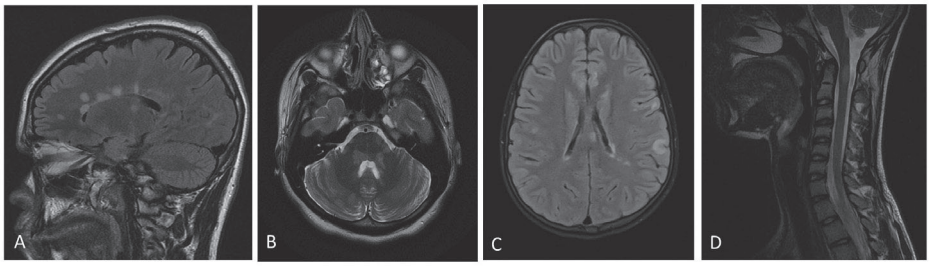

Figure 1.1: MRI predilection sites for multiple sclerosis
Figure 1.1A: periventricular lesions, typically perpendicular on the corpus callosum. Figure 1.1B: extensive infratentorial lesions (midbrain/pons and right cerebellum). Figure 1.1C: a typical juxtacortical 'U-fiber' lesion in the left parietal lobe. Figure 1.1D: cervical spinal cord lesion with a typical oval configuration.

Applicability of the adult 2010 criteria in children was supported by several studies.[30–34] The International Pediatric MS study group (IPMSSG) implemented the McDonald 2010 criteria in the international consensus diagnostic criteria for pediatric MS, with provisions for children <12 years and patients with ADEM.[9]

Recently, the international panel on diagnosis of MS proposed the McDonald 2017 criteria, after revision of the prior 2010 McDonald criteria. [29,35] These revised criteria include modifications to make the criteria easier to apply and to facilitate earlier MS diagnosis, while attempting to preserve the diagnostic accuracy of the criteria. Important modifications included re-introducing CSF oligoclonal bands (OCB) into the criteria and allowing symptomatic lesions to contribute to DIS and DIT.[3,35] Furthermore, cortical lesions have been added to demonstrate DIS. The performance of the McDonald 2017 criteria need to be validated in clinical practice. The McDonald 2010 and revised McDonald 2017 criteria are displayed in *Table 1.1*.

Table 1.1: McDonald 2010 and McDonald 2017 criteria

	McDonald 2010 criteria	Revised McDonald 2017 criteria
DIS	**Clinical** a) Objective clinical evidence of ≥2 lesions, or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack involving a different CNS site	**Clinical** a) Objective clinical evidence of ≥2 lesions, or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack involving a different CNS site
	MRI b) ≥ 1 T2 lesion in at least 2 out of 4 typical regions: periventricular lesion, juxtacortical lesion, infratentorial lesion, spinal cord lesion.	**MRI** b) ≥ 1 T2 lesion in at least 2 out of 4 typical regions: periventricular lesion, juxtacortical and/or cortical lesion, infratentorial lesion, spinal cord lesion.
	(symptomatic brain stem syndromes or spinal cord lesions are excluded)	**(brain stem syndromes or spinal cord lesions are included irrespective of clinical symptoms)**
DIT	**Clinical** a) ≥2 attacks separated by a period of at least month	**Clinical** a) ≥2 attacks separated by a period of at least month
	MRI b) Simultaneous presence of gadolinium-enhancing and non-enhancing lesions at any time c) A new T2 and/or gadolinium-enhancing lesion on follow-up MRI, irrespective of its timing with reference to a baseline scan	**MRI** b) Simultaneous presence of gadolinium-enhancing and non-enhancing lesions at any time c) A new T2 and/or gadolinium-enhancing lesion on follow-up MRI, irrespective of its timing with reference to a baseline scan
		OR **d) Demonstration of CSF-specific OCBs (as substitute for demonstration of DIT)**
	(symptomatic brain stem syndromes or spinal cord lesions are excluded)	**(brain stem syndromes or spinal cord lesions are included irrespective of clinical symptoms)**

Overview of the McDonald 2010 and revised 2017 diagnostic criteria for DIS and DIT. Based on the 2017 revisions to the McDonald 2010 criteria.[35] DIS = dissemination in space, DIT = dissemination in time, OCB = oligoclonal bands. Changes compared to the 2010 criteria are made bold.

What are the disease course and outcome of pediatric MS?

Although pediatric onset relapsing remitting MS (RRMS) resembles adult-onset RRMS in clinical symptoms, a few differences in features of the disease in children should be noted:

Children have a more inflammatory disease course than adults as observed in the higher annualized relapse rate (2–3 times more frequent and more severe relapses than adults early in the disease course) and a higher MRI lesion burden at baseline.[36,37] Notably, a high proportion of children with a future MS diagnosis has T1 hypointense lesions on baseline MRI.[15] In addition, pediatric onset MS patients show a reduced head and brain size for age, have disproportionally smaller thalami, and fail to reach their age-expected brain growth over serial MRI scans.[38,39] These data imply the presence of a prominent neurodegenerative aspect in pediatric onset MS, but additional exploration is needed.[38] One biomarker of interest for axonal damage is neurofilament light chain (NfL).[40] NfL can be tested in CSF and serum, but are not explored

earlier in pediatric ADS. In contrast to the implications of axonal damage, compensatory mechanisms seem to play a role considering the overall well recovery of relapses, and the delayed progression to secondary progressive MS.[41–44]

Considering pediatric MS manifests in the key formative years of education and brain maturation, cognitive function cannot be neglected. While knowing that children have a more inflammatory disease than adults and have signs of neurodegeneration early in the disease course, this population is at risk of cognitive and physical sequelae. Significant cognitive deficits are detected in approximately 30% of the children early in the disease course, but the outcome is heterogeneous.[45–47] Also fatigue is a disabling complaint that is frequently reported by pediatric and adult patients with MS, and is associated with a reduced quality of life.[48–50] The cause of fatigue in MS remains unclear. In a Canadian study, pediatric MS patients were less physically active and scored higher on fatigue scales than patients with monophasic ADS.[51] One hypothesis is that due to fatigue, patients become less physically active and this may lead to decreased exercise capacity. On top of this, accrual of motor disabilities can also affect motor performance. The Expanded Disability Status Scale (EDSS), which was designed for adult patients, is widely used to evaluate disease severity and monitor disease progression.[52] Despite the longer disease duration in pediatric patients to the secondary progressive disease phase of MS, these patients will reach disability milestones at younger ages than their adult-onset counterparts.[42–44] Whether EDSS is an optimal metric to determine disabilities in the daily life of these children and whether there is a correlation between fatigue, exercise capacity, motor performance and quality of life are yet to be investigated.

What are risk factors for pediatric MS?

MS has been identified as a complex and multifactorial auto-inflammatory disease, with involvement of genetic susceptibility and environmental exposure.[53] However the exact cause is unknown.

The greatest contribution to genetic risk in both children and adults is conferred by HLA-DRB1*15 allele.[54] In addition, in large-scale genome wide association studies, up to 200 non-HLA MS risk loci, which are mainly located in genes with immunological functions, are identified that increase MS susceptibility.[55,56] In children, 57 of these risk loci have been validated to contribute to MS risk in pediatric patients.[57]

Migration studies have shown that children who were born in high MS prevalence countries and who migrated to countries with low MS prevalence before age 15, adapt to the low risk of the country where they live.[58–60] For example, if a 13-year old child born in The Netherlands (with high prevalence of MS) moves to Africa (with low prevalence of MS), the child will have a low risk of MS adapting to the African population. On the contrary, if the child migrates at age 17,

the child will keep the risk of the country of origin (in this case the high Dutch risk of MS). The place of residence during childhood, rather than ancestry, influences MS risk.[61] This supports the hypothesis that exposure to environmental risk factors for MS occur for the most part before age 15.[60] As such, studying risk factors in pediatric MS during the putative window of disease susceptibility is highly relevant, considering the shorter time between time of exposure and time of disease onset compared to adults. This hypothesis is displayed in *Figure 1.2*.

Whether these risk factors purely play a role in immune mechanisms, or whether they also influence the target tissue (brain and spinal cord) to become more vulnerable to damage and have reduced repair possibilities, remains unknown.

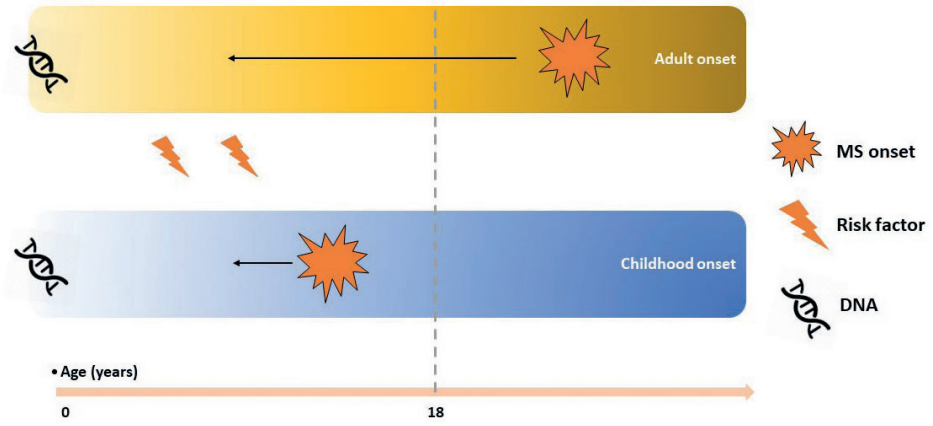

Figure 1.2: Putative window of disease susceptibility and the age of onset.
As indicated before, MS patients have a genetic susceptibility but environmental risk factors also convey a risk, leading to disease onset. The time from exposure to risk factors and disease onset is shorter in childhood onset MS than in adult onset MS. Therefore the chances to identify these pivotal environmental factor are better in patients with childhood onset than in patients with adult onset.

Several environmental risk factors are identified. Epidemiological studies have shown a relationship between latitude and MS prevalence, and low vitamin D has been shown as a risk factor for MS in children [25,59,62,63] and increased risk of relapses.[25,64] An increased BMI is also associated with a higher MS risk.[65–67] Recently a causal and independent association between low serum vitamin D concentration, increased BMI and increased risk of pediatric MS is reported, after adjusting for sex, ancestry and HLA-DRB1*15.[68] Other risk factors for MS include a remote infection of Epstein-Barr virus (EBV)[25,69–72] and the exposure to parental smoking and the duration of this exposure.[73,74] Whereas a remote infection of EBV increases MS risk, exposure to cytomegalovirus decreases the risk of MS.[75] Earlier menarche in girls increases

MS risk and leads to an earlier age of onset, particularly in association with obesity.[67,76] Also, the gender distribution is equal in pre-pubertal pediatric MS patients, whereas females are more often affected after puberty.[42,69] This suggests that hormonal influences are correlated with disease onset.

Several new potential risk factors are mentioned, for example high dietary salt intake. This may contribute in risk of MS diagnosis and a higher relapse rate.[77–79] The effect on MS risk of delivery method, being breast fed and environmental quality (for example air pollution) during childhood needs further exploration.[80,81] The latter also includes the relatively new concept of third-hand smoking (THS). Third-hand tobacco smoke contains residual tobacco smoke and particles that is left on indoor surfaces and dust, which might interact with environmental compounds to form new pollutants.[82,83] Consequences on human health and the influence of THS on MS risk needs to be determined in the future. Another new field of pediatric ADS is the association of MS risk with the difference in gut colonization by non-pathogenic microflora: the gut microbiota.[84] These findings need to be replicated.

Growing evidence supporting the genetic and environmental interplay has implications on the pathophysiological insights and potential prevention strategies in children. The exact mechanisms need to be elucidated.

How are pediatric ADS treated and managed?

Acute treatment

First choice of acute treatment in ADS is consensus based and contains high-dose intravenous corticosteroids (methylprednisolone for 3-5 consecutive days; IvMP).[69,85–87] In case children do not respond to the first cycle of IvMP, a second cycle can be considered. Intravenous immunoglobulins (IvIG) or plasmapheresis could be considered in the acute phase when children do not tolerate or do not respond to corticosteroids. Severely affected children with a NMOSD-like phenotype who do not respond to IvMP are more likely to benefit from plasmapheresis than IvIG.[88]

Chronic treatment of ADS subtypes

The international pediatric MS study group made a consensus statement in 2012 about the initiation of immunomodulatory therapy in every child that is diagnosed with pediatric MS.[89] The evidence for treatment effects and potential side effects for first line injectable treatment with interferon beta and glatiramer acetate are well established in adults, but less evaluated in children.[89] The number of the studies conducted in pediatric MS are retrospective or open-label and there is a lack of randomized controlled trials. This is also the case for Natalizumab treatment (Tysabri), the only registered second-line option for pediatric MS. Despite this, a

similar (if not better) efficacy is observed as in adults.[89–92] Limited information is available about the long term (adverse) effects of these drugs on the immune system, development and growth of the CNS and endocrine systems of children.[93]

The lack of oral agents affects the adherence to currently available injectable therapy and also the limited second-line options for children are challenges in daily practice.[94,95] In adults, oral first-line therapies (dimethylfumarate and teriflunomide) are now widely used.[96,97] Recently, the first double-blinded randomized controlled trial in children (PARADIGMS study) was successfully completed. Fingolimod, an oral second line agent, was compared to interferon beta injections and showed superior efficacy on reduction of relapses and of inflammatory and neurodegenerative signs on MRI over a period of two years.[98] The efficacy seemed even higher than in adult patients with a similar safety profile.[98] This second-line drug will soon be available for pediatric MS patients. New clinical drug trials for children with MS have been launched, including oral teriflunomide (Terikids study) and alemtuzumab (Lemkids study).[99] Chronic treatment of other ADS subtypes, including NMSOD and MOG-IgG associated disorders, are discussed later in this chapter.

What is the Outcome of pediatric ADS

Regarding recovery in ADS in general, it is reported that about 90% of the patients recovered physically from their initial event.[100] However, monophasic illness, especially longitudinally extended transverse myelitis (LETM) and moderate/severe deficits at onset were associated with poor recovery.[100]

ADEM is considered as a monophasic and benign illness that occurs mainly in young children who show good motor recovery.[5,13,14] Despite this, a Canadian study showed the presence of lower brain volume, and impaired age-expected brain growth especially in ADEM patients compared to controls in longitudinal measurements.[101] Moreover, these findings also applied for monophasic ADS with more CNS-region restricted presentations, like ON and TM. [101] This implies that, even in absence of chronic insults to the brain, one single demyelinating attack already causes permanent and irrevocable changes to the developing brain. Cognitive studies in ADEM are limited and show varying results.[102] However, long-term deficits including affected attention, executive function, verbal processing and lower IQ scores are reported; especially in children with an early onset <5 years of age.[103,104]

An American study with anti-aquaporin 4 antibodies (AQP4-IgG) seropositive NMOSD children reported that 94% of these children had a second attack and that residual deficits are seen frequently in >40% of the patients after a median follow-up of 1 year, including visual impairment and motor deficits.[19] Long term follow-up of NMOSD children is necessary to gain more insight in their outcomes in this era with immunosuppressive drugs.

The potential cognitive and physical sequelae of pediatric MS are mentioned in the *'Disease course and outcome of MS'* earlier in this chapter.[1,2,10] Even though outcome studies of monophasic ADS and non-MS chronic disorders are emerging, the evidence is not as extensive as in pediatric MS patients. Additional studies on the outcomes and follow-up of pediatric ADS are needed.

NEUROMYELITIS OPTICA SPECTRUM DISORDERS

Back in the 19th century, Eugene Devic and his pupil Fernand Gault described a case of a patient with simultaneous bilateral ON and TM, and subsequently provided a series of 16 cases in literature.[105–107] The presence of ON and TM in the absence of brain involvement were emphasized. Almost a century later, diagnostic criteria for neuromyelitis optica (NMO) were put forward with the same emphasis on ON, TM and absence of brain MRI lesions.[108] Due to the overlap in affected localizations of the CNS, NMO was considered as a rare subtype of MS, with demyelinating, recurrent and often severe attacks.[108]

Antibody discovery

However, the pivotal discovery of the pathogenic serum AQP4-IgG in 2004 made the distinction between NMO and MS possible by laboratory findings.[109] This led to an important change in perspective about NMO. NMO is now considered as a separate disease entity rather than a rare and severe subtype of MS.[109] The identification of AQP4-IgG has brought insight in the pathophysiology of NMO as a primary B-cell mediated astrocytopathy with secondary demyelination. [4,110,111]

After the discovery of serum AQP4-IgG, the diagnostic criteria for NMO were revised and included the use of the AQP4-IgG antibodies.[112] Due to the clinical implications of AQP4-IgG antibodies, a lot of attention has been paid to the test characteristics of antibody detection.[113,114] In the Netherlands, already early in 2008, a highly specific cell-based assay (CBA) was developed at Sanquin Diagnostic Services in Amsterdam, together with the MS center ErasMS in Rotterdam.[115] Sanquin has since this collaborative initiative been the Dutch centralized laboratory for AQP4-testing.

Clinical spectrum and diagnosis

Thanks to the identification of APQ4-IgG in patients with demyelination, the clinical spectrum of NMO has broadened and is now rather called NMO spectrum disorders (NMOSD). NMOSD includes the classical involvement of the optic nerve(s) and spinal cord, but also limited forms such as isolated or recurrent ON, TM, acute brainstem syndromes,[116] area postrema syndrome with intractable nausea, vomiting and/or hiccups,[117] and cerebral syndromes (including diencephalic brain syndromes and symptomatic narcolepsy).[4] Epidemiological estimates of

incidence and prevalence were not available till recently, mostly thanks to the identification of AQP4-IgG antibodies. NMOSD is a rare disorder with incidence figures in other countries ranging between 0.05-0.4 per 100.000 persons.[118–121] The incidence figures of the Netherlands are yet to be investigated.

Diagnosis of NMOSD can be made based on clinical presentation, aided by the serological AQP4-IgG status and radiological features.[4,122,123] AQP4-IgG positive patients are more often female, and have their initial presentation with a median in the late 30s, with a predominance for the African and Caribbean population.[118,124,125]

In *Table 1.2* the current diagnostic criteria for NMOSD are displayed. Some typical MRI features are presented in *Figure 1.3*.

Table 1.2: Neuromyelitis optica spectrum disorders (NMOSD) diagnostic criteria (Wingerchuk et al., 2015)

NMOSD with AQP4-IgG	1. At least 1 core clinical characteristic 2. Positive serological 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 longitudinally extensive transverse myelitis (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 transverse myelitis (TM) 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 non-specific white matter lesions, or (b) optic nerve MRI with T2-hyperintense lesion or T1-weighted gadolinium enhancing lesion extending over >50% optic nerve length or involving the chiasm 2. Acute TM: 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

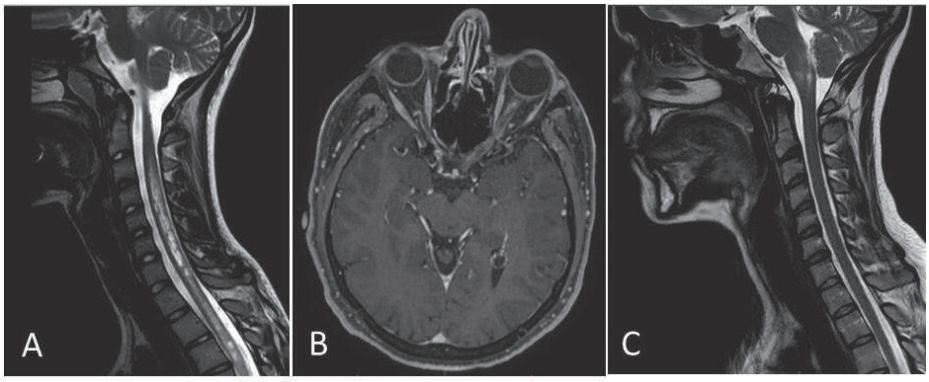

Figure 1.3: Radiological features in AQP4-IgG positive neuromyelitis optica patients
Figure 1.3A: severe and extensive cervical transverse myelitis with >3 contiguous segments. Figure 1.3B: longitudinal involvement of the left optic nerve with swelling and gadolinium enhancement from intra-orbital area till the optic chiasm. Figure 1.3C: dorsal medulla lesion in a patient with area postrema syndrome.

Despite the most recent diagnostic criteria for NMOSD, the diagnostic process remains challenging at times. Although 75%-90% of the patients with an NMOSD phenotype are tested seropositive for AQP4-IgG, the other patients still remain AQP4-IgG negative.[126] Another relevant antibody in NMOSD is anti-myelin oligodendrocyte glycoprotein (MOG-IgG). Despite these two antibodies, a proportion of patients with an NMOSD phenotype remains seronegative for both. MOG-IgG positive NMOSD will be discussed further in *'MOG-IgG spectrum diseases'* later in this chapter.

Treatment

AQP4-IgG positive NMOSD are likely to have a relapsing disease course, with usually severe attacks.[108] The accumulation of disability is associated with relapses.[108,127] Yet, much of the neurological damage in NMOSD patients is caused during the first attack, often with a delayed diagnostic period. Therefore early identification of AQP4-IgG related disease and prompt initiation of acute treatment in these patients is important in order to minimize injury and accelerate recovery. Intravenous methylprednisolone is first choice in treatment of new relapses. However if insufficient improvement is seen during treatment, plasmapheresis is shown to be beneficial.[88,128,129] The main goals of initiating chronic treatment is to reduce attack frequency and severity, in order to prevent progression of disability. Initiating MS treatment in NMOSD patients can worsen NMOSD disease activity and should therefore be avoided.[130–135] Azathioprine and mycophenolate mofetil are considered first-line treatment.[88,128,129] In case the patient is refractory to the given chronic treatment, Rituximab should be considered and is currently the most effective for patients with NMOSD.[136,137] Promising therapeutical agents

are under research in ongoing clinical trials (for example anti-CD19 agent Inebilizumab, complement inhibitor Eculizumab, anti-IL6 receptor agent SA237).[128]

MOG-IGG SPECTRUM DISEASES

In the recent years, new candidate biomarkers have gained attention in acquired CNS demyelination, especially anti-myelin oligodendrocyte glycoprotein IgG subtype antibodies (MOG-IgG).

Antibody testing

MOG is a protein expressed on the outer surface of the myelin sheath and oligodendrocytes. [138–140] The protein is a minor component of myelin (0.05%) and is found in the outermost lamella of the myelin sheath. In vivo studies demonstrated induction or contribution to CNS inflammation by MOG-IgG.[141] MOG-IgG might mediate a complement-dependent immune reaction and therefore causing inflammation and demyelination.[142] Serum cell-based assays (CBA) are most reliable for detection of MOG-IgG.[138,143] These autoantibodies are highly specific for acquired CNS demyelination, since they are not detected in healthy controls or other neurological diseases.[144–147] Epidemiological studies of MOG-IgG associated CNS demyelination are scarce and need further investigation.

Clinical spectrum

MOG-IgG are consistently identified in a spectrum of ADS in both adults and children. In both adults and children, these antibodies are predominantly found in ADEM and NMOSD-like phenotypes, especially with involvement of the optic nerves, and in lesser degree LETM and brain stem syndromes.[138,146–148] Multiple studies support that MOG-IgG demyelination is a separate entity from MS and the presence of MOG-IgG pleads against MS diagnosis.[149,150] Even though the presence of MOG-IgG pleads against MS diagnosis, MOG-IgG seropositivity is found in adults with an MS phenotype, though very rare, and is associated only with low antibody titers.[145,146] This can lead to diagnostic dilemmas. How to handle patients with persisting low MOG antibody titers and a clinical MS phenotype needs to be investigated.

MOG-IgG associated demyelination has its own distinct clinical and radiological profile.[149,150] The lesions are often fluffy, poorly demarcated, and are often located infratentorially, in the brainstem or cerebellar peduncles.[151,152] A few examples are shown in *Figure 1.4*.

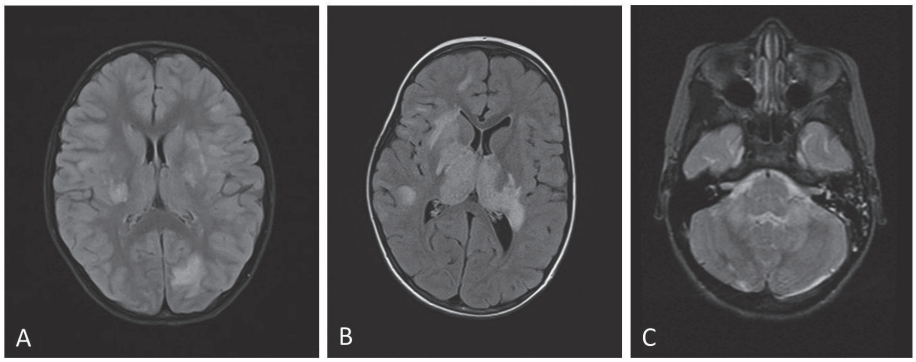

Figure 1.4: MOG-IgG associated MRI abnormalities. Figure 1.4A and 1.4B: supratentorial large confluent white matter abnormalities, some are poorly demarcated. 1.4C: white matter abnormalities in the bilateral cerebellar peduncles.

Up to 39% of the AQP4-IgG negative NMOSD patients are seropositive for MOG-IgG.[142,152–157] Studies suggested that MOG-IgG positive NMOSD patients usually have a monophasic disease course and benign outcome compared to AQP4-IgG positive patients.[142,152–155] A comparison between AQP4-IgG and MOG-IgG positive adult patients can be found later in this thesis.

In pediatric ADS patients, MOG-IgG are present in 18%-32% at first presentation.[144,149,150] Furthermore, age-dependent clinical phenotypes seem to be present, with ADEM-like presentation in childhood and NMOSD like phenotypes in adulthood.[158] Several studies described the prognostic relevance of MOG-IgG. In children, MOG-IgG are found in 36%-40% of the ADEM patients.[149,159] Children with monophasic ADEM often have transient serum MOG-IgG: high serum antibodies are detected at onset and at serial samples titers often reduce to undetectable levels.[146] In contrast, ADS patients with persisting MOG-IgG are at risk of a recurrent disease course[160], including MDEM, ADEM-ON and recurrent NMOSD-like phenotypes.[6,148,161,162] In children, often widespread bilateral grey matter and white matter lesions in the brain and extensive involvement of the spinal cord (LETM) are seen.[163,164] Compared to AQP4-IgG positive NMOSD children, MOG-IgG children less often present with area postrema syndrome, have lower disability, a longer time to relapse and more cerebellar peduncle lesions.[165]

Treatment of MOG-IgG patients

The high proportion of MOG-IgG patients with a monophasic disease course supports the decision against initiating immunosuppressive treatment after the first clinical event. Despite the seemingly benign course, a proportion still relapses and shows accumulation of disability, sometimes despite chronic immunosuppressive treatment. This raises the question how

relapsing MOG-IgG associated diseases need to be treated. The current treatment of MOG-IgG associated disorders has been influenced by AQP4-IgG positive NMOSD treatment guidelines.[88,128] However, evaluation of treatment responses in different subtypes of relapsing MOG-IgG disorders is lacking and is urgently needed.

MS CENTER IN ROTTERDAM

The National pediatric MS center and NMO expert center are both part of the Academic Center of Excellence (ACE) ErasMS Center Rotterdam.

As mentioned in the previous sections, pediatric MS is a highly heterogeneous disease and affects multiple functional domains, including both cognitive and physical functions of a developing child or adolescent. This requires a multidisciplinary approach in the treatment of pediatric MS patients.[166] The Dutch National Pediatric MS center in Rotterdam operates with a multidisciplinary team *(Figure 1.5)*.

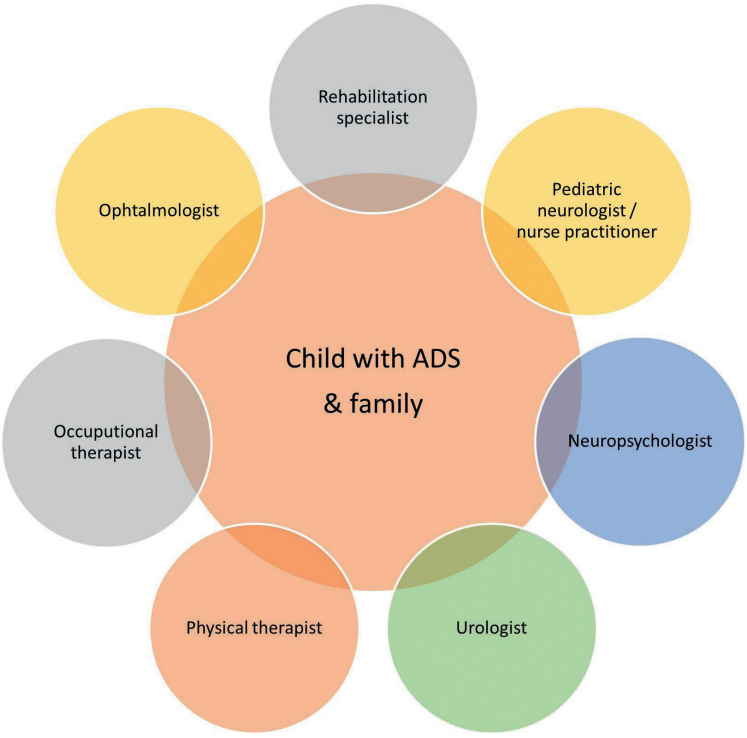

Figure 1.5: Multidisciplinary team of the National pediatric MS center.

The Dutch NMO expert center (ErasMS Rotterdam and Sanquin Diagnostic Services) aims to combine clinical care with scientific research in order to further delineate the spectrum of NMOSD and its pathophysiology.

Towards colleagues in the Netherlands and beyond, both centers have an advisory role in diagnostic and therapeutic decisions. Patients are referred by (pediatric) neurologists and pediatricians: some patients will be treated primarily in the our ErasMS center, whereas others are followed in collaboration with the referring center (executing the 'shared care' concept). In addition, due to the rarity of pediatric MS, ADS and NMOSD, apart from coordinating care and providing accurate counselling to patients and parents, raising awareness and providing opportunities for patients and parents to connect with each other via a specialized center is essential.

SCOPE

This thesis focuses on two groups of patients in the ADS spectrum: NMOSD in adults and the spectrum of childhood onset ADS. The NMO expert center and the National pediatric MS center, as part of the ACE ErasMS Center Rotterdam, render us the unique opportunity to collect data from both groups. We aimed to further delineate the spectrum of ADS in children and NMOSD in adults by keeping two questions in mind:

1. Can we improve on the early identification ADS subtypes?
2. Can we shed more light on the disease course and outcome of ADS subtypes in adults and children?

The first part of this thesis focuses on NMOSD in adults **(chapter 2)**. In **chapter 2.1** we compared the clinical phenotypes of AQP4-IgG positive patients with MOG-IgG positive and seronegative patients with NMOSD. In **chapter 2.2 and 2.3** the incidence estimates of AQP4-IgG and MOG-IgG seropositive NMOSD in the Netherlands are presented respectively. Moreover, the distribution of clinical phenotypes of MOG-IgG seropositive adults and children is described in **chapter 2.3**.

The second part of this thesis focuses on childhood onset ADS (**chapter 3)** and is divided in two sections:
Section *TWO-A* focuses on finding biomarkers for early and accurate diagnosis of pediatric onset MS (**chapter 3.1-3.4**). The recently proposed McDonald 2017 criteria are validated in the spectrum of childhood onset ADS in **chapter 3.1**. In **chapter 3.2** we investigated and validated the predictive value of the immunological biomarker soluble CD27 for CDMS diagnosis at first attack of childhood demyelination. Another promising biomarker is neurofilament light chain (NfL), a marker for axonal damage. NfL in CSF predicts MS diagnosis in adults, but the predictive value in children is unknown. We studied the difference CSF NfL levels and predictive value for

MS diagnosis in in adults and children with ADS in **chapter 3.3**. Subsequently in **chapter 3.4,** we tested NfL levels in serum of our pediatric patients to investigate the correlation with CSF NfL levels and the predictive value of these serological levels for CDMS diagnosis.

Section *TWO-B* of this thesis focuses on the disease course and outcome of childhood onset ADS (**chapter 3.5-3.10**). In order to investigate the clinical spectrum of relapsing MOG-IgG associated disorders, we collaborated with the European pediatric demyelination consortium. The results are presented in **chapter 3.5** and **3.6**. In **chapter 3.5** we describe the disease course and outcome of children with ADEM followed by optic neuritis, one of the relapsing subtypes with relapsing MOG-IgG disorders. In **chapter 3.6** we investigated the clinical phenotypes, treatment responses and outcomes of children with relapsing MOG-IgG associated disease. In **chapter 3.7** we provide an update on the incidence estimates of ADS subtypes in the Netherlands and give an overview of the long-term outcome of ADS patients. Fatigue and motor function of children with MS and ADEM are studied in **chapter 3.8**. In addition, **chapter 3.9** shows the assessment of urological dysfunction early in the disease course in children with MS. Patients with ADEM usually have quick clinical recovery, however, the evolution of MRI lesions during follow-up is rarely investigated. The results are presented in **chapter 3.10**.

The main findings of thesis, the interpretation and the potential clinical implications of these results are discussed in **chapter 4**. In this chapter suggestions for future directions are provided.

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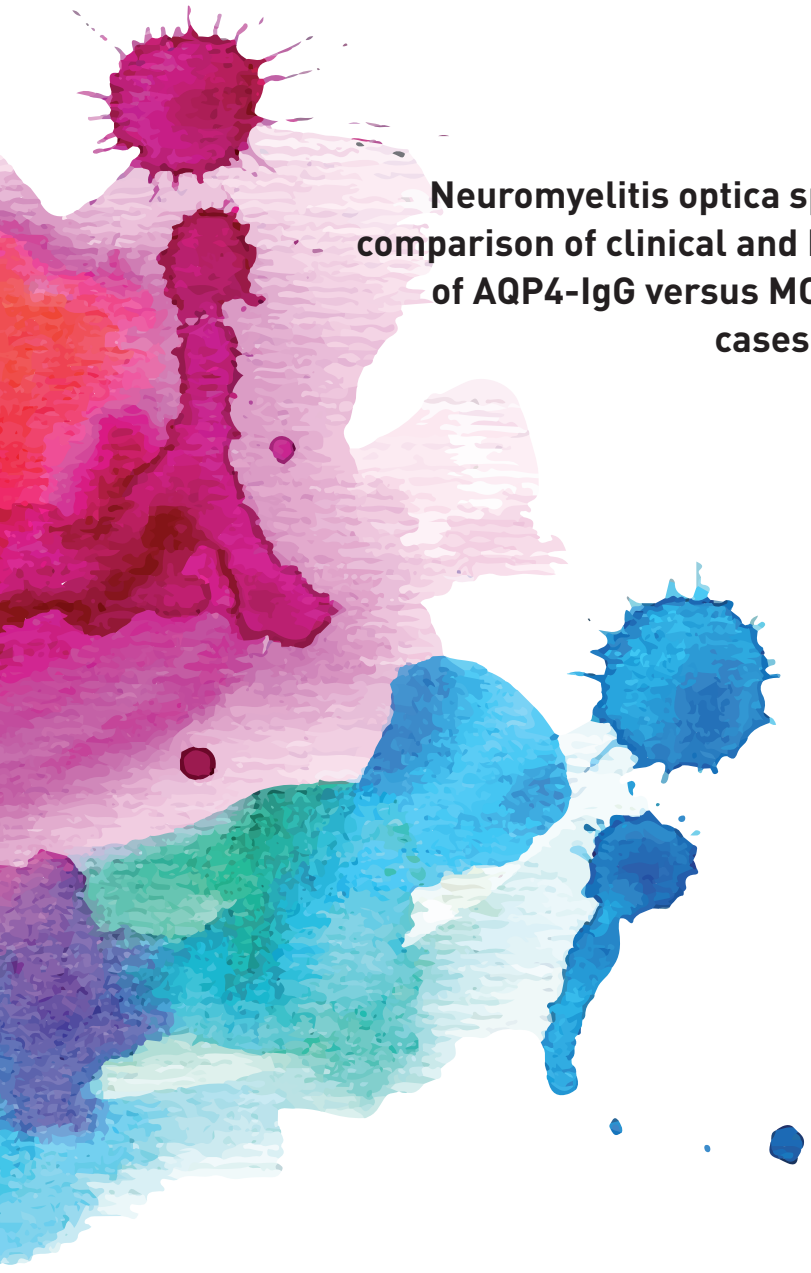
PART ONE

NEUROMYELITIS OPTICA SPECTRUM DISORDERS IN ADULTS



2.1

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

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ABSTRACT

Background

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). We investigated whether the clinical characteristics of these patients differ.

Methods

Using a cell based assay, we analysed serum of 61 AQP4-IgG seronegative patients and 41 AQP4-IgG seropositive patients with clinically NMOSD. 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 (RR 2.4, 95% CI 1.2 – 4.7). AQP4-IgG seropositive patients had higher EDSS levels at last follow-up (p <0.01) and suffered more from residual complaints.

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 ADEM 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

Study participants

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. Patients with NMOSD referred to the Dutch NMO expert centre at Erasmus MC between 2000 and 2015 were included 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: 1) age at first presentation at Erasmus MC ≥ 18 years; 2) 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 AQP-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 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 average deltaMFI + 10 standard deviations of 8 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 into three groups: AQP4-IgG seropositive, MOG-IgG seropositive and double seronegative NMOSD. Statistical analyses were performed using SPSS 21.0. 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 A P-value <0.05 was considered significant.

RESULTS

Hundred and two 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 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 2.1.1* The median time to sampling was 10.7 months (0 – 401.5). Thirteen patients (12.7%) received chronic treatment while the sample was collected. In *table 2.1.1* clinical characteristics are presented for MOG-IgG seropositive (n=20), AQP4-IgG seropositive (n=41) and double seronegative (n=41) NMOSD patients.

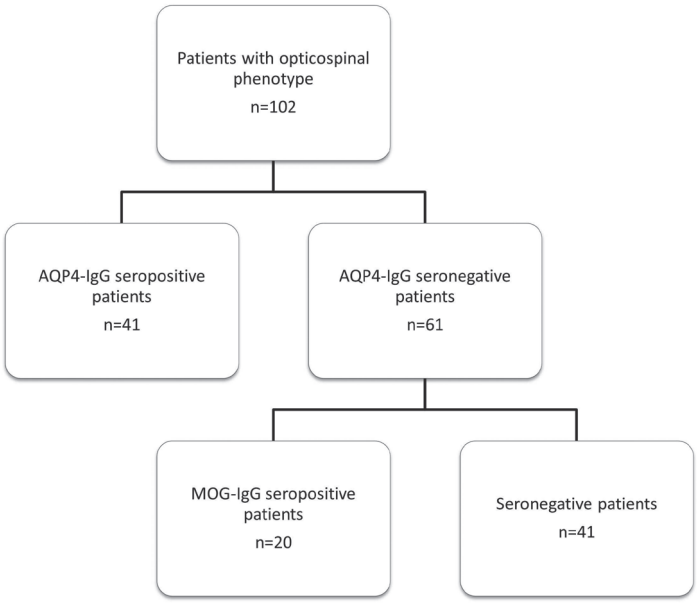

Figure 2.1.1: Overview of included patients.

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%, 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 2.1.2* MRI features are presented.

Table 2.1.1: 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)	0.001	0.238
Mean age at onset, years (SD)	36.2 (14.2)	42.0 (16.1)	40.4 (13.0)	0.233	0.258
Autoimmune comorbidity, n (%)	1 (5)	8 (20)	6 (15)	0.249	0.407
Familial autoimmune disease in first and/or second degree relatives, n (%)	5/18 (28)	19/38 (50)	22/38 (58)	0.117	0.035
Caucasian, n (%)	18 (90)	26 (63)	34 (83)	0.030	0.704
Follow-up, median, months (range)	23.9 (2.7-463.8)	61.5 (4.3-312.9)	23.9 (5.8-267.6)	0.089	0.485
Monophasic disease course	8.0 (2.7 – 463.8)	11.9 (4.3 – 61.5)	17.8 (5.8 – 202.4)	0.432	0.055
Relapsing disease course	106.4 (30.5 – 234.3)	115.8 (14.3 – 312.9)	34.6 (17.7 – 267.6)	0.810	0.068
Monophasic disease, n (%)	14 (70)	12 (29)	25 (61)	0.003	0.491
Phenotype at onset, n (%)					
ON	8 (40)	16 (39)	15 (37)	0.942	0.796
Bilateral ON	6/8 (75)	6/16 (38)	11/15 (73)	0.193	1.000
LETM	4 (20)	20 (49)	15 (37)	0.031	0.189
ON and TM	8 (40)	5 (12)	11 (27)	0.020	0.297
Bilateral ON	7/8 (88)	4/5 (80)	8/11 (73)	1.000	0.603
Phenotype at last follow-up, n(%)					
ON	6 (30)	4 (10)	10 (24)	0.066	0.640
LETM	3 (15)	11 (27)	12 (29)	0.353	0.344
ON and TM	11 (55)	26 (63)	19 (46)	0.528	0.525
CSF Elevated protein >0.60, n (%)	4/14 (29)	12/32 (38)	9/32 (28)	0.739	1.000
CSF Oligoclonal bands, n (%)	3/16 (19)	10/28 (36)	7/35 (20)	0.314	1.000
CSF IgG index >0.68, n (%)	4/13 (31)	13/27 (48)	15/34 (44)	0.298	0.404
EDSS at onset, median (range)	4.0 (2.0-8.0)	4.0 (1.5-9.0)	3.0 (1-9.5)	0.397	0.158
EDSS at best recovery, median (range)	1.0 (0-8.0)	2.0 (0-8.0)	3.0 (1.0-8.0)	0.063	0.013
EDSS at last FU, median (range)	1.0 (0-10)	3.0 (1.0-10.0)	3.0 (1.0-8.0)	0.005	0.021
Nadir visual acuity, median (range)	0.05 (0.003-0.7)	0.003 (0-1.0)	0.05 (0.002-1.0)	0.155	0.832
Chronic treatment, n (%)[c]	4 (20)	34 (83)	17 (43)	0.000	0.098
Duration, months (range)	40.3 (2.8 -79.1)	30.4 (1.3 – 152.4)	32.3 (3.5 – 213.3)	0.982	1.000

[a] Comparison between MOG-IgG and AQP4-IgG seropositive patients. [b] MOG-IgG Comparison between seropositive and seronegative patients.
[c] Fifty-five patients received chronic therapy: 4 MOG-IgG seropositive patients (1 azathioprine, 1 mycophenolate, 1 immunomodulatory treatment, 1 mycophenolate and rituximab), 34 AQP4-IgG seropositive patients (27 azathioprine, 5 mycophenolate, 2 rituximab) , 17 seronegative patients (11 azathioprine, 4 mycophenolate, 1 low dose oral prednisone, 1 mitoxantrone).
Abbreviations: AQP4-IgG aquaporin-4 immunoglobulin G, CSF cerebrospinal fluid, EDSS expanded disability scoring system, LETM longitudinally extensive transverse myelitis, MOG-IgG myelin oligodendrocyte glycoprotein immunoglobulin, NMOSD neuromyelitis optica spectrum disorder, ON optic neuritis, TM transverse myelitis.

Table 2.1.2: 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)*	**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

* Magnetic resonance imaging was reviewed for the presence of NMOSD 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.
Abbreviations: AQP4-IgG aquaporin-4 immunoglobulin, MOG-IgG myelin oligodendrocyte glycoprotein immunoglobulin, MRI magnetic resonance imaging, NMOSD neuromyelitis optica.

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 2.1.2*. 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 2.1.1)* (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) and they more often suffered from residual visual, motor and sensory complaints *(Table 2.1.1)*. Presenting phenotype was not predictive for a relapsing disease course. *Table 2.1.3* 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).

Table 2.1.3: 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] Thirty-seven patients received chronic therapy: 3 MOG-IgG seropositive patients (1 azathioprine, 1 mycophenolate, 1 mycophenolate and rituximab). 26 AQP4-IgG seropositive patients (23 azathioprine, 1 mycophenolate, 2 rituximab), 8 seronegative patients (4 azathioprine, 2 mycophenolate, 1 low dose oral prednisone, 1 mitoxantrone).
Abbreviations: AQP4-IgG aquaporin-4 immunoglobulin, MOG-IgG myelin oligodendrocyte glycoprotein immunoglobulin, ON optic neuritis, TM transverse myelitis.

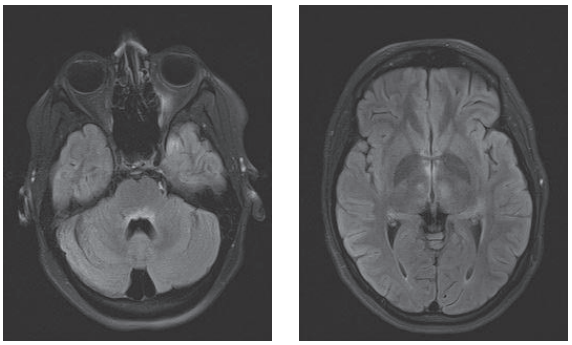

Figure 2.1.2: Transversal MRI FLAIR images presenting typical NMO dorsal brainstem (A) and midbrain (B) lesions of one of our NMO patients with AQP4-IgG seropositivity.

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 2.1.4*.

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 a relatively high rate of a monophasic disease course was observed 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.[23,24] 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 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 2.1.4)*.[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 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. The limitation of our study is a relatively small sample size and therefore statistical corrections could not be made. Also sequential samples were not collected. 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.

Table 2.1.4: Overview of Cell Based Assays (CBA) on MOG-IgG in NMO and NMO-SD.

Study	Patients (age at first presentation)	NMO inclusion criteria	Assay	Number of MOG-IgG seropositive cases*	MOG-IgG seropositivity as percentage of the AQP-IgG seronegative cases*	MOG-IgG seropositivity as percentage of the total included group*	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
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**
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

Table 2.1.4: continued

Study	Patients (age at first presentation)	NMO inclusion criteria	Assay	Number of MOG-IgG seropositive cases*	MOG-IgG seropositivity as percentage of the AQP-IgG seronegative cases*	MOG-IgG seropositivity as percentage of the total included group*	Number of double seropositive (AQP4-IgG and MOG-IgG) patients
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 = 1109	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, NMO-SD, ON**	**CBA**	**179**	**11%**	**9%**	**5**

* Patients seropositive for both AQP4-IgG and MOG-IgG were excluded
** not applicable -only AQP4-IgG seronegative patients were included
Abbreviations: 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.

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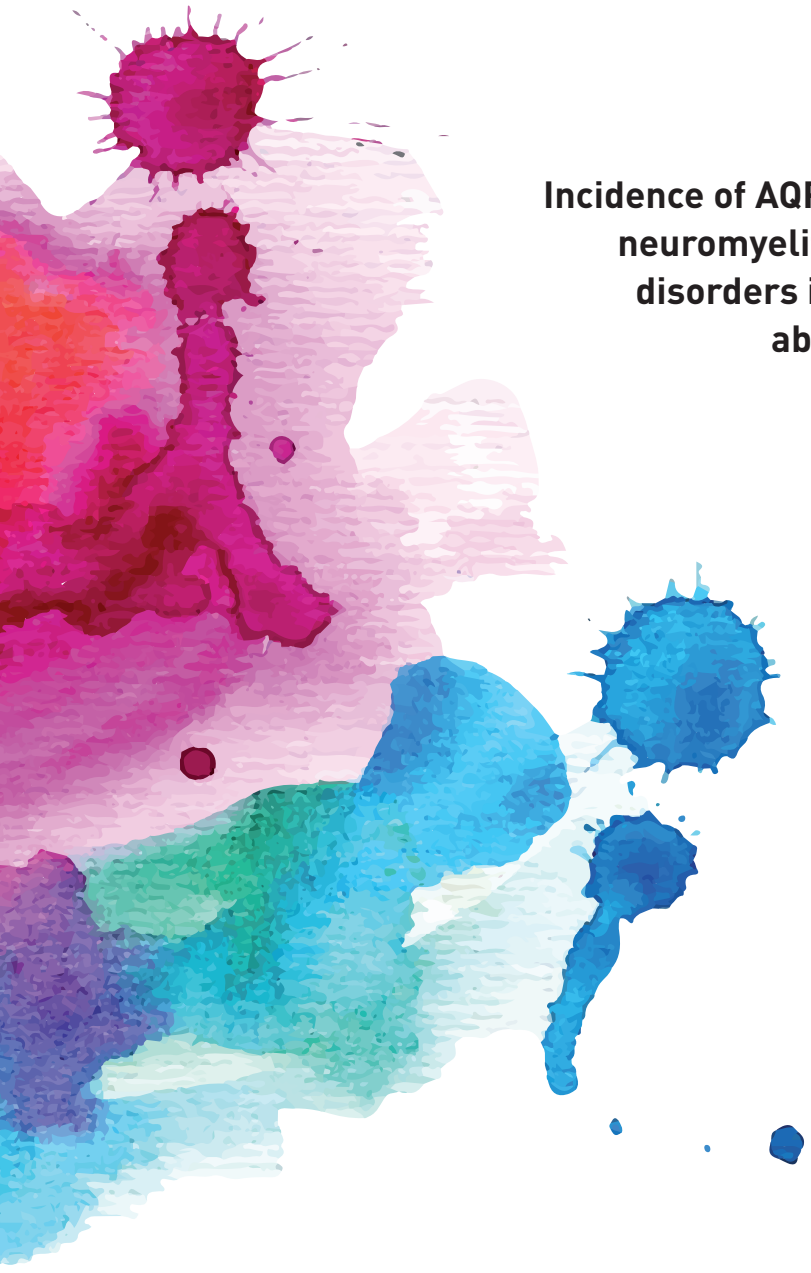

2.2

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 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 an 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

Study participants

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 2.2.1*.

Table 2.2.1: Incidence rates of 6 consecutive years of AQP4-IgG seropositive NMOSD in the Netherlands. Populations 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***	**16,676,000**	**0.09**

* Results rounded to integer.
AQP4-IgG aquaporin-4 immunoglobulin G, NMOSD neuromyelitis optica spectrum disorders.

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 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.*2 Seventy-eight percent of 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]

Table 2.2.2: 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 APQ4-IgG assay, median months (range)	7.9 (0.3 – 248.8[1])
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 (%)*	17/34 (50%)
NMO like[5]	4 (12%)
Aspecific	13 (41%)
MRI spinal cord lesions, n (%)*	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%)

[1]In our cohort we report a patient with an extraordinary time from onset to sampling of 248.8 months. This particular patient suffered from recurrent unilateral optic neuritis in 1988, 2004 and in several years afterwards. Using the current diagnostic criteria[2] NMOSD diagnosis could not have been made in this unique case prior to the AQP4-IgG testing.

*MRIs performed at onset and/or follow-up.

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

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 NMOSD understanding.

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2.3

The clinical spectrum and incidence of anti-MOG associated acquired demyelinating syndromes in children and adults

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ABSTRACT

Objectives

To assess the Dutch nationwide incidence of anti-MOG antibodies (MOG-IgG) associated demyelinating syndromes of the CNS and to describe clinical and serological characteristics of MOG-IgG positive patients.

Methods

All serum samples for routine diagnostics from February 2014 until December 2017 were sent to the single central reference laboratory for MOG-IgG in the Netherlands. Serum was tested with a full-length MOG-IgG cell-based assay. Incidence figures were calculated using population data. Clinical data from patients known in our National ADS center were available.

Results

1414 samples of 1277 patients were received and of these,92 patients (7%) were MOG-IgG seropositive. The mean incidence was 0.16/100,000 people, with higher seropositivity in children (0.31/100,000) than in adults (0.13/100,000). In MOG-IgG positive patients at the National ADS center (61/92, 66%), the most common presenting phenotype is ADEM (56%) in children and ON (44%) in adults. Relapsing disease occurred in 9/34 (26%) children and 11/27 (41%) adults during median follow-up (FU) of 27.5months. The median time-to-first-relapse (TTFR) was 5.8 months (IQR 2.5-10.1 months). Three patients were tested MOG-IgG positive >200 months after the initial attack, suggesting an extended TTFR. Longitudinal analysis of MOG-IgG (26/61,43%) showed that 64% of the monophasic patients remain seropositive (median onset-to last-sampling time 21.7 months) and 66% in relapsing patients. Majority of seronegative patients had no relapses (89%).

Conclusion

This nationwide study of a typical western European country shows that the overall incidence of MOG-IgG seropositive disease in children and adults is 0.16 per 100,000 people. The distribution over the clinical phenotypes differs between adults and children. Seropositivity can be maintained over years even without clinical activity, while seronegative patients generally had no relapses.

INTRODUCTION

Myelin oligodendrocyte glycoprotein (MOG) is a protein expressed on the surface of myelin sheaths and oligodendrocytes.[1,2] Anti-MOG antibodies (MOG-IgG) can cause demyelination in vitro and induce experimental autoimmune encephalomyelitis.[3,4] MOG-IgG are found in subtypes of central nervous system (CNS) acquired demyelinating syndromes (ADS) in both adult and pediatric patients, for example in neuromyelitis optica spectrum disorders (NMOSD)[5] and acute disseminated encephalomyelitis (ADEM).[6] Since the optimization of the MOG-IgG cell-based assay (CBA), this CBA has become available for routine clinical practice.[7] Although research on the clinical aspect of MOG-IgG associated demyelinating syndromes has taken a great leap in the recent years, the incidence figures of MOG-IgG seropositivity in the general population has not yet been investigated. In the Netherlands, one single centralised laboratory performs the diagnostic testing of MOG-IgG.[6] This provides an unique opportunity to gain insight into the nationwide incidence of MOG-IgG seropositivity in both children and adults presenting with CNS demyelinating diseases. In addition, a recent study showed that the distribution of clinical phenotypes differs between MOG-IgG seropositive children and adults.[8] We here aim to provide an incidence estimate of MOG-IgG seropositivity in a typical western European country and to describe the clinical and serological characteristics of patients within the MOG-IgG spectrum.

METHODS

Study participants

The Dutch National ADS center includes the NMOSD center and Pediatric MS center (Rotterdam) and Sanquin diagnostic services (Amsterdam). Basic demographic data (age and treating center) were available from all serum samples sent in for routine MOG-IgG diagnostics. All serum samples were tested blindly, centrally and in duplicate at Sanquin Diagnostics with a CBA.[6] All patients were tested negative for anti-aquaporin 4 antibodies (AQP4-IgG). Details on the CBAs will follow in the next section. The CBA for MOG-IgG has become available nationwide since February 2014. Data was collected from four consecutive years (Feb 1st 2014 until December 31st 2017). Samples that were sent in from abroad, mainly from the Dutch Caribbean and Belgium, were excluded from this study (n=121). Incidence rates were calculated as the number of MOG-IgG-seropositive patients per year divided by the number of Dutch inhabitants. This was done for the pediatric and adult patients together, and separately. Population figures were extracted from Statistics Netherlands.[9] To calculate representative mean incidence figures for MOG-IgG seropositivity, incidence figures of 2015-2017 were used as the incidence of 2014 was exceptionally low compared to the following years.

From the patients known in the National ADS center and whose serum were tested between Feb 2014 and Dec 2017, clinical data were available. Patients were diagnosed with NMOSD or ADEM by the international consensus criteria.[10,11] MRI lesions were scored on T2 and FLAIR sequences: poorly demarcated (deep) grey and/or white matter lesions, gyral filling, extensive confluent white matter lesions, well-demarcated ovoid lesions (MS-like), non-specific lesions.[12,13] Spinal MRIs were evaluated if available and scored for lesion location (cervical, thoracic, lumbar) and presence of longitudinally extensive transverse myelitis (LETM; ≥3 segments).[11] For the serial sample analyses, only samples ≥3 months after the previous sample were taken into account.

Cell based assays

CBAs were used for MOG-IgG and AQP4-IgG detection as described elsewhere. [6, 14] Briefly, patient serum was incubated with HEK 293 cells transiently transfected with AQP4-M23 (eGFP tagged; final serum dilution 1:20) or LN18 cells stably transfected with full-length MOG (eGFP tagged; final serum dilution 1:200). After washing, cells were subsequently incubated with goat anti-human IgG allophycocyanin conjugated secondary antibody (specific for human IgG; Jackson ImmunoResearch Laboratories, Brunschwig Chemie B.V., Amsterdam, The Netherlands) and analysed after washing using fluorescence-activated cell sorting. We expressed the quantitative levels of antibody titers as the difference in median fluorescence intensity (ΔMFI) between the AQP4-transfected and MOG-transfected with respectively the untransfected HEK 293 cells and untransfected LN18 cells.The cut-off was determined in every assay as the average mean fluorescence intensity + 10 standard deviations of eight individual negative control sera. Both assays have an anti-IgG specific detection antibody and thus no IgM anti-MOG or IgM anti-AQP4 were detected.

Ethics approval

This study was approved by the Medical Ethical Committee of the Erasmus MC in Rotterdam. All patients from the Erasmus MC provided written informed consent.

STATISTICAL ANALYSIS

Demographic data of the general Dutch population were provided by Statistics Netherlands and were used to calculate the incidence rates. For statistical analyses we used SPSS software, version 24.0(SPSS Inc.) and GraphPad Prism5. The Chi-Square test and Mann-Whitney U test were used to compare categorical and continuous data, respectively.

RESULTS

Incidence figures

From February 1st 2014 until December 31st 2017, 1414 serum samples of 1277 patients were tested for MOG-IgG. Of all included patients, 92 (7%) were seropositive. In total 196 children and adolescents younger than 18 years old were tested. Thirty-four (17%) of these pediatric patients were MOG-IgG seropositive. To calculate the representative mean incidence of MOG-IgG seropositivity in the Netherlands, data from 2015-2017 were used resulting in an incidence of 0.16 per year per 100,000 people, approximately two per million people per year. After dividing the MOG-IgG patients into children and adults, we observed a mean incidence of 0.31 per 100,000 children and 0.13 per 100,000 adults per year. The incidence figures for children, adults and all patients of the included years are shown in *Table 2.3.1*.

Baseline characteristics

Sixty-one of 92 (66%) MOG-IgG seropositive patients were treated in the Dutch ADS center (pediatric disease onset n=34, adult disease onset n=27). The clinical characteristics of this subgroup are displayed in *Table 2.3.2*. No difference was found between the MOG-IgG results and the time 'from most recent disease activity to sampling' (p=0.8). The median age of onset in adult patients was 32.6 years and in children 8.7 years with an equal gender distribution in both adults and children. MOG-IgG seropositivity was seen mostly in patients from Caucasian origin (45/92, 75%). In children, the most common presenting phenotypes were ADEM (19/34, 56%), ON (7/34, 21%, of them 2/7, 29% bilateral ON) and NMO (5/34, 15%) with simultaneous ON and TM, comprising 92% of all presenting phenotypes. In adults, common presentations were ON (12/27, 44%, of them 7/12, 58% bilateral ON), TM (11/27, 41%) and NMO (3/27, 11%). Brainstem manifestations were rare.

Table 2.3.1: Incidence figures of anti-MOG seropositivity in the Netherlands.

Year	Incidence in children per 100,000 people	Number of Dutch pediatric inhabitants	Number of MOG-IgG seropositive pediatric patients/year	Incidence in adults per 100,000 people	Number of Dutch adult inhabitants	Number of MOG-IgG seropositive adult patients/year	Overall incidence per 100,000 people
2014	0.06	3.442.802	2	0.05	13.386.487	7	0.05
2015	0.20	3.429.193	7	0.11	13.471.533	15	0.13
2016	0.26	3.416.581	9	0.08	13.562.539	11	0.12
2017	0.47	3.404.098	16	0.18	13.677.409	25	0.24
Mean/year 2015-2017	**0.31**	**3.416.624**	**11**	**0.13**	**13.570.493**	**17**	**0.16**

Table 2.3.2 – Clinical characteristics of MOG-IgG seropositive patients

	Children (n= 34)	Adults (n= 27)	All (n=61)	p-value*
Age, median (IQR), years	8.7 (5.7-12.3)	32.6 (25.4-48.6)	16.6 (7.9-31.9)	<0.001
Sex female, n (%)	16 (47)	12 (44)	28 (46)	0.8
AID comorbidity, n (%)	1 (3)	0 (0)	1 (2)	1.0
Caucasian, n (%)	23 (70)	22 (82)	45 (75)	0.3
Type of onset, n (%)				<0.001
ON	7 (21)	12 (44)	19 (31)	
- Bilateral ON	- 2/7 (29)	- 7/12 (58)	- 9/19 (47)	
TM	1 (3)	11 (41)	12 (20)	
- LETM	- 1/1 (100)	- 5/11 (42)	- 6/12 (50)	
Brainstem syndrome	1 (3)	1 (4)	2 (3)	
CIS other	1 (3)	0	1 (2)	
NMO	5 (15)	3 (11)	8 (13)	
ADEM	19 (56)	0 (0)	19 (31)	
MRI brain lesions, n (%)	23/32 (72)	7/27 (26)	30/59 (51)	0.001
- *Poorly demarcated (deep) grey and white matter involvement*	16 (70)	0	16 (53)	<0.001
- *Gyral filling*				
- *Extensive confluent white matter lesions*	11 (48)	0	11 (37)	0.003
- *Well-demarcated ovoid lesions (MS-like)*	2 (9)	0	2 (7)	0.5
- *Non-specific lesions*	4 (17)	3 (43)	7 (23)	1.0
	3 (13)	5 (71)	8 (27)	0.1
MRI spine lesions, n (%)	13/22 (59)	13/22 (59)	26/44 (59)	1.0
- *Cervical*	10 (77)	9 (69)	19 (73)	1.0
- *Thoracic*	12 (92)	8 (57)	20 (77)	0.3
- *Lumbar*	6 (46)	0 (0)	6/26 (23)	0.02
≥ 2 CSF unique OCB, n (%)	3 (11)	5 (28)	8 (18)	0.262
Relapsing disease, n (%)	9 (27)	11 (41)	20 (33)	0.2
Time to first relapse, median (range), months	10.5 (1.8-322.1)	7.7 (0.4-218.7)	8.0 (0.4-322.1)	0.5
Use of IMT, n (%)	12 (36)	8 (30)	20 (33)	0.6
Follow-up time, median (IQR), months	30.7 (10.3-71.2)	20.2 (11.3-43.0)	27.5 (11.3-61.9)	0.5

Type at last follow-up, n (%)				<0.001
ON	6 (18)	10 (37)	16 (26)	
- Bilateral	- 3/6 (50)	- 6/10 (60)	- 9/16 (56)	
TM	1 (3)	8 (30)	9 (15)	
- LETM	- 1/1 (100)	- 3/8 (38)	- 4/9 (44)	
Brainstem syndrome	0 (0)	1 (4)	1 (2)	
Monophasic ADEM	14 (41)	0 (0)	14 (23)	
NMO	6 (18)	8 (30)	13 (21)	
Other**	7 (21)	0 (0)	7 (12)	

This table related to the subgroup of 61 out of the 92 MOG-IgG seropositive patients (66%) for which we had clinical data available at the National ADS center. Abbreviations: interquartile range (IQR), auto-immune diseases (AID), optic neuritis (ON), transverse myelitis (TM), longitudinal extended transverse myelitis (LETM), clinically isolated syndrome other than ON or TM (CIS), neuromyelitis optica (NMO) with ON and TM involvement , acute disseminated encephalomyelitis (ADEM), cerebrospinal fluid (CSF), oligoclonal bands (OCB), immunomodulatory treatment (IMT).

*Considered significant if p-value <0.05. Comparison between adults and children.

**Other (n=7): children were diagnosed with ADEM-ON (n=3), ADEM-TM (n=1), ON followed by cortical phenotype (epilepsy and hemiparesis, n=1), CIS (n=1), relapsing remitting MS (n=1).

Follow-up characteristics

The most common phenotypes at end of FU in children were monophasic ADEM (14/34, 41%), followed by ON (6/34, 18%) and NMO phenotype (6/34, 18%). One MOG-IgG seropositive child was diagnosed with relapsing remitting multiple sclerosis (RRMS, low delta-MFI 330; cut-off 104). Another child had a first episode of ON and had multiple relapses with a cortical phenotype (epilepsy, hemiparesis) with new corresponding lesions on brain MRI. In adults, the ON phenotype was most common(10/27, 37%), followed by TM (8/27, 30%) and NMO phenotype (13/27, 30%). During FU, optic nerve involvement was observed at least once in 16/34 children (47%) and in 17/27 adults (63%). Two out of 34 children showed relapsing ON (6%) and three out of 27 adults (11%). One of these adults patients (4%) was diagnosed with chronic relapsing inflammatory optic neuritis (CRION), suffering from multiple relapses when tapering off oral prednisone and showing quick and complete recovery after steroid administration. The distribution of clinical phenotypes at presentation and at last follow-up are presented in *Figure 2.3.1* for adults and children.

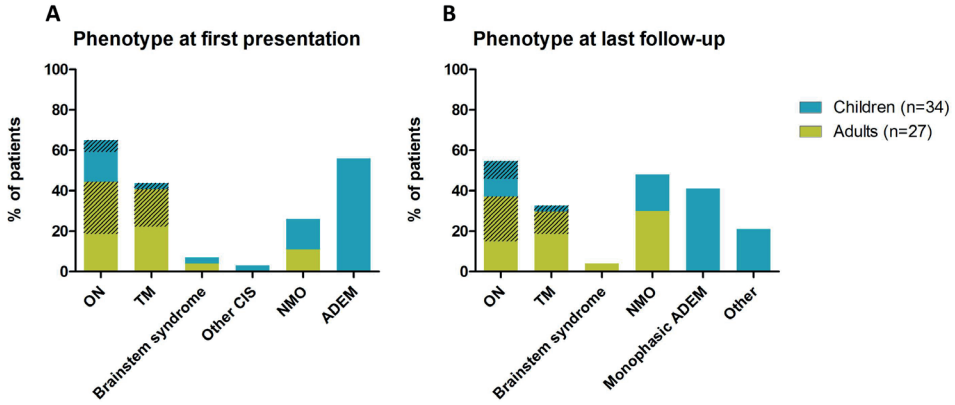

Figure 2.3.1: Distribution of clinical phenotypes in adults and children.

A: phenotypes at first presentation. B: phenotypes at last follow-up. Other (n=7) children were diagnosed with ADEM-ON (n=3), ADEM-TM (n=1), ON followed by cortical phenotype (epilepsy and hemiparesis, n=1), clinically isolated syndrome (CIS) (n=1), relapsing remitting MS (n=1). Striped areas: bilateral optic neuritis and longitudinal extensive transverse myelitis respectively.
Abbreviations: optic neuritis (ON), transverse myelitis (TM), clinically isolated syndromes other than ON or TM (other CIS), neuromyelitis optica (NMO), acute disseminated encephalomyelitis (ADEM).

At the end of FU (median 27.5 months; IQR 11.3-61.9), 20/61 (33%) patients showed a relapsing disease course (pediatric onset 9/34 (26%), adult onset 11/27 (41%)). The proportion of patients with a first relapse within the first year after onset was 75% (median time to first relapse of 5.8 months, IQR 2.5-10.1 months). After two and three years this proportion was 80% (16/20) for both time intervals. Within five years, 17/20 (85%) of the relapsing patients had a relapse. The

remaining 3/20 relapsing patients were tested MOG-IgG positive >200 months after the initial attack, suggesting an extended TTFR. Of these 20 relapsing patients, 15 (75%) were given immunomodulatory or immunosuppressive treatment (IMT), including Azathioprin, Rituximab (RTX), Mycophenolate, Cyclophosphamide, monthly intravenous immunoglobulins (IvIG) and Teriflunomide.

Longitudinal analysis of MOG-IgG

Serial sampling were performed in 25/61 (41%) National ADS center patients for routine clinical care (40 serum samples) with a median interval between first and last sample of 13.7 months (IQR 7.2-25.4) with a median follow-up of 31.9 months (IQR 12.3-57.7).

In *Figure 2.3.2* we divided the 25 patients with serial samples into 4 groups based on their disease course and the last available test result: **1.** relapsing patients with persisting MOG-IgG (n=6) , **2**. relapsing patients who turned seronegative for MOG-IgG (n=4), **3**. monophasic patients with persisting MOG-IgG (n=10) and **4**. monophasic patients who turned seronegative for MOG-IgG (n=5).

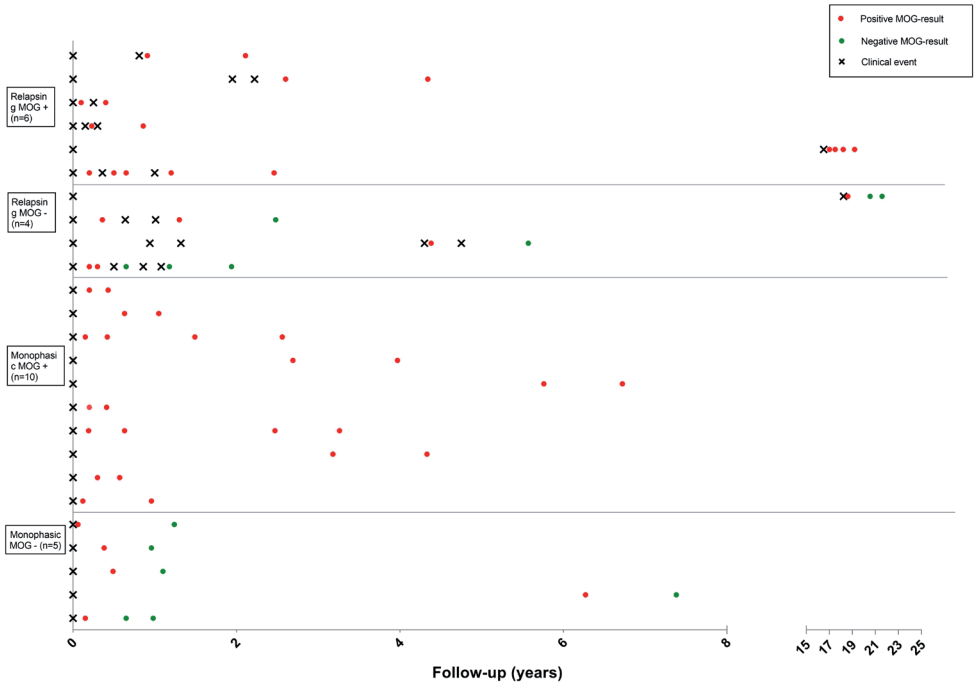

Figure 2.3.2: Longitudinal analysis of clinical and serological data

Twenty-six of the 92 MOG-IgG positive patients who were known at the National ADS center were tested serially for MOG-IgG. Patients are classified into four groups according to their disease course (relapsing or monophasic) and the results of the last tested serum sample of that patient.

We observed that 10/15 (67%) monophasic patients remained seropositive (with a median FU time of 23.6 months, IQR 9.7-50.1) (median onset-to last-sampling time 21.7 months). The 5/15 (33%) monophasic who turned seronegative had a median FU of 30.4 months (IQR 19.5-75.4). Relapsing patients (n=10) remained seropositive in 6/10 (60%) during a median FU of 38.1 months (IQR 22.4-121.7). Four relapsing patients became seronegative during FU 62.2 months, IQR 33.0-215.6). In three out of these four patients, no relapses occurred after turning seronegative. One out of these four patients had new clinical disease activity after turning seronegative while being on RTX and monthly IvIG: the first blood sample with a negative result was taken four months after the previous attack while being on oral prednisone and azathioprine, and the second negative blood sample was taken two days after two cycles of RTX therapy. Two out of the three patients who did not show relapses anymore after turning seronegative were treated with IMT, including cyclophosphamide and MMF and Azathioprin.

IMT was initiated in 6/16 MOG-IgG positive patients who remained positive during FU, and in 4/9 patients who were initially tested MOG-IgG positive and turned seronegative during FU. No difference was found between the patients who did and did not use IMT and the last serostatus (p=1.0).

DISCUSSION

We report the incidence estimate of MOG-IgG seropositivity in both adults and children with CNS acquired demyelinating syndromes in the Netherlands, from data derived from the National ADS center. All samples were tested in one central laboratory, resulting in a nationwide MOG-IgG seropositivity incidence of 0.16/100,000 people per year, with a higher incidence in children (0.31/100.000) than in adults (0.13/100,000), reflecting the rarity of MOG-IgG seropositivity. These incidences result in 11 MOG-IgG positive children and 17 MOG-IgG positive adults per year. Previous studies showed that 18%-32% of the pediatric ADS patients are MOG-IgG positive at baseline.[6,15,16] Recently, we reported an update of the pediatric ADS incidence in the Netherlands of 0.80/100,000 children.[17] When extrapolating these results, the incidence of MOG-IgG positive patients in the pediatric population could vary between 0.14-0.26 per 100,000 children. Our incidence figure of 0.31/100,000 approximates this range, though somewhat higher. A slight difference is that in the current study only patients with a a positive test result were included. Incidence figures of MOG-IgG testing bear relevance for clinical care and counselling, but also for survey of these ailments, implementation of assays in regional/national laboratories, planning with health care authorities and organisation of potential future clinical trials.

We had access to the clinical data of 61 MOG-IgG seropositive patients who were all known in the National ADS center (66%). In keeping with previous studies, the optic nerve was frequently involved in children (47%) and adults (63%) with MOG-IgG.[5,18-21] On the contrary brainstem manifestations were rare in both children and adults. The clinical phenotypes that our patients showed at the end of follow-up were similar to previously published literature. Yet, one girl in our study experienced one ON and relapsing disease with predominantly cortical presentations (unilateral cerebral cortical epilepsy and hemiparesis). This presentation with seizures was described earlier in a case report with 4 MOG-IgG seropostive adult patients (of whom two also experienced ON prior to seizures) and in a recent study two children are identified.[8,22] The clinical spectrum of MOG-IgG positive patients may further expand since the CBAs are now being established worldwide and more patients with MOG-IgG related diseases are being identified. Clinicians should be aware of less frequently reported clinical manifestations asscociated with MOG-IgG positivity.

A relapsing disease course was observed in 33% of our patients, in line with other studies.[5,23] In children, the proportion of MOG-IgG positive patients with relapses (27%) is somewhat lower than in previous literature (up to 50%).[6,15,16] In adults, 41% of the patients had a relapsing disease course which was comparable to other studies.[5,18,20,23] However, a few studies reported a much higher proportion of relapsing patients (62%-80%) after a longer period of follow-up.[13,24,25] This high proportion could be explained by longer follow-ups and the retrospective character of the studies. Our current figures of relapsing patients support the current clinical practice not to start immunomodulatory treatment after the first attack of MOG-IgG associated CNS demyelination in order to prevent overtreatment.

Of note is that the majority of our relapsing patients (75%) have their first attack within one year after onset with a median TTFR of 5.8 months, comparable with other studies.[18,26] Compared to anti-aquaporin 4 positive patients, the TTFR in the MOG-IgG patients is similar.[18] In our previous work we reported a significantly longer TTFR of 28 months which can be explained by the small sample size.[5] A minor subgroup of the MOG-IgG positive patients showed a relapse after an exceptional long time interval of >20 years after onset, in line with other studies.[5,26,27]

The longitudinal analysis of MOG-IgG patients showed that a considerable proportion of relapsing patients remains seropostive (64%). Yet, in our study one patient had new relapses after turning seronegative, while being treated with potent immune suppression with RTX and monthly IvIG. A possible explanation is the timing of the blood sampling, as the first blood sample with a negative result was taken four months after the previous attack while being treated with immunesuppression, and the second negative blood sample was taken two days after a two cycles of RTX therapy. This might have influenced the MOG-IgG results. Beside from this patient, no relapses were observed in relapsing patients who eventually converted to

seronegativity. This is in line with another study that described 10 MOG-IgG positive patients with serial sampling, and observed that no relapses occur after patients turned seronegative.[28]

One child was diagnosed with relapsing remitting MS with a typical MS disease course, including MS-like MRI fulfilling both the McDonald 2010 and 2017 criteria and had unique CSF oligoclonal bands, and was therefore treated with teriflunomide. The delta-MFI was low (330; cut-off 104). Low levels of MOG-IgG are observed in pediatric RRMS patients in previous studies.[6,29].

Our study has several limitations. First, these current incidence estimates are minimum figures, since mild cases and forme fruste types of the disease could have been missed. The incidence of 2014 is excluded from the mean incidence calculation, since the number is exceptionally low compared to the following years. This is most likely due to the low awareness of CBA availability in the first year after launching the CBA as a routine diagnostic test. Increased awareness of treating physicians could potentially contribute to even higher incidence figures in the future by testing more patients more quickly, although an asymptote may have been reached in this relatively small country. Second, it has not been feasible to have access to full clinical data of all MOG-IgG seropositive patients in this country apart from the patient's gender, as we did not have ethical permission to approach the patients who have not visited the Erasmus MC. Still, the more detailed analysis of this study covered 66% of the study population as these patients were referred to our National ADS center. It is possible that certain phenotypes are over-represented in this group, for example because of a lower threshold for referral in case of recurrent disease. Third, for definite conclusions on the temporal dynamics of MOG-IgG and the effect of immunosuppressive drugs on MOG-IgG serostatus, we lacked sufficient sample size and systematic schedule for blood sampling. Well-designed prospective studies including blood sampling at pre-defined time points will be needed to address these important questions.

In conclusion, this nationwide study of a typical western European country shows that the overall incidence of MOG-IgG seropositive disease in children and adults is low, with an average of 0.16 per 100,000 people. The distribution over the different clinical phenotypes differs between adults and children. Seropositivity can be maintained over years even without clinical activity, while patients who converted seronegative during follow-up generally had no new relapses. Further studies with detailed clinical and serological follow-up in an international collaborative setting will set the stage for further improved insight on the use of this diagnostic test in clinical practice.

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PART TWO A

EARLY and ACCURATE DIAGNOSIS for PEDIATRIC MS

3.1

Real world validation of the 2017 McDonald criteria for pediatric multiple sclerosis

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ABSTRACT

Objective:

To compare the diagnostic accuracy of the McDonald 2017 vs the McDonald 2010 criteria to predict a second attack of multiple sclerosis (clinically definite multiple sclerosis, CDMS) at first attack of acquired demyelinating syndromes(ADS).

Methods

Hundred and sixty-four children (<18 years) with an incident attack of ADS were included in a prospective multicenter study between June 2006 and December 2016. Brain (and spinal if available) MRI was performed ≤3 months after symptom onset. Sensitivity, specificity, positive predictive value, negative predictive value, accuracy were compared at baseline between the 2010 and 2017 criteria.

Results

Among the 164 patients,110 (67%) patients presented without encephalopathy (ADS-, female 63%; median age 14.8 years, IQR 11.3-16.1) and 54 (33%) with encephalopathy (ADEM, female 52%; median age 4.0, IQR 2.6-6.1). Of the 110 ADS- patients, 52 (47%) were diagnosed with CDMS within a median FU of 4.5 years (IQR 2.6-6.7). The sensitivity was higher for the 2017 criteria than for the 2010 criteria (83%; 95% CI 67-92, vs 49%; 95% CI 33-65; p<0.001), but the specificity was lower (73%; 95% CI 59-84 vs 87%; 95% CI 74-94, p=0.02). At baseline, 48 patients fulfilled the 2017 criteria compared to 27 patients when using the 2010 criteria. The results for children without encephalopathy <12 years were similar. In ADEM patients, 8% fulfilled the 2010 criteria and 10% the 2017 criteria at baseline, but no patient fulfilled the criteria for CDMS.

Conclusions:

The McDonald 2017 criteria are more sensitive than the McDonald 2010 criteria for predicting CDMS at baseline. These criteria can also be applied in children <12 years without encephalopathy, but not in children with ADEM.

INTRODUCTION

Multiple sclerosis (MS) is a chronic demyelinating disorder of the central nervous system.[1] Up to 10% of all MS patients have their first symptoms before the age of 18.[2,3] Recently, the international panel on diagnosis of MS proposed the McDonald 2017 criteria, by reviewing and revising the prior 2010 McDonald criteria.[4,5] These revised criteria include modifications to facilitate earlier MS diagnosis, while attempting to preserve the diagnostic accuracy of the criteria.[5] Important modifications included re-introducing CSF oligoclonal bands into the criteria as a substitute for dissemination in time (DIT), and allowing symptomatic lesions to contribute to dissemination in space(DIS) and DIT. Furthermore cortical lesions have been combined with the juxtacortical lesion component to demonstrate DIS.

The applicability of the adult McDonald 2010 criteria in children was supported by several studies.[6-12] These criteria were implemented in the revised 2012 diagnostic criteria for children with acquired demyelinating syndromes (ADS) proposed by the International pediatric MS study group(IPMSSG).[13] ADS encompasses the first attack of demyelination in the central nervous system(CNS).[13,14] According to the IPMSSG criteria, MS diagnosis based on the first baseline MRI in children with a first attack should be taken with caution for children aged <12 and patients presenting with acute disseminated encephalomyelitis(ADEM) due to a lower specificity and sensitivity of the McDonald 2010 criteria in these groups.[13]

A Canadian study reported that the revised McDonald 2017 criteria apply well in children with a first attack of ADS across the age-span.[15] Validation of these criteria in different study populations is imperative because of the long-term administration of disease modifying treatment(DMT) after MS diagnosis. Overtreatment in patients must be prevented, as well as under-treatment due to delayed diagnosis. Early and accurate identification of MS patients is therefore essential.

We aimed to evaluate the diagnostic accuracy of the revised 2017 MS criteria in children with ADS at time of first presentation. Extra attention is paid to children below the age of 12 years and to patients presenting with ADEM.

METHODS

Patients and design

Children younger than 18 years with a first attack of demyelination were consecutively included in the nationwide, multicenter and prospective study for children with ADS between June 2006 and December 2016 (PROUD-kids study).[16,17] Patients were assessed at baseline and were prospectively followed. MRI was performed within 3 months after symptom onset as

part of routine diagnostic process to rule out alternative diagnoses. Patients with alternative non-demyelinating disorders were excluded from our study. All patients had a follow-up(FU) duration of at least 1 year, as the interval between first and second attack in pediatric MS is typically less than one year.[18-21]

Patients were included for analysis when presenting with ADS without(ADS-) and with encephalopathy(ADEM).[13] Patients with neuromyelitis optica spectrum disorders(NMOSD) or with relapsing disease other than MS were excluded from analyses (for example patients presenting with relapsing anti-MOG antibody related disorders), because there is emerging evidence that these patients have a distinct clinical phenotype.[22,23]

Standard protocol approvals and patient consents

The PROUD-kids study protocol was approved by the Ethics Committee Erasmus MC Rotterdam and by the other participating centers in the Netherlands. Written informed consent was obtained from all patients and/or their families.

Definitions

Acquired demyelinating syndromes in children encompass the first attack of demyelination in the CNS, including patients presenting with (ADS+) and without encephalopathy (ADS-). ADEM was defined as a polyfocal onset with encephalopathy (ADS+).[13] Clinically definite multiple sclerosis (CDMS) was defined as a second attack of MS, with two non-encephalopathic confirmed attacks with clinical evidence of two separate lesions.[13] ADS- patients who remained monophasic were defined as monophasic ADS-.[13] Patients were re-assessed annually. The patients were instructed to contact the outpatient clinic if new symptoms occurred in order to be clinically assessed. A relapse was defined as new neurological deficits or subacute worsening of existing symptoms after 30 days of improvement or stable disease, without evidence of an alternative diagnosis.[24]

Procedures

Brain and spine MRI scans were performed at 1.5 Tesla scanners. Available T1-, axial T2-, axial and/or sagittal fluid attenuated inversion recovery(FLAIR)-, and T1-weighted images with gadolinium administration were evaluated centrally. The MRI scan closest to the date of symptom onset was evaluated as the baseline scan.

For DIS, all baseline MRI scans were scored using the McDonald 2010 criteria, and the modified components as described in the revised McDonald 2017 criteria for the first MRI scan (*Table 3.1.1*). MRI techniques which are required to reliably demonstrate cortical lesions, such as double inversion recovery, were not part of the routine MRI protocol. Therefore the cortical lesion component was not taken into account in our analyses. If a spinal MRI was performed

within 30 days after or before brain MRI, this scan was taken into account in scoring the DIS components. For DIT, all MRI scans with post-gadolinium T1 images were used, or scans that did not have gadolinium administered, but did not show any FLAIR/T2 hyperintense lesions either. CSF analyses for oligoclonal bands(OCB) were performed in local laboratories using isoelectric focusing.[25] OCB status was considered as positive if there were ≥2 unique bands in CSF compared to serum.

Table 3.1.1: Baseline MRI criteria for MS diagnosis derived from the McDonald 2010 and revised 2017 criteria.

McDonald 2010 criteria for baseline MRI	Revised McDonald 2017 for baseline MRI
DIS	**DIS**
At least 2 out of 4 of: - ≥1 periventricular lesion - ≥1 juxtacortical lesion - ≥1 infratentorial lesion - ≥1 spinal cord lesion (symptomatic brain stem syndromes or spinal cord lesions are excluded)	At least 2 out of 4 of: - ≥1 periventricular lesion - ≥1 juxtacortical **or cortical** lesion - ≥1 infratentorial lesion - ≥1 spinal cord lesion **(asymptomatic and symptomatic brain stem syndromes or spinal cord lesions are included)**
DIT	**DIT**
- Simultaneous presence of asymptomatic gadolinium-enhancing lesions	At least one: - Simultaneous presence of asymptomatic **or symptomatic** gadolinium-enhancing lesions - **Presence of unique CSF oligoclonal bands compared to serum as substitute for DIT**

Changes in the McDonald 2017 criteria compared the 2010 criteria are made bold.

Abbreviations: dissemination in space (DIS), dissemination in time (DIT), cerebrospinal fluid (CSF).

Rationale

We set out to analyze our data in a manner that is representative for clinical practice. As acknowledged by the International Panel on Diagnosis of MS, spinal cord MRI and/or lumbar puncture were not performed in every case depending on clinical presentation and were left to the decision of the local treating physician.[5] We did not exclude patients without a spinal cord MRI or lumbar puncture, as this would probably introduce selection bias in our study.

DIS was based on three parameters(periventricular, juxtacortical, infratentorial), or four including spinal localization if a spinal MRI was performed. We allowed OCB status to contribute to the fulfillment of DIT 2017 in patients with no gadolinium enhancement.

STATISTICAL ANALYSIS

For statistical analyses we used SPSS software, version 24.0 (SPSS Inc.) and GraphPad Prism5. CDMS diagnosis was used as endpoint for all following analyses. For group comparisons, the

Chi-square test and Fisher Exact test were used for categorical data. The Mann-Whitney U test was used for continuous data.

Diagnostic performance and accuracy

Patients fulfilling the diagnostic criteria at time of the first attack with a subsequent diagnosis of CDMS during FU were considered as true positives (TP). False positives (FP) did fulfil the diagnostic criteria for MS at baseline MRI, but were not diagnosed with CDMS during FU. Patients who did not fulfil the diagnostic criteria at baseline MRI and who were not diagnosed with CDMS during FU were considered as true negatives (TN). False negatives (FN) were patients who did not fulfil the diagnostic criteria on baseline MRI, but were diagnosed with CDMS during FU.

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated, including a 95% confidence interval(CI).

Comparison between the sensitivity and specificity for the 2010 and 2017 criteria were made using the McNemar's test. Kaplan Meier analysis was used to analyze the time to CDMS diagnosis using the 2010 and 2017 criteria(log-rank test). Patients who did not meet the criteria for CDMS diagnosis during FU were considered as censored observations. Cox hazard regression analyses were performed to calculate hazard ratios(HR) for time to MS diagnosis based on the McDonald 2010 criteria (applied at baseline), McDonald 2017 criteria (applied at baseline) and CDMS diagnosis. P-values <0.05 were considered significant.

RESULTS

Patient characteristics

The inclusion and exclusion process of eligible ADS children is displayed in *Figure 1*. Among the 164 included patients with a first demyelinating event, 54 patients(33%) presented with encephalopathy (ADS+) and were diagnosed with ADEM. The other 110 (67%) were ADS- at time of inclusion. Of these, 52/110 (47%) were diagnosed with CDMS during FU (median FU time 4.5 years, IQR 2.6-6.6). Median time to CDMS diagnosis was 10.2 months(IQR 3.8-20.7). None of the ADEM patients had a second attack within a median FU of 5.1 years (IQR 2.7-7.8). Patient characteristics and statistical comparisons between monophasic ADS- and CDMS are displayed in *Table 3.1.2*.

Table 3.1.2: Patient characteristics

	ADS- (n=110)	Monophasic ADS- (n=58)	CDMS (n=52)	**ADS+ (n=54)**	**All (n=164)**	**P-value[a]**
Sex, female, n (%)	69 (63)	32 (55)	37 (71)	28 (52)	97 (59)	0.11
Age at onset, median (IQR), years	14.8 (11.3-16.1)	13.3 (9.1-16.0)	15.4 (13.7-16.2)	4.0 (2.6-6.1)	12.1 (5.1-15.8)	<0.004
Age <12 years, n (%)	30 (27)	23 (40)	7 (14)	51 (94)	81 (49)	0.002
Presenting phenotype, n (%)						
- Optic neuritis	36 (33)	23 (40)	13 (25)	0	36 (22)	0.001
- Transverse myelitis	18 (16)	15 (26)	3 (6)	0	18 (11)	
- Other monofocal ADS-	25 (23)	11 (19)	14 (27)	0	25 (15)	
- Polyfocal ADS-	31 (28)	9 (16)	22 (42)	0	31 (19)	
- Polyfocal ADS with encephalopathy (ADEM)	0 (0)	0 (0)	0 (0)	54 (100)	54 (33)	
Spinal MRI, n (%)	61 (56)	34 (59)	27 (52)	14 (26)	75 (46)	.57
Spinal cord lesions present, n (%)	45/61 (74)	23/34 (68)	22/27 (82)	11/14 (79)	56/75 (75)	0.27
Symptomatic spinal cord lesion, n (%)	29/45 (64)	18/23 (78)	11/22 (50%)	7/11 (64%)	36/56 (64)	0.07
Gadolinium administration, n (%)	89 (81)	48 (83)	41 (79)	49 (91)	138 (84)	0.63
Gadolinium enhancement, n (%)	38/89 (43)	12/48 (25)	26/41 (63)	8/49 (16)	46/138 (33)	<0.001
OCB tested, n (%)	86/110 (78)	44/58 (76)	42/52 (81)	33/54 (61)	119 (73)	0.65
OCB present, n (%)	54/86 (49)	17/44 (39)	37/42 (88)	1/33 (3)	55 (34)	<0.001
Time to baseline MRI, median (IQR), weeks	1.6 (0.6-3.4)	1.4 (0.4-3.1)	2.3 (0.8-4.7)	1.4 (0.7-2.4)	1.4 (0.6-3.1)	0.02
Time to lumbar puncture, median (IQR), weeks	2.5 (0.7-9.3)	1.8 (0.4-8.4)	3.3 (1.0-10.1)	1.1 (0.4-2.4)	2.0 (0.7-6.5)	0.11
Time to CDMS, median (IQR), months	10.2 (3.8-20.7)	n/a	10.2 (3.8-20.7)	n/a	n/a	n/a
Follow-up time, median (IQR), years	4.5 (2.6-6.7)	3.4 (2.1-5.2)	6.0 (4.2-7.8)	5.1 (2.7-7.8)	4.6 (2.6-7.1)	<0.001
DMT use, n (%)	63/110 (57)	15/58 (26)	48/52 (92)	1/54 (2)	64 (39)	<0.001
DMT use before CDMS diagnosis, n (%)	14/63 (22)	N/A	14/48 (30)	N/A	14 (9)	N/A
Presence of MOG antibodies, n (%)	5/69 (7)	5/34 (15)	0/35 (0)	16/35 (46)	21/104 (20)	0.03
Presence of AQP4 antibodies, n (%)	0/61 (0)	0/40 (0)	0/21 (0)	0/27 (0)	0/88 (0)	N/A

Patient characteristics for patients with acquired demyelinating syndromes without encephalopathy (ADS-), clinically definite MS (CDMS) and acute disseminated encephalomyelitis (ADEM; ADS+). Abbreviations: oligoclonal bands (OCB), disease modifying treatment (DMT), anti-myelin oligodendrocyte glycoproteins (MOG), anti-aquaporin 4 (AQP4), not applicable (N/A).
In the Dutch pediatric setting, patients DMTs are prescribed when the patient fulfills criteria for MS, either clinically or radiologically.
[a] Comparison between monophasic ADS- and CDMS. Statistical significance p<0.05.

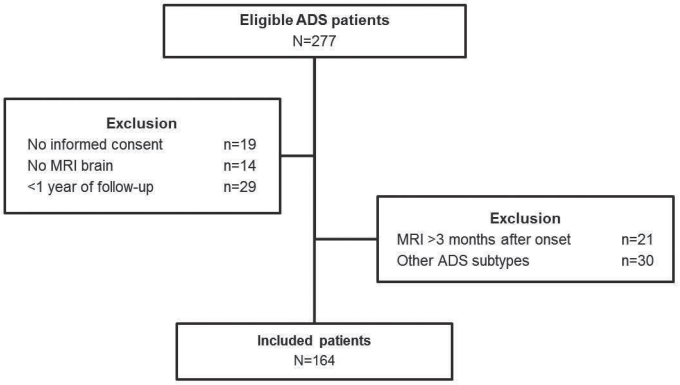

Figure 3.1.1: Flow chart for patient inclusion

DIS and DIT performance on baseline MRI

All test characteristics of analyses with and without children <12 years are presented in *Table 3.1.3*.

Dissemination in space

Hundred and ten ADS- patients were included for this analysis, of whom 52 were diagnosed with CDMS during FU(47%). Spinal MRI was included when available. The 2010 DIS criteria were fulfilled in 54 patients(49%). Of these, 41 were diagnosed with CDMS(76%). Additional 16 patients fulfilled the 2017 DIS criteria (n=70, 64%) and 48 of these 70 patients were diagnosed with CDMS(69%). The 2017 DIS criteria yield an increase in sensitivity of 13% compared to the 2010 criteria(p=0.1), and a loss in specificity of 16%(p=0.008).

A sub-analysis evaluating the fulfillment of DIS in patients who had T1 images with gadolinium administration (n=93) yielded no difference in the results.

Of the 110 ADS- patients, 61 patients (55%) had spinal cord imaging and spinal lesions were detected in 45/61 scans(74%). In a subgroup analysis we only included patients who underwent a spinal scan. The test characteristics for DIS were comparable with the results above.

Dissemination in Time

To evaluate the DIT for both criteria we selected ADS- patients who had T1 images with gadolinium administration (n=93). Of these patients, 41 were diagnosed with CDMS during FU(44%). The 2010 DIT criteria were fulfilled in 35 patients(38%). Of these, 23 were diagnosed with CDMS(66%). Regarding the difference between symptomatic and asymptomatic lesions for the DIT-component, we observed that the 2017 DIT criteria (excluding OCB) yielded 3 more

Table 3.1.3: Test characteristics of the McDonald 2010 and McDonald 2017 criteria.

Patients with ADS- and CDMS, excluding ADEM	DIS 2010 (n=110)	DIS 2017 (n=110)	DIT 2010 (n=93)	DIT 2017 (OCB excluded) (n=93)	DIT 2017 (OCB included) (n=93)	McDonald (DIS+DIT) 2010 (n=93)	McDonald (DIS+DIT) 2017 (OCB excluded) (n=93)	McDonald (DIS+DIT) 2017 (OCB included) (n=93)
Sensitivity %, (95%CI)	79 (65-89)	92 (81-98)	56 (40-71)	63 (47-77)	90 (76-97)	49 (33-65)	61 (45-75)	83 (67-92)
Specificity %, (95%CI)	78 (64-87)	62 (48-74)	77 (63-87)	77 (63-87)	60 (45-73)	87 (74-94)	83 (69-91)	73 (59-84)
PPV %, (95%CI)	76 (62-86)	69 (56-79)	66 (48-80)	68 (51-82)	64 (50-76)	74 (53-88)	74 (55-87)	71 (56-83)
NPV %, (95%CI)	80 (67-89)	90 (75-97)	69 (55-80)	73 (59-84)	89 (72-96)	68 (55-79)	73 (60-83)	84 (70-93)
Accuracy %, (95%CI)	78 (71-86)	76 (69-84)	68 (58-77)	71 (62-80)	73 (64-82)	70 (61-79)	73 (64-82)	77 (69-86)
Patients with ADS- and CDMS <12 years (excluding ADEM)	**n=30**	**n=30**	**n=28**	**n=28**	**n=28**	**n=28**	**n=28**	**n=28**
Sensitivity %, (95%CI)	86 (42-99)	100 (56-100)	71 (30-95)	71 (30-95)	100 (56-100)	57 (20-88)	71 (30-95)	100 (56-100)
Specificity %, (95%CI)	87 (65-97)	78 (56-92)	90 (68-98)	90 (68-98)	81 (57-94)	95 (74-100)	95 (74-100)	91 (68-98)
PPV %, (95%CI)	67 (31-91)	58 (29-84)	71 (30-95)	71 (30-95)	64 (32-88)	80 (30-99)	83 (37-99)	78 (40-96)
NPV %, (95%CI)	95 (74-100)	100 (78-100)	90 (68-98)	90 (68-98)	100 (77-100)	87 (65-97)	91 (69-98)	100 (79-100)
Accuracy %, (95%CI)	87 (75-99)	83 (70-96)	86 (73-99)	86 (73-99)	86 (73-99)	86 (73-99)	89 (78-100)	93 (83-100)

Test characteristics of dissemination in space (DIS) criteria, dissemination in time (DIT) criteria, full McDonald 2010 and McDonald 2017 criteria (DIS+DIT) for CDMS diagnosis in patients without encephalopathy (ADS-). DIT and DIS+DIT: patients were included for analysis if gadolinium was administered or when no T2 lesions were present at baseline MRI. Spinal MRI was used in the evaluation of DIS and DIS+DIT if available. Sub-analyses are presented of patients including acute disseminated encephalomyelitis (ADEM), and patients younger than 12 years old (excluding ADEM)

patients fulfilling DIT criteria (n=38, 41%), of whom 26/38 were diagnosed with CDMS(68%). A major increase in patients fulfilling DIT with the 2017 criteria is caused by allowing OCB to contribute to DIT when no gadolinium enhancement was present: an additional 20 patients fulfilled 2017 DIT at baseline(n=58 patients, 62%). Thirty-seven(64%) of these 58 patients were diagnosed with CDMS.

The test characteristics for 2010 and 2017 DIT criteria, excluding OCB status, were similar. When adding OCB status, the DIT criteria yield 27% in sensitivity (p<0.001), but lost 17% in specificity(p=0.004).*(Table 3.1.3)*

McDonald 2010 vs McDonald 2017 criteria

To evaluate the McDonald 2010 and 2017 criteria we selected ADS- patients who had T1 images with gadolinium administration (n=93). The McDonald 2010 DIS+DIT criteria were fulfilled in 27/93 patients at baseline (29%), of whom 20 were diagnosed with CDMS (74%) after a median FU of 4.5 years (IQR 2.6-7.1).

The McDonald 2017 criteria identified 21 additional patients who fulfilled the criteria at baseline (n=48, 52%) compared to the 2010 criteria, and 34/48 patients (71%) were diagnosed with CDMS. The sensitivity was higher in the McDonalds 2017 criteria (83% vs 49%; p<0.001) and the specificity was lower (73% vs 87%; p=0.02)(*Table 3.1.3*).

The seven patients who caused the loss in specificity were identified (fulfilling the 2017 criteria and not the 2010 criteria at baseline, but not having a second attack during a FU of median 2.7 years (IQR: 1.2-6.5)). New lesions on subsequent MRI were observed in 4 out of these 7 patients during FU. The other 3 patients did not undergo a second MRI.

Of the 41 patients diagnosed with CDMS, the McDonald 2010 criteria led to the identification of 20 patients (49%) at baseline. A second attack occurred in 17/20 (85%) within 3 years of FU and 19/20 (95%) within 5 years. With the McDonald 2017 criteria 34/41 (83%) CDMS patients were identified at baseline. At 3 and 5 years of FU, 31/34 (91%) and 33/34 (97%) had a second attack within 3 and 5 years.

Only one patient who fulfilled the 2010 and 2017 diagnostic criteria at baseline did not have a second attack (CDMS) within 5 years of FU, yet this patient showed new MRI lesions on FU scans.

The survival curves for CDMS diagnosis, the McDonald 2010 criteria and the revised McDonald 2017 criteria on baseline MRI are presented in *Figure 2*. MS diagnosis could be made earlier in ADS patients using the 2017 than the 2010 criteria. For both criteria the hazard ratios for the DIS, DIT and full criteria at baseline are displayed in *Table 3.1.4.*

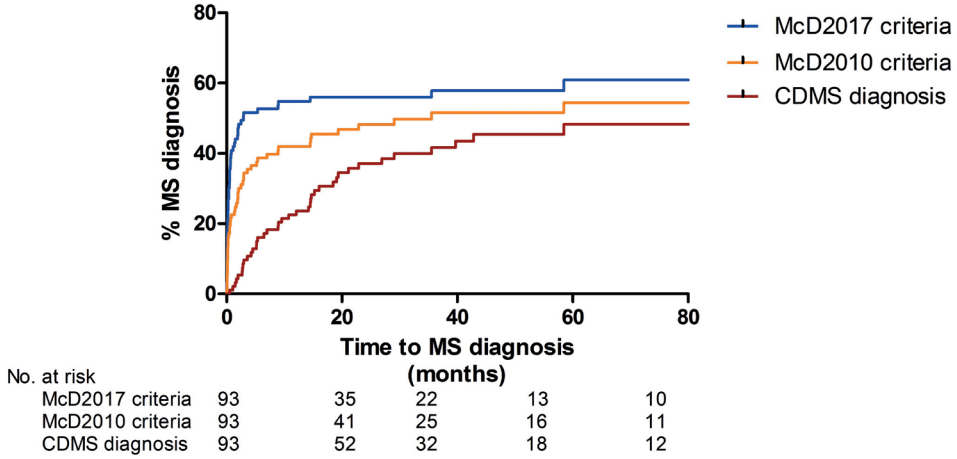

Figure 3.1.2: Time from incident ADS event to MS diagnosis

Survival curves for time from incident ADS event to MS diagnosis according to the McDonald 2010 (at baseline), McDonald 2017 (at baseline) criteria and CDMS. Abbreviations: acquired demyelinating syndromes (ADS), clinically definite multiple sclerosis (CDMS).

Contribution of symptomatic enhancing lesions, OCB and spinal cord imaging

As described before, the McDonald 2017 criteria identified 21 additional patients(n=48) compared to the 2010 criteria (n=27).

These 21 patients were identified with the contribution of two major changes in the criteria. First, symptomatic lesions can be included to demonstrate DIS and DIT for the McDonald 2017 criteria. This led to 7 more MS diagnoses (n=34) at baseline compared to the 2010 criteria (n=27). Of these 34 patients, 25 were diagnosed with CDMS (74%).

Table 3.1.4: Hazard ratios for CDMS diagnosis after applying DIS, DIT and full criteria (DIS+DIT) for McDonald 2010 and 2017 on baseline MRI.

	McDonald 2010	p-value	McDonald 2017	p-value
DIS (n=110)	HR 5.8 (95% CI 3.0-11.4)	p<.001	HR 11.3 (95% CI 4.0-31.6)	p<.001
DIT (n=93)	HR 2.9 (95% CI 1.5-5.4)	p=.001	HR 8.3 (95% CI 3.0-23.4)	p<.001
DIS+DIT (n=93)	HR 3.1 (95% CI 1.7-5.8)	p<.001	HR 8.5 (95% CI 3.5-20.4)	p<.001

Abbreviations: clinically definite multiple sclerosis (CDMS), dissemination in space (DIS), dissemination in time (DIT)

Second, OCB are allowed to be taken into account when assessing DIT. This led to the other 14 patients who fulfilled the McDonald 2017 criteria at baseline (total n=48) and caused the major increase in sensitivity of the criteria. Among these 48 patients, 34 (71%) were diagnosed with CDMS.

No additional patients fulfilled the McDonald 2010 criteria depending on spinal imaging. In contrast, the presence of a spinal cord lesion contributed to fulfilling McDonald 2017 at baseline in 6 patients. Three of these patients were eventually diagnosed with CDMS. Thus, 3/41 (7%) patients with future CDMS fulfilled the DIS component at baseline by performing spinal cord imaging.

Relevant subgroup analyses

DMT use before CDMS

DMTs have the potency to postpone a second attack, and could therefore influence CDMS diagnosis. We performed a subgroup analysis after excluding patients who received DMT before CDMS diagnosis (exclusion n=12). In the included patients (n=81), test characteristics for the full criteria (DIS+DIT) remained comparable with the total group (including DMT use before CDMS) for both the 2010 (sensitivity 41% vs 49%; specificity 87% for both selections) and 2017 criteria (sensitivity 83% and specificity 73% for both selections).

Analysis in ADS- patients <12 years old

The IPMSSG recommended that the McDonald 2010 criteria at baseline should not be used in patients <12 years. We applied the novel 2017 criteria to the group of patients below age 12 only, after exclusion of ADEM cases.

Seven of 28 ADS- patients (25%) were diagnosed with CDMS. Five patients (18%) fulfilled baseline criteria for 2010, 4 of them (80%) fulfilled the criteria for CDMS. Nine patients fulfilled the 2017 criteria (32%), and 7 (78%) were diagnosed with CDMS.

The test characteristics were even better in children <12 years old than in the total group, for both McDonald 2010 criteria (sensitivity 57% vs 49%; specificity 95% vs 87%) and 2017 criteria(sensitivity 100% vs 83%; specificity 91% vs 73%).

Analysis in ADEM subgroup

Only ADEM patients are included in this analysis (n=54). DIS was fulfilled for the 2010 criteria and 2017 criteria in respectively 24 (44%) vs 28 ADEM patients (52%).

Regarding DIT, 7/49 (14%) ADEM patients fulfilled the 2010 DIT criteria and 9/49 (18%) fulfilled the 2017 DIT criteria, including OCB status. 4/49 (8%) of the patients would have fulfilled the McDonald 2010 criteria, and 5/49 (10%) the McDonald 2017 criteria(gain of 1 patient due to OCB). None of the patients fulfilled the criteria for CDMS during FU.

DISCUSSION

We investigated the application of the novel McDonald 2017 criteria for pediatric MS in clinical practice. We show that the McDonald 2017 criteria have a higher sensitivity than the previous 2010 criteria for CDMS diagnosis for pediatric patients (83% vs 49%, p<0.001). However, the specificity was lower (73% vs 87%, p=0.02). Overall the diagnostic accuracy of the 2017 criteria was higher than for the 2010 criteria (77% vs 70%). The revised criteria are easier to apply than the McDonald 2010 criteria, mainly because of the major change of accepting all lesions to contribute to DIS and DIT, without taking the clinical symptoms into account. Moreover, we show that the 2017 McDonald criteria lead to more MS diagnosis at baseline, therefore MS diagnosis can be made earlier using the 2017 criteria.

A high sensitivity is important to start DMT as soon as possible.[26] which might lead to overtreatment in the group of patients who have a less active clinical disease course. Our main findings are supported by a recent extensive study in a cohort of ADS patients that also included evaluation of the applicability of the McDonald 2017 criteria.[15] We validate their finding that the sensitivity is increased and specificity is decreased mainly by including OCB status into the 2017 criteria. However, specificity of the McDonald 2017 criteria in our study is somewhat lower. This is probably due to our study design by taking CDMS, a more clinical primary endpoint instead of new T2 lesions on a second MRI. We identified a minor subgroup of 7 patients that was responsible for the loss in specificity, who had a FU duration of 2.3 years. New lesions on subsequent MRI were observed in 4 of these patients. The other 3 patients did not undergo a second MRI. However, given the presence of typical MS lesions at baseline and OCB positivity in these 3 patients, it is quite likely that these patients would also have developed new MRI lesions after a longer FU. Taken together, we believe that initiation of DMT based on the novel criteria is warranted. Yet, clinicians should be aware that with the McDonald 2017 criteria, more patients will be identified at baseline, and that a proportion of these patients will clinically have a less active disease course.

Performing spinal MRI led to fulfillment of the McDonald 2017 criteria at baseline in 3/41 (7%) CDMS patients, but did not have additional value in the McDonald 2010 criteria, in line with another study that indicated limited value of spinal cord imaging in the McDonald 2010 criteria in children.[6] Fadda et al argued whether spinal cord acquisition would meaningfully add to the performance of the McDonald 2017 criteria, as only a real small proportion of their patients fulfilled the criteria based on spinal cord MRI. [15] However, in our cohort 7% of the CDMS patients could have been identified at baseline, which could significantly reduce the time to diagnosis in these patients. The exact place of spinal cord imaging as part of the MS diagnostic procedure deserves further investigation.

Our data shows that about 10% of the ADEM patients fulfill the McDonald 2010 and 2017 criteria at baseline. However, no ADEM patient was diagnosed with CDMS in our cohort. The IPMSSG 2012 criteria explicitly mention not to apply the McDonald 2010 criteria to patients with ADEM. Our data supports this view for the McDonald 2017 criteria, in order to prevent incorrect initiation of treatment in these monophasic patients.

Regarding age, ADS- patients <12 years seemed to have better accuracy for the McDonald 2017 criteria than the total group (sensitivity 100% vs 90%, specificity 81% vs 60%, PPV both 64%, NPV 100% vs 89%), despite the small sample size for this analysis. This implies that both the McDonald 2010 criteria and McDonald 2017 criteria can be used across the age span, including children <12 years old with ADS, excluding patients with ADEM, in keeping with results from previous studies. [12,15,27]

Our study has several limitations. The choice of brain and/or spinal MRI, inclusion of contrast or not, and the decision to include testing for CSF oligoclonal bands, were left to the discretion of the treating physician. For example, spinal imaging is not always justified (sedation may be needed), CSF is not always tested in isolated optic neuritis and a paired serum sample is not always available with CSF (e.g. in case of exclusion of suspected infection). Therefore, like in other studies on the diagnostic criteria for pediatric MS, there was not complete coverage of all potentially relevant parameters. However, our main goal was to evaluate the revised 2017 criteria in real world data, therefore we did not exclude patients from the analyses. Instead a few sub analyses have been performed. Despite our considerable FU duration of median 4.6 years, it is possible that some patients are going to develop a second attack in the future. DMT could have postponed CDMS diagnosis, however our sub analysis excluding these patients showed no differences in test characteristics. Second, the PROUD-kids study did not have a standardized MRI follow-up, therefore we did not take FU MRIs into account for the analysis of the 2010 and 2017 criteria. However, the scope of this article was to analyze the diagnostic accuracy for CDMS at first attack of ADS.

In conclusion, our data suggests that the McDonald 2017 criteria perform well in children. MS diagnosis can be made earlier and leads to a higher number of MS patients at baseline. Both the McDonald 2010 and 2017 criteria show similar results for the patients <12 years presenting with ADS, and can therefore be applied in this population as well. As proposed by the IPMSSG for the McDonald 2010 criteria, application of both 2010 and 2017 criteria in ADEM patients should be avoided.

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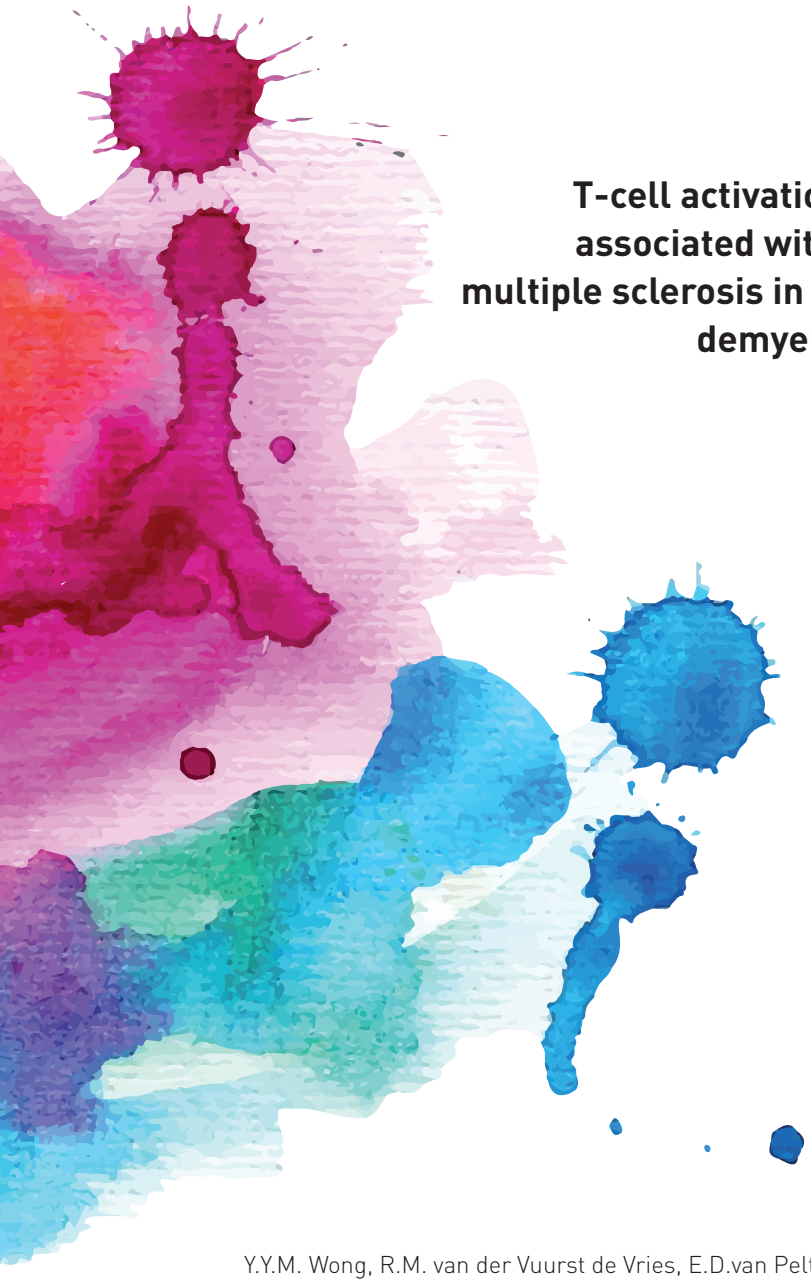

3.2

T-cell activation marker sCD27 is associated with clinically definite multiple sclerosis in childhood acquired demyelinating syndromes

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ABSTRACT

Background

Cerebrospinal fluid (CSF) levels of T-cell activation marker soluble CD27 (sCD27) are associated with subsequent disease activity after a first attack of suspected MS in adults. The predictive value for disease course in children with acquired demyelinating syndromes (ADS) is unknown.

Objectives

To assess the predictive value of sCD27 levels for clinically definite MS (CDMS) diagnosis in childhood ADS.

Methods

Children <18 years with a first demyelinating event were prospectively included and followed. sCD27 was determined in CSF using an enzyme-linked immunosorbent assay (ELISA). Cox regression analyses were used to calculate hazard ratios (HR) for CDMS.

Results

Ninety-four ADS children were included (ADS with encephalopathy (ADS+) n=33 and ADS without encephalopathy (ADS-) n=61). Twenty-nine of 61 ADS- children(48%) were diagnosed with CDMS during follow-up. At baseline, sCD27 levels were higher in patients with a future CDMS diagnosis (n=29) than in monophasic ADS+(n=30), monophasic ADS- (n=28) and relapsing non-MS patients (n=7)($p<0.001$). In ADS- patients, sCD27 was associated with CDMS (HR 1.8 per 100 U/mL increase in sCD27 levels, $p=0.031$), after adjustments for age, oligoclonal bands and presence of dissemination in space on baseline MRI.

Conclusion

CSF sCD27 levels at first attack of demyelination is associated with CDMS diagnosis in children. This makes sCD27 a potential clinically relevant quantitative marker when performing routine CSF diagnostics.

INTRODUCTION

Clinical manifestations of acute onset inflammatory demyelinating disease of the central nervous system (CNS) in children are termed acquired demyelinating syndromes (ADS).[1,2] ADS encompasses for example optic neuritis (ON), transverse myelitis (TM) as well as other presentations that localise to monofocal or polyfocal locations in the CNS, such as acute disseminated encephalomyelitis (ADEM). Up to one third of the children with ADS receive a later diagnosis of multiple sclerosis (MS).[1,3,4 5] At the time of a first attack, it can be a challenge to determine the disease course of these patients. Early identification of children who will have an active disease course is important and can have therapeutic implications.[6]

Soluble CD27 (sCD27) is a soluble form of CD27 secreted by activated T-cells after activation via the T-cell receptor, and is introduced as a potential biomarker for T-cell mediated inflammation.[7] CD27 and sCD27 have a role in maturation, activation and proliferation of T- and B-cells.[8,9] High sCD27 levels are reported in autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus and MS.[10-12] Komori et al, validated CSF sCD27 as a biomarker for intrathecal T-cell activation in MS, using an extensive and validated battery of biomarkers for CNS inflammation.[13] Serum sCD27 does not discriminate between healthy individuals and MS patients.[14] In a recent study, high sCD27 levels in CSF associate with MS diagnosis and disease course in adult patients with clinically isolated syndromes.[15]

These observations in adults have not yet been validated in the paediatric population with CNS demyelination. It is shown that children with MS tend to have a more inflammatory disease course than adults.[16,17] Therefore we hypothesized that the predictive value of sCD27 levels for a second attack of MS will be equal or even higher in children than in adults. Furthermore, ADS with encephalopathy (ADEM) are known to have extensive intracerebral inflammation on MRI scans and may have severe clinical presentations.[18] The levels of sCD27 might therefore differ between ADS subtypes.

Here we examined whether sCD27 levels at first attack in children differ between ADS subtypes and assessed the predictive value of sCD27 for a second attack of MS in paediatric ADS patients.

METHODS

Study participants

Patients <18 years were included in the Dutch prospective and multicentre study for children with acquired demyelinating syndromes (ADS) (PROUD-kids study). [2] All patients with a lumbar puncture and baseline MRI, performed for routine diagnostics <6 months after onset of first symptoms, were included between June 2006 and February 2017. Patients with alternative

diagnosis were excluded. Patients were assessed at baseline and reassessed regularly. Patients were instructed to contact the hospital in case of suspected exacerbation.

Definitions

Acquired demyelinating syndromes in children encompass the first attack of demyelination in the central nervous system, including patients presenting with encephalopathy (ADEM, defined as ADS+) and ADS without encephalopathy (defined as ADS-).[2 3] CDMS was defined as two non-encephalopathic attacks, based on the clinical criteria proposed by the International Pediatric MS study group for paediatric MS diagnosis.[3] Relapsing patients who have a distinct clinical phenotype other than CDMS were also included in this study, such as ADEM followed by relapsing optic neuritis (ADEM-ON)[19], anti-aquaporin 4 antibody (AQP4-IgG) positive and negative relapsing disease.[3,20,21]

A relapse was defined as acute worsening of existing symptoms or new symptoms after 30 days of improvement or stable disease and no evidence of an alternative diagnosis. The symptoms should exist for more than 24 hours and not be preceded by fever.[22] Exacerbations were confirmed by neurological examination.

Follow-up (FU) duration was calculated by subtracting the date of first symptoms from the last visit date. Disability was expressed by the Expanded Disability Status Scale (EDSS).[23]

CSF samples and sCD27 ELISA

CSF samples were centrifuged for 10 minutes at 3000 rpm to separate the supernatant from cells and cellular components. After centrifugation, all samples were stored in -80 degrees Celsius until use.[24] Routine diagnostics of CSF included OCB, IgG index, cell count and total protein. Soluble CD27 levels were measured in duplo using the available commercial ELISA kit (Pelikine compact human sCD27 kit) manufactured by Sanquin in Amsterdam, the Netherlands.[7] The manufacturer's instructions were followed when performing the sCD27 ELISA. Levels of sCD27 were expressed by units/mL by reference to a standard curve supplied with the ELISA kit. The clinical diagnosis was blinded for the analysts who performed the ELISA. The detection limit of the ELISA was 6 units/mL.

Standard protocol approvals, registrations and patient consents

The PROUD-kids study was approved by the Erasmus MC ethical committee and by the ethical committees of the other participating centres. Written informed consent was obtained from patients and/or their families.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS 24.0. Kolmogorov-Smirnov test was used to assess the normality of the data. Figures are made in Graphpad Prism5. Soluble CD27 levels were not normally distributed, and were therefore log transformed to attain normally distributed data. Due to log-transformation, geometric means were calculated. For group comparisons, Student's t-test and Mann-Whitney U test were used for continuous variables when appropriate. Student's t-test was performed to compare the sCD27 levels in different ADS subgroups. Chi-square and Fisher exact test were used for categorical data. Correlation analyses were done for two continuous variables.

Cox proportional hazard regression models were used to calculate univariate and multivariable hazard ratios (HR) in the ADS- group, with CDMS set as endpoint. The Cox proportional hazard assumption was tested by including a time dependent covariate in the model. Known predictors for MS diagnosis such as age of onset, OCB and fulfilling dissemination in space (DIS) at baseline MRI are used for adjustments in the multivariable analysis for sCD27 levels.

Annualised relapse rate (ARR) for CDMS patients was compared between groups with high and low levels of sCD27 using a binomial regression model with the natural logarithm of number of FU years after a second clinical attack as offset. This offset corrects for the difference in FU duration between patients. The data were overdispersed and therefore the Poisson regression model was not suitable for our data set. P-value of <0.05 was considered significant.

RESULTS

Patients characteristics

A total of 94 children with a first attack of ADS were included in this study. Of these children, 33 presented with ADS+ and 61 with ADS-. The median age for ADS+ was 4.5 years (IQR 2.6-6.3) and for ADS- patients 14.5 years (IQR 11.3-16.0). During follow-up, 30/33 (91%) of the ADS+ patients remained monophasic. Three ADS+ patients (9%) had a relapsing disease and fulfilled the criteria for ADEM-ON. No patient presenting with ADS+ was diagnosed with CDMS. Within the ADS- patients, 33/61 (54%) had a second attack. Of these 33 relapsing patients, 29/33 (88%) children were diagnosed with a second attack fulfilling the criteria for CDMS and the other 4/33 (12%) were diagnosed with a relapsing demyelinating disorder other than MS (2 AQP4-IgG positive and 2 AQP4-IgG negative patients). The median time to CDMS was 10.3 months (IQR 4.3-15.7). The median FU duration for all included patients was 2.5 years (IQR 1.4-4.9).

The following flowchart *(Figure 3.2.1)* illustrates the presenting phenotypes (ADS+ and ADS-) and diagnoses during follow-up.

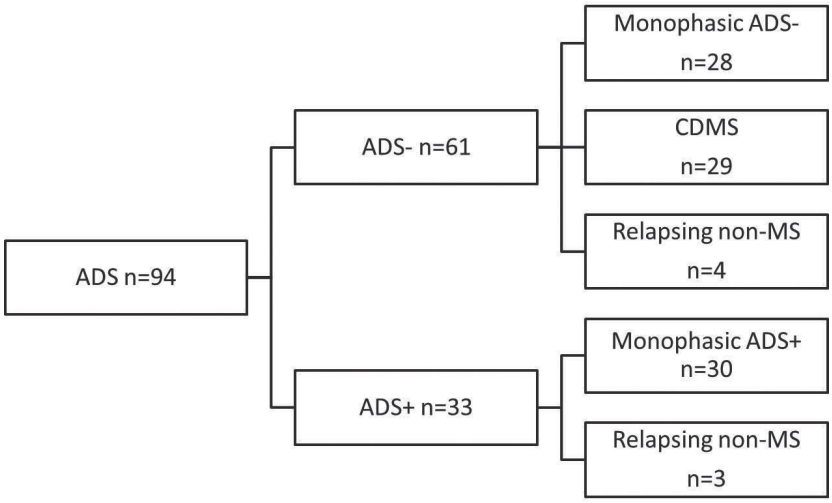

Figure 3.2.1: Flowchart of included ADS patients.
Patients presented as ADS without encephalopathy (ADS-) and ADS with encephalopathy (ADS+; ADEM). The disease course during follow-up are shown dividing patients in monophasic ADS-, monophasic ADS+, CDMS and relapsing non-MS. The relapsing non-MS patients included 3 ADEM followed by optic neuritis, 2 AQP4 positive NMOSD and 2 patients with AQP4 negative NMOSD. *Abbreviations: acquired demyelinating syndromes (ADS), ADS without encephalopathy (ADS-), ADS with encephalopathy (ADS+), acute disseminated encephalomyelitis (ADEM), anti-aquaporin 4 antibodies (AQP4), clinically definite MS (CDMS).*

Table 3.2.1: Patient characteristics

Characteristic	Mono- ADS+ (n=30)	Mono-ADS- (n=28)	CDMS (n=29)	Relapsing non-MS (n=7)	All (n=94)	p-value[a]
Female, no. (%)	20 (67)	13 (46)	19 (66)	4 (57)	56 (60)	0.380
Age, median (IQR), years	4.1 (2.5-6.1)	11.7 (6.2-16.0)	15.1 (13.8-16.0)	10,7 (6.0-16.3)	11.3 (5.1-15.2)	<.001
Follow-up time, median (IQR), years	3.7 (1.3-6.1)	2.1 (1.1-3.9)	2.6 (1.8-4.6)	2.3 (1.2-4.3)	2.6 (1.4-4.9)	0.343
Clinical presentation, no.(%)						
- Isolated optic neuritis no.(%) Isolated bilateral ON, no.(%)	0 N/A	7(25) 2/7 (22)	7(24) 0	0 N/A	14 (15) 2/14 (14)	0.940[b]
- Isolated transverse myelitis no.(%) Isolated LETM, no.(%)	0 N/A	8(29) 4/8 (50)	7(24) 0	1(14) 1/1 (100)	16(17) 5/16 (31)	0.704[b]
- Other CIS, no.(%)	0	6 (21)	3 (10)	1 (14)	10 (11)	0.251[b]
- Polyfocal CIS, no.(%) LETM and ON, no.(%)	0	7 (25) 3/7 (60)	12 (41) 0	2 (29) 2/2(100)	21(22) 5 (5)	0.190[b]
- Polyfocal CIS with encephalopathy, no.(%)	30 (100)	0	0	3 (43)	26 (37)	N/A[b]
CSF OCB, (≥ 2 bands), no.(%) (n=78)	0/21	14/24 (58)	25/27 (93)	0/6	39/78 (48)	<0.001
IgG index, median (IQR) (n=67)	0.57 (0.53-0.74)	0.57 (0.53-0.77)	1.11 (0.88-1.84)	0.63 (0.52-0.66)	0.72 (0.56-1.02)	<0.001
CSF WBC count, median (IQR) (n=90)	22 (7-48)	5 (3-10)	14 (7-28)	43 (9-77)	10 (5-35)	0.491
- % of CSF mononuclear WBC, median (IQR) (46/90)	77 (55-95)	75 (45-90)	100 (90-100)	90 (70-95)	90 (65-100)	0.004
Time from symptom onset to CSF sampling, median (IQR), weeks	1.6 (0.6-2.8)	1.3 (0.4-5.9)	5.0 (2.4-12.9)	2.6 (1.3-10.6)	2.3 (0.7-5.9)	<.001
CSF sampling prior to acute treatment, no.(%)	23 (77)	21 (75)	23 (79)	3 (43)	70 (75)	0.249

[a]Comparison between all subgroups. [b]Comparison between monophasic ADS- and CDMS. *Abbreviations: monophasic acquired demyelinating syndromes with encephalopathy (mono-ADS+), monophasic acquired demyelinating syndromes without encephalopathy (mono-ADS-), clinically definite multiple sclerosis (CDMS), relapsing ADS not diagnosed as MS (relapsing non-MS), optic neuritis (ON), clinically isolated syndrome (CIS), longitudinally extended transverse myelitis (more than 2 segments involved; LETM), oligoclonal bands (OCB), interquartile range (IQR), white blood cell (WBC), not applicable (N/A)*

The median time between onset of symptoms and CSF sampling was 2.2 weeks (IQR 0.7-5.9). No correlation was found between the levels of sCD27 and time between first symptoms and CSF sampling in the total group and in all groups separately. Twenty-four out of the 94 patients (26%) received acute treatment (intravenous corticosteroids) before CSF sampling. No difference in sCD27 was found in patients who did or did not receive intravenous corticosteroids. No patients were on disease modifying therapy (DMT) or oral steroids before CSF sampling. Patient characteristics are shown in *Table 3.2.1*.

Soluble CD27 levels in subgroups of ADS

Soluble CD27 levels at first attack of ADS were higher in patients with a future second attack of MS (n=29) than in ADS-patients who remained monophasic (n=28) (geometric means 65 U/mL; 95% CI 47-89 vs 13 U/mL; 95% CI 9-18, p<0.001). Patients with monophasic ADS+ (n=30) did not differ in sCD27 levels from monophasic ADS- patients (n=28) (geometric mean 18 U/mL; 95% CI 11-30 vs 13 U/mL; 95% CI 9-18), but did differ from ADS- patients with future CDMS diagnosis (geometric mean 13 U/mL; 95% CI 9-18 vs 65 U/mL; 95% CI 48-89; p<0.001). Patients with a relapsing non-MS disease course (n=7; 4 patients with ADS- onset and 3 with ADS+ onset) had lower sCD27 levels at onset than patients with a future CDMS diagnosis (geometric mean 18 U/mL; 95% CI 8-42 vs 65 U/mL; 95% CI 48-89 U/mL; p=0.001), but did not differ from monophasic ADS+ and monophasic ADS- patients. These results are displayed in *Figure 3.2.2*. No differences were found in sCD27 levels between anti-MOG positive (n=13) and anti-MOG negative (n=81) patients (data not shown).

Fourteen of the 59 ADS- patients (24%) (after excluding the 2 AQP4 positive patients) fulfilled MS diagnosis at baseline by fulfilling the IPMSSG 2012 criteria for MS on first MRI.[3] The sCD27 levels of these 14 children are significantly higher than ADS- patients who did not fulfil the criteria at baseline (geometric mean 78 U/mL; 95% CI 53-115 vs 20 U/mL; 95% CI 15-30, p<0.001).

Ten out of the 28 monophasic ADS- patients showed sCD27 levels that exceed the upper bound of the 95% confidence interval of the geometric mean (13 U/mL; 95% CI 9-18), as shown in *Figure 3.2.2*. Of these patients, 8/10 fulfilled the diagnosis of MS by follow-up MRI scans, but did not experience a second attack during FU. The sCD27 geometric mean of these 8 patients was higher than the other 20 monophasic ADS- patients (geometric mean 35 U/mL; 95% CI 27-45 vs 9 u/mL; 95% CI 6-12; p<0.001), but lower than the patients with future CDMS diagnosis (geometric mean 35 U/mL; 95% CI 27-45 vs 65 U/mL; 95% CI 48-89 U/mL; p=0.04).

A subgroup analysis (n=59) was performed after excluding patients with <2 years of FU (patients with CDMS n=20). This did not change our observation that sCD27 levels are elevated in patients with a future second attack of MS compared to the three other subgroups (geometric mean 61 U/mL, 95% CI 42-89 vs 15 U/mL, 95% CI 10-22; p<0.001).

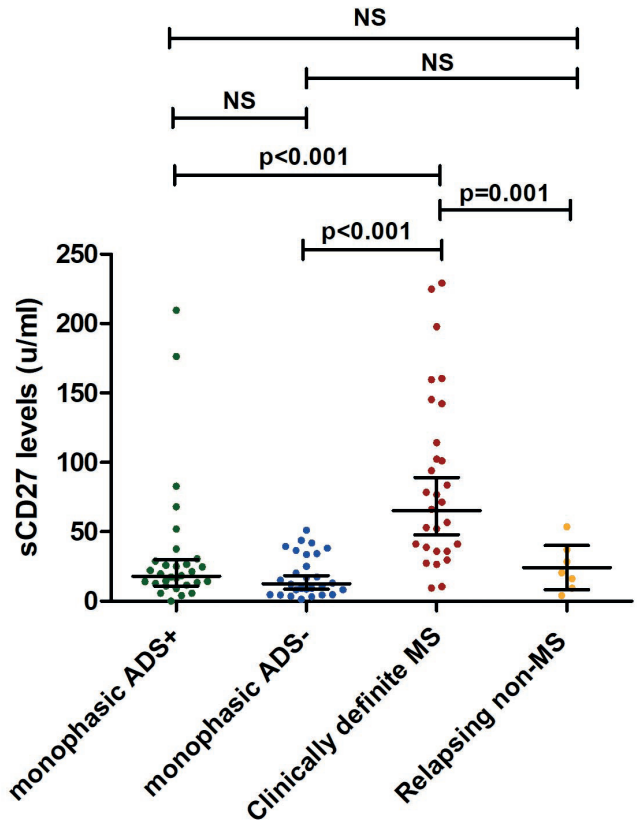

Figure 3.2.2: Comparison of CSF sCD27 levels in ADS at time of first demyelinating event
Comparison of CSF soluble CD27 levels between patients with ADS+ (ADEM), monophasic ADS-, CDMS and relapsing non-MS patients. The relapsing non-MS patients included 3 ADEM followed by optic neuritis, 2 AQP4 positive NMOSD and 2 patients with AQP4 negative NMOSD. Horizontal lines with error bars indicate geometric means with 95% CI. *Abbreviations: acquired demyelinating syndromes (ADS), ADS without encephalopathy (ADS-), ADS with encephalopathy (ADS+), acute disseminated encephalomyelitis (ADEM), clinically definite MS (CDMS).*

Soluble CD27 correlates with CSF and MRI parameters

Patients with OCB (n=39) had higher sCD27 levels compared to patients without OCB (geometric means 42 U/mL; 95% CI 30-60 vs 17 U/mL; 95% CI 12-23, p<0.001). Soluble CD27 levels were positively correlated with IgG index (Spearman rho 0.695, p<0.001) as well as white blood cell count (Spearman rho 0.444, p<0.001).

Patients fulfilling DIS on baseline MRI (n=50) showed higher sCD27 levels than patients without DIS (geometric mean 35 u/mL; 95% CI 26-46 vs 16 u/mL; 95% CI 11-24, p=0.003).

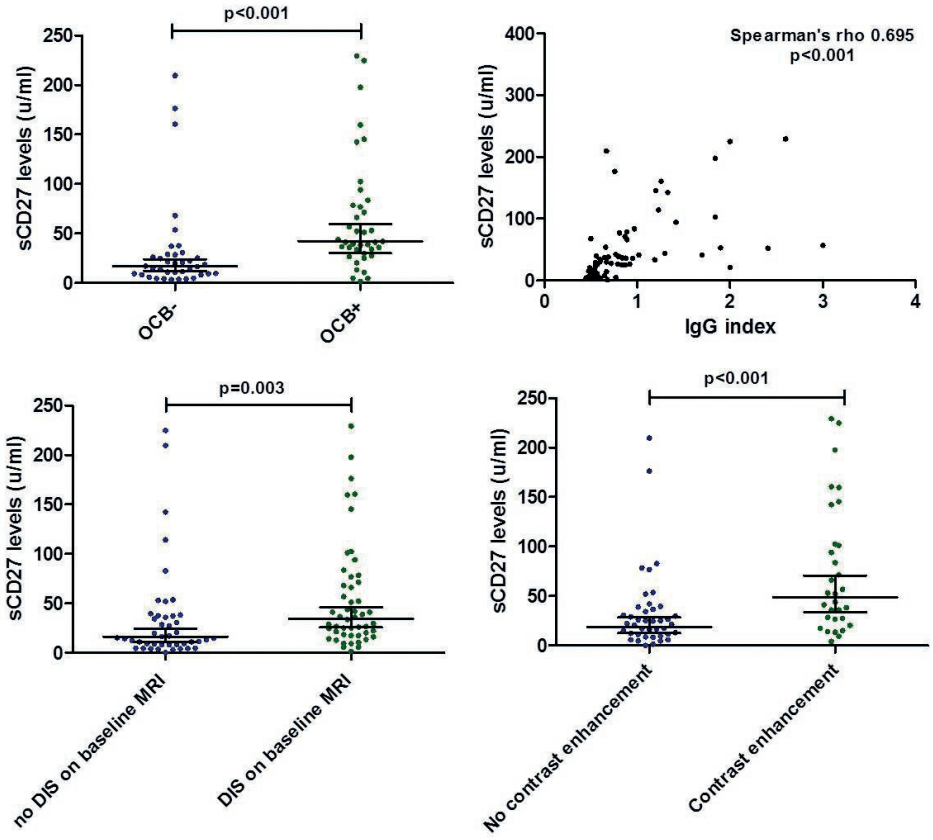

Figure 3.2.3: Comparison of sCD27 levels with CSF and MRI parameters
Comparing CSF and MRI parameters with the levels of sCD27 in ADS patients. Horizontal lines with error bars indicate geometric means with 95% CI. *Abbreviations: acquired demyelinating syndromes (ADS), oligoclonal bands (OCB), dissemination in space (DIS)*

Seventy-two patients (77%) received gadolinium when the first MRI was performed. The sCD27 levels were significantly higher in children who showed enhancement (n=31, 58%) than patients without enhancement (geometric mean 49 U/mL; 95% CI 34-71 vs 19 U/mL; 95% CI 12-28, p=0.001).

For the analyses depicted above, all patients were analysed. Exclusion of patients with ADS+ (ADEM) and relapsing non-MS from the analysis (thus only including patients with monophasic ADS- and CDMS), did not alter the results. Results are shown in *Figure 3.2.3*.

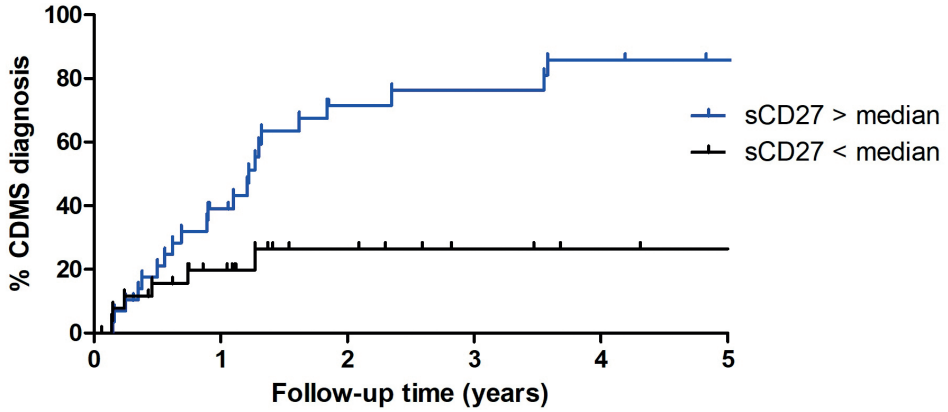

Figure 3.2.4: Kaplan Meier curve for time to CDMS diagnosis in ADS without encephalopathy
Kaplan Meier curve for time to CDMS diagnosis. All ADS- patients were stratified into two groups by the median CSF sCD27 level of all CIS patients; median 36 u/mL. *Abbreviations: acquired demyelinating syndromes (ADS), ADS without encephalopathy (ADS-), clinically definite MS (CDMS).*

High levels of sCD27 at first attack in ADS- patients are independently associated with a shorter time to CDMS.

As described above, all patients with a second attack of MS had an ADS- presentation. No patient with ADS+ was diagnosed with CDMS. Therefore, in the following analyses ADS+ patients were excluded as well as the relapsing non-MS patients with AQP4-IgG.

A Kaplan Meier curve was obtained after dichotomizing the sCD27 levels, using the median of the included ADS- patients (n=59); median sCD27 36.0 U/ml. Six out of 29 patients in the low sCD27 group were diagnosed with CDMS (21%) versus 23/30 (79%) in the high level group. In *Figure 3.2.4* shows the Kaplan Meier curve for time to CDMS diagnosis (Log rank test, p=0.006).

The univariate HR for CDMS was 2.8 (95% CI 1.7-4.6) per 100 U/mL increase in sCD27 levels (p<0.001). In the multivariable COX regression analyses, we corrected for age of onset, OCB and dissemination in space (DIS). After these corrections, sCD27 was independently associated with time to CDMS diagnosis with an HR of 1.8 (95% CI 1.0-3.3) per 100 U/mL increase in sCD27 levels (p=0.031).

Eight out of 29 patients (28%) who received MS diagnosis based on MRI received DMT before CDMS and this might have postponed the second attack. We performed a sub-analysis using the COX-regression analysis where we excluded these patients, resulting in the same HR for the univariate (2.8, 95% CI 1.7-4.9; p<0.001) and multivariable analyses after adjusting for age of onset, OCB and DIS (1.8, 95% CI 0.96-3.4; p=0.061).

Annualised relapse rate and disability

In patients who were diagnosed with CDMS, we used a negative binomial regression model for analyzing the ARR. The median of sCD27 levels in CDMS patients (n=29; 71 U/mL) was used to stratify patients into two groups with either high or low sCD27 levels. There was no difference found in ARR between high and low level group after correcting for follow-up duration. No correlation was found between sCD27 levels and EDSS score during follow-up.

DISCUSSION

Here we show that the T-cell activation marker sCD27 in CSF at first attack in paediatric ADS differs among ADS subtypes. Soluble CD27 levels in patients with a future diagnosis of CDMS were higher than in monophasic ADS+, monophasic ADS- and relapsing non-MS patients. There was no difference between the latter three groups. We analyzed ADS+ patients separately from ADS- patients as MS diagnoses after ADS+ is extremely low and also in our cohort no patients with ADS+ were diagnosed with CDMS during follow-up. Soluble CD27 was associated with a shorter time to CDMS diagnosis independently of known relevant clinical parameters such as MRI and CSF characteristics.

An interesting observation was that 80% of the monophasic ADS- patients, whose sCD27 levels exceeded the 95% CI intervals of the whole monophasic ADS- group, fulfilled the IPMSSG diagnostic criteria for MS based on FU MRI. The sCD27 levels of this group were higher than monophasic ADS- and somewhat lower than patients with CDMS diagnosis. This may be related to the fact that these patients (who are diagnosed with MS by MRI alone) have a less active clinical disease course, compared to CDMS patients with higher levels of sCD27.

In line with Van der Vuurst de Vries et al, we observed higher soluble CD27 levels in patients with a future CDMS diagnosis than in monophasic ADS patients without encephalopathy (including clinically isolated syndromes).[15] The levels were even higher in children than in adults with MS (geometric mean 65 U/ml; 95% CI 48-89 versus 42 U/mL; 95% CI 29-51 respectively).[15] Our data not only validates the conclusion of Van der Vuurst et al, but is also congruent with previous observations that paediatric MS patients have a more inflammatory disease course compared to adults. [16,25-30]

The higher levels in patients with a future CDMS diagnosis most likely correspond to a higher intrathecal T-cell activation and higher inflammatory activity. The correlation we found between sCD27, OCB and IgG index is in line with previous adult studies. [13-15] In vitro, a functional role of sCD27 on stimulation and differentiation of B-cells is described earlier. [31,32] However the exact role for sCD27 on IgG production remains to be investigated.

In adult MS patients, a higher ARR was found in patients with high sCD27 levels at time of clinically isolated syndrome.[15] One would expect also a higher relapse rate in paediatric MS patients with high sCD27 levels at time of the first attack, however, no association with ARR was found. This finding might be explained by a ceiling effect, as the overall relapse rate in our paediatric MS cohort is high.[16]

No association was found with EDSS, which is little surprising given the slower disease progression in paediatric MS.[27,33] Longer follow-up duration will be needed to investigate a possible relationship between sCD27 levels and chronic disease progression.

There were a few limitations of this study. First, the FU duration varied between patients. We addressed this problem by correcting for FU duration in the survival analyses. We also performed a sub-analysis in which we excluded patients with less than two years of follow-up. This did not alter the conclusions. Second, we did not perform a follow-up MRI on a regular basis. However, we aimed to assess the value of sCD27 levels on the clinical disease course and therefore chose CDMS as a study endpoint instead of the McDonald criteria. In addition, the use of DMTs may have delayed a second attack and could have influenced the results of the survival analyses. Therefore we performed sub-analyses after excluding patients with ADS- who used DMT before CDMS diagnosis. After excluding these patients, the multivariable analysis was not significant (p=0.061), however there was still a clear trend and the univariate analysis remained significant. Lastly, patients who remained monophasic had a shorter time to CSF sampling compared to patients who were diagnosed with CDMS during follow-up. This may have been caused by the difference in severity of the presenting symptoms and the differential diagnosis at onset, for example in ADEM, where acute non-demyelinating pathology needs to be ruled out. Yet, we found no correlation between the time to CSF sampling and the levels of sCD27, making it unlikely that this influenced our results.

In summary, we show that CSF sCD27 in children with ADS at time of the first attack is associated with a future CDMS diagnosis, independently of MRI and CSF parameters. This result is in line with the earlier finding in adult CIS patients that higher sCD27 was associated with subsequent MS diagnosis.[14] Therefore we can conclude that sCD27 is a potential clinically relevant quantitative marker when performing routine CSF diagnostics not only in adults but also in children with ADS. The next step will be validation of these findings in international cohorts.

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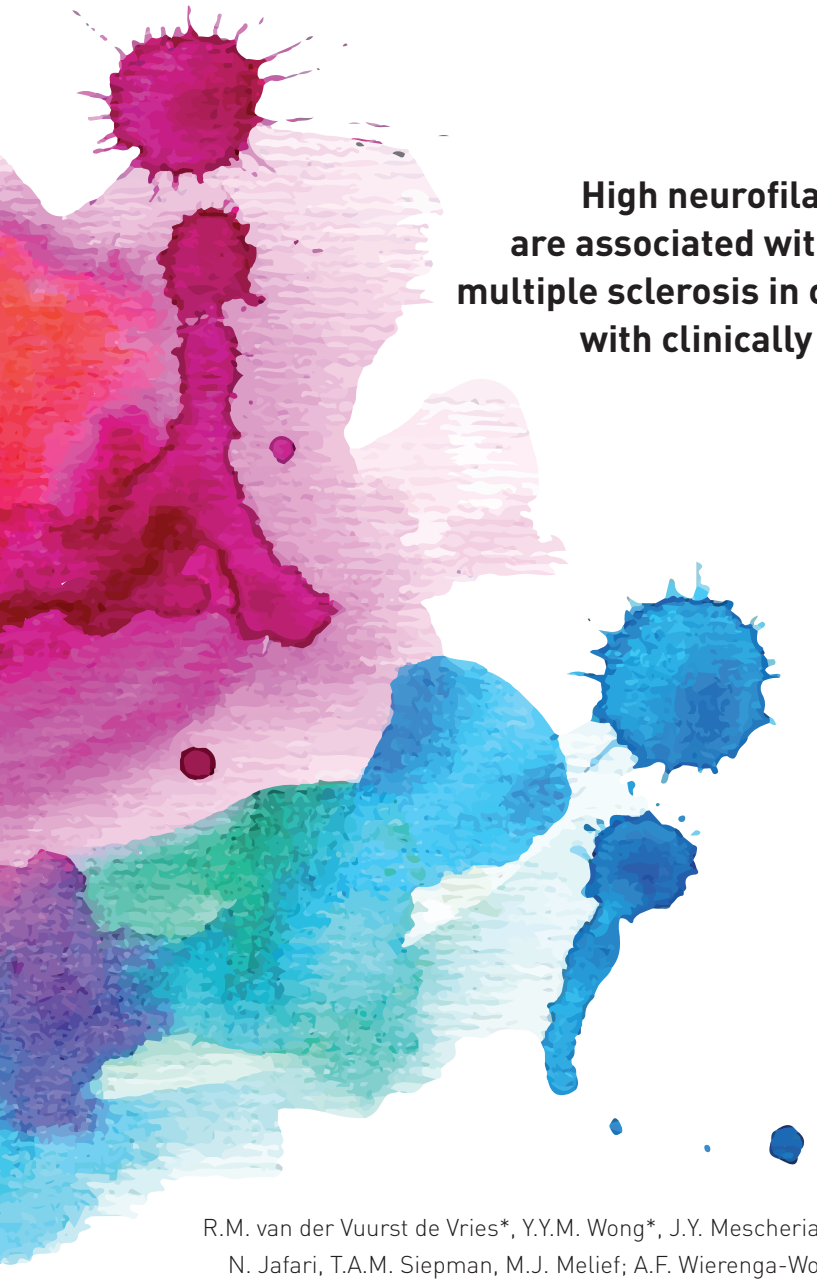

3.3

High neurofilament levels in CSF are associated with clinically definite multiple sclerosis in children and adults with clinically isolated syndrome

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ABSTRACT

Background

A promising biomarker for axonal damage early in the disease course of MS is neurofilament light chain (NfL). It is unknown whether NfL has the same predictive value for MS diagnosis in children as in adults.

Objective

To explore the predictive value of NfL levels in CSF for MS diagnosis in paediatric and adult CIS patients.

Methods

88 adult and 65 paediatric patients with a first attack of demyelination were included and followed (mean follow up-time in adults: 62.8 months (SD±38.7) and 43.8 months (SD±27.1) in children). Thirty control patients were also included. Lumbar puncture was done within 6 months after onset of symptoms. NfL was determined in CSF using ELISA. COX regression analyses were used to calculate hazard ratios (HR) for clinically definite MS (CDMS) diagnosis.

Results

After adjustments for age, oligoclonal bands, and asymptomatic T2-lesions on baseline MRI, increased NfL levels in both paediatric and adult CIS patients were associated with a shorter time to CDMS diagnosis (children HR 3.7; p=0.007, adults HR 2.1; p=0.032). For CIS patients with a future CDMS diagnosis, children showed higher NfL levels than adults (geometric mean 4888 vs 2156 pg/mL; p=0.007).

Conclusion

CSF NfL levels are associated with CDMS diagnosis in children and adults with CIS. This makes NfL a promising predictive marker for disease course with potential value in clinical practice.

INTRODUCTION

Childhood-onset multiple sclerosis (MS) occurs in 3-5% of all MS patients. [1,2] Although children with MS have a more inflammatory disease course with a higher relapse rate than adult patients, [3-5] clinical follow-up studies suggest that disability progression is slower in children than in adults. [4,6,7] However, impairment of age-expected brain growth was seen early in the disease course of paediatric MS patients. [8] This indicates that not only neuroinflammation but also neurodegeneration occurs early in childhood-onset MS.

Axonal damage is considered one of the major causes for persisting neurological disability in MS. [9] A promising biomarker for axonal damage is neurofilament light chain (NfL).

Neurofilament light chain is an element of the neuron cytoskeleton, and is released in the extracellular space after neuronal cell death. [10] In healthy individuals, NfL levels increase with age, which reflects neurodegeneration and is part of the physiological aging process. [11]

High NfL levels in CSF in adults with clinically isolated syndrome (CIS) have been reported as an independent risk factor for MS diagnosis. [12] Furthermore, NfL levels have been associated with brain volume changes in adult CIS patients. [12] However, whether NfL is also increased at disease onset in children is still unknown.
The primary purpose of this prospective study was to investigate whether NfL levels can predict diagnosis of clinically definite MS (CDMS) in children with CIS. Our second aim was to compare NfL levels in CSF at time of a first demyelinating event between children and adults. Finally, we examined the association between NfL and signs of axonal loss on MRI.

METHODS

Study participants

Children and adults were included in either our prospective cohort of adult patients with CIS (PRedicting the OUtcome of a Demyelinating event, PROUD study) or in our prospective cohort of children with acquired demyelinating syndromes (ADS) (PROUD-kids study). [13,14] Both studies are ongoing multicentre studies initiated by Erasmus MC in Rotterdam, The Netherlands, which is a tertiary referral centre for adult and paediatric MS patients (MS Centre ErasMS, and National Paediatric MS Centre).

All patients were included between February 2002 and December 2015 within 6 months after a first event of demyelination of the CNS. Adult patients were younger than 50 years of age, and paediatric patients were younger than 18. No patients had a history of previous neurological

symptoms suggestive for CNS demyelination. Patients with alternative diagnoses were excluded from analyses.

The included patients underwent a baseline brain MRI and routine laboratory tests to rule out other possible diagnoses. A lumbar puncture was performed and extra CSF was collected and stored at –80°C until use.

Patients were assessed at baseline and were reassessed regularly. At baseline instructions were given to the patients to contact the hospital in case of suspected exacerbation.

Cerebrospinal fluid of adult control samples (n=30) was obtained in the Erasmus MC from patients with neurological symptoms but no objective clinical or paraclinical findings to define a specific neurological disease (symptomatic controls). [15]

Standard protocol approvals and patient consents

The study protocol was approved by the Medical Ethics Committee of Erasmus MC Rotterdam and of the other participating centres. Written informed consent was obtained from all patients and/or their families.

Definitions

Clinically isolated syndrome (CIS) was defined as a first attack of demyelination in the CNS without encephalopathy. [16] Clinically definite MS (CDMS) was defined by the Poser criteria as two non-encephalopathic attacks with clinical evidence of two separate lesions. [17] Acquired demyelinating syndromes (ADS) in children encompass the first attack of demyelination, including CIS and acute disseminated encephalomyelitis (ADEM).[14] Patients presenting with other ADS subtypes than CIS or ADEM were excluded from the analyses. Children were diagnosed with CIS, ADEM and CDMS according to the diagnostic criteria proposed by the International Paediatric Multiple Sclerosis Study Group. [13] CDMS was used as the primary outcome. Patients who remained CIS during follow-up are referred to as CIS-CIS and patients who were diagnosed with CDMS during follow-up are referred to as CIS-CDMS. In both children and adults an exacerbation is defined as sub-acute worsening of existing symptoms, or new symptoms after at least 30 days of improvement or stable disease. Symptoms should exist for more than 24 hours, not be preceded by fever, and not be caused by an alternative diagnosis.[18] All exacerbations were confirmed by neurological examination.

Expanded Disability Status Scale (EDSS) was used to assess disability. [19] When patients were diagnosed with CDMS an EDSS was done annually. EDSS scores performed within 3 months after an exacerbation were not considered. Follow-up was calculated by subtracting the date of first symptoms from the last visit date. Baseline MRI scans were performed at 1.5 Tesla scanners

and reviewed blindly. Available T1-, axial T2-, axial and/or sagittal fluid attenuated inversion recovery (FLAIR)- images were used. The MRIs were scored on ≥9 T2 lesions, dissemination in space and time, and asymptomatic T2 lesions. The presence of T1-hypointense lesions on baseline MRI were assessed in CDMS patients. T1-hypointense lesions were defined as non-enhancing lesions being hypointense relative to cortical grey matter. [20] Patients who did not receive gadolinium were excluded for the analysis of T1-hypointense lesions.

CSF sampling and NfL ELISA

Routine CSF diagnostics including IgG index, oligoclonal bands (OCB), cell count and total protein were performed. The remaining CSF was immediately centrifuged for 10 minutes at 3000 rpm to separate the supernatant from cells and cellular elements. After centrifugation, samples were aliquoted and stored at –80°C until use.

CSF analyses for oligoclonal bands (OCB) were performed in local laboratories using isoelectric focusing. [21] OCB status was regarded as positive if there were ≥2 unique bands in CSF compared to serum. IgG index above 0.66 was considered as elevated.

Neurofilament light chain levels in CSF were measured batch-wise in two rounds, according to the manufacturer's instructions, using a stable commercially available solid phase sandwich ELISA (UmanDiagnostics, Umea, Sweden). [22] NfL concentrations (picogram per millilitre (pg/mL)) were calculated using a standard curve according to manufacturer's instructions. All samples were tested double blind and measured in duplicate. The detection limit of the ELISA was 150 pg/ml.

Data analysis

We used SPSS software, version 21.0 (SPSS Inc) and GraphPad Prism5 to perform statistical analyses. After log transformation, NfL levels showed a parametric distribution in both children and adult samples. Therefore we calculated geometric means for NFL levels. Group comparisons for continuous data were performed using 2-tailed t test for normally distributed variables (NfL, age at onset, follow-up time) and Mann-Whitney U test was used for non-parametric data (time between CIS and lumbar puncture). We used one-way ANOVA and post-hoc Bonferroni correction for parametric data to analyse differences between multiple groups. Chi-square or Fisher exact test were performed for categorical variables (gender, type of clinical onset, OCB, elevated IgG-index, asymptomatic T2 lesions, ≥9 T2-lesions, and disease modifying therapies (DMT) after CIS and before CDMS). Spearman rank correlation was used for correlation analyses between non-parametric continues variables. Time to CDMS diagnosis was determined by subtracting the date of the first symptoms from the date of diagnosis. COX proportional hazard regression analyses were used to calculate univariate and multivariable hazard ratios (HR) for time to CDMS diagnosis. Known predictors for MS diagnosis were used in

the multivariable analyses (OCB, asymptomatic T2-lesions). Patients who were not diagnosed with CDMS during follow-up were considered as censored observations. We used the median of NfL levels to establish cut-off values for high and low levels of NfL in children and adults separately. P-values less than 0.05 were considered significant.

RESULTS

Patients characteristics

Sixty-five children with a first demyelinating event of the CNS, 88 adult patients with CIS, and 30 age and gender matched adult control individuals were included in this study.

Of the 65 children, 24 presented with ADEM and 41 with CIS. Twenty-five out of 41 (61%) children with CIS were diagnosed with CDMS during a mean follow-up time of 38.4 months (SD: 21.0). The mean follow-up time in adult CIS patients was 68.2 months (SD: 39.3) in this period 43 (49%) patients were diagnosed with CDMS.

The median time from CIS to CDMS in adult patients was 36.4 months (IQR 14.4-48.9) and in children 10.8 months (IQR 5.0-15.7).

The time between CIS and lumbar puncture was not significantly different between children and adult patients. No patients were receiving disease modifying therapies (DMTs) at time of lumbar puncture.

Sixteen adult patients (18%) and 14 (34%) children who were not yet diagnosed with CDMS received DMT (glatiramer acetate (n=11), interferon (n=19), natalizumab (n=1)).
The patient characteristics for adults and children are shown in *Table 3.3.1* and *Table 3.3.2*.

NfL levels at time of the first demyelinating event per clinical subgroup

Patients (children and adults) at time of a first attack of demyelination showed higher NfL levels than control individuals; geometric mean 2040 vs 444 pg/mL; p<0.001. In *Figure 3.3.1*, NfL levels from controls, adult, and paediatric patients are shown.

In adult patients, NfL levels at time of CIS were higher in the group that was diagnosed with CDMS (CIS-CDMS, n=43 (49%)) compared to the group that remained CIS during follow-up (CIS-CIS); geometric mean 2156 vs 1342 pg/mL; p=0.012. *(Figure 3.3.2)*

Table 3.3.1: Patient characteristics (adults)

Adults	Controls (n=30)	CIS-patients (n=88)	CIS-CDMS (n=43)	CIS-CIS (n=45)	p-value [a]
Female sex, no. (%)	20 (66.7)	59 (67.0)	34 (79.1)	25 (55.6)	0.02
Age [b], mean (SD), years	33.4 (±9.5)	31.2 (±7.2)	31.9 (±7.1)	33.6 (±7.3)	0.28
Follow-up time, mean (SD), months	na	62.8 (±38.7)	89.0 (±36.8)	48.3 (±30.7)	<0.01
Type of clinical onset, no. (%)					
-Optic nerve	na	41 (46.6)	21 (48.8)	20 (44.4)	0.68
-Spinal cord	na	23 (26.1)	11 (25.6)	12 (26.7)	0.91
-Other localization	na	24 (27.3)	11 (25.6)	13 (28.9)	0.73
OCB, (≥2 bands), (%)	na	63/83 (75.9)	36/41 (87.8)	27/42 (64.3)	0.01
Elevated IgG index (cut-off: 0.66), no. (%)	na	44/85 (51.8)	22/41 (53.7)	22/44 (50.0)	0.74
Time CIS to LP, median (IQR), weeks	na	6.1 (2.7-13.2)	6.0 (2.9-12.6)	6.7 (2.6-14.1)	0.97
≥9 lesions on T2-weighted images, no. (%)	na	27 (30.7)	18 (41.9)	9 (20.0)	0.03
Asymptomatic T2-lesions, no. (%)	na	76 (86.4)	38 (88.4)	38 (84.4)	0.59
MS based on first MRI [b], no (%)	na	16 (18.2)	11 (25.6)	5 (11.1)	0.08
DMT before CDMS diagnosis, no. (%)	na	16 (18.2)	12 (27.9)	4 (8.9)	0.02
Time between CIS and start DMT, median (IQR), months	na	27.8 (9.7-43.1)	29.5 (9.9-46.6)	14.4 (5.9-29.0)	0.25

Abbreviations: CIS = Clinically isolated syndrome; CIS-CDMS = patients who are diagnosed with CDMS during follow-up after CIS defined by Poser criteria; CIS-CIS = not diagnosed with CDMS; na = not applicable; DMT, disease modifying therapy; OCB = oligoclonal bands; Ig = Immunoglobulin; LP= lumbar puncture; pg/mL = picogram/millilitre. [a] P value calculated between CIS-CDMS and CIS-CIS [b] For patients with CIS: age at CIS, for Controls: age at lumbar puncture. [b] Dissemination in space and time at baseline based on McDonald 2010 criteria

Table 3.3.2 Patient characteristics (children)

Children	ADS-patients (n=65)	ADEM (n=24)	CIS-CDMS (n=25)	CIS-CIS (n=16)	p-value [a]
Female sex, no. (%)	38 (58.8)	16 (66.7)	16 (64.0)	6 (37.5)	0.10
Age, median (IQR), years	12.5 (5.4-15.5)	4.1 (2.6-7.2)	15.0 (13.8-16.0)	14.2 (9.0-16.4)	0.52
Follow-up time, mean (SD), months	43.8 (±27.1)	53.1 (±33.7)	44.1 (±22.3)	29.5 (±15.5)	0.03
Type of clinical onset, no. (%)					
-Optic nerve	11 (16.9)	0 (0.0)	5 (20.0)	6 (37.5)	0.22
-Spinal cord	11 (16.9)	0 (0.0)	6 (24.0)	5 (31.2)	0.61
-Other localization	7 (10.8)	0 (0.0)	4 (16.0)	3 (18.8)	1.00
-Polyfocal without encephalopathy	12 (18.4)	0 (0.0)	10 (40.0)	2 (12.5)	0.08
-Polyfocal with encephalopathy	24 (36.9)	24 (100.0)	0 (0.0)	0 (0.0)	1.00
OCB, (≥2 bands), (%)	28/54 (51.9)	0/17 (0.0)	21/23 (91.3)	7/14 (50.0)	0.01
Elevated IgG index (cut-off: 0.66), no. (%)	33/50 (66.0)	4/12 (33.3)	22/23 (95.7)	7/15 (46.7)	<0.01
Time first symptoms to LP, median (IQR),weeks	2.3 (0.8-7.6)	1.57 (0.57-2.64)	6.0 (1.9-12.8)	1.8 (0.6-7.8)	0.10
≥9 lesions on T2-weighted images, no. (%)	27 (41.5)	6 (25.0)	16 (64.0)	5 (31.2)	0.04
Asymptomatic T2-lesions, no. (%)	55 (8.6)	22 (91.7)	24 (96.0)	9 (56.2)	0.003
MS based on first MRI [b], no. (%)	10 (15.4)	na	9 (36.0)	1 (6.2)	0.03
DMT before CDMS diagnosis, no. (%)	14/41 (34.1)	na	9 (36.0)	5 (31.3)	0.75
Time between CIS and start DMT, median (IQR), months	6.4 (3.3-12.1)	na	6.3 (1.7-14.8)	6.5 (3.5-10.4)	0.72

Abbreviations: CIS = Clinically isolated syndrome; CIS-CDMS = patients who are diagnosed with CDMS during follow-up after CIS defined by Poser criteria; CIS-CIS = not diagnosed with CDMS; na = not applicable; DMT, disease modifying therapy; OCB = oligoclonal bands; Ig = Immunoglobulin; LP= lumbar puncture; pg/mL = picogram/millilitre.

[a] *P value calculated between CIS-CDMS and CIS-CIS*

[b] *Dissemination in space and time at baseline based on McDonald 2010 criteria*

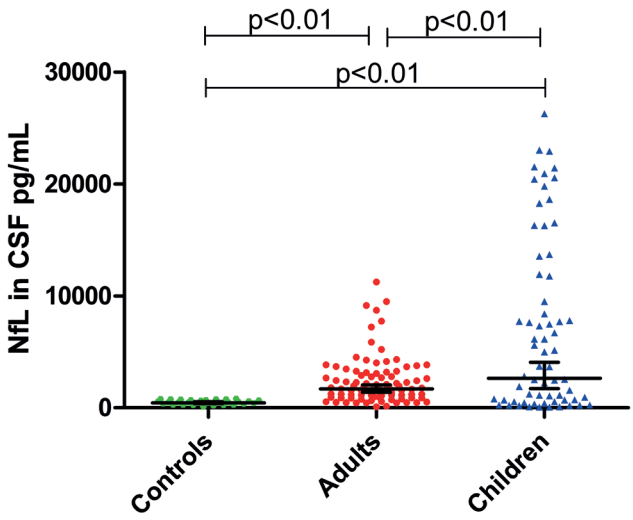

Figure 3.3.1: CSF NfL levels in Controls vs Adults vs Children
CIS and ADEM patients are included in children.
Horizontal lines with error bars indicate geometric mean with 95% CI.
Abbreviations: NfL = Neurofilament light chain; pg/mL = picogram/millilitre

In children, we compared NfL levels between CIS-CIS, CIS-CDMS, and ADEM patients. NfL levels at time of CIS in paediatric CIS-CDMS patients (n=25; 61%) were higher than in paediatric CIS-CIS patients; geometric mean 4888 vs 967 pg/mL; p=0.01. Children with ADEM did not differ in NfL levels from CIS-CIS and CIS-CDMS children; geometric mean 2683 pg/mL *(Figure 3.3.2)*. There was no correlation between time from onset of symptoms to lumbar puncture and NfL levels in both children and adults.

NfL levels compared between children and adults

Next, we compared NfL levels between children and adult patients at time of CIS. In the CIS-CDMS group, NfL levels were higher in children compared to adults; geometric mean 4888 vs 2156 pg/mL; p=0.007. NfL levels were not different between adults and children in the CIS-CIS groups *(Figure 3.3.2)*.

Association of NfL levels with time to CDMS diagnosis in children and adults with CIS

To analyse time to CDMS diagnosis, we used median CSF NfL levels in CIS patients as cut-off. This resulted in a cut-off of 1802 pg/mL for adults and 2537 pg/mL for children. These cut-offs were used to divide CIS patients (ADEM excluded) into groups with high and low NfL levels, and were subsequently used in the COX regression analysis.

The univariate COX regression analysis showed a HR for CDMS diagnosis of 2.1; p=0.024 in adults and 3.8 in children with CIS; p=0.003. Kaplan-Meier curves are shown in *Figure 3.3.3A/B*. In a multivariable COX regression analysis, we corrected for clinically relevant parameters; the presence of asymptomatic T2 lesions on the baseline MRI and OCB. We also corrected for age at onset, based on correlation with NfL levels in control individuals (spearman rho 0.59, p=0.001). The HR in the multivariable COX regression analysis for high NfL levels was 2.1 in adults (p=0.032) and 3.7 in children (p=0.007). *(Table 3.3.3)*

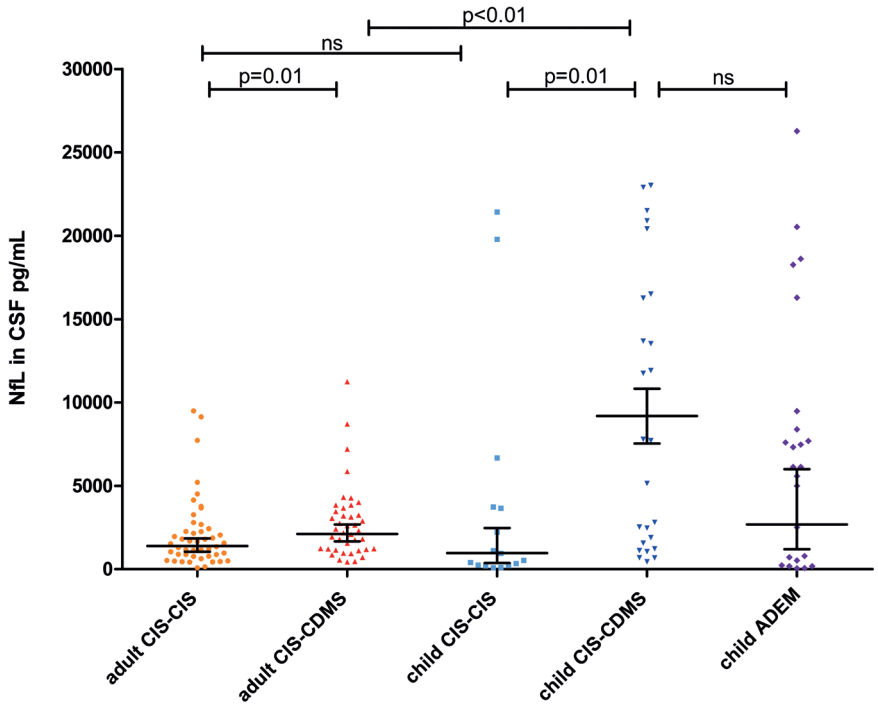

Figure 3.3.2: NfL levels in clinical subgroups of adults and children
Horizontal lines with error bars indicate geometric mean with 95% CI.
Abbreviations: NfL = Neurofilament light chain; ns = not significant; pg/mL = picogram/millilitre

A total of 16 (18%) adult CIS patients and 14 (34%) children received DMT before CDMS diagnosis. This could have postponed the second attack. The HR in children increased after excluding patients who received DMT before CDMS diagnosis. In adults the univariate HR did not change, and the HR in the multivariable analysis showed a trend towards significance.

When we add DMT before CDMS diagnosis into the COX regression model, the HRs did not change.

Thirteen (11%) adults and nine (22%) children were treated with methylprednisolone within three months before LP. When we corrected for this in the COX regression model, the results did not change.

In another subanalysis, we excluded CIS-CIS patients who had less than 2 years follow-up (children: n=5, adults: n=10). After this exclusion, HRs were not altered in adults and increased in children. *Table 3.3.3* shows the univariate and multivariable HRs including those of the subanalyses.

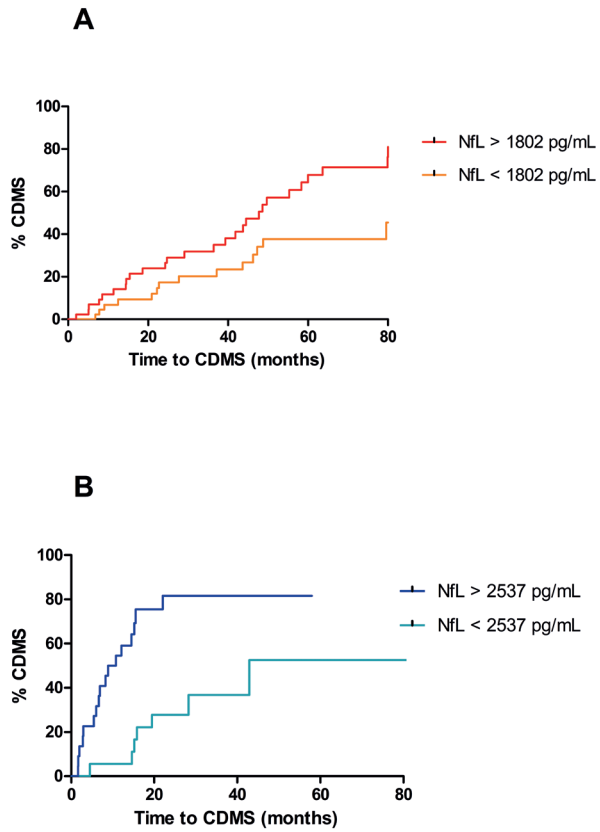

Figure 3.3.3: Time from CIS to CDMS in CIS patients with high and low CSF NfL levels
A. Adults (log-rank test p=0.02).
B. Children (log-rank test p=0.001). Kaplan-Meier curves showing time to CDMS diagnosis for CIS patients (ADEM excluded) with either high or low CSF NfL levels.
Abbreviations: CDMS = Clinically definite multiple sclerosis; NfL = Neurofilament light chain; pg/mL = picogram/millilitre

Table 3.3.3: Cox regression (univariate and multivariable) hazard ratios for CDMS diagnosis in adults and children with CIS

	Univariate analysis HR (95% CI)	P-value	Multivariable analysis HR (95% CI)	P-value
Adults				
Total group (n=88)	2.1 (1.1-3.9)	0.024	2.1 (1.1-4.1)	0.032
After excluding patients with DMT before CDMS diagnosis (n=72)	2.2 (1.1-4.7)	0.035	2.2 (1.0-4.9)	0.061
After excluding CIS-CIS patients with FU <2 years (n=78)	2.0 (1.1-3.8)	0.027	2.1 (1.1-4.1)	0.034
Children				
Total group (n=41)	3.8 (1.6-9.2)	0.003	3.7 (1.4-9.3)	0.007
After excluding patients with DMT before CDMS diagnosis (n=27)	19.8 (2.5-155.5)	0.005	13.7 (1.6-114.3)	0.015
After excluding CIS-CIS patients with FU <2 years (n=36)	3.8 (1.6-9.2)	0.003	4.2 (1.6-11.3)	0.004

Hazard ratios for CDMS diagnosis in subgroups for adults and children with CIS (ADEM excluded)
Abbreviations: NfL = Neurofilament light chain, HR = hazard ratio, CI = confidence interval, DMT = disease modifying treatment, CDMS = clinically definite multiple sclerosis, FU = follow-up. Multivariable analyses: corrected for presence of asymptomatic T2 lesions on baseline MRI, presence of OCB and age of onset

Association of CSF NfL levels with disability

We did not find a correlation between CSF NfL levels and EDSS scores after CDMS diagnosis. We collected EDSS data from 55/68 (81%) patients who were diagnosed with CDMS. Only 6 patients (4 adults and 2 children) reached an EDSS of 3.0 or more.

Association of CSF NfL levels with signs of axonal damage on MRI

CSF NfL levels were increased in CDMS patients showing T1-hypointense lesions on baseline MRI (adults 20/39, 51%; children 15/23, 65%). We found this in adults (geometric mean 3188 vs 1588 pg/mL; p=0.001) and in children (geometric mean 8920 vs 1668; p=0.001). *(Figure 3.3.4A/B)*

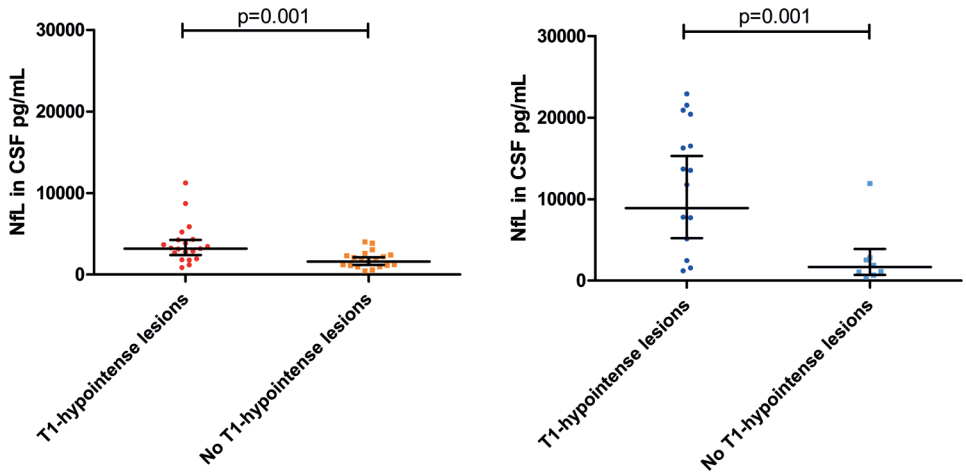

Figure 3.3.4: CSF NfL levels in CIS-CDMS adults and children with and without T1-hypointense lesions on baseline MRI

A. Adults: T1-hypointense lesions vs no T1-hypointense lesions on baseline MRI.
B. Children: T1-hypointense lesions vs no T1-hypointense lesions on baseline MRI.
Horizontal lines with error bars indicate geometric mean with 95% CI.
Abbreviations: NfL = Neurofilament light chain; pg/mL = picogram/millilitre; CDMS = Clinically definite multiple sclerosis

DISCUSSION

In this prospective study, we demonstrate that CSF NfL levels in children and adults with a first attack of suspected MS are predictive for CDMS diagnosis. Furthermore at time of CIS, CSF NfL levels in patients with a future CDMS diagnosis are higher in children than in adult

patients. This underlines that not only inflammation is more severe in children [3-5] but that children also have more axonal damage early in the disease course of MS than adult patients. [8]

To our knowledge, we are the first to show that CSF NfL levels are associated with a subsequent diagnosis of CDMS in children with CIS. In addition, the results validate the predictive value of CSF NfL levels for CDMS diagnosis in adult CIS patients. [12,23] Both in adults and children, these findings were independent of known predictive factors for CDMS, i.e. asymptomatic T2 lesions on baseline MRI and unique OCBs in CSF. Moreover, CSF NfL levels predicted a second attack even better than these currently used markers.

It is essential to improve currently available routes to prediction to prevent unnecessary treatment of patients with low clinical disease activity, especially because these immunomodulatory therapies can have serious side effects. Recently, other potential CSF biomarkers for a future MS diagnosis have been identified. [24,25] Our findings draw further attention to the relevance of including CSF analyses as part of routine diagnostics.

Since NfL is considered a biomarker for axonal damage, neurodegeneration, and brain atrophy, [26,27] we reasoned that its presence in CSF could be associated with T1-hypointense lesions on MRI, which are signs of axonal loss. [28] In children with CIS, these T1-hypointense lesions have been reported to be highly predictive for MS diagnosis. [20] Here, we demonstrate that children and adult CIS patients with T1-hypointense lesions on baseline MRI have higher NfL levels than patients without these lesions.

CSF NfL levels in children with ADEM were not significantly different from levels in patients who remained CIS or who were eventually diagnosed with CDMS. Nevertheless, these levels were high in ADEM patients (geometric mean 2683 pg/ml), indicating considerable axonal damage. Studies have reported cognitive impairment and persistent motor dysfunction in children with ADEM. [29,30] Moreover, it has been shown that subsequent white matter maturation and age-expected brain growth is disturbed not only in paediatric MS, but also in monophasic ADS, including ADEM. [31,32] These findings support the occurrence of damage during the acute phase with a lasting impact. Which stresses the importance of adequate follow-up and support after the acute event.

NfL levels have been reported to be also increased in serum of adult CIS and MS patients , [33,34] but the predictive value in CIS patients for MS diagnosis of this marker seems limited to CSF.[12] As we here aimed to assess and compare prediction in both children and adults with CIS, we restricted in this study to CSF samples.

There are some limitations in this study. First, the range of follow-up is rather wide. We did correct for this in the COX regression analyses, and we also performed a subanalysis after excluding CIS-CIS patients with a follow-up less than 2 years, which did not change our findings. Second, in both the adult and paediatric study, we did not perform a follow-up MRI on a regular basis. Therefore, the Poser criteria were used instead of the McDonald 2010 criteria. In this way, we could show an effect on clinical disease activity (second attack) instead of disease activity measured with MRI. Third, in order to prove an association of CSF NfL levels with EDSS, we will need a longer follow-up period since disability occurs later in the disease course especially in children. [6] Fourth, we did not have access to advanced imaging techniques for quantification of neurodegeneration (e.g. T1-hypointense lesion volumes, total brain volume and brain tissue integrity). However, we used the presence of T1-hypointense lesions as an MRI marker for axonal damage, [28] because it is easily assessable. Furthermore, we did not include paediatric controls since we did not receive ethical permission to collect paediatric control CSF samples, which made the collection of this rare material not possible. Yet, control groups in other paediatric studies indicate low physiological levels of NfL, in the same range as the adult controls in the present study.[35,36]

Last, although our sample size was relatively small due to limited availability of CSF, we were still able to correct our results for other predictive factors for MS diagnosis. Our findings in children were also compatible to those in adults, further stressing the robustness of the observations.

In conclusion, we show that high levels of CSF NfL are associated with CDMS diagnosis independently of known predictive factors (i.e. asymptomatic T2 lesions and OCB) in both children and adults. CSF NfL levels at time of a first demyelinating event are higher in children than in adults with a future CDMS diagnosis. In addition, this marker for axonal damage, is associated with MRI signs of neurodegeneration in both groups. Given that therapeutic interventions might delay disease progression and accumulation of disability, [37,38] it is essential to accurately predict MS diagnosis, not the least in the paediatric population. Hence, NfL in CSF is a promising predictive marker for the disease course in both adults and children with a first demyelinating event, with a potential value in future clinical practice.

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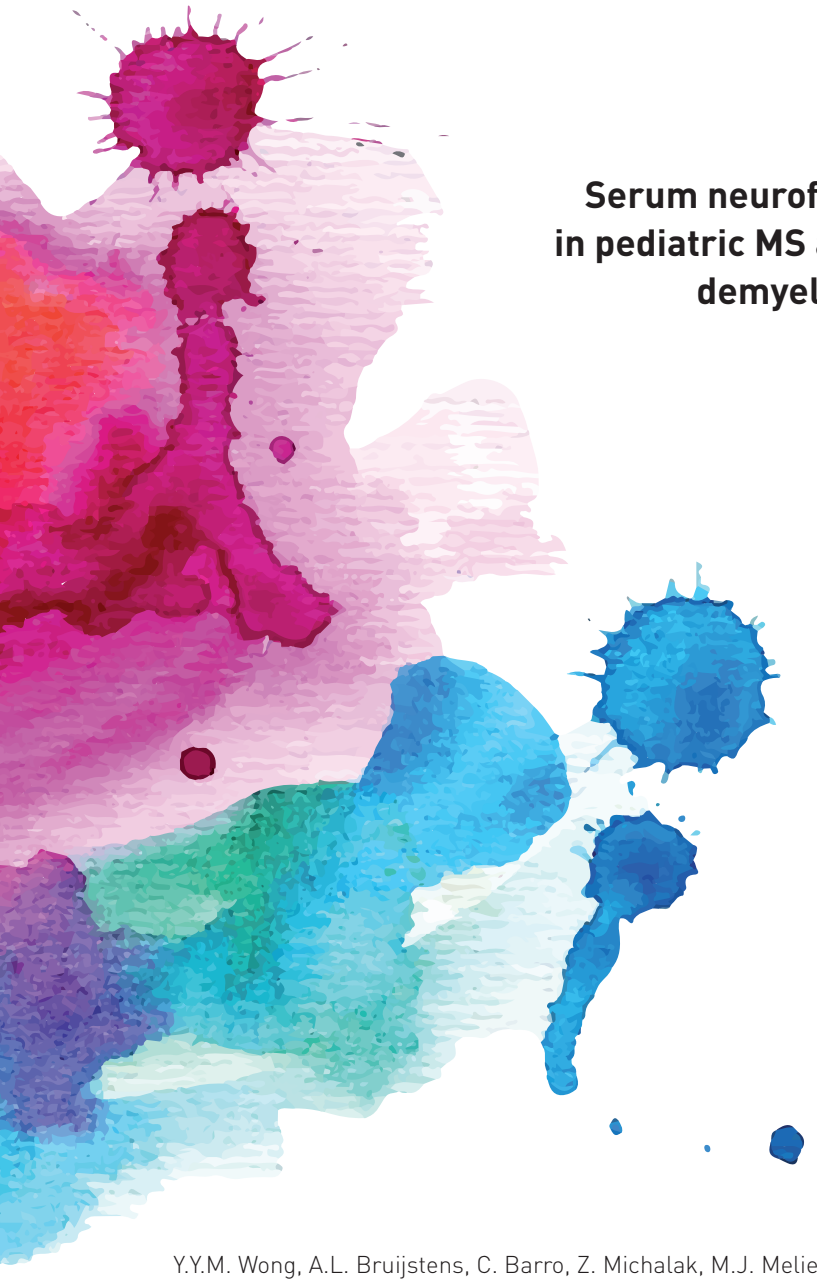

3.4

Serum neurofilament light chain in pediatric MS and other acquired demyelinating syndromes

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ABSTRACT

Introduction

Neurofilament light chain (NfL) is a biomarker for neuro-axonal damage and can reliably be measured in serum using an ultrasensitive single-molecule array (Simoa) assay. In adult multiple sclerosis (MS), CSF and serum NfL (sNfL) are highly correlated and the levels are associated with future disease activity. We aimed to explore these associations in pediatric patients with acquired demyelinating syndromes (ADS).

Methods

In total, 102 children<18 years with a first attack of CNS demyelination and 23 age-matched controls were included. Clinically definite MS (CDMS) was set as an endpoint for analysis. CSF NfL was tested by the commercially available ELISA (Uman Diagnostics); sNfL was tested with a Simoa assay. Hazard ratios (HR) were calculated with Cox regression analysis.

Results

Of the 102 patients, 47 (46%) were tested for CSF NfL. CSF and sNfL correlated significantly in the total group (ρ 0.532, p<0.001) and even stronger in the subgroup of patients with future CDMS diagnosis (ρ 0.773, p<0.001). sNfL was higher in patients than in controls (geometric mean 6.1 pg/mL, p<0.001), and was highest in ADS presenting with encephalopathy (ADEM,n=28, 100.4 pg/mL), followed by patients without encephalopathy (ADS-) with future CDMS diagnosis (n=40, 32.5 pg/mL), and ADS- who remained monophasic (n=34,17.6 pg/mL). sNfL levels higher than a median of >26.7pg/mL at baseline are associated with a shorter time to CDMS diagnosis in ADS- (p=0.045).HR for CDMS diagnosis was 1.09 for each 10 pg/mL increase of sNfL, after correction for age, OCB and MRI parameters (p=0.012).

Conclusion

The strong correlation between CSF and sNfL strengthens its reliability as a peripheral marker of neuroaxonal damage. Higher sNfL levels at baseline were associated with higher probability of future CDMS diagnosis in ADS-.

INTRODUCTION

Childhood-onset multiple sclerosis (MS) occurs in 3-10% of all MS patients.[1,2] Despite the higher inflammatory activity in MS children with a higher relapse rate and MRI lesion load at baseline than adults[3-5], children have slower disease progression than adults.[4,6,7] Yet, there are suggestions that point towards prominent neurodegeneration not only in pediatric MS patients, but also in other forms of acquired demyelinating syndromes (ADS) including impaired brain growth.[8-10] A neurodegenerative biomarker of interest is neurofilament light chain (NfL), which is an element of the neuron cytoskeleton and is released in the extracellular space after neuronal cell death.[11] In adult MS patients, CSF and serum NfL (sNfL) were well correlated with a novel ultrasensitive single molecule array (Simoa) assay, suggesting that sNfL could be a relevant peripheral biomarker for axonal injury in MS.[12-15] In our previous study we showed that high CSF NfL levels in adults and children are independent predictors for MS diagnosis at the first attack of demyelination and are associated with the amount of T1 hypointense lesions on baseline MRI as reflection of axonal loss.[16] Moreover, in adult MS patients the NfL levels in serum are associated with current and future clinical and radiological disease activity, disability (EDSS) and are influenced by disease modifying treatment (DMT).[13,14,17] However, sNfL in children with a first attack of demyelination has not yet been investigated. Patients with pediatric ADS have a long disease duration ahead if diagnosed with MS and quantitative biomarkers are needed in easily assessable biomaterial, such as serum, for better prediction of disease course. We aimed to provide data on sNfL in children with ADS by (a) studying the correlation between CSF and sNfL levels, (b) explore the differences in sNfL levels between different ADS subtypes and symptomatic controls, (c) whether high sNfL levels predict clinically definite MS (CDMS) diagnosis.

METHODS

Study participants

Patients <18 years were included in the Dutch prospective and multicentre study for children with ADS (PROUD-kids study). All patients who were tested for CSF NfL for our previous work were included in order to investigate the correlation between CSF and serum NfL (n=47).[16] Additionally, patients who underwent a venipuncture <3 months after symptom onset between June 2006 and February 2017 (n=55) were also included in this study. Patients with alternative diagnosis were excluded. Patients were assessed at baseline and reassessed at least annually. Patients were instructed to contact the hospital in case of suspected exacerbation. Serum samples of pediatric age-matched control children (n=23) were obtained in the Erasmus MC from patients with neurological symptoms but no objective clinical or paraclinical findings to define a specific neurological disease (symptomatic controls, SCs).[18]

Definitions

Acquired demyelinating syndromes in children encompass the first attack of demyelination in the central nervous systems, including ADEM (ADS with encephalopathy: ADS+) and ADS without encephalopathy (ADS-).[19,20] Patients who remained monophasic during follow-up are referred to as mono-ADS. Patients who had a second clinical attack were diagnosed with clinically definite MS (CDMS).[21] Patients with other ADS subtypes, for example relapsing demyelinating disease other than MS, were not analyzed in this study.

Relapse was defined as acute worsening of existing symptoms or new symptoms after 30 days of improvement or stable disease and no evidence of an alternative diagnosis. The symptoms should exist for more than 24 hours and not be preceded by fever.[22] Exacerbations were confirmed by neurological examination.

Disability was measured by the Expanded Disability Status Scale (EDSS).[23] EDSS scores performed within 3 months after an exacerbation were not considered. Follow-up (FU) duration was calculated by subtracting the date of first symptoms from the last FU date. Baseline MRI scans were performed at 1.5 Tesla scanners and reviewed blindly. Available T1-, axial T2-, axial and/or sagittal fluid attenuated inversion recovery (FLAIR)- images were used. The MRIs were scored on the presence of T2 hyperintense lesions and of ≥9 T2 hyperintense lesions. The presence of T1-hypointense lesions on baseline MRI were assessed in CDMS patients. T1-hypointense lesions were defined as non-enhancing lesions being hypointense relative to cortical grey matter.[24] Patients who did not receive gadolinium were excluded for the analysis of T1-hypointense lesions.

CSF and serum samples

Routine CSF diagnostics including IgG index, oligoclonal bands (OCB), cell count and total protein were performed. The remaining CSF was immediately centrifuged for 10 minutes at 3000 rpm to separate the supernatant from cells and cellular elements. After centrifugation, samples were aliquoted and stored at –80°C until use.[25] CSF analyses for OCB were performed in local laboratories using isoelectric focusing.[26] OCB status was regarded as positive if there were ≥2 unique bands in CSF compared to serum. IgG index above 0.66 was considered as elevated.

Serum samples were collected, and processed by following standard procedures and stored in -80 degrees until use.[25]

CSF and serum NfL measurements

CSF NfL levels were measured batch-wise in two rounds, according to the manufacturer's instructions, using a commercially available solid phase sandwich ELISA (UmanDiagnostics,

Umea, Sweden).[27] NfL concentrations (picogram per millilitre (pg/mL)) were calculated using a standard curve according to manufacturer's instructions. All samples were tested double blind and measured in duplicate. The detection limit of the ELISA was 150 pg/ml.

sNfL levels were measured with a validated Simoa NfL assay using the capture monoclonal antibody (mAB) 47:3 and the biotinylated detector mAB 2:1 from UmanDiagnostics,[28] transferred onto the Simoa platform as described before[14].

Standard protocol approvals, registrations and patient consents
Erasmus MC ethical committee approved this study. Written informed consent was obtained from patients and/or their families.

STATISTICAL ANALYSIS

For statistical analyses we used SPSS software, version 24.0 (SPSS Inc.) and GraphPad Prism7. Kolmogorov-Smirnov test was used to assess the normality of the data. NfL levels for both CSF and serum were not normally distributed, and were therefore log transformed to attain normally distributed data. Due to log-transformation, geometric means (GM) were calculated. For group comparisons, Student's t-test and Mann Whitney U test were used for continuous variables. Student's t-test was performed to compare the NfL levels in mono-ADS (ADS+ and monophasic ADS-) and ADS- patients with future CDMS diagnosis. Chi-square and Fisher exact test were used for categorical data. Correlation analyses were done for two continuous variables.

Cox proportional hazard regression models were used to calculate univariate and multivariable hazard ratios (HR) in the ADS- patients for CDMS diagnosis. The Cox proportional hazard assumption was tested by including a time dependent covariate in the model. Known predictors for MS diagnosis such as age of onset, OCB, presence of ≥ 9 T2 lesions on baseline MRI and gadolinium enhancing lesions are used

RESULTS

Patient characteristics

In total, 102 children with a first demyelinating event of the CNS and 23 symptomatic controls (SCs) were included in this study and were tested for sNfL. Patient characteristics and basic demographics of the SCs are shown in *Table 3.4.1* Of the 102 ADS patients, 28 presented with encephalopathy (ADS+) and 74 without encephalopathy (ADS-). Within a median follow-up of 3.5 years (IQR 1.5-5.7), 40/74 (54%) patients who presented with ADS- were diagnosed with CDMS during follow-up. All ADS+ patients remained monophasic and none were diagnosed with CDMS. CSF and serum NfL were available in 47/102 patients (46%), including ADS+ (n=11), mono ADS- (n=11) and ADS- patients with future CDMS (n=23). In the SCs, no correlation or difference was found between the age (ρ -0.263, p=0.225), gender (p=0.883) and sNfL. The time between ADS onset and serum sampling were not significantly different between the different subtypes of ADS, as displayed in *Table 3.4.1*. No patients received disease modifying therapies at time of biosampling.

Correlation CSF and serum NfL in ADS patients

Data on NfL levels in CSF were available in 47/102 (46%) patients. Median time from onset to first CSF and serum sampling was 2.6 weeks (IQR 0.9-7.6 weeks) and 6.0 weeks (IQR 2.0-16.0) respectively. No correlations were found between time to biosampling and NfL levels for both serum and CSF.

We analyzed the correlation between CSF and serum NfL in these 47 patients and observed a positive correlation (Pearson rho 0.532, p<0.001). When only analyzing patients who were diagnosed with CDMS during FU (n=23), a strong correlation was found (Pearson rho 0.773, p<0.001).

sNfL levels at time of first demyelinating event

sNfL obtained at first event was higher in patients (n=102) than pediatric controls (n=23) (geometric mean 36.1 pg/ml, 95% CI 27.5-47.4 vs 6.1 pg/ml, 95% CI 5.-7.3; p<0.001). When comparing the ADS subgroups, sNfL was highest in ADS+ (100.4 pg/mL, 95% CI 60.8-165.9) and was significantly higher than ADS- patients with a future diagnosis of CDMS (32.5 pg/mL, 95% CI 22.3-47.2; p<0.001) and mono ADS- (17.6 pg/mL, 95% CI 11.6-26.8; p<0.001). The latter two groups also differed significantly from each other (p=0.031). The sNfL levels of the symptomatic controls and the ADS subgroups are displayed in *Figure 3.4.1*.

Table 3.4.1: Patient characteristics

	Controls (n=23)	ADS+ (n=28)	ADS- (monophasic) (n=34)	ADS- (CDMS) (n= 40)	All patients (n=102)	P-value*
Female, n(%)	19 (39)	14 (50)	12 (35)	29 (73)	55 (54)	0.005
Age at onset, yrs, median IQR**	9 (5-14)	4.2 (2.3-8.7)	14.4 (9.7-16.3)	15.4 (13.8-16.1)	13.7 (7.0-16.0)	0.091
Presenting symptoms, n (%)	n/a					0.225
ON		0	14 (41)	9 (23)	23 (23)	
- Bilateral ON			- 3/14 (21)	- 0	- 3/23 (13)	
TM		0	9 (27)	9 (23)	18 (18)	
- LETM			- 1/9 (11)	- 0	- 1/18 (6)	
Monofocal ADS- other than ON and TM		0	3 (9)	7 (18)	10 (10)	
Polyfocal ADS-		0	8 (24)	15 (38)	23 (23)	
Polyfocal ADS+		28 (100)	0	0	28 (28)	
≥2 unique CSF OCB, n (%)	n/	0/20	10/26 (39)	33/35 (94)	43/61 (70)	<0.001
MP at first event, n(%)	n/a	23 (82)	25 (74)	24 (60)	72 (71)	0.134
Onset to serum sampling, weeks median (IQR)	n/a	2.1 (1.3-5.4)	3.4 (1.2-7.6)	9.5 (3.4-13.5)	4.7 (1.8-10.4)	0.069
FU duration, years median (IQR)	n/a	4.5 (1.8-6.8)	2.1 (1.3-3.9)	4.2 (2.0-5.6)	3.5 (1.5-5.7)	0.031
EDSS at last FU, median (range)	n/a	n/a	n/a	1.0 (0.-6.5)	1.0 (0.-6.5)	n/a

Abbreviations: Acquired demyelinating syndromes presenting without encephalopathy (ADS-), Acquired demyelinating syndromes presenting with encephalopathy (ADS+), clinically definite multiple sclerosis (CDMS), cerebral spinal fluid (CSF), Expanded disability status scale (EDSS), follow-up (FU), interquartile range (IQR), longitudinal extensive transverse myelitis (LETM), methylprednisolone (MP), optic neuritis (ON), transverse myelitis (TM).
**Comparison between patients with ADS- (monophasic) and ADS- (CDMS). ** Age at serum sampling for controls.*

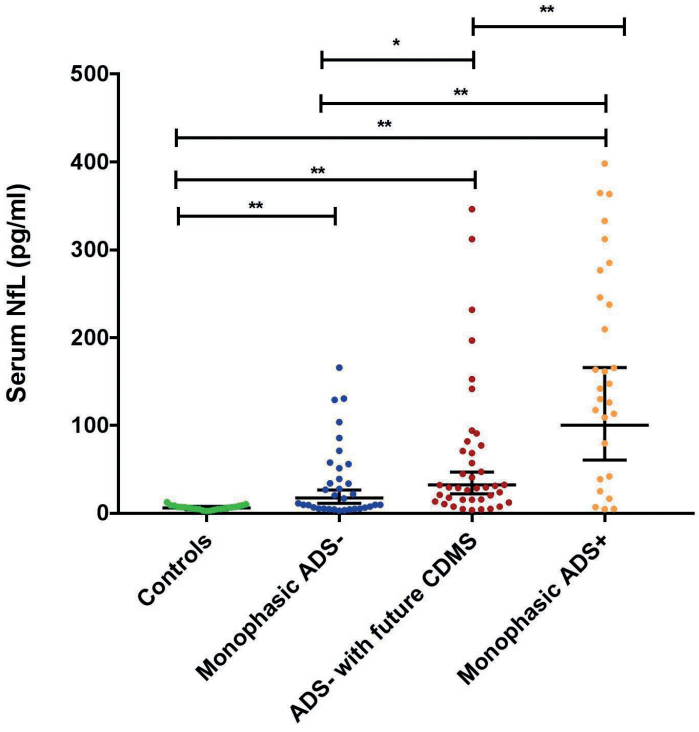

Figure 3.4.1: Levels of serum Neurofilament light chain in controls and in ADS patients at first attack.

Abbreviations: Acquired demyelinating syndromes presenting without encephalopathy (monophasic ADS-), clinically definite multiple sclerosis (CDMS), Acquired demyelinating syndromes presenting with encephalopathy (monophasic ADS+). Neurofilament light chain (NfL). * p<0.05, ** p<0.001.

No correlation was found between sNfL and time from ADS onset to serum sampling. Acute therapy with methylprednisolone (IvMP) was given to 72/102 patients (71%). No difference in sNfL was found between patients who did (47/72, 65%) and did not (25/72, 35%) receive IvMP before serum sampling (45.8 pg/mL, 95% CI 29.8-92.3 vs 39.4 pg/mL, 95% CI 22.0-70.4).

Presenting phenotypes and sNfL levels (ADS+ excluded)

ADS+ patients show a wide variation in sNfL levels, and differentiate clinically from ADS- patients. We therefore only included ADS- (n=74) in the following analyses.

Regarding the presenting phenotypes and sNfL levels, we found a higher level of sNfL in patients with a non-optic neuritis phenotype (n=51) compared to patients with an isolated ON onset (n=23) (29.6 pg/ml, 95% CI 20.8-42.0 vs 16.2 pg/ml, 95% CI 10.3-25.3; p=0.047). Patients with an isolated transverse myelitis (n=18, TM) do not differ in sNfL levels from non-TM patients n=56 (24.6 pg/ml, 95% CI 17.7-34.3 vs 24.1 pg/ml, 95% CI 13.4-43.2; p=0.987. Moreoever,

no differences in sNfL were found between other presenting phenotypes (monofocal ADS-including brainstem, p=0.119, and polyfocal ADS-, p=0.416).

Correlation with CSF and MRI parameters (ADS+ excluded)

For the reason mentioned above, ADS+ patients were excluded from this analysis, leaving 74 ADS- for analysis. OCB and IgG index were tested in 61/74 (82%). OCB were positive in 43/61 patients (70%). No difference in sNfL levels were found in patients with and without OCB. Forty-five of 61 patients (74%) had an elevated IgG index of >0.66. No difference was found between patients with an elevated and normal IgG index, nor was there an correlation between the IgG index levels and sNfL levels.

Patients with at least one T2 lesion on MRI brain or spine (n=66, 89%) had a higher sNfL than patients without T2 lesions (27.5 pg/ml, 95% CI 20.6-36.8 vs 6.6 pg/ml, 95% CI 3.7-11.8; p=0.002). Patients with a high lesion load (≥9 T2 lesions, n=35) had higher sNfL levels than patients with less than 9 lesions (40.6 pg/ml, 95% CI 27.7-59.5 vs 14.8 pg/ml, 95% CI 10.3-21.3; p<0.001).

In the 61/74 (82%) patients who received gadolinium, 29 of them (48%) showed gadolinium enhancement. In patients with contrast enhancing lesions a higher sNfL level was found than in non-contrast enhancing lesions (43,7 pg/ml, 95% CI 30,1-63,3 vs 14,3 pg/ml, 95% CI 9,6-21,5; p<0.001).

For correlation of sNfL with T1 hypointense lesions only patients with gadolinium administration are included in this analysis (n=61), as T1 hypointense lesions cannot be evaluated adequately without gadolinium administration. Higher sNfL levels were found in patients with T1 hypointense lesions on baseline MRI (n=27) than in patients without these lesions (44.9 pg/ml, 95% CI 30.3-66.4 vs 13.8 pg/ml, 95% CI 9.1-21.0; p<0.001). In addition, a positive correlation was seen between the number of T1 hypointense lesions on baseline MRI and sNfL levels (Spearman rho 0.489, p<0.001).

Predictive value of sNfL in ADS- patients for CDMS diagnosis (ADS+ excluded)

As described above, all patients with a second attack of MS had an ADS- presentation. No patient with ADS+ was diagnosed with CDMS. Therefore, in the following analyses only ADS- (n=74) were included. A Kaplan Meier analysis was performed after dichotomizing the sNfL levels, using the median of the included patients (26.7 pg/mL). During follow-up, 17/37 patients (46%) were diagnosed with CDMS in the low level group vs 23/37 (62%) in the high level group. Higher levels of sNfL >26.7 pg/mL are associated with a shorter time to a second attack (CDMS diagnosis) (Log-Rank test, p=0.045), shown in *Figure 3.4.2*.

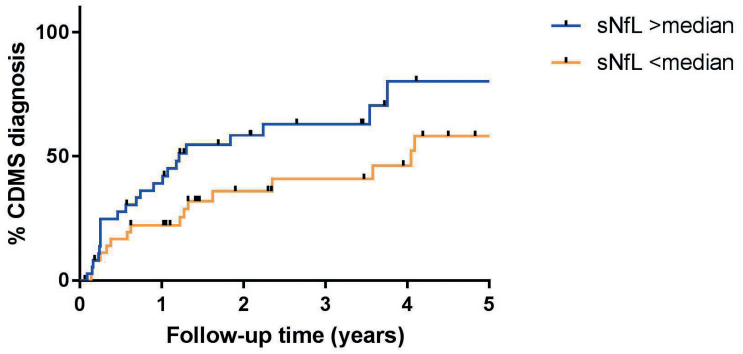

Figure 3.4.2: Kaplan-Meier curves showing time to CDMS diagnosis for ADS- patients (ADS+ excluded).

Patients are divided in having high or low CSF NfL levels using the median sNfL level of ADS- as cut-off (26.7 pg/mL). Abbreviations: CDMS = Clinically definite multiple sclerosis; sNfL = Neurofilament light chain in serum; pg/mL = picogram/millilitre

Cox proportional hazard analyses were performed. The univariate analysis showed a hazard ratio (HR) of 1.04 for each 10 pg increase of sNfL (p=0.057). After adding age, presence of ≥9 T2 lesions at baseline MRI, presence of contrast enhancing lesions and OCB into the multivariable analysis, the HR was 1.09 per 10 pg increase of sNfL (p=0.012).

Thirteen of the 40 CDMS patients (33%) received MS diagnosis based on MRI and received DMT before CDMS, which could have postponed the second attack. We did a subanalysis using the COX regression analysis after excluding these 13 patients. The univariate HR remained similar 1.05 per 10 pg increase of sNfL (p=0.062), however the multivariable analysis remained non-significant after adding age, OCB and MRI parameters.

DISCUSSION

In this study we demonstrate that NfL levels correlate well in serum and in CSF of pediatric ADS patients, in line with adult CIS/MS studies.[12-15] In addition, sNfL levels are higher in pediatric ADS patients at onset than in controls, and the levels are highest in ADS+ patients (ADEM), followed by ADS- with future CDMS diagnosis and are lowest in monophasic ADS-. Furthermore, high sNfL levels have predictive value for CDMS diagnosis after correction for age, OCB, ≥9 T2 lesions and contrast enhancing lesions at baseline MRI.

Multiple MRI parameters were associated with sNfL levels, presence of ≥9 T2 lesions, gadolinium enhancement and T1 hypointense lesions. As we found an association between

CSF NfL levels and T1 hypointense lesions in our previous study[16], we hypothesized that sNfL would correlate with T1 hypointense lesions as well. Indeed the current study showed that the presence of these lesions is highly predictive for MS diagnosis in children with ADS, and suggests the presence of a chronicity in disease activity and damage.[24]

We showed that high sNfL levels at first attack is predicts a shorter time to CDMS diagnosis, even after adjustments for parameters that are currently being used in clinical practice, in line with our previous study on CSF NfL in children.[16] Especially in children it is preferred to have non-invasive metrics to predict disease activity. Our study shows that sNfL may be an potential quantitative biomarker for MS diagnosis in childhood.

In the current study, sNfL levels are highest in patients with encephalopathy (ADEM). Our previous study investigating NfL in CSF showed that levels of ADEM patients did not differ between monophasic ADS- and future CDMS patients, but still showed high levels.[16] The data of these two studies complement each other and indicate that neuro-axonal damage is a prominent feature in ADEM. The long-term sequelae of ADEM have been reported on earlier, including cognitive impairment and persistent motor dysfunction.[29-31] Advanced and longitudinal imaging studies have shown that these patients also have a disrupted white matter maturation and impaired age-expected brain growth.[9] The association between NfL levels in CSF and serum and cognitive and radiological outcome measures need to be investigated.

Our study has several limitations. First, we were not able to evaluate the association between DMT treatment and sNfL levels as all patients had serum sampling prior to DMT initiation. Moreover, our current PROUD-kids protocol does not include regular serial sampling. Treatment evaluation and disease activity monitoring is executed by clinical and radiological evaluation, which both requires time of both physician and patient and is costly. Finding a clinically meaningful and easily assessable biomarker to monitor disease activity and treatment effects is urgently needed. Longitudinal serum sampling in order to investigate these correlations is necessary for future projects. Second, we did not have access to advanced imaging techniques to quantify MRI parameters for neurodegeneration such as brain atrophy. However, we used T1 hypointense lesions as an easily assessable MRI marker for axonal damage.[24] Lastly, our sample size is relatively small, thus further validation of our data in international cohorts is needed.

In conclusion, we show that a strong correlation is present between CSF and serum NfL. This strengthens its reliability as a peripheral marker of neuroaxonal damage in children with ADS. Higher sNfL levels at baseline were associated with a higher probability of and shorter time to future CDMS diagnosis in ADS- patients, after adjustments for existing predictive factors including age, OCB and MRI abnormalities. Replication in larger international cohorts with longer follow-up is needed.

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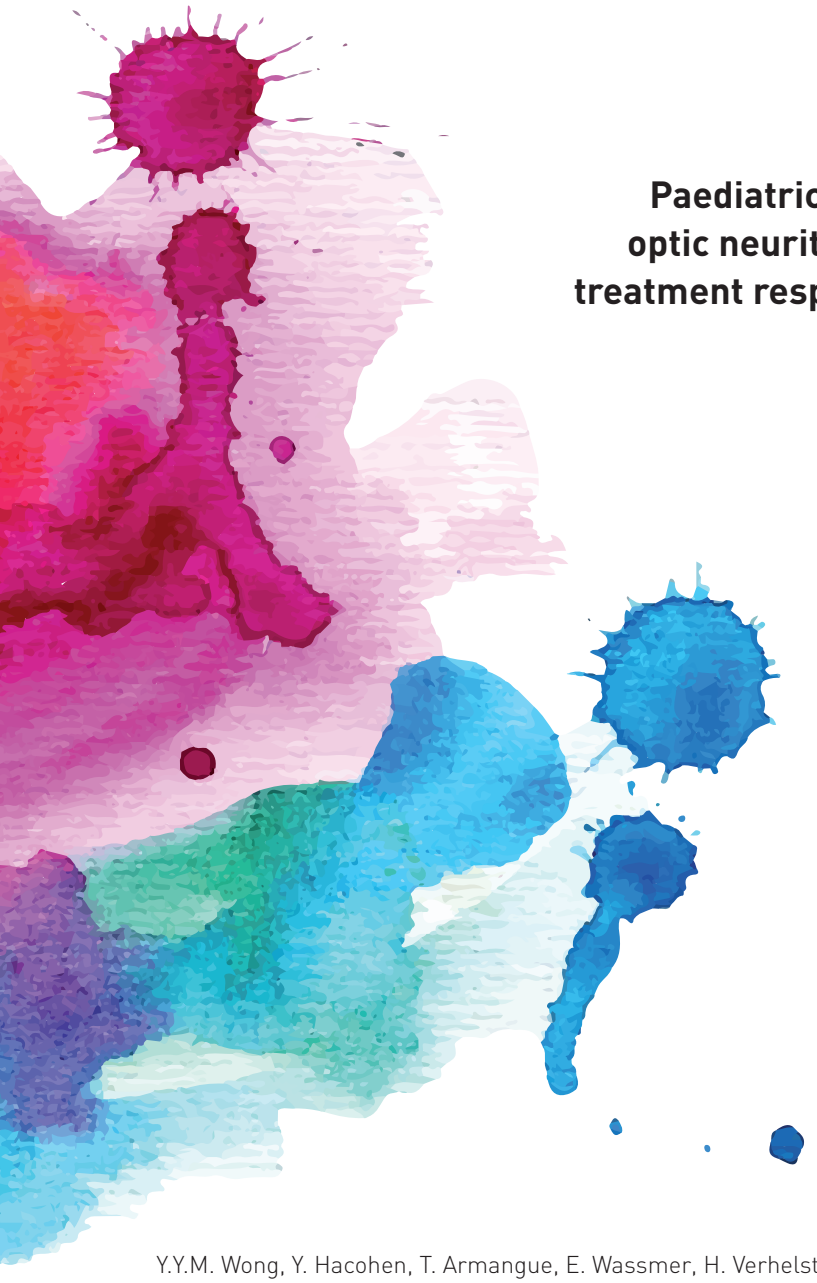
PART TWO B

DISEASE COURSE and OUTCOME of CHILDHOOD ONSET ADS



3.5

Paediatric ADEM followed by optic neuritis: disease course, treatment response and outcome

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ABSTRACT

Background

Acute disseminated encephalomyelitis, followed by optic neuritis (ADEM-ON) is a rare demyelinating syndrome different than MS and neuromyelitis optica spectrum disorder. We aim to describe the disease course, treatment response and outcome of these children.

Methods

Children <18 years were identified from 6 countries of the EU Paediatric Demyelinating Disease Consortium. Patients fulfilled the diagnostic criteria for ADEM, followed by at least one ON. Anti-MOG antibodies (MOG-IgG) were tested in all patients.

Results

In this study of 17 patients (9 boys) with ADEM-ON, MOG-IgG were identified in 16/17. Age at onset was 6.1 years (IQR 5.1-9.2). Twelve patients received oral prednisolone and 10 received maintenance immunosuppression (e.g. azathioprine, intravenous immunoglobulins, Rituximab). During a follow-up of 5.3 years (IQR 1.8-10.2), 54 relapses occurred with a median 3 relapses/patient (range 1-9). Patients relapsed on all treatments but no relapses occurred on a prednisolone dose >10mg/day. Visual and cognitive residual deficits were common in this group.

Conclusion

ADEM-ON is a MOG-IgG associated relapsing disorder and can have a heterogeneous disease course. Patients were refractory for maintenance immunosuppression and appeared to be corticosteroid-dependent. Further international collaborations are now required to unify guidelines in this difficult to manage group of patients.

INTRODUCTION

Acute disseminated encephalomyelitis (ADEM) is a well-recognized acquired demyelinating syndrome (ADS) characterized by a polyfocal onset and encephalopathy. It is most commonly seen in young children.[1] Although predominantly monophasic, some children relapse and may fulfill the diagnostic criteria of multiple sclerosis (MS) or neuromyelitis optica spectrum disorders (NMOSD).[1] More recently, a distinct clinical phenotype has been recognized, different than MS and NMOSD, with patients relapsing with single or recurrent episode of ON following ADEM.[2] The majority of these children have myelin oligodendrocyte glycoprotein antibodies (MOG-IgG). [3-5] In a Dutch paediatric cohort of acquired demyelinating syndrome (ADS) only 1.2% of children were diagnosed with ADEM-ON.[6] Despite the rarity of ADEM-ON in ADS, ADEM-ON has been the final diagnosis in about 40% of MOG-IgG positive relapsing patients who initially presented with ADEM.[7,8]

As no treatment guidelines are currently available for patients with ADEM-ON the aim of this European collaboration study was to describe the disease course, treatment response and outcome of ADEM-ON patients in a multinational approach.

METHODS

Patients and design

We collected demographic, clinical, radiological and serological data of 17 patients under age 18 from 6 countries of the EU Paediatric Demyelinating Disease Consortium (The Netherlands n=7, United Kingdom n=6, Germany n=1, France n=1, Spain n=1, Belgium n=1). This consortium was initiated as a component of the ERN-RITA (European Reference Network for Rare Immunodeficiency, Autoinflammatory and Autoimmune Disease). Patients were identified by reviewing local or national paediatric demyelination registries and included after fulfilling the following criteria: i) presenting with ADEM as a first demyelinating event in accordance with the IPMSSG criteria ii) experiencing ≥1 subsequent attack of optic neuritis (ON) without encephalopathy ≥3 months after ADEM onset, with or without additional episodes of ADEM in between (MDEM-ON).[1]

A unified case reporting form was distributed to all participating sites to collect de-identified patient data. Assessments of visual function were carried out by ophthalmology departments at the respective centres, including high contrast visual acuity measured by the logarithm of the minimum angle of resolution (logMAR) and colour vision measured by Ishihara plates.

All patients were tested locally in the respective reference laboratories of the referring countries for serum MOG and AQP4 antibodies, using live cell-based assays, as part of standard clinical care while clinically symptomatic.[3,4,9-11]

Institutional review board and/or national research ethics approval was obtained at individual centers or national programs respectively.

STATISTICAL ANALYSIS

Chi square and Mann-Whitney U test were used for group comparison. Time to first ON-relapse (TTFR) was calculated by subtracting the date of ON-relapse from the onset date of the last ADEM attack. ARR after the first ON was analyzed with a negative binomial regression, with the natural logarithm of follow-up years after the first ON as offset. This offset was used to correct for the different follow-up durations between patients. Statistical significance was set at p-value of 0.05.

RESULTS

Seventeen ADEM-ON patients were included in this study. Clinical, paraclinical features, treatment response and outcome are summarised in *Table 3.5.1* and *Figure 3.5.1*. MOG-IgG were identified in 16/17 (94%). This seronegative patient had a typical ADEM-ON disease course and serum was tested in the acute phase at onset and re-tested during relapses. MOG-ab were persistently positive in all patients who were retested at follow-up (n=8) irrespective of the presence of disease activity. All patients were AQP4-IgG negative. Brain MRI at follow-up showed improvement or complete resolution of ADEM brain lesions in all patients.

A total of 54 relapses were reported in the cohort (median 3 relapses per patient, range 1-9), during a median follow-up of 5.3 years (IQR 1.8-10.2). Of which 51/54(94.4%) were ON (10/51 bilateral) and 3/54(5.6%) ADEM-relapses *(Figure 3.5.1)*. Nadir visual acuity (VA) at relapses (n=32) was median 0.08 (IQR <1/300 – 0.38). No differences in nadir VA or residual deficits was observed between treated and untreated patients. Oral prednisolone taper was used in 12/17 (71%) patients (starting dose 1-2 mg/kg/day). Twenty-seven of the 54 relapses occurred when oral corticosteroids were tapered off to a low dose (median 8.5 mg, range 1-10 mg) or in the four weeks following discontinuation (median 1.1 weeks; range 0.5-5 weeks). Oral corticosteroid was re-introduced using a higher dose when relapse occurred (median 20 mg, range 10-60 mg), resulting in prolonged corticosteroid exposure (median 6 months, IQR 1.7-14.8 months). No relapses occurred while using a dose>10mg.

Maintenance immunotherapy was commenced in 10/17(59%); Azathioprine 2-3 mg/kg/day (n=6), Mycophenolate 1200 mg/m^2/day (n=1), regular IVIG 0.4-1.0 g/kg/dosis (n=2) and Cyclophosphamide 750 mg/m^2/month for 6 months (n=1). Median time from acute treatment to initiating maintenance therapy was 3 days (range 0-42 days). Treatment response was evaluated at least 6 months after initiation (time of treatment range 6-61 months). Six patients

relapsed on maintenance treatment (total 13 relapses) and were switched to another therapy or another agent was added to the treatment regime, including Rituximab (500 mg/m^2/dosis, 2 dosis per cycle; every 6 months) and IviG *(Figure 3.5.1)*. Of these 6/13 relapses occurred when the oral corticosteroids were tapered off to a low dose (≤10 mg/day) despite ≥6 months of treatment with maintenance therapy. Seven relapses occurred in patients on maintenance treatment only. The disease course differed between patients in the number of relapses and the length of disease-free intervals, up to many years (median 5.7 months, range 3-247 months), in both treated and untreated patients *(Figure 3.5.1)*.

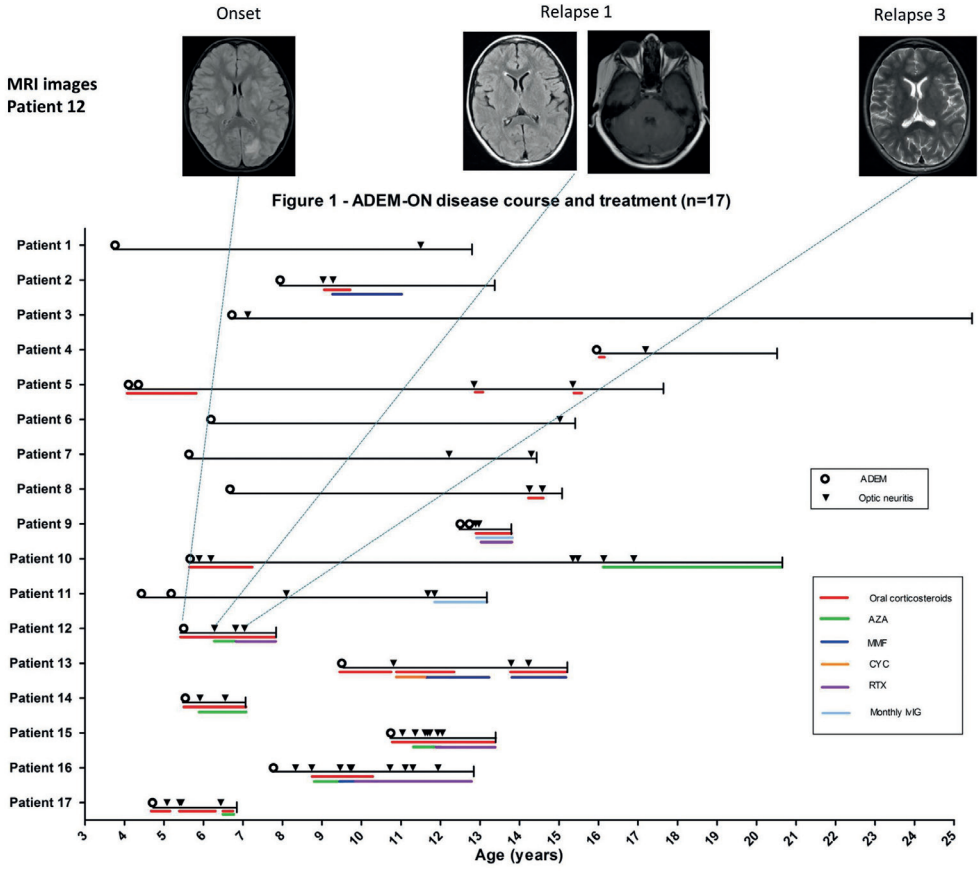

Figure 3.5.1 ADEM-ON disease course and treatment (n=17).

Disease course of acute disseminated encephalomyelitis followed by optic neuritis (ADEM-ON) and treatments given per patient. Magnetic resonance imaging from patient 12. Onset: bilateral multiple large and hazy lesions on fluid-attenuated inversion recovery (FLAIR) imaging sequence. Relapse 1: resolution of white matter lesions on FLAIR imaging and a thickened left optic nerve on T1 sequence without gadolinium enhancement. Relapse 3: relapse of ON without new T2 lesions. AZA, azathioprine; CYC, cyclophosphamide; IVIG, intravenous immunoglobulins; MMF, mycophenolate mofetil; RTX, Rituximab. °, ADEM; ▼, ON.

Table 3.5.1: Patient characteristics

Clinical parameters	**Patients n=17**
Male, n (%)	9 (56)
Age of onset, years, median (IQR)	6.1 (5.1-9.2)
Caucasian, n (%)	13 (81)
Other autoimmune diseases, n (%)	0
Admission to ICU, n (%)	7 (41)
Preceding event <4 weeks before onset, n (%) - Vaccination - Infection/fever	 - 5 (29) - 11 (65)
CSF oligoclonal bands, n (%)	0/14
MOG-IgG	16/17
MRI brain at baseline*	
ADEM-like[a]	15
Unspecific lesions[b]	1
Unavailable[c]	1
MRI Spine at baseline	12/15 (80%)
Presenting symptoms	
Seizures, n (%)	7 (41)
Vision disturbances, n (%)	6 (35)
Autonomic features, n (%)	7 (41)
Cranial nerves, n (%)	3 (18)
Bulbar dysfunction, n (%)	1 (6)
Sensory, n (%)	3/15 (20)
Motor dysfunction, n (%)	9 (53)
Cerebellar symptoms, n (%)	9 (53)
Headache, n (%)	10 (59)
Acute treatment, n (%) - IvMP 3-5 days - Dexamethasone - IvIG 5 days - Additional treatment o 2nd IvMP 3-5 days o 2nd IvIG 5 days	13 (77) - 10/13 (77) - 2/13 (15) - 1/13 (8) - 7/13 (54) o 4/7 o 3/7
Outcome, n (%)	
- Residual deficits	12 (71)
- Visual impairment	8 (47)
- Visual acuity impairment	7 (41)
- Visual field impairment	7 (41)
- Colour vision	4 (24)
- Cognitive impairment	8 (47)
- Seizures	2 (12)

Definitions. IQR: interquartile range. ICU: intensive care unit. CSF: cerebral spinal fluid. ADEM: acute disseminated encephalomyelitis. IvMP: intravenous methylprednisolone. IvIG: intravenous immunoglobulins. MOG-IgG: myelin oligodendrocyte glycoprotein antibodies (IgG subtype).
** Baseline MRI brain performed within 3 months after onset of symptoms. [a]ADEM-like MRI: predominantly confluent, hazy and poorly demarcated involving both grey and white matter.[b]Unspecific lesions: non-specific white matter lesions not fulfilling MAGNIMS criteria for MS specific lesions. [c]Unavailable: patient was retrospectively identified. MRI was not assessable. However, the patient had encephalopathy at presentation and patient charts mentioned lesions fitting the diagnosis of ADEM.*

A shorter TTFR was associated with more relapses after the first ON during follow-up (spearman rho -0.531,p=0.028). The median TTFR was 7 months(IQR 4-85). There was a trend to have higher ARR in patients who relapsed within 7months (mean ARR 1.24 vs 0.35,p=0.061).

At last follow-up, residual deficits were reported in 12/17(71%) patients and included visual impairments (n=8), cognitive impairments (n=8), seizures (n=2), behavioural problems (n=3), weakness in extremities (n=1) and bladder/bowel dysfunction (n=1) were less common. The median EDSS score at last follow-up was 1.0 (IQR 0-3.0).

Residual deficits did not correlate with number of relapses or TTFR.

DISCUSSION

Here we report the disease course, treatment and outcome of ADEM-ON patients identified from six European countries. MOG-IgG were identified in 16/17. This rare group of patients was characterized by large heterogeneity in disease course and applied treatment regimens. By contrast to the reported literature in adults with MOG-IgG associated disease, residual deficits such as visual and cognitive impairments were common.[11-15]

Despite heterogeneity in treatment we observed that relapses occurred on all treatments even under more potent therapies such as Rituximab and/or IVIG. Oral prednisone, on the contrary, was effective if given in doses >10mg daily. This indicates a certain degree of corticosteroid dependence in these children, although the worrisome side effects and unpredictable disease course of ADEM-ON patients warrant against long-term use of high doses (>10mg) prednisone. Corticosteroid dependence may be typical for relapsing MOG-IgG relapsing ON.[16]

We observed a prolonged interattack intervals (>5 years) which is not typically seen in children with RRMS and may result in potential overtreatment and exposure to long-term immunosuppression. This stresses the importance of identifying early predictors for future disease course. TTFR may be good marker, as children with a short TTFR tended to have a higher ARR. Monitoring of MOG-IgG during the course of disease may aid in predicting the future disease course and guiding treatment.[17]

Although this study was the result of a multinational EU collaboration, the sample size remained relatively small due to the extreme rarity of ADEM-ON. Some patients with solely one ON-relapse within the first three months of ADEM were excluded as we followed the IPMSSG 2012 criteria[1]. However this three months duration merits further study as it is arbitrary. International collaborations are now required to unify treatment guidelines to inform on early prognostic markers and treatment effect in this rare demyelinating syndrome.

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SUPPLEMENTAL FILE 3.5.1.

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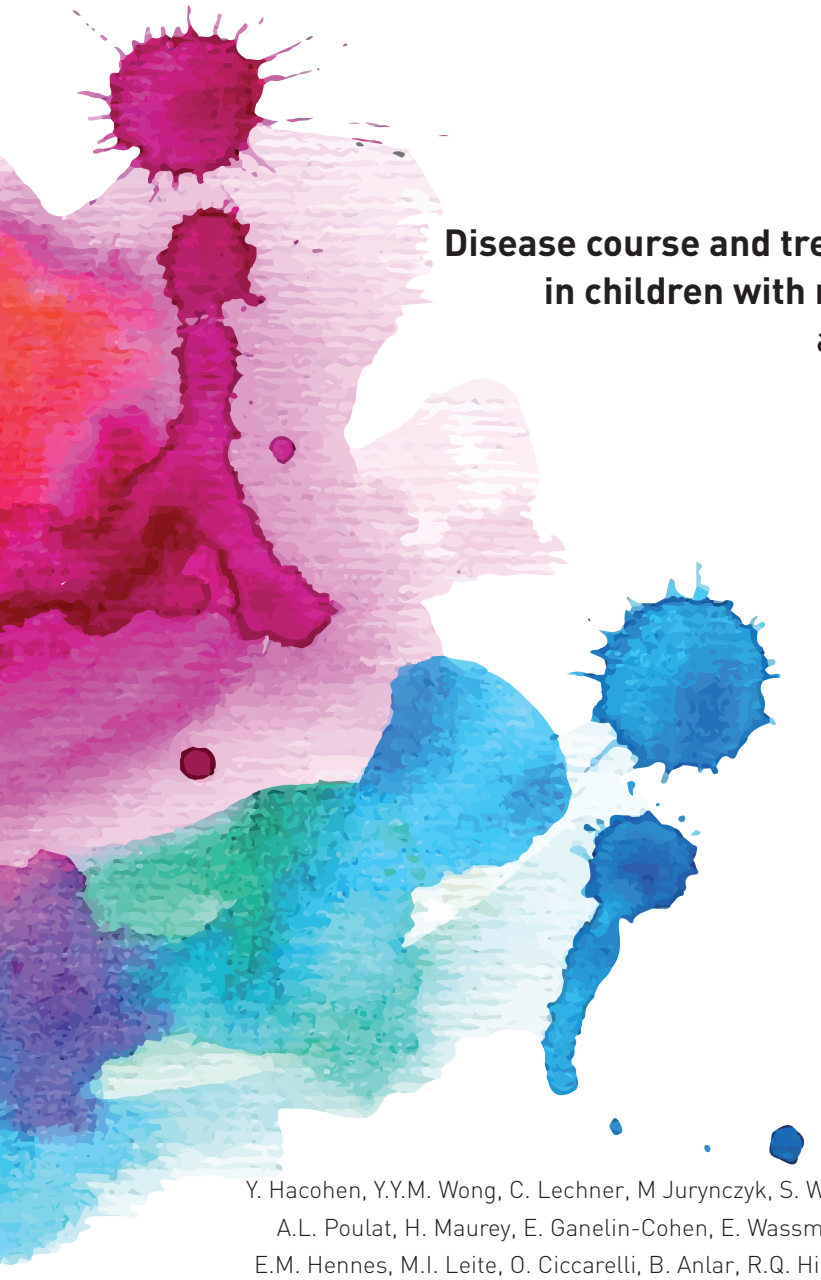

3.6

Disease course and treatment responses in children with relapsing MOG-IgG associated disease

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ABSTRACT

Importance

Myelin oligodendrocyte glycoprotein antibodies (MOG-IgG) are consistently identified in a range of demyelinating disorders in adults and children. Current therapeutic strategies are largely center-specific and no treatments have been formally evaluated.

Objective

To examine the clinical phenotypes, treatment response and outcome of children with relapsing MOG-IgG associated disease.

Design, Setting, and Participants

We prospectively collected demographic, clinical, and radiological data of 102 patients from 8 countries of the EU Paediatric Demyelinating Disease Consortium. Patients were treated according to local protocols.

Main outcomes and measures

Annualised relapse rate (ARR) and Expanded Disability Status Scale (EDSS) score before and during treatment with disease-modifying therapies (DMT).

Results

102 children were identified (median age 7.0, range 1.5-7.9 years; male to female ratio 1.0:1.8; white to other race/ethnicity ratio 3.6/1.0). Original diagnoses were neuromyelitis optica spectrum disorder (NMOSD, 43.1%), acute disseminated encephalomyelitis followed by optic neuritis (ADEM-ON, 19.6%), multiphasic disseminated encephalomyelitis (MDEM, 19.6%), and relapsing optic neuritis (RON, 17.6%). A total of 464 demyelinating events were reported. Treated patients had more relapses (median, 3.0; range, 1.0-17.0) than untreated patients (median, 1.0; range 1.0-7.0) ($P = 0.009$) and higher EDSS scores (median, 1.5; interquartile range, 0-2.5) than untreated patients (median, 1.0; interquartile range, 0-1.5) ($P < 0.001$). Fifty-two children (51.0%) received DMT: 28 (53.8%) were treated with 1 DMT, 17 (32.7%) with 2, and 7 (13.5%) with 3 or more sequential DMT. Patients relapsed during all treatments, with a total of 127 relapses on treatment reported. No changes in median ARR and EDSS score were observed between the pre-initiation and post-initiation phases of interferon beta and glatiramer acetate treatment (n = 11). The median ARR was reduced from 1.84 to 1.0 with azathioprine (n = 20, $P < 0.001$), 1.79 to 0.52 with mycophenolate mofetil (n = 15, $P = 0.003$), and 2.12 to 0.67 with rituximab (n = 9, $P < 0.001$), although the median EDSS score remained unchanged. An improvement in ARR (from 2.16 to 0.51, $P < 0.001$) and EDSS score (from 2.2 to 1.2, $P = 0.01$) was observed in the 12 patients treated with regular intravenous immunoglobulins.

Conclusions

Although commonly used to treat patients with multiple sclerosis, DMT were not associated with clinical improvement in children with MOG-IgG associated disease, whereas azathioprine, mycophenolate mofetil, rituximab, and particularly intravenous immunoglobulins were associated with a reduction in relapse frequency. A correct diagnosis of relapsing MOG-IgG associated disorders is therefore important to optimize immune treatment.

INTRODUCTION

Myelin oligodendrocyte glycoprotein antibodies (MOG-IgG) are consistently identified in a range of acquired demyelinating syndromes (ADSs) in adults and children[1] and in up to 50% of children at first presentation of ADSs.[2-4] Although MOG-IgGs were initially reported in predominantly monophasic disease, a recent report[5] of 210 children with ADS who were followed up for at least 2 years observed that 22 of 65 MOG-IgG positive children (33.8%) experienced clinical relapse and were diagnosed with multiphasic disseminated encephalomyelitis (MDEM),[6] recurrent optic neuritis (RON),[7] acute disseminated encephalomyelitis followed by optic neuritis (ADEM-ON),[8] or neuromyelitis optica spectrum disorder (NMOSD).[9-11] Two recent reports identified MOG-IgGs in 22 of 35 children (62.8%)[5] and 26 of 48 children (54%) 12 with non–multiple sclerosis (MS) relapsing demyelination, which is more than 3 times more common than the aquaporin 4 antibody (AQP4-IgG) (4 of 35 patients[5] and 8 of 48 patients[12]).The MOG-IgG positive children had distinctive clinical and magnetic resonance imaging (MRI) features different from MS and AQP4-IgG NMOSD.[12] Treatment of MOG-IgG associated disease has been influenced by protocols used for NMOSD with AQP4-IgG,[13] although these 2 disorders are thought to be clinically and biologically different.[14,15]

The high proportion of monophasic courses in patients with MOG-IgGs[5] supports the decision against commencing maintenance immunosuppression after the first clinical event of MOG-IgG associated disease. Furthermore, because some patients with MOG-IgGs seem to have a milder NMOSD phenotype than patients with AQP4-IgG,[16] with good short-term response to corticosteroids, many of the relapsing cases were also not treated with maintenance immunosuppression. Recent reports[1,17] also highlight that patients with MOG-IgGs continue to relapse and accrue disability, sometimes despite maintenance treatment, raising important questions about how patients with relapsing MOG-IgG associated disease should be treated. Current therapeutic strategies are largely center specific, formal consensus guidelines are yet to be formulated, and no clinical trials have been performed. We therefore conducted this retrospective, multicenter study to describe the first attack features, paraclinical characteristics, disease course, and responses to different treatment strategies in children with MOG-IgG associated relapsing demyelinating syndromes.

METHODS

Study participants

From January 1, 2014, through December 31, 2016, we collected demographic, clinical, and radiologic data from102 patients from 8 countries of the EU Paediatric Demyelinating Disease Consortium (United Kingdom [n = 57],Germany/Austria [n = 18], the Netherlands [n = 12], France [n = 10], Turkey [n = 3], Switzerland [n = 1], and Israel [n = 1]), a component of the

European Reference Network for Rare Immunodeficiency, Autoinflammatory, and Autoimmune Disease. Participants were retrospectively identified from those prospectively recruited into the respective national demyelination programs or centers and fulfilled the following inclusion criteria: (1) a diagnosis of relapsing demyelination syndrome, (2) presence of MOG-IgGs detected at onset or at the time of a clinical relapse, and (3) age younger than 18 years at first presentation.

Institutional review board and/or national research ethics approval was obtained at individual centers or national programs, respectively. Patients included in this study had been enrolled in national programs with respective review board/ethical committee approvals (France [Hopital Bicetre, Paris], the Netherlands[Medische ethische toetsings commissie Erasmus Medical Centre, Rotterdam], Germany and Austria [University of Innsbruck Ethics Committee], United Kingdom [West Midlands–South Birmingham Research Ethics Committee],and Turkey [Hacettepe University, Ankara]) or provided verbal and/or written informed consent to the respective referring physician. All data were de-identified.

Procedure

Clinical data were de-identified and entered by each participating investigator onto a unified case reporting form (CRF), detailing selected demographics, clinical findings, and laboratory results (MOG-IgG and AQP4-IgG, cerebrospinal fluid white blood cell count, protein level, number of oligoclonal bands, virologic test results, erythrocyte sedimentation rate, and Epstein-Barr virus serologic test results), first and subsequent attacks characteristics, and treatment information. All CRFs were initially reviewed by the respective national leads (5 of us, M.B., K.R., R.N., K.D., and M.L.) and subsequently analyzed by 2 of our investigators (Y.H., M.L.). Demyelinating phenotype at onset was determined from the patient's clinical features, according to established criteria.[18] All patients had undergone brain and spinal cord MRI according to local MRI protocols (which do not routinely include orbital MRI). Cases were assigned by participating investigator and subsequently confirmed by national leads based on clinical and radiologic information provided on the CRF to one of the following diagnostic categories: (1) MS, fulfilling the 2013 International Pediatric Multiple Sclerosis Study Group consensus criteria[18]; (2) NMOSD, fulfilling the 2015 International Panel for NMO diagnosis criteria[19]; (3) MDEM and ADEM-ON, fulfilling the 2013 International Pediatric Multiple Sclerosis Study Group consensus criteria[18];and (4) recurrent demyelination in a single central nervous system area without evidence of clinically silent disease,[18] such as RON.

Annualized relapse rates (ARRs) were calculated as the number of relapses per year before treatment (excluding index event) and during treatment only in patients with at least 6 months of follow-up after initiation of treatment.[20] Relapses were analyzed for up to 2 years before initiation of therapy and for the duration of the time undergoing therapy.

Outcomes at last follow-up were retrieved from the patient's medical records to represent the most contemporary assessment of disability. If unavailable, this assessment was obtained directly from the patient's primary treating physician. The Expanded Disability Status Scale (EDSS) scores were documented at point of disease stability at least 3 months from acute or relapsing events.

MOG-IgG testing

Within 1 month of an acute event (onset or relapse), clinically symptomatic children underwent testing for serum MOG-IgGs, using a live cell-based assay optimized to reduce IgM cross-reactivity[5,21] in the respective reference laboratories (detailed in the supplemental file) of the referring countries, as part of routine assessments of children with demyelinating diseases (antibody testing in the cerebrospinal fluid was not routinely performed).

STATISTICAL ANALYSIS

Parametric or nonparametric statistical tests (Mann-Whitney and Kruskal-Wallis tests) were used for continuous distributions, as appropriate, and $\chi 2$ or Fisher exact tests for nominal data to compare the demographics, presenting symptoms, demyelinating phenotypes, and radiologic and serologic characteristics across the different groups and between those who received or did not receive maintenance immunotherapy. Receiver operating characteristic analysis was used to identify the cutoff age associated with phenotype change. A paired 2-tailed *t* test was used to compare ARRs and EDSS scores before and during treatment. A 2-sided $P < 0.05$ was considered to be significant. Data were analyzed using GraphPad Prism 5 (GraphPad Software).

RESULTS

Patients group

A total of 102 children with relapsing MOG-IgG associated disease were studied (median [range] age, 7.0[1.5-7.9] years; male to female ratio, 1.0:1.8; white to other race/ethnicity ratio, 3.6:1.0). All patients were tested for AQP4-IgG, and none were double positive. The median length of follow-up (from first clinical presentation) was 5 years (interquartile range [IQR], 3-9 years). The original diagnoses were NMOSD in 44 children (43.1%), MDEM in 20 (19.6%), ADEM-ON in 20 (19.6%), and RON in 18 (17.6%). None of the patients had a final diagnosis of relapsing-remitting MS. Patients presenting with ADEM were younger than patients presenting with other ADS (mean [SD] age, 5.6 [0.4] years vs 10.7 [0.6] years; $P < 0.001$). Mean (SD) age at onset was 3.8 (1.7) years in patients with MDEM, 6.9 (2.6) in patients with ADEM-ON, 9.1 (4.5) years in patients with NMOSD, and 11.7 (4.0) years in patients with RON. Clinical events and radiologic changes in patients 9 years or younger were more likely to affect the brain, whereas events in patients older than 9 years were more likely to affect the optic nerve *(Figure 3.6.1)*.

Patients' demographic, clinical, and paraclinical features and EDSS scores according to each relapsing demyelination syndrome phenotype are summarized in *Table 3.6.1*.

First Attack Features

The most frequent demyelinating phenotype at onset was ADEM (53 [52.0%]) followed by optic neuritis (41 [40.2%]). Of the children presenting with optic neuritis, 18 (43.9%) had bilateral optic neuritis, 15 (36.6%) had unilateral optic neuritis, and 8 (19.5%) had simultaneous optic neuritis and transverse myelitis. Visual symptoms were reported in 55 patients (53.9%) and encephalopathy in 53 (52.0%). Of the 58 patients with abnormal brain MRI findings at onset, cerebellar symptoms were found in 29 (50.0%) and seizures in 19 (32.8%).

Paraclinical Features

Cerebrospinal fluid lymphocytosis was reported in 43 of 73 tested patients (58.9%) (lymphocyte count, 10-624/μL; to convert to×10^9/L, multiply by 0.001). Intrathecal oligoclonal bands were seen in only 6 of 54 tested patients (11.1%) across the phenotypes *(Table 3.6.1)*. Erythrocyte sedimentation rate was increased in 21 of 36 patients (58.3%), and evidence of remote Epstein-Barr virus infection was seen in 11 of 43 (25.6%).

Disease Course

A total of 464 demyelinating events were reported in the cohort *(Figure 3.6.1)*. No differences were found in time to first relapse, total number of relapses, and EDSS scores among the different original diagnoses. Despite no differences in EDSS scores detected in the different phenotypes, cognitive problems were seen more frequently in patients with MDEM and ADEM-ON

(16 of 40 patients [40.0%]) vs NMOSD and RON (4 of 62 patients [6.5%], $P < 0.001$). Similarly, patients with abnormal intracranial MRI findings (18 of 65 patients [27.7%]) were more likely to have cognitive problems than patients with normal intracranial MRI findings (2 of 37 patients [5.4%], $P = 0.008$).

Patients receiving immunotherapy had more clinical relapses and worse EDSSs than untreated patients (Table 3.6.2). One patient died. Good recovery, defined as an ARR of 0 at last follow- up (>6 months) and having no neurologic sequelae (EDSS score of 0), was reported in 32 of the 102 patients (31.3%; of these 10 were treated patients).

Table 3.6.1: Demographics, clinical and paraclinical features of children according to their original RDS diagnosis[a]

	MDEM (n=20)	ADEM-ON (n=20)	NMOSD (n=44)	RION (n=18)	All Patients (n=102)
Age (median, range)	3.6 (1.6-8)	6.0 (3.9-15)	8.0 (1.5-17.5)	11.4 (3.8-17.9)	7.0 (1.5-7.9)
Sex (M:F)	1:1.5	1:1.2	1:2.7	1:1	1:1.8
Ethnicity (white:other)	4:1	4:1	3:1	5:1	3.6:1
Family history of autoimmunity	2 (10.0)	2 (10.0)	5 (11.4)	2 (11.1)	11(10.8)
Demyelinating phenotype at onset					
ADEM	20 (100)	20 (100)	13 (29.5)	0	53 (52)
ON	0	0	15 (34.1) (9 bilateral)	18 (100) (9 bilateral)	33 (32.4) (18 bilateral)
TM	0	0	6 (13.6)	0	6 (5.9)
ON+TM	0	0	8 (18.2)	0	8 (7.8)
Brainstem syndrome	0	0	2 (4.5)	0	2 (2.0)
Symptoms at onset					
Vision	3 (15.0)	8 (40.0)	26 (59.1)	18 (100)	55 (53.9)
Encephalopathy	20 (100)	20 (100)	13 (29.5)	0	53 (52.0)
Motor	8 (40.0)	11 (55.0)	21 (47.7)	0	40 (39.2)
Cerebellar syndrome	11 (55.0)	9 (45.0)	9 (20.5)	0	29 (28.4)
Seizures	6 (30.0)	8 (40.0)	5 (11.4)	0	19 (18.6)
Sensory	0	4 (20.0)	12 (27.3)	0	16 (15.7)
Cranial nerve involvement	3 (15.0)	6 (30.0)	4 (9.1)	0	13 (12.7)
Autonomic features	2 (10.0)	5 (25.0)	5 (11.4)	0	12 (11.8)
Paraclinical features					
Intrathecal OCB	3/12 (25.0)	1/10 (100)	2/25 (8.0)	0/7	6/54 (11.1)
CSF WBC count >10/µL	13/17 (76.5)	13/17 (76.5)	15/27 (55.6)	2/12 (16.7)	43/73 (58.9)
CSF protein >0.4 g/L	7/15 (46.7)	1/17 (5.9)	12/30 (40.0)	1/10 (10.0)	21/72 (29.2)

Table 3.6.1: continued

	MDEM (n=20)	ADEM-ON (n=20)	NMOSD (n=44)	RION (n=18)	All Patients (n=102)
ESR >10 mm/h	7/9 (8.8)	8/11 (72.7)	6/12 (50.0)	0/4	21/36 (58.3)
EBV IgG	1/10 (10.0)	0/10	10/19 (52.6)	0/4	11/43 (25.6)
Abnormal brain MRI at onset	20 (100)	20 (100)	18 (40.9)	0	58 (56.9)
Outcome					
Follow-up duration (years)	6.3(2.0-10.2)	7.0 (3.6-9.2)	5.0 (3.1-7.6)	4.3 (3.0-6.7)	5.5 (3.1-9.0)
TTFR (months)	5.5 (3.5-28.2)	10.0 (3.0-28.0)	5.0 (2.0-19.0)	12.0 (4.0-27.0)	6.0 (3.0-22.0)
Total number of relapses (median, IQR)	2.5(1.0-5.0)	2.0 (2.0-4.0)	2.0 (1.0-4.5)	2.0 (1.0-4.0)	2.0 (1.0-4.0)
EDSS (median, range)	1.5 (0-5.0)	1.0 (0-4.0)	1.2 (0-10.0)	1.0 (0-2.0)	1.0 (0-10.0)
Good recovery (EDSS=0 and no relapse>6month)	5 (25)	5 (25)	14 (31.8)	8 (44.4)	32 (31.4)
Cognitive problems	10 (50)	6 (30)	4 (9.1)	0	20 (19.6)

Demographics, clinical and paraclinical features of children according to their original relapsing demyelinating syndrome diagnosis.). Five patients had organisms identified in the CSF (polymerase chain reaction analysis: Enterovirus and Mycoplasma pneumoniae; CSF IgM positivity: cytomegalovirus, human herpesvirus 6, and Borrelia). Of these, 4 presented with ADEM and 1 presented with optic neuritis (human herpesvirus 6). All patients had negative serologic test results when retested at time of relapse.

a Data are presented as number (percentage) of patients unless otherwise indicated.

Abbreviations: ADEM-ON, acute disseminated encephalomyelitis followed by optic neuritis; CSF, cerebrospinal fluid; EBV, Epstein-Barr virus; EDSS, Expanded Disability Status Scale; ESR, erythrocyte sedimentation rate; IQR, interquartile range; MDEM, multiphasic disseminated encephalomyelitis; MRI, magnetic resonance imaging; NMOSD, neuromyelitis optica spectrum disorder; OCBs, oligoclonal bands; RON, relapsing optic neuritis; TTFR, time to first relapse; WBC, white blood cell. SI conversion factors: To convert WBCs to ×109/L, multiply by 0.001.

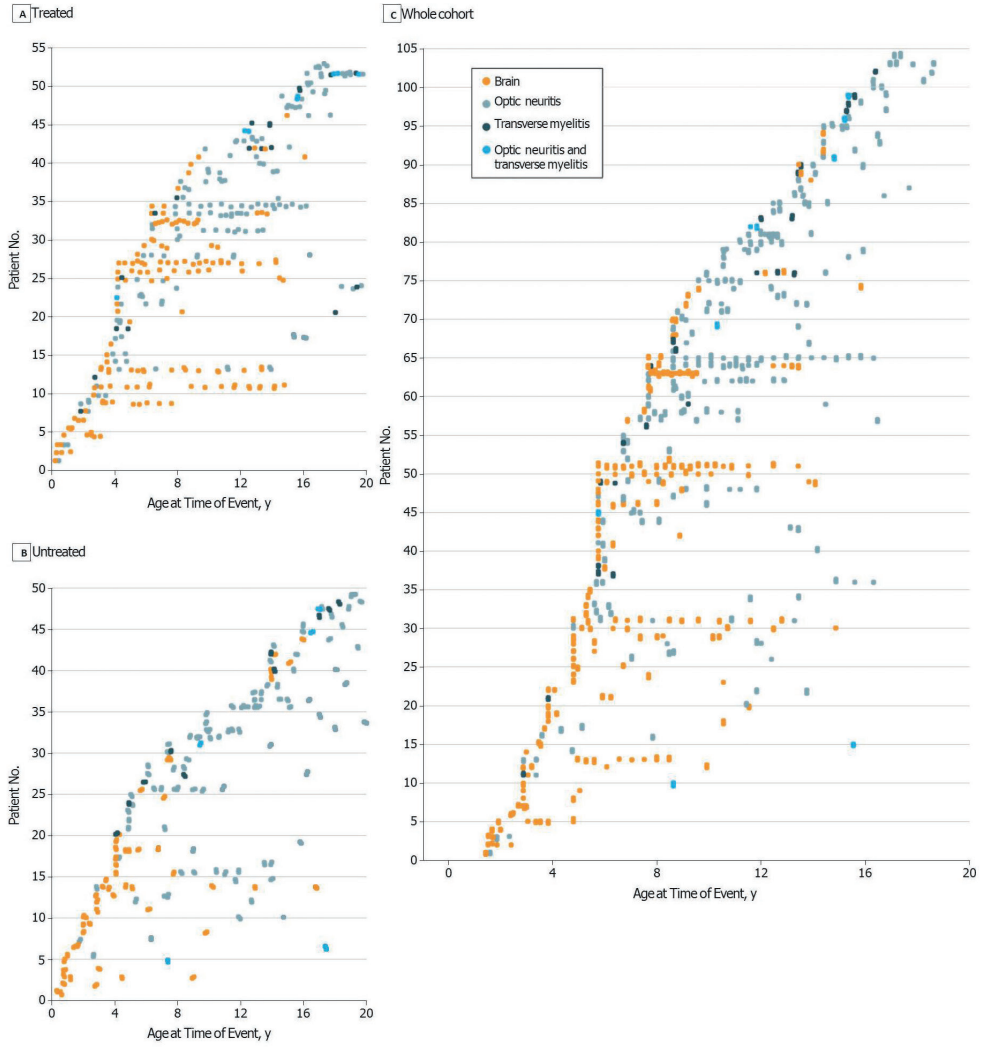

Figure 3.6.1: Demyelinating phenotypes of the first attack and subsequent relapses.
A total of A total of 464 demyelinating events were reported in 102 patients presenting with MOG-IgG associated relapsing demyelination syndrome. Receiver operating characteristic analysis identified age 9 to be the best cut off age associated with phenotype change. Clinical events in patients of 9 years or younger were more likely to affect the brain, whereas events in patients older than 9 years were more likely to affect the optic nerve ($p<0.001$). Brain MRI abnormalities were also more common in the younger group ($p<0.001$). There was no sex predisposition of differences (female:male ratio in patients under or over the age of 9 years was 1:1.64 vs 1:1.6, $p>0.99$).

Table 3.6.2: Comparison between patients who were treated and not treated with disease modifying drugs.[a]

	Treated (n=52)	Untreated (n=50)	p-value
Age median (IQR), years	6.0 (5.0-9.2)	7.0 (5.0-13.0)	0.22
Female to male ratio	2:1	1.5:1	0.84
White to other race/ethnicity ratio	1.9:1	1.4:1	0.54
Demyelinating phenotype at onset:			
- ADEM	29 (55.8)	23 (46.0)	0.32
- Optic neuritis	14 (26.9)	19 (38.0)	0.40
- Transverse myelitis	3 (5.8)	3 (6.0)	>0.99
- Optic neuritis and transverse myelitis	3 (5.8)	5 (10.0)	0.72
- Brainstem syndrome	2 (3.8)	0	0.24
Original RDS diagnoses:			
- MDEM	10 (19.2)	10 (20.0)	>0.99
- ADEM-ON	10 (19.2)	10 (20.0)	>0.99
- NMOSD	25 (48.1)	19 (38.0)	0.32
- RON	6 (11.5)	12 (240.)	0.19
Follow-up time, Median (IQR), years	5.0 (3.0-9.0)	5.0 (3.0-8.0)	0.78
EDSS at last follow-up, median (IQR)	1.5 (0-2.5)	1.0 (0-1.5)	0.009
Total number of relapses throughout the follow-up, median (range)	3 (1-19)	1(1-7)	<0.001

Abbreviations: ADEM, acute disseminated encephalomyelitis; ADEM-ON, ADEM followed by optic neuritis; EDSS, Expanded Disability Status Scale; IQR, interquartile range; MDEM, multiphasic disseminated encephalomyelitis; NMOSD, neuromyelitis optica spectrum disorder; RDS, relapsing demyelination syndrome; RON, relapsing optic neuritis. [a] Data are presented as number (percentage) of patients unless otherwise indicated

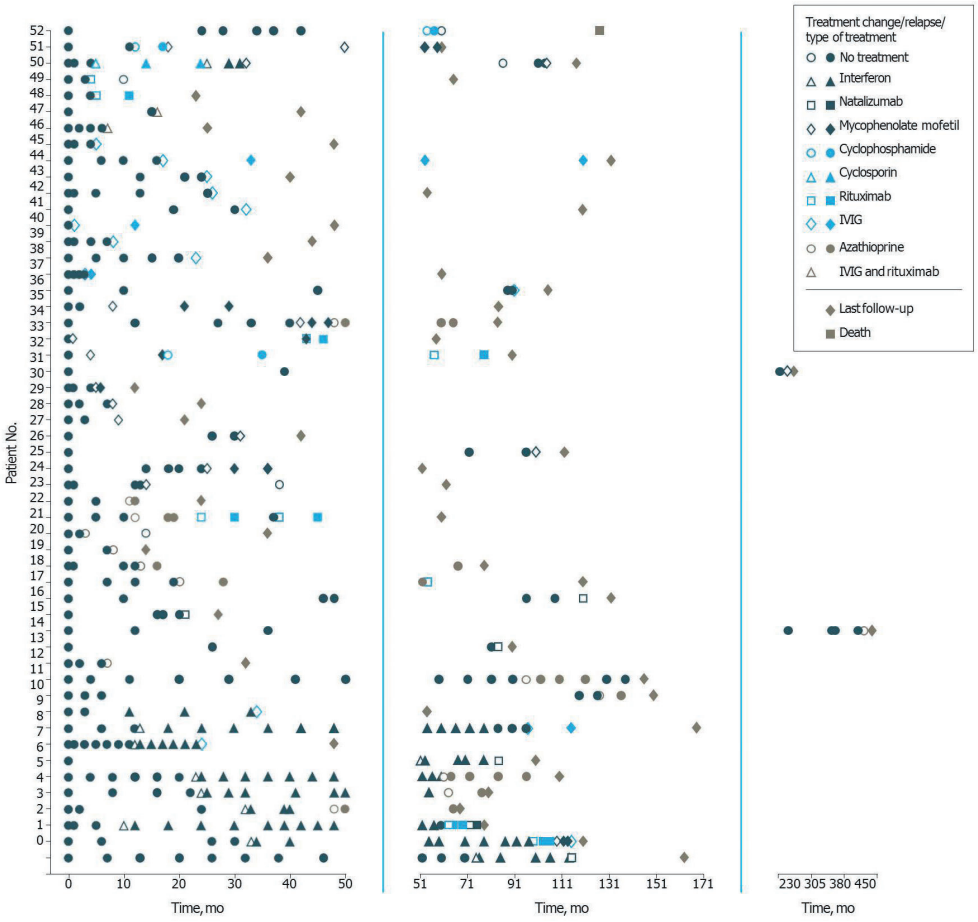

Figure 3.6.2: Disease course in relation to respective therapies.
Each solid marker denotes a demyelinating event, with the color in the figure key denoting respective treatment, whereas an open marker denotes initiation of therapy. Patient relapsed while undergoing all treatments, with a total of 127 relapses during treatment reported in the cohort. All patients treated with first-line injectable multiple sclerosis treatment continued to relapse. Twenty-eight patients remained relapse free while receiving treatment; 7 of 15 (46.7%) treated withmycophenolate mofetil, 10 of 20 (50.0%) treated with azathioprine, 1 of 7 (14.2%) treated with rituximab alone, 6 of 10 (60.0%) treated with intravenous immunoglobulin (IVIG), and 2 of 2 (100%) treated with rituximab and IVIG together. Patient 52 presented initially with bilateral optic neuritis, relapsed 2 years later with transversemyelitis, experienced cognitive and psychiatric problems, and died at 20 years of age of progressive encephalopathy and respiratory failure.

Response to Immunotherapy

The short-term treatment for each of these patients at presentation and during subsequent episodes of relapses was directed by the treating pediatricians based on protocols influenced

by their regional and/or national reference center for central nervous system demyelination, guided by severity and persistence of symptoms. Disease-modifying drugs (i.e., all forms of maintenance immunomodulation or immunosuppression therapies) were given in 52 children (51.0%): 28 patients (53.8%) were treated with 1 DMT, 16 (30.7%) with 2, and 7 (13.5%) with 3 or more sequential DMT, with only 2 patients receiving combinational treatment (intravenous immunoglobulin [IVIG] and rituximab) at any time point. All treatments were optimized at their respective regional or tertiary treating center. The clinical course and disease activity in patients who underwent therapy with maintenance treatment are illustrated in *Figure 3.6.2*. Median time from disease onset to DMT treatment was 1.64 years (IQR, 0.50-3.60 years). Patient relapsed while receiving various treatments, with a total of 127 relapses while receiving treatment reported in the cohort *(Figure 3.6.2)*; Interferon beta and glatiramer acetate (total relapses, 71; 2.1 relapses during treatment), azathioprine (total relapses, 20.0; 0.5 relapse during treatment), mycophenolate mofetil (total relapses, 13.0; 0.5 relapse during treatment), rituximab (total relapses, 10.0;0.7 relapse during treatment), IVIG (total relapses, 6.0; 0.1 relapse during treatment), cyclophosphamide (total relapses, 3.0; 2.0 relapses during treatment), cyclosporine (total relapses, 2.0; 2.0relapses during treatment), and natalizumab (total relapses, 2.0; 0.3 relapse during treatment). The ARRs and EDSS scores before and during treatment are depicted in *Figure 3.6.3*.

Conventional MS treatment (interferon beta and glatiramer) was given as first-line treatment in 10 children and as a second-line treatment in 1 child and was discontinued in all in view of lack of response and ongoing treatment adverse effects. Two patients were initially switched to an alternative interferon preparation before changing treatment. All patients relapsed while receiving treatment. There was no change in the ARRs before and during treatment, with a mean difference of 0.02 (mean ARR before treatment, 2.40; mean ARR during treatment, 2.38; $P >$ .99). There was no change in EDSS score (mean EDSS score before treatment, 2.2; mean EDSS during treatment, 3.0; $P = 0.23$). No severe or life-threatening relapses have been reported with conventional MS treatment. Three patients received natalizumab (2 with good response and 1 who continued to relapse), and no patients received fingolimod or alemtuzumab.

Eleven patients began therapy with mycophenolate mofetil of whom 3 were switched in view of treatment failure, with 1 having additional adverse effects. Four patients were switched to mycophenolate mofetil after cyclophosphamide (n = 2), azathioprine (n = 1), rituximab (n = 1), and cyclosporine treatment followed by interferon beta-1a (n = 1). Eight of 15 patients (53.5%) relapsed while receiving treatment. Mycophenolate mofetil treatment was associated with a mean reduction in the ARR of 1.27 (mean ARR before treatment, 1.79; mean ARR during treatment, 0.52; $P = 0.003$), with no change in EDSS score (mean EDSS score before treatment, 1.7; mean EDSS score during treatment, 1.9; $P = 0.59$).

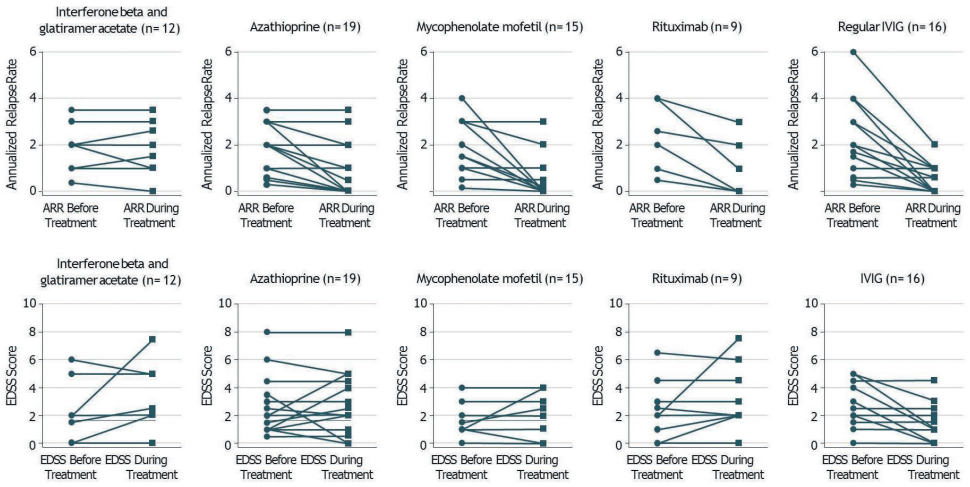

Figure 3.6.3: Efficacy of various disease-modifying therapies in patients with MOG-IgG associated relapsing demyelination

Only 2 patients receiving combinational treatment (IVIG and Rituximab) and were included for both treatment analysis. No differences were detected in the pre-treatment EDSS and ARR between the different treatment groups

Twelve patients began therapy with azathioprine of whom 2 were switched in view of treatment failure and 2 stopped treatment (1 because of treatment failure and 1 because of adverse effects). Eight patients received azathioprine as second line treatment; first-line MS treatment failed in 5 (1 receiving mycophenolate mofetil and 2 after 1 year of corticosteroid treatment). Ten of 20 patients (50.0%) relapsed while receiving treatment. Azathioprine treatment was associated with a mean reduction in the ARR of 0.84 (mean ARR before treatment, 1.84; mean ARR during treatment, 1.0; $P < 0.001$), with no change in EDSS score (mean EDSS score before treatment, 2.5; mean EDSS score during treatment, 2.6; $P = 0.74$).

Rituximab was given as first-line treatment in 4 patients (with additional IVIG in 2), as second-line treatment in 4 patients, and as third-line treatment in 1 patient. Of the patients treated with rituximab as first-line treatment, no additional immunotherapy was used after treatment. Six of 9 patients (66.7%) relapsed during treatment. Two of 3 patients who did not relapse were additionally receiving maintenance IVIG. One child had a severe life-threatening relapse while receiving therapeutic doses of rituximab and had depleted B cells. Rituximab was associated with a mean reduction in the ARR of 1.61 (mean ARR before treatment, 2.12; mean ARR during treatment, 0.67; $P < 0.001$), with no change in EDSS score (mean EDSS before treatment, 2.4; mean EDSS during treatment, 3.2; $P = 0.23$).

Intravenous immunoglobulin (regular infusion every 4 weeks) was given as first-line maintenance treatment in 12 patients (2 received additional rituximab) and in 4 patients as a second-line treatment after revision of the diagnosis. All patients continued to receive IVIG, but in 2 the infusion was reduced to every 8 weeks. Four of 12 patients (33.3%) relapsed while undergoing treatment. The IVIG treatment was associated with a reduction in the ARR of 1.71 (2.16 to 0.51, $P < 0.001$). The EDSS was also reduced (mean EDSS before treatment, 2.2; mean EDSS during treatment, 1.2; $P = 0.01$).

A total of 8 patients received oral prednisolone for more than 6 months; 5 (62.5%) relapsed while receiving treatment (3 while weaning from corticosteroids), and 1 patient relapsed 1 week after treatment with corticosteroids was stopped. Two patients started treatment with cyclophosphamide and 1 with cyclosporine; all relapsed while receiving treatment. Overall, we did not identify any phenotype that was more responsive to any specific treatments *(Figure 3.6.3)*.

Fifty patients (49.0%) were not treated. The median number of relapses in the untreated group was 1.0 (range, 1.0-7.0), and the median EDSS score was 1.0 (IQR, 0-1.5). No differences were found in patient demographics and clinical symptoms at onset and final demyelinating phenotype between the patients who were treated and those who were not *(Table 3.6.2)*. Overall, the treated patients had more relapses (median, 3.0; range, 1.0-17.0) and higher EDSS scores (median, 1.5; IQR, 0-2.5) than the untreated patients (median number of relapses, 1.0; range, 1.0-7.0; $P = 0.009$ and median EDSS score, 1.0; IQR, 0-1.5; $P < 0.001$).

DISCUSSION

Although MOG-IgG associated disease is now well recognized in children and adults, few comparative studies have been performed of their clinical and investigative features, treatment response, or outcomes. In this large multicenter study of 102 children with relapsing MOG-IgG associated disease, the original diagnoses were various, and overall the treated patients had a more severe disease. Although treatments were heterogeneous, injectable MS drugs were not associated with improvement, and maintenance IVIG was found superior to other treatments.

We observed an age-dependent phenotype, with brain manifestation in younger children and optic neuritis and/or transverse myelitis with normal intracranial imaging findings in the older child. This finding is in keeping with the physiologic, age-dependent white matter maturation that occurs from infancy to adulthood and may suggest susceptibility of the uncompacted myelin[22] to an antibody that targets the outermost layer of the myelin sheath.[23] A progressive loss of tissue integrity occurs over time in patients with recurrent brain demyelination, which is

likely to result in secondary neuro-axonal injury and could explain the poor cognitive outcome seen in this group and the reduced response to immunotherapies over time.
In this cohort, we observed the treatment paradox described in similar disorders,[24,25] whereby the higher relapse rate and poorer outcome in the group receiving more therapy is simply reflected by the a priori threshold for initiating such treatments. In the 52 patients who were treated with DMT, treatment was associated with a reduction in the ARR in patients treated with regular IVIG, rituximab, mycophenolate mofetil, and azathioprine in descending order. Care is also required when interpreting the ARR, which is susceptible to artefactual elevation, for example, when there is a short time to first relapse and a short time to treatment (increasing pretreatment ARR). Although we ensured therapeutic DMT doses by including treatment length of at least 6 months, lag time to therapeutic effect of specific treatments may lead to an artefactually elevated posttreatment ARR.

The unresponsiveness to conventional MS therapy is reminiscent of a report[26] in AQP4-IgG NMOSD, although none of these children were reported to have life-threatening relapses after MS therapy as reported in some patients with NMOSD. None of the patients received alemtuzumab, which was reported to cause disease worsening in patients with NMOSD[27] and MOG-IgG associated disease.[28] Interestingly, 6 of 7 patients (85.7%) who received rituximab alone continued to relapse despite B-cell depletion.

A key finding of our study is that IVIG as maintenance therapy was associated with the greatest improvements in ARR and EDSS score. Intravenous immunoglobulin is the only treatment that does not induce immunosuppression. Its mechanisms of action may go beyond the known immunomodulatory effect and may also be beneficial in patients with secondary inflammation.[29] Interestingly, in a recent study[30] using organotypic cerebellar section cultures from transgenic mice and MOG-IgG–induced demyelination, treatment with IVIG was protective from demyelination in a dose dependent manner.

A major limitation of this study is that disease was not systematically managed in all patients, with possible biases in treatment initiation and/or escalation. Because testing for MOG-IgG has only recently become available, the patients described in this article were frequently misdiagnosed with MS, viral encephalitis (in view of the cerebrospinal fluid leukocytosis), and central nervous system vasculitis (in view of the increased erythrocyte sedimentation rate), which resulted in heterogeneous treatment and management regimens across the multiple centers. Because a significant number of cases were retrospectively tested, with diagnosis only considered at relapse and often many years later, this study could not provide information on the utility of serial measurements and/or antibody titers in predicting disease course or directing DMT. This cohort was not adequately powered to evaluate potential differences of immunotherapy responses across the different relapsing phenotypes and was not optimal for

a direct evaluation of an individual or sequence of treatment effect, which is better suited to a study design in which the lag phase of efficacy or washout period of specific therapies could be prospectively controlled. One particular treatment that deserves specific attention is the cumulative use of corticosteroids, often used in conjunction with DMT and at low doses but also during relapses. Prolonged corticosteroid maintenance, which appears to be effective in adults with NMOSD, is less commonly used in the pediatric population in view of the adverse effects.[13]

In conclusion, despite the limitations, this post hoc evaluation and analysis of data previously collected and published allowed us to make important observations about the treatment responsiveness of patients with relapsing MOG-IgG associated disease, which has to be carefully and pragmatically considered alongside the safety of many of these treatments. Importantly, because most children with MOG-IgGs remain monophasic, the data reported here do not evaluate treatment for patients with monophasic ADS; hence, these treatment strategies should not be applied to children after the first clinical event. The important questions our study raises are whether the treatment resistant group represents a selected group of patients who are biologically or immunologically different and whether earlier intervention with more specific maintenance immunotherapy would lead to a better neurologic outcome. However, to achieve this, studies must initially elucidate many key aspects of the MOG-IgG associated disorders, such as disease heterogeneity, early biomarkers of relapsing and/or severe disease, and optimal outcome measures, after which controlled trials could be performed

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SUPPLEMENTAL FILE 3.6.1.

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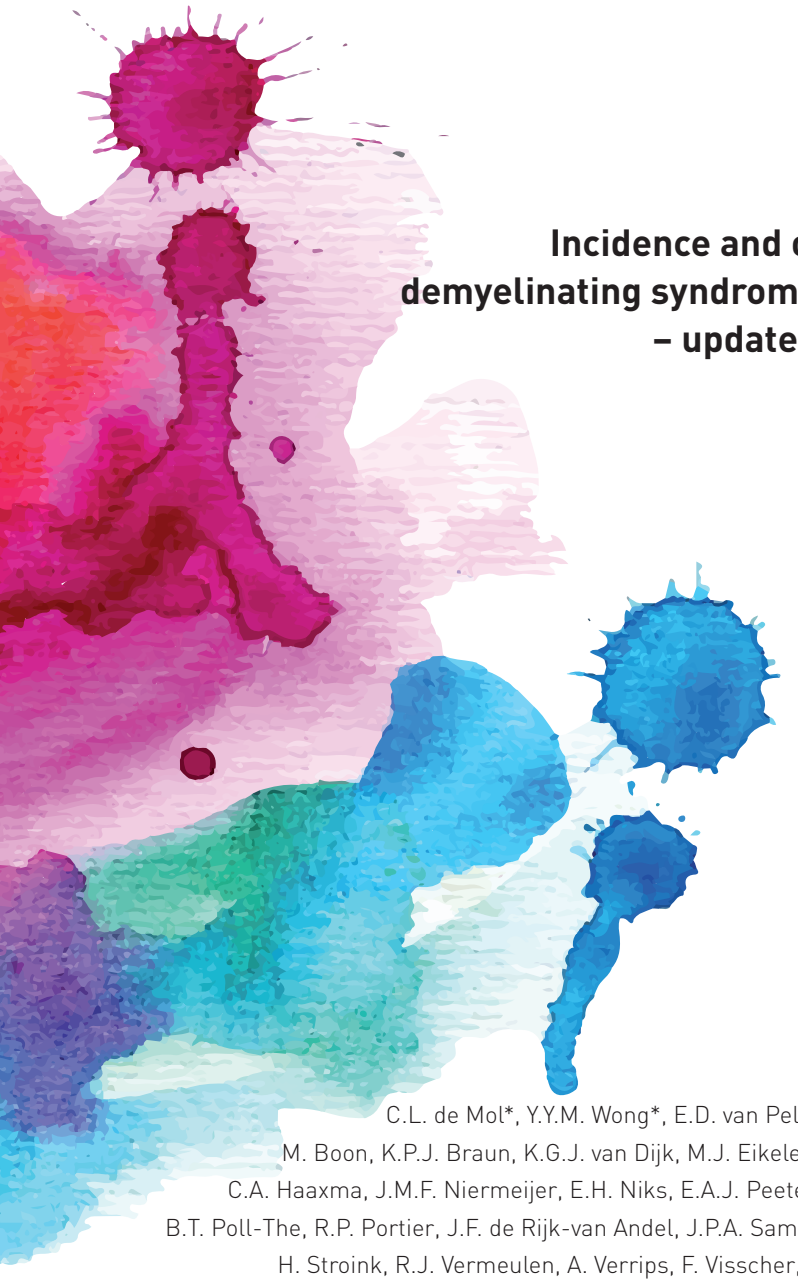

3.7

Incidence and outcome of acquired demyelinating syndromes in Dutch children – update of a nationwide and prospective study

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ABSTRACT

Introduction

Acquired demyelinating syndromes (ADS) are immune-mediated demyelinating disorders of the central nervous system in children. A nationwide, multicentre and prospective cohort study was initiated in the Netherlands in 2006, with a reported ADS incidence of 0.66/100,000 per year and MS incidence of 0.15/100,000 per year in the period between 2007 and 2010. In this study, we provide an update on the incidence and the long-term follow-up of ADS in the Netherlands.

Methods

Children <18 years with a first attack of demyelination were included consecutively from January 2006 to December 2016. Diagnoses were based on the International Paediatric MS study group consensus criteria. Outcome data were collected by neurological and neuropsychological assessments, and telephone call assessments.

Results

Between 2011 and 2016, 55/165 of the ADS patients were diagnosed with MS (34%). This resulted in an increased ADS and MS incidence of 0.80/100,000 per year and 0.26/100,000 per year respectively. Since 2006 a total of 243 ADS patients have been included. During follow-up (median 55 months, IQR 28-84), 137 patients were diagnosed with monophasic disease(56%), 89 with MS(37%) and 17 with multiphasic disease other than MS (7%). At least one form of residual deficit including cognitive impairment was observed in 69% of all ADS patients, even in monophasic ADS. An Expanded Disability Status Scale(EDSS) score of ≥5.5 was reached in 3/89 MS patients (3%).

Conclusion

The reported incidence of ADS in Dutch children has increased since 2010. Residual deficits are common in this group, even in monophasic patients. Therefore, long-term follow-up in ADS patients is warranted.

INTRODUCTION

Acquired demyelinating syndromes (ADS) are immune-mediated demyelinating disorders of the central nervous system (CNS) in children.[1,2] ADS encompass a wide spectrum of neurological symptoms depending on the location of inflammation and the severity of demyelination. As the clinical symptoms overlap in this spectrum, international consensus criteria have been proposed in 2007 to aid in diagnosis and distinction between subtypes.[3] These criteria were revised in 2012.[4] In addition, new findings in the past few years added valuable insights in paediatric ADS and its subtypes, including the identification of new biomarkers such as anti-myelin oligodendrocyte glycoproteins antibodies (MOG-IgG)[2,5] and the identification of new clinical subtypes as acute disseminated encephalomyelitis followed by optic neuritis (ADEM-ON).[6]

ADS may remain monophasic after the first event. Yet, 15-32% of these children will fulfil the diagnostic criteria for paediatric MS within five years after the initial attack.[1,2,7,8] Multiple aspects of outcome of paediatric MS patients have been described before, including the rate of disease progression in Expanded Disability Status Scale (EDSS) scores [9,10], cognitive performance[11-13], decreased motor performance[14,15], and neuropsychiatric complaints like fatigue and mood disorders.[14,16] However, studies describing the long-term outcome of other ADS subtypes are scarce.

In the Netherlands, a multicentre and prospective study was established in 2006 with national coverage for children with a first demyelinating event. Incidence estimates of paediatric ADS and multiple sclerosis have been reported in our prior work for the period between 2007-2010.[17] However, the number of patients who will be diagnosed with MS will likely increase with longer follow-up time. Furthermore, an increasing MS incidence in children has been reported in specific regions.[18,19] Therefore, we aim to re-assess the incidence and presenting characteristics of ADS and its subtypes in the Netherlands. Second, we aim to provide long-term follow-up data of the patients included in our prospective and multicentre cohort in the Netherlands.

METHODS

Study participants

Children younger than 18 years, residing in the Netherlands, and experiencing a first inflammatory demyelinating event of the CNS in the period from 2006 to 2016 have been included in this study. All patients are participants of the PROUD-kids study (*PRedicting the OUtcome of a Demyelinating event in children*), a prospective, multicentre and observational

cohort study. Paediatric neurologists of the eight Dutch academic hospitals and of ten non-academic hospitals took part in this study and included patients to reach nationwide coverage.

Diagnoses were made using the revised criteria proposed by the International Pediatric Multiple Sclerosis Study Group (IPMSSG).[4] Patients with alternative diagnoses were excluded (e.g. systemic autoimmune diseases, infectious diseases or metabolic diseases). Patients were classified as neuromyelitis optica spectrum disorder (NMOSD) as presenting phenotype if either patients were tested seropositive for anti-aquaporine 4 antibodies (AQP4-IgG), or when AQP4-IgG negative patients presented with simultaneous optic neuritis (either unilateral or bilateral) and transverse myelitis (TM) with at least three segments.

Baseline parameters

At inclusion, demographic and clinical information of each patient was gathered. Demographic characteristics consisted for example of ethnic background, date and place of birth and family history on familial autoimmune diseases. Clinical characteristics consisted of presenting symptoms, reported infection or vaccination in the preceding four weeks, acute treatment and hospitalization. MRI images, serum and CSF parameters were also reviewed when available for diagnostics or evaluation.

Follow-up parameters

If patients were not referred to the paediatric MS centre for follow-up, the follow-up data of the patients were provided by the treating physician (f.e. clinical letters) and by interviewing the parents through telephone every 2 years after disease onset.

Cognitive impairment (CI) and residual neurological deficits were assessed using the most recent neuropsychological assessment (NPA) performed by a paediatric clinical neuropsychologist, and neurological examination by a paediatric neurologist. NPAs were being performed appropriately for age. During the NPA, at least six of the following cognitive domains were being assessed for the presence of cognitive deficits: behaviour, language, intelligence, attention and concentration, memory, executive control functions and visuospatial abilities. Children were classified as cognitive impaired if at least one of these domains was affected.

If data on neurological examination or NPA were not available, a standardised questionnaire was administered asking parents or patients about the presence of sensory complaints, motor deficits (e.g. complaints regarding paresis, ataxia, balance problems), bladder complaints (e.g. urge incontinence), maximum walking distance and cognitive impairment (including negatively affected school performance).

The Expanded Disability Status Scale is widely used to express disability of patients with MS diagnosis.[20] EDSS 5.5 stands for a walking distance of maximum 100 metres, without aid or rest.

Antibody testing

Serum AQP4-IgG and MOG-IgG were tested with cell-based assays (CBA) provided in a central laboratory as described previously.[21,22] Patients were tested for regular diagnostics, or retrospectively when serum of the patient was still available.

Ethics approval

This study was approved by the Medical Ethical Committees of the Erasmus MC in Rotterdam and the other participating centres. Written informed consent was obtained from parents and also from patients if aged >12 years at presentation.

STATISTICAL ANALYSIS

Demographic data of the general Dutch population were provided by Statistics Netherlands.[23] These data were used to calculate the incidence of ADS and its subtypes in the period of 2011-2016 in the Netherlands. Statistical analyses were performed using IBM SPSS 21. Figures were made using Graphpad Prism 5.

Chi-square and when appropriate Kruskal-Wallis tests, were used to test differences in demographic and clinical characteristics between the different subtypes. For differences in numerical data between subtypes the ANOVA test was used, and when necessary the Mann-Whitney U test. To compare the ethnic background of the patients with the Dutch population we used a Z-test, with data provided by Statistics Netherlands.[23] Results were considered significant if $p < 0.05$. Missing data was removed from the analyses in all subgroups.

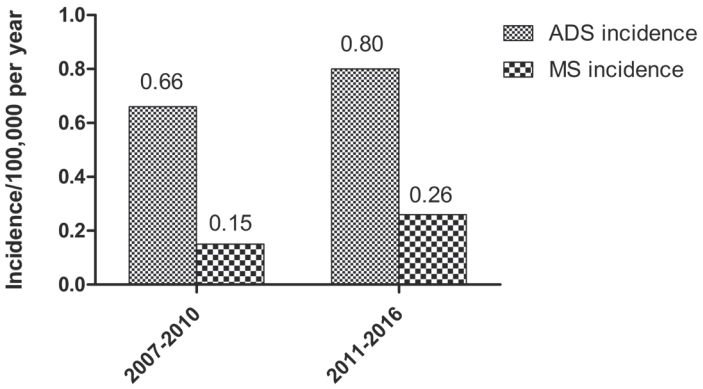

Figure 3.7.1: Incidence of acquired demyelinating syndromes (ADS) and multiple sclerosis (MS) in the Netherlands in 2007-2010 and 2011-2016

RESULTS

Incidence

From January 1 2011 to December 31 2016, 165 ADS patients were reported of which 55 received an MS diagnosis during FU. In this period, the incidence of ADS was 0.80/100,000 per year, ADEM incidence was 0.23/100,000 per year and MS incidence was 0.26/100,000 per year. An overview of the calculated incidences is shown in *Figure 3.7.1*.

First presentation of ADS

From January 1 2006 to December 31 2016, 353 patients were eligible. Of these patients, 243 patients with a first demyelinating event were included in the study *(Figure 3.7.2)*. Presenting phenotypes consisted of optic neuritis (ON; n=55, 23%; from which 16/55 bilateral ON, 29%), transverse myelitis (TM; n=23, 9%); other monofocal clinically isolated syndromes (CIS; n=37, 15%), polyfocal CIS (n=47, 19%), acute disseminated encephalomyelitis (ADEM; n=70, 29%) and NMOSD (n=11, 5%).

Regarding the age of onset, children with ADEM were significantly younger than the other presenting phenotypes ($p<0.001$) and presented more often after a reported preceding infection ($p<0.001$). The latter also applied to children who experienced a TM as first event ($p=0.01$).

The ratio between females and males in all ADS patients did not differ significantly between the presenting phenotypes. When the ADS patients are divided in a group aged < 11 years (n=104) and a group > 11 years (n=139), the female:male ratio differed significantly (1.02:1 versus 1.76:1, $p=0.04$).

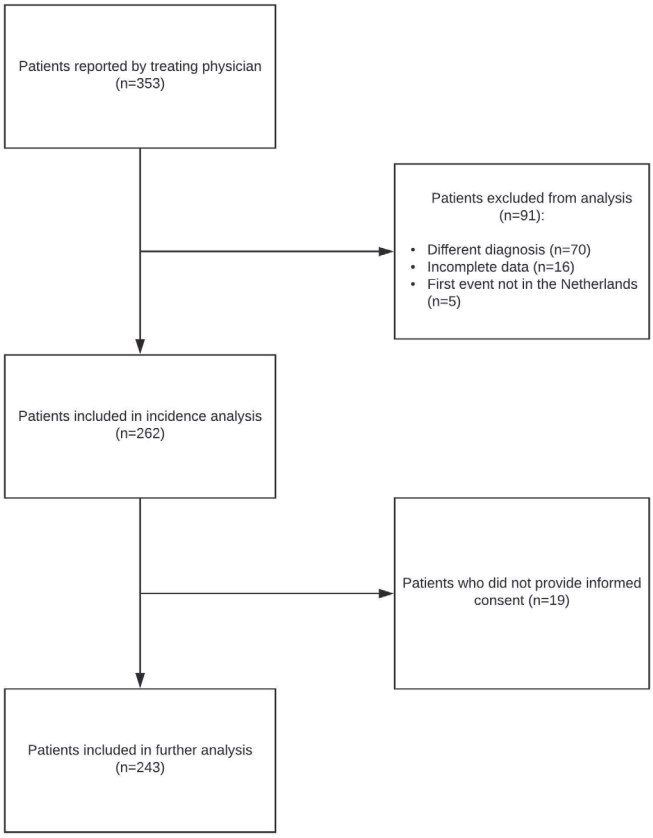

Figure 3.7.2: Flowchart describing the patient selection process

Seventy-eight patients (32%) were of non-Caucasian origin. This proportion was significantly higher than the proportion of children of non-Caucasian origin (17%) in the general paediatric population in the Netherlands ($Z = 5.1$, $p<0.001$) [23]. Most of the non-Caucasian patients were of African (29%) or Middle-eastern (23%) ethnicity.

Forty-eight percent of all patients had a positive familial history of autoimmune diseases (first and second grade family members). Forty-eight (20%) patients reported the presence of rheumatoid arthritis in their family, 40 (16%) reported thyroid diseases, 35 (14%) the presence of other autoimmune diseases (e.g. Wegener's disease and Crohn's disease), 15 (6%) the presence of MS, 14 (6%) diabetes mellitus type 1 and 4 (2%) the presence of optic neuritis (ON). No significant difference was observed between the presenting phenotypes considering the familial history ($p=0.3$).

Table 3.7.1: Presenting phenotypes and demographic characteristics.

	ON (n=55)	TM (n=23)	CIS monofocal (n=37)	Polyfocal CIS (n=47)	ADEM (n=70)	NMOSD (n=11)	P-value*
Female. n (%)	30/55 (55)	15/23 (65)	23/37 (62)	29/47 (62)	35/70 (50)	7/11 (64)	0.7
Age at onset. years. median (IQR)	13.0 (9.6-15.8)	12.7 (4.5-16.1)	14.9 (12.0-16.2)	14.3 (9.4-15.9)	4.2 (2.6-6.1)	12.1 (9.7-16.3)	<0.001[a]
Reported infection < 4 weeks prior to first event. n (%)	11/52 (21)	11/22 (50)	6/34 (18)	14/45 (31)	40/69 (58)	2/11 (18)	<0.001 [a] 0.01[b]
Reported vaccination < 4 weeks prior to first event. n (%)	1/53 (2)	1/22 (5)	1/36 (3)	1/43 (1)	3/69 (4)	0/11 (0)	0.9
Presence of familial autoimmune diseases. n (%)	26/54 (48)	9/22 (41)	21/34 (62)	18/47 (38)	33/69 (48)	7/11 (64)	0.3
Use of acute immunomodulatory treatment. n (%)	37/55 (67)	19/23 (83)	15/36 (42)	30/44 (68)	61/70 (87)	11/11 (100)	<0.001
Average amount of days in the hospital. median (IQR)	3.0 (0.0-5.0)	11.0 (5.0-22.0)	3.0 (0.0-6.5)	5.0 (0.0-10.5)	12.0 (6.8-21.0)	23.0 (5.0-23.0)	<0.001
Total MS cases. n (%)	23/55 (42)	5/23 (22)	30/37 (81)	30/47 (64)	1/70 (1)	0/11 (0)	<0.001
Relapsing disease. n (%)	23/55 (42)	4/23 (17)	25/37 (68)	27/47 (57)	8/70 (11)	3/11 (27)	<0.001
Presence of MOG antibodies. n (%)	4/31 (13)	1/15 (7)	1/20 (5)	3/34 (9)	17/39 (44)	5/7 (71)	<0.001
Presence of AQP4 antibodies. n (%)	0/37 (0)	0/17 (0)	0/12 (0)	0/23 (0)	0/36 (0)	5/11 (46)	<0.001

Abbreviations: optic neuritis (ON), transverse myelitis (TM), acute disseminated encephalomyelitis (ADEM), neuromyelitis optica spectrum disorder(NMOSD), clinically isolated syndrome (CIS), multiple sclerosis (MS), anti-myelin oligodendrocyte glycoproteins (MOG), anti-aquaporine 4(AQP4), interquartile range (IQR), number (n).

[a] *ADEM compared to the other presenting phenotypes.*

[b] *TM compared to the other presenting phenotypes (excluding ADEM).*

** P-value <0.05 is considered statistically significant*

In all ADS patients disease onset in winter was most prevalent (32%), compared to spring (28%), summer (22%) and autumn (17%). MOG antibodies were found positive in 31 of the 146 tested patients (21%). When comparing the presenting phenotypes, MOG antibodies were most frequently found in patients who presented with ADEM and NMOSD (p<0.001).

Detailed patient characteristics are displayed in *Table 3.7.1*.

Follow-up

For the follow-up analysis, we divided all patients into the following categories: monophasic disease, MS and multiphasic non-MS disease *(Table 3.7.2)*. The median follow-up time of all patients was 55 months (IQR 28-84).

Monophasic patients

One hundred thirty-seven patients remained monophasic (137/243, 56%), including ADEM (n=62, 45%), ON (n=27, 20%; from which 11/27 bilateral ON, 41%), TM (n=17, 12%), CIS (n=7, 5%), polyfocal CIS (n=16, 12%), and monophasic NMOSD (n=8, 6%). Of these NMOSD patients, three were tested seropositive for AQP4-IgG and four were seropositive for MOG-IgG. Seven monophasic patients received chronic immunosuppressive therapy: this was initiated in all AQP4-IgG positive patients, and in one of the MOG-IgG positive patients due to the disease severity at onset *(Table 3.7.2)*. Two CIS patients received disease modifying treatment (DMT) because of suspected risk of future MS. One patient had a LETM that required ICU admission and ventilation, and was therefore given chronic immunosuppressive therapy for 1 year.

MS patients

Eighty-nine patients were diagnosed with MS in our cohort (37%), of which 87 received the diagnosis within 5 years of follow-up. Of the 89 MS patients, 70 individuals developed a second attack during follow-up, and were thus diagnosed with clinically definite MS (CDMS).
In these patients, the median time to CDMS was 9 months (IQR: 4-27). After 2 years of follow-up, 74 percent of the MS patients developed CDMS. No patients with MS had a primary progressive disease course. Only one MS patient had an ADEM as first presentation.

After dividing the MS patients into two groups, aged over or below 11 years, the sex ratio showed a trend towards significance (p=0.07), with more girls in the older MS group.
Compared to monophasic ADS, patients who received MS diagnosis during follow-up were more often of non-Caucasian origin (p<0.001) *(Table 3.7.2)*. The calculated MS incidence for patients of non-Caucasian origin was 0.78/100,000 per year in the period from 2011 to 2016, compared to 0.16/100,000 per year in children of Caucasian origin.

Table 3.7.2: Follow-up characteristics of the ADS patients.

	Monophasic disease (n=137)	MS (n=89)	Multiphasic non-MS (n=17)	P-value*
Amount of relapses. median (IQR)	n/a	2.0 (1.0-3.5)	3.0 (1.5-4.0)	0.12
Length of follow-up in months. median (IQR)	47 (22-81)	61 (38-90)	71 (32-102)	0.01
Ethnicity. n (%)				<0.001[a]
- European	106 (77)	44 (49)	14 (82)	
- Middle-eastern	7 (5)	11 (12)	0 (0)	
- African	5 (4)	19 (21)	0 (0)	
- South-American	1 (1)	1 (1)	2 (12)	
- Caribbean	1 (1)	3 (3)	0 (0)	
- Asian	3 (2)	2 (2)	0 (0)	
- Mixed	13 (10)	9 (10)	1 (6)	
- Unknown	1 (1)	0 (0)	0 (0)	
Use of immunomodulatory treatment >1 year. n (%)	7/137 (5)	73/89 (82)	12/17 (71)	<0.001 [a]
Use of second-line immunomodulatory treatment. n (%)	1/137 (1)	28/89 (32)	5/17 (29)	<0.001 [a]
Presence of anti-MOG antibodies. n (%)	24/82 (29)	0/55 (0)	7/9 (78)	<0.001 [a]
Presence of anti-AQP4 antibodies.n (%)	3/83 (4)	0/37 (0)	2/16 (13)	0.09 [a]

[a] *Comparison between monophasic patients and MS*

Abbreviations: multiple sclerosis (MS), interquartile range (IQR), anti-myelin oligodendrocyte glycoprotein (MOG), anti-aquaporin 4 (AQP4), number (n).

** P-value <0.05 is considered statistically significant*

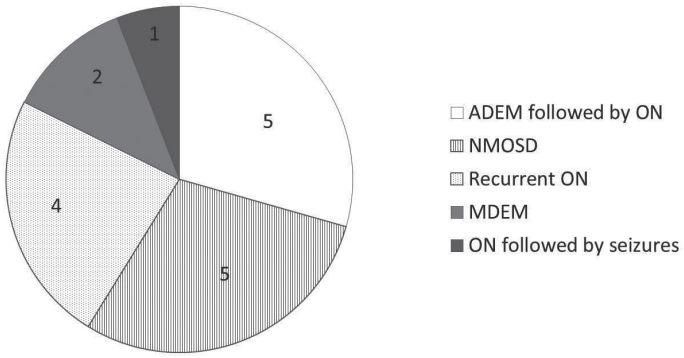

Figure 3.7.3: Distribution clinical subtypes of patients diagnosed with multiphasic non-MS (n=17).
Abbreviations: acute disseminated encephalomyelitis (ADEM), optic neuritis (ON), neuromyelitis optica spectrum disorder (NMOSD), multiphasic acute disseminated encephalomyelitis (MDEM)

At last follow-up, 73/89 MS patients were on DMT for the duration of at least 1 year and in 28 patients second-line treatment was started *(Table 3.7.2)*. Of these 28 patients, 22 started using second-line treatment because of high MS activity, either on MRI or clinically, five because of side effects of first-line treatment and one due to the participation in an international paediatric MS drug trial.

Multiphasic non-MS patients

The patients with multiphasic non-MS disease consisted of five patients who were diagnosed with ADEM-ON, five with NMOSD (AQP4: n=2, MOG: n=1), four with recurrent ON, two with multiphasic disseminated encephalomyelitis (MDEM) and one with ON followed by seizures (once secondary generalized convulsion, once focal epilepsy) *(Figure 3.7.3)*. Of these 17 patients, 12 used chronic immunomodulatory treatment > 1 year. Seven remained on first-line treatment (e.g. azathioprin, myfocenolate) and five patients were switched to second-line treatment (f.e. rituximab n=3, monthly intravenous immunoglobulins (IVIG) n=2).

Residual neurological deficits

Overall, physicians or parents reported at least one form of residual deficit at the last follow-up in 162 (69%) ADS patients, including 71 (83%) of the MS patients, 76 (57%) of the monophasic patients and 15 (94%) of the multiphasic non-MS patients. Residual neurological deficits were significantly more observed in MS patients compared to monophasic patients ($p<0.001$).

In the mono-ADS group, residual deficits were most often present in patients with TM and NMOSD ($p=0.02$). From the monophasic patients with a TM and residual deficits 11/14 had suffered from a longitudinal extended transverse myelitis. In MS and multiphasic non-MS

patients, no significant difference was found in residual deficits between the presenting phenotypes.

Specific differences between the three categories were observed: MS patients reported significantly more sensory deficits and motor deficits compared to monophasic patients ($p<0.05$) *(Figure 3.7.4)*. Yet in multiphasic non-MS patients visual deficits and cognitive impairment were most reported at last follow-up.

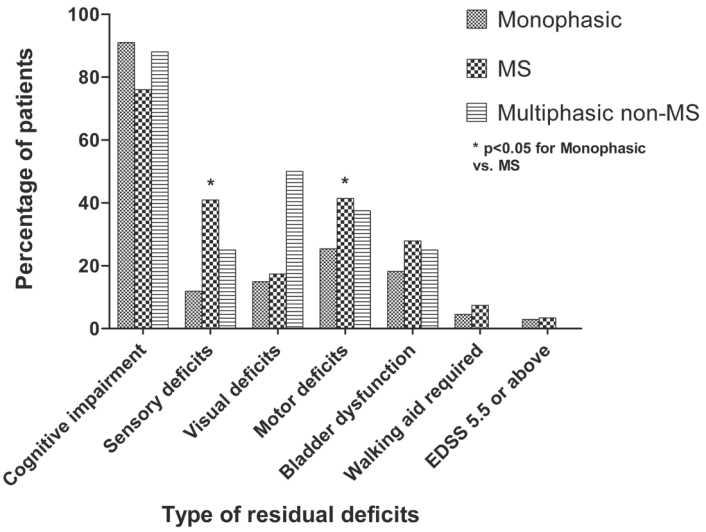

Figure 3.7.4: Residual deficits of ADS patients at last follow-up.
Definition: patients were classified as cognitive impaired if they had a deficit in at least one cognitive domain, tested by a neuropsychological assessment. EDSS: expanded disability status scale of 5.5 stands for a walking distance of about 100 metres, without aid or rest

Cognitive deficits

Thirty-two percent of all included patients (78/243) underwent an NPA (32/137 mono-ADS, 38/89 MS and 8/17 multiphasic non-MS).

At least one of the cognitive domains was affected in 65 of the tested patients (83%), respectively in 29/32 (91%) monophasic ADS, 29/38 (76%) MS and 7/8 (88%) multiphasic non-MS. Three or more cognitive domains were affected in 18/32 (56%) monophasic patients, 22/38 (58%) MS patients and in 6/8 (75%) multiphasic non-MS patients. The three most commonly affected domains in monophasic patients were intelligence, attention and concentration and memory, in MS patients language, attention and concentration and memory and in multiphasic non-MS

patients attention and concentration, memory and executive control functions. Median time till NPA from disease onset was 15 months (IQR 6-32). In monophasic patients the median time was 25 months (IQR 11-62), in MS patients 11 (IQR 6-24) and in multiphasic non-MS patients 10 (IQR 3-22).

Data on school performance was available in 216/243 patients (monophasic patients n=124, MS n=76, multiphasic non-MS patients n=16). Negatively affected school performance was reported by 63/216 (29%) of the participants: 37/124 (30%) of the monophasic patients, 16/76 (21%) of the MS patients and 10/16 (63%) of the multiphasic non-MS patients. This included children to require academic accommodations, for instance extra assistance at school, extra time to complete examinations and change to special education. Of those who reported negatively affected school performance, 19/37 (51%) of the monophasic patients had CI assessed through an NPA, 7/16 (44%) of the MS patients and 6/10 (60%) of the multiphasic non-MS patients.

Furthermore, a total of 122/243 patients reported attention deficits in the standardized questionnaire.

Disease progression in MS patients
Three of the MS patients had an EDSS of 5.5 or above at the last moment of follow-up. All three patients received acute treatment at presentation. They all presented with a polyfocal CIS at the first event, including brainstem as well as spinal involvement (n=3). These patients had a follow-up time of 24, 52 and 96 months and reached EDSS 5.5 at 6, 48 and 66 months respectively. The first patient declined DMT. DMT was commenced in the other two patients, and both were escalated to second-line treatment (natalizumab) because of high MS disease activity.

DISCUSSION

We showed that the incidence of ADS and MS is higher in the period of 2011-2016 than of 2007-2010 [17]. Thirty-seven percent of the patients received a diagnosis of MS during follow-up, which is in line with previous reports about the proportion of MS diagnosis in ADS. Residual deficits are often reported not only in MS, but in all ADS subtypes at last follow-up, irrespective of the presenting phenotype.

The improved awareness of ADS in The Netherlands, aided by a more stable and extended referral network, likely attributed to the increase in incidence compared to 2007-2010. Notably, the small increase in ADS incidence was mainly driven by the rise in MS incidence. Our extended follow-up may have contributed to this higher MS incidence. We cannot exclude that the true incidence of paediatric MS is increasing in the Netherlands, as has been reported on overall

MS incidence in other regions [18,19]. Prolonged assessment of the incidence will be necessary to answer this question. Our new ADS incidence estimates are comparable to previous prospective studies that reported ADS and MS incidence in children [1,24]. Moreover, our study confirms the skewed ethnic distribution in paediatric MS patients towards non-Caucasian ethnicities [1,17,25,26].

A non-MS multiphasic disease course was observed in a minority of the patients (17/243, 7%). Remarkably 78% of these patients were tested seropositive for MOG-IgG, in line with previous findings that MOG-IgG positivity pleads against MS diagnosis and that these patients tend to have a relapsing disease course [27,28].

MOG-IgG and AQP4-IgG seropositivity may be underestimated in this cohort, as the CBAs for both antibodies were developed and validated after the start of our prospective study. Serum was not retrospectively available of every patient who was included before the CBAs were implemented in routine diagnostics.

Over a median follow-up time of 61 months, only three MS patients reached an EDSS of 5.5 or above. However, residual neurological deficits are common in patients with MS (83%), in line with previous studies [11-16]. Cognitive deficits are commonly encountered in MS, but are also described in ADEM [29,30]. Our results show similar results, as 34% of the ADEM patients show CI assessed by an NPA. A limitation here is that only one-third of our patients underwent an NPA in a standardised way. As part of the nationwide epidemiological orientation of our study testing all patients was not feasible. Still, every patient who underwent an NPA had at least six cognitive domains tested. Furthermore, since 2013 all ADEM and MS patients who were presented in the paediatric MS centre in Rotterdam, have been consecutively referred for an NPA. Therefore, any selection bias within these two groups would have been minimal, leading to a more representative view on cognitive impairment in these patients. Also the multiphasic non-MS patients reported cognitive impairment and visual problems. These findings can be explained by the relatively high proportion of ADEM-ON patients in this group [6].

Our data further feed the impression that one single hit of ADEM can leave considerable intracerebral damage and may be considered less benign than previously thought [31-34]. A recent study showed reduced age-expected brain growth in monophasic ADS patients, especially ADEM, indicating irreversible and continuing changes occurring in the CNS even in absence of chronicity [35]. Furthermore, recent studies have shown long-term residual deficits in ADEM and monophasic patients, such as a higher prevalence of motor problems, lower physical activity and fatigue [14,15].

The high number of children with long-term residual deficits in the total group is concerning in relationship with school performances and psychomotor development, especially taking into account the cumulative nature of acquired disabilities after a longer disease duration in chronic demyelinating syndromes [9,36]. Future participation in society, including work-related activities, is likely to be affected. Indeed, in a large proportion of adult patients, MS had negatively affected their employment situation [36,37]. These effects could even be worse in paediatric onset ADS. Therefore adequate detection and guidance of ADS patients is important to preserve and improve societal functioning, and is essential during follow-up of these patients into adulthood.

There are some limitations to this study. Despite our quite unique and extensive national paediatric MS network with full geographical coverage, it is still possible that we have missed a few cases and thus have an underestimation of our incidence figures. Adolescents with CIS may have been assessed and followed-up by an adult neurologist and therefore have not been referred to take part in our study. In addition, the negatively affected school performance in ADS patients may be correlated with other problems than CI. Fatigue, mood disorders, anxiety and negative coping strategies could correlate with a negative school performance in these patients, and may interact with cognitive impairment as well [14-16].

In conclusion, the reported incidence of ADS and MS in the Netherlands has increased during the previous years. Across all ADS subtypes the observed residual neurological deficits are considerable. Long-term follow-up studies of ADS patients will be needed to provide more insight into the risks involved and to identify possibilities for timely intervention.

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3.8

Fatigue and physical functioning in children with multiple sclerosis and acute disseminated encephalomyelitis

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ABSTRACT

Background and objective

Fatigue and physical impairments are a major concern in children with multiple sclerosis (MS) and after acute disseminated encephalomyelitis (post-ADEM). We here aimed to evaluate the interaction between fatigue, exercise capacity, motor performance, neurological status and quality of life (HRQoL).

Methods

In this cross-sectional study, data of thirty-eight children (MS n=22, post-ADEM n=16), aged 4-17 years attending our national pediatric MS-center, were studied. Fatigue was measured with the Pediatric Quality of life-Multidimensional Fatigue Scale, exercise capacity with the Bruce-protocol, motor performance with the Movement-Assessment-Battery-Children-II, HRQoL with the Pediatric Quality of Life questionnaire and extent of disability with the Expanded Disability Status Scale (EDSS).

Results

Children with MS and post-ADEM experienced more fatigue ($p<0.001$), reduced exercise capacity ($p<0.001$) and impaired motor performance ($p<0.001$), despite low scores on the EDSS. Fatigue, but not the other parameters, was significantly correlated with HRQoL. Fatigue was not correlated with exercise capacity.

Conclusion

We confirm the major impact of fatigue on quality of life in children with MS and post-ADEM. Fatigue was not explained by reduced exercise capacity or impaired motor performance. An important finding for clinical practice is that the low EDSS score did not reflect the poor physical functioning.

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. It is generally considered as a disease of young adults in their twenties and thirties, although 2-10% of all MS patients experience their first attack before the age of 18.[1-3] Pediatric onset MS (POMS) shows parallels with adult MS, but runs a more severe course because the relapse rate is higher, next to a higher lesion load on MRI.[4,5] Despite time to the secondary progressive phase in POMS is longer, patients are disabled at a younger age than patients with adult onset MS.[1,6]. In addition to mental and cognitive problems, many patients struggle with physical impairments and fatigue.[7,8] These problems do not limit themselves to chronic forms of demyelinating disease like MS, but are also reported in children after acute disseminated encephalomyelitis (post-ADEM), although rapid motor function recovery after the acute phase in most of the patients seems to be the case.[9-11]. The Expanded Disease Status Scale (EDSS) is commonly used to evaluate disability of adult and pediatric patients with demyelinating disorders.[12] However, it is originally intended for adult MS patients.

As mentioned above, fatigue is a frequently occurring problem in patients with POMS and post-ADEM. The cause of fatigue still remains unclear. In a Canadian study of children with MS and monophasic acquired demyelinating syndromes (mono-ADS), a correlation was found between fatigue and physical activity.[13] POMS patients were less physically active and scored higher on fatigue scales than patients with mono-ADS. Possibly, due to complaints of fatigue, patients become less physically active and this may lead to decreased exercise capacity.

We assume that physical disturbances as motor problems, fatigue and decreased exercise capacity affect a child's physical and psychosocial development and hence their quality of life. To the best of our knowledge, there is no data available of motor development in children with POMS and ADEM and maximal exercise capacity in children with ADEM.

Therefore, we primarily aimed to evaluate whether children with MS and ADEM have a reduced exercise capacity and whether this correlates with fatigue. Second, we analyzed the possible relations between quality of life and fatigue, exercise capacity or motor performance. Lastly, we aimed to investigate whether the EDSS is an optimal measurement to determine disabilities in daily life in these children.

METHODS

Study participants

Children under the age of eighteen were eligible for this cross-sectional study when diagnosed with POMS or ADEM in consensus with the International Pediatric Multiple Sclerosis Study Group 2012 diagnostic criteria.[14] Patients with other demyelinating syndromes (e.g. clinically isolated syndromes, neuromyelitis optica) were excluded. All children were evaluated in our national-multidisciplinary-pediatric-MS-center as part of routine medical care between 2013 and December 2015. Assembled clinical parameters consist of patient history and neurological examination performed by a pediatric neurologist. Disability was expressed by the EDSS score.[12] Measurements of exercise capacity, motor development, fatigue and quality of life were administered by a pediatric physical therapist. After the evaluation, tailor made advice concerning physical therapy and rehabilitation was given.

Written informed consent and permission to use the data for research purposes was obtained from all parents and/or children between 12 and 18 years of age.

Fatigue – Pediatric Quality of life Multidimensional Fatigue Scale (PedsQL-MFS)

The PedsQL-MFS was designed as a generic symptom-specific, and standardized instrument to measure fatigue in healthy children and in children with acute and chronic health conditions aged 2–18 years, also validated in Dutch children.[15] The PedsQL-MFS comprises three subscales: general fatigue, sleep/rest fatigue and cognitive fatigue. A total fatigue score is calculated from the subscales. A scale score and total fatigue score of one standard deviation (SD) below the mean of healthy age-related reference norm was considered abnormal.

Exercise capacity - Bruce-protocol

The Bruce-protocol was used to test maximal exercise capacity.[16] Children were encouraged to perform to exhaustion. The maximal endurance time on the treadmill was used as criterion of exercise capacity. Before and during the test, heart rate (HR) and transcutaneous oxygen saturation were monitored (motion artifact system, type 2001, Respironics Novametrics, Murrysville, PA, USA). HR of ≥185 beats per minute or loss of coordination, because of excessive fatigue was taken as maximal performance.[17] The standard deviation score (SDS) of the maximal endurance time is calculated using age-related reference values for healthy Dutch children.[18,19]

Motor performance - MABCII

Motor performance was examined with the Movement-Assessment-Battery-Children second edition (MABCII). The MABCII is a standardized and aged-related norm referenced test, validated for Dutch children, and developed to classify children according to degree of motor

performance.[20] The MABCII has three domains: manual dexterity, ball- and balance skills. For each child, the raw item scores were transformed into a domain percentile score and a total percentile score. Scores ≤ fifth percentile denote a definite motor problem; scores between the sixth to 16th percentiles denote borderline performance; scores > 16th percentile indicate normal performance.

Quality of Life - Pediatric Quality of Life Inventory (PedsQL) 4.0

Health related quality of life (HRQoL) data were collected using the PedsQL 4.0.[21] Patients as well as one of their parents are asked to fill in the questionnaires. It encompasses 23 items on four Generic Core Scales: physical, emotional, social, and school functioning. A psychosocial functioning scale can be derived from the emotional, social, and school functioning items. All 23 items together provide the total functioning score. The children indicate on a five-point scale the frequency in which they experience a problem and these scores are linearly transformed to a zero to 100 scale. Higher scores indicate better functioning. The Dutch version of the PedsQL has adequate psychometric properties.[21] A scale score and total functioning score of one SD below the mean of healthy age-related reference norm was taken to indicate impaired HRQoL.[22]

STATISTICAL ANALYSIS

Analyses were performed using SPSS 23.0 (IBM, Chicago, IL, USA). The Kolmogorov-Smirnov test was used to test whether the data was normally distributed. One sample T-test was used when comparing continuous data of each measurement to expected average of the reference group. A Chi-square test was applied to test whether the distribution of motor performance scores in our population differed significantly from that in the normative population. For correlation analyses Pearson correlation and Spearman correlation were used when appropriate. Mann-Whitney U test was used for group comparison. A p-value was considered significant <0.05.

Body Mass Index (BMI) was calculated, and the Dutch Growth Analyzer version 3.5 served to calculate SDS for BMI on the basis of Dutch references.[23]

RESULTS

Between June 2013 and December 2015, 40 children were diagnosed with MS(n=24) or ADEM (n=16). Two children with MS, had no standard assessment by the pediatric physical therapist, due to disability caused by serious ataxia *(Figure 3.8.1)*. Thus, data of 38 children were eligible for analysis. The median age at time of assessment was 13.4 years (range 4.3-17.6). Fifteen children had disease activity within the sixth months before the FU visit. Twenty-two children were participating in sports activities.

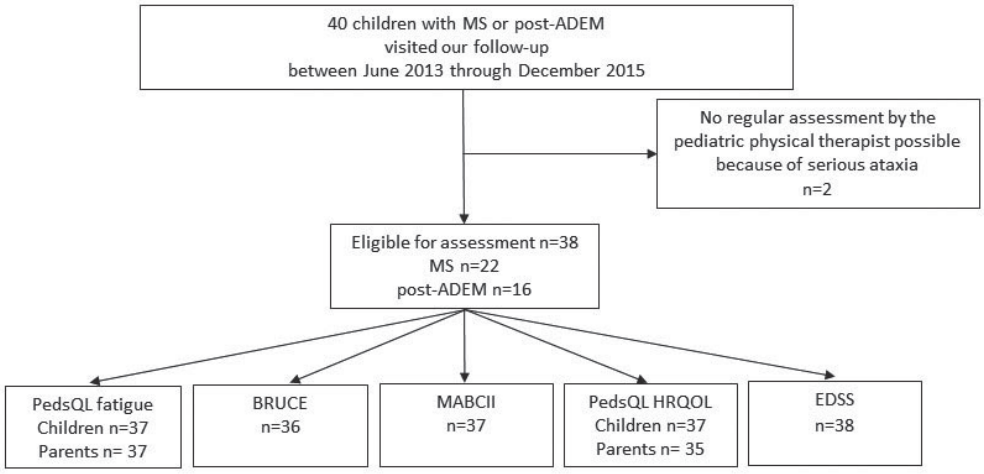

***Figure 3.8.1*: Patient eligibility**
Abbreviations: MS=Multiple Sclerosis, post-ADEM=after Acute Disseminated Encephalomyelitis, MABCII=Movement Assessment Battery for Children, second version; PedsQl=Pediatric Quality of Life Inventory, HRQOL=Health-related quality of life; EDSS= Expanded Disability Status Scale

Relevant baseline characteristics are listed in *Table 3.8.1*. None of the children had concomitant diagnosis influencing cardiopulmonary function.

Fatigue - PedsQL-MFS

The PedsQL-MFS was filled in by 37/38 of the children. One child could not fill in the questionnaire due to his young age (<5 years). All parents returned the questionnaire. Fifteen children and nineteen parents indicated fatigue (<-1 SD). Scores on all subscales were significantly lower than the reference group in both patients and parents *(Table 3.8.2)*. Parents indicated more often and more severe fatigue for their children than the children themselves. Both children with MS as children post-ADEM experienced greater fatigue than healthy related peers: eight of 22 children with MS and seven of fifteen children post-ADEM.

Table 3.8.1 Baseline characteristics of participants.

	Total group n = 38	**MS n = 22**	**Post-ADEM n = 16**
Boys, n (%)	13 (34)	4 (18)	9 (56)
Age in years	13.4 (9.2 - 15.7)	14.0 (13.0 - 15.0)	4.5 (2.3 - 5.9)
SDS BMI	0.8 (0.3 - 1.4)	1.2 (0.3 - 1.6)	-0.3 (-0.9 - 1.0)
Age first symptoms, years	11.8 (5.0 - 14.1)	14 (9.0 - 17.0)	4.5 (1.5 - 11.5)
Time from onset to assessment, months	16.3 (5.1 - 40.5)	10.2 (4.6 - 21.5)	40.1 (11.4 - 63.5)
DMT in MS at time of assessment, YES, n (%)	18 (47%)	18 (82%)	-
Number of episodes within 12 months to assessment, n (%)			
0	15 (40)	3 (14)	12 (75)
1	18 (47)	14 (64)	4 (25)
2	3 (8)	3 (13)	-
3	2 (5)	2 (9)	-
EDSS			
0	20 (53)	10 (46)	9 (56)
1.0	8 (21)	4 (18)	5 (31)
1.5	7 (18)	6 (27)	1 (6)
2.0	2 (5)	2 (9)	-
3.0	1 (3)	-	1 (6)
Sports participation at time of assessment, YES, n (%)	22 (58)	11 (50)	11 (69)

Data are presented as median (IQR) unless otherwise stated. MS = Multiple Sclerosis, post-ADEM = after Acute Disseminated Encephalitis, SDS = Standard Deviation Score, BMI = Body Mass Index, DMT = Disease Modifying Therapy, IQR = Interquartile Range.

Exercise capacity - Bruce-protocol

Exercise capacity data were analyzed for 36/38 children. Data of two children were not analyzed because they could not reach maximal performance (HR <185/min). The 36 children performed significantly below reference values (p<0.001) as shown in *Table 3.8.2*. Especially children with MS had a limited exercise capacity. Seventeen out of twenty (85%) children with MS and nine out of sixteen (56%) children with post-ADEM scored below average (<-1 SD).

Motor performance - MABCII

Thirty-seven children were tested with the MABCII. One child was not tested because of logistic reasons. Nineteen of these 37 children (51% vs 84% expected based on reference values) had a total impairment score (TIS) within the normal range, five children (14% vs 11% expected) were classified as borderline and another thirteen (35% vs 5% expected) as having a definite motor impairment. This distribution is significantly different from reference values (p≤0.001).

Problems were encountered in all three subscales: manual dexterity, ball- and balance skills as well (p<0.001) as displayed in *Table 3.8.2 and Figure 3.8.2*.

Table 3.8.2: Test results: fatigue, exercise capacity, motor performance, quality of life and EDSS

Measurement	Total group children	Total group parents	MS child	MS parents	Post-ADEM child	Post-ADEM parents
PedsQL Fatigue	Mean SDS (SD) n = 37	Mean SDS (SD) n = 38	n (%) < -1 SD n = 22	n (%) < -1 SD n = 22	n (%) < -1 SD n = 15	n (%) < -1 SD n = 16
Total fatigue score	-0.76 (1.25)***	-1.22 (1.63)***	8 (36)	10 (46)	7 (47)	9 (56)
General fatigue	-0.74 (1.17)***	-1.06 (1.54)***	8 (36)	7 (32)	6 (40)	9 (56)
Sleep-rest fatigue	-0.56 (1.13)**	-1.04 (1.46)***	6 (27)	9 (41)	8 (53)	7 (44)
Cognitive fatigue	-0.60 (1.37)**	-0.84 (1.25)***	7 (32)	7 (32)	6 (40)	9 (56)
Exercise capacity **BRUCE**	Mean SDS (SD) n=36 -1.37 (1.09)***		n (%) < -1 SD n=20 17 (85)		n (%) < -1 SD n = 16 9 (56)	
MABCII	n (%) n = 37		n (%) n = 21		n (%) n = 16	
Total impairment score,						
Normal	19 (51.4)***		10 (48)		9 (56)	
Borderline	5 (13.5)***		1 (5)		4 (25)	
Motor problem	13 (35.1)***		10 (48)		3 (19)	
Manual dexterity, n (%)						
Normal	24 (64.9)*		13 (62)		11 (69)	
Borderline	8 (21.6)*		6 (29)		2 (13)	
Motor problem	5 (13.5)*		2 (10)		3 (19)	
Bal skills, n (%)						
Normal	21 (56.8)***		11 (52)		10 (63)	
Borderline	7 (18.9)***		5 (24)		2(12)	
Motor problem	9 (24.3)***		5 (24)		4 (25)	
Balance skills, n (%)						
Normal	17 (45.9)***		5 (24)		12 (75)	
Borderline	8 (21.6)***		7 (33)		1 (6)	
Motor problem	12 (32.4)***		9 (43)		3 (19)	

Table 3.8.2: continued

Measurement	Total group children	Total group parents	MS child	MS parents	Post-ADEM child	Post-ADEM parents
PedsQL-HRQoL	Mean SDS (SD) n = 37	Mean SDS (SD) n = 35	n (%) < -1 SD n = 22	n (%) < -1 SD n = 22	n (%) < -1 SD n = 15	n (%) < -1 SD n = 13
Total functioning score	-0.83 (1.54)***	-0.73 (1.18)***	9 (41)	8 (36)	5 (33)	4 (31)
Physical functioning	-1.07 (1.83)***	-0.53 (1.30)*	10 (45)	8 (36)	6 (40)	5 (38)
Emotional functioning	-0.43 (1.34)	-0.79 (1.07)***	4 (18)	9 (41)	6 (40)	6 (46)
Social functioning	-0.55 (1.48)*	-0.38 (1.08)*	7 (32)	8 (36)	6 (40)	3 (23)
School functioning	-1.13 (1.70)***	-0.89 (1.14)***	10 (46)	10 (46)	10 (67)	6 (46)
Psychosocial functioning	-0.85 (1.52)***	-0.85 (1.04)***	10 (46)	10 (46)	6 (40)	6 (46)
EDSS	n (%) n = 38		n (%) n = 22		n (%) n = 16	
0	20 (53)		10 (46)		10 (63)	
1.0	8 (21)		4 (18)		4 (25)	
1.5	7 (18)		6 (27)		1 (6)	
2.0	2 (5)		2 (9)		0 (0)	
3.0	1 (3)		0 (0)		1 (6)	

Data are presented as number (%) of patients or mean (SD)
*One sample t-test (compared with zero) or Chi-square test (observed vs expected distribution): ***: $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$*
MS=Multiple Sclerosis, post-ADEM=after Acute Disseminated Encephalomyelitis, MABCII=Movement Assessment Battery for Children, second version; PedsQl=Pediatric Quality of Life Inventory, HRQoL=Health-related quality of life, EDSS=Expanded Disability Status Scale, SDS=Standard Deviation Score

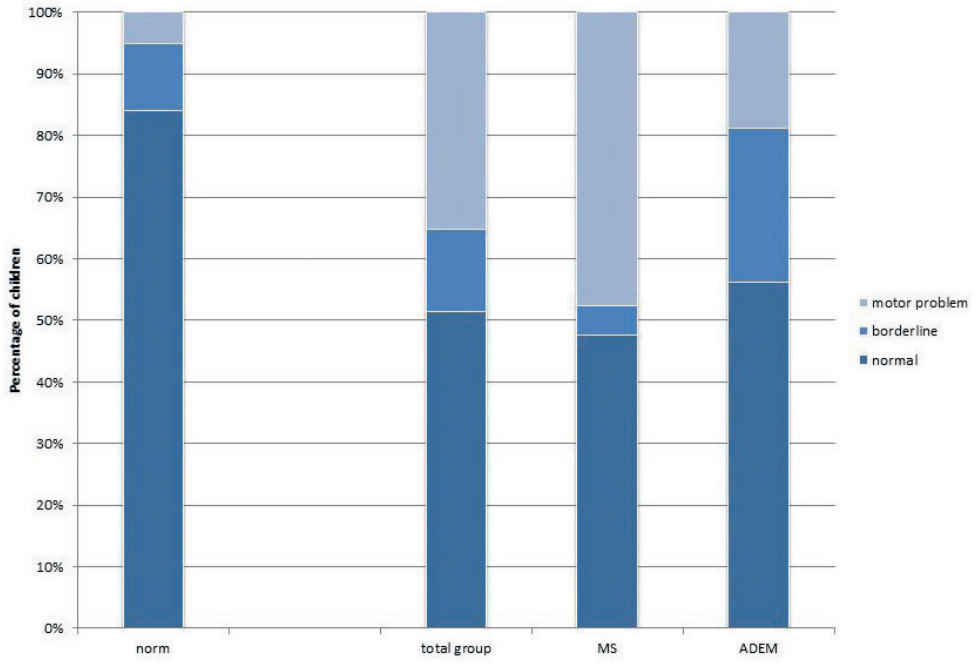

Figure 3.8.2 MABCII - Total Impairment Score (TIS): Left bar 'norm': percentage of children from Dutch reference group. Total group: data of MS and ADEM children

MABCII - Total Impairment Score (TIS).

When we added the two children who were excluded from analysis due to serious ataxia, fifteen of the 40 children (38%) had a definite motor problem.

Motor performance between the diagnosis groups differs: children with MS have more severe and more often motor impairments than children post-ADEM. Children with MS encountered most problems in balance skills, followed by ball skills and manual dexterity. Children post-ADEM encounter problems in all three subscales, but these problems are more equally distributed over the three subscales.

Health-related quality of life - PedsQl-HRQoL

The PedsQl-HRQoL was completed by 37/38 of the children and 35/38 parents. One child could not fill in the questionnaire because of his age (<5 years) and three parents did not return the questionnaire. Fourteen children and twelve parents indicated an impaired HRQOL (<-1 SD). Compared with the reference values, scores on all PedsQL-scales, except emotional

functioning, were significantly lower *(Table 3.8.2)*. In contrast, the parental scores were significantly lower on all subscales.

See *Table 3.8.2* for descriptive results of children with MS and post-ADEM patients separately.

Expanded Disability Status - EDSS

All of the children had an EDSS ≤3.0 *(Table 3.8.2)*. Thirty-five patients (92%) did not have disabilities or were not aware of it in daily life (EDSS <2.0).See *Table 3.8.2* for results of children with MS and post-ADEM separately.

Correlations between parameters

The mean SDS of the child reported PedsQL total fatigue score was significantly correlated with the mean SDS of the PedsQl-HRQoL total score reported by the child (r=0.704, p<0.001) and the parent (r=0.554, p=0.001). There was no significant correlation between the mean SDS of the PedsQL total fatigue score of the child and mean SDS exercise capacity, MABCII, BMI or sports participation. Furthermore, there were no correlations found between the subscales of the PedsQL fatigue and above mentioned parameters (data not shown).

No significant correlation was found between the EDSS and the mean SDS of the PedsQl fatigue total score of the child and parent, exercise capacity, MABCII, PedsQl-HRQoL total score of the child and the parent, BMI or sports participation (data not shown).

A significance correlation was found between exercise capacity and sports participation (r= 0.365, p=0.034).

Differences between children with and without fatigue

A significant difference in mean SDS of the PedsQl-HRQoL total score was found between children with and without fatigue (p<0.001). For the other parameters (exercise capacity, MABCII, BMI and sports participation), no significant difference was found (data not shown).

Impact of disease duration on outcome parameters

Disease duration differed for children with MS and post-ADEM *(Table 3.8.1)*, therefore we analyzed the groups separately. Within both groups there was no significant correlation between any of the outcome parameters and disease duration (data not shown). Disease duration was calculated per group as beneath or above the median.

Impact of disease activity on outcome parameters

The number of demyelinating events within the year to assessment was assessed to express disease activity. This did not correlate with any of the outcome parameters (data not shown).

DISCUSSION

In this cohort of 38 children with MS and post-ADEM we found that a large proportion of the children experienced fatigue and had reduced exercise capacity. In addition many children had motor impairments and impaired HRQoL. However, the hypothesis that fatigue and exercise capacity are related in children with MS and post-ADEM was not confirmed. HRQoL was related with fatigue, but not with motor performance, the EDSS score or exercise capacity. Despite the physical impairments measured by the MABCII, in no more than three children (8%) the disability interfered with activities of daily living according to the EDSS results.

Exercise capacity was reduced in both MS and post-ADEM patients. Durstine et al. describe that most individuals with a chronic disease or disability become less physically active.[24] This is in line with Grover et al, who describe less physical activity in POMS and mono-ADS patients.[13] Physical inactivity can lead to a reduced exercise capacity, which in turn can lead to further inactivity and a decrease in participation in daily life activities. A negative spiral of reduced exercise and physical inactivity may arise.[24]

In our study, sports participation served as a measure of physical activity and was found related with exercise capacity. This may suggests that exercise capacity and the level of physical activity of children with MS and post-ADEM can be improved with exercise interventions. In other studies of children with chronic systemic inflammatory conditions, exercise interventions indeed improved children's exercise capacity and physical functioning.[24] High intensity exercise programs appeared to be safe in adult MS patients, but need to be investigated in children with MS and post-ADEM.[25,26]

We did not found a difference in exercise capacity between fatigued and not-fatigued patients, as initially hypothesized. This suggests that fatigue itself is not explained by reduced exercise capacity in our patients, and vice versa. The process of inactivity, decreased exercise capacity and fatigue probably involves a complex interaction including other factors as well. As Grover et al. not only found a correlation between physical activity and fatigue, but also with depression, we argue here that psychosocial factors and reduced psychosocial participation may play an important additional role in diminished physical activity and in turn decreased exercise capacity.

This is supported by our findings in HRQoL; children who experienced fatigue reported more problems in not only physical functioning, but also in emotional, social, scholastic, and psychosocial functioning. From clinical experience we observe that children with MS and post-ADEM experience psychological distress and difficulties with coping with the diagnosis or residual deficits. It is described that chronic ill young adolescents feel their chronic condition as 'disrupting normal life' and they perceive 'discomfort in their own body'.[27] Literature of adult

patients with MS suggested that exercise could improve physical activity, depression, fatigue and HRQoL.[25] In children without chronic illness literature shows a positive correlation between physical activity and HRQoL.[28] Interventions for these problems in pediatric demyelinating disorders have not been evaluated to date. Disease perception, disease acceptance and coping might be potential areas in understanding the mechanism of fatigue, diminished physical activity and reduced exercise capacity.

Physical ability in children with MS and post-ADEM is frequently evaluated with the EDSS. The EDSS contains items on different functional systems that reflect disability of adult patients with MS and includes for example sexual functioning.[12] In our experience, administration of this scale requires the subject to have adequate language perception and expression, which may not always be the case in a pediatric ADS cohort. In this study we used the MABCII to measure motor functioning, including manual dexterity, ball- and balance skills. Children with MS showed motor impairments, particularly of balance skills. Children post-ADEM showed motor impairments as well, but these were more equally distributed over the three subscales. Forty-nine percent of all children showed severe or borderline deficits on the total impairment score, which is strikingly high. However, if compared to EDSS scores, only 8% of our cohort has a score ≥2, which reflects disability which the patient is aware of in daily life.[12] Nine percent of the MS patients scored EDSS ≥2 versus 6% of the patients post-ADEM, which is low in contrast to the high total MABCII impairment scores (52% and 44% respectively). This discrepancy confirms that the EDSS is not an optimal measurement for motor deficits in pediatric MS patients, and especially not in young patients post-ADEM. The MABCII seems to be a more sensitive measurement for expressing motor deficits and its severity.

In children it is important to acquire motor skills being able to participate in physical activities and therewith being able to participate with peers. Lack of participation in physical activity has contributed to a decrease in fitness, and an increased risk for disease.[29] Monitoring of motor performance is important to enable timely intervention.

Several limitations of this study need to be addressed. First, the sample size was relatively small. Second, only sports participation was taken as a measurement of physical activity. Physical activity includes not only sports, but also other activities which involve bodily movement, such as playing, home-school transfers, and recreational activities.[30] Monitoring physical activity in children is difficult. As far as we know, reference values of activity trackers in Dutch children are not available yet. The use of questionnaires on physical activity is debated, because children and adolescents tend to overrate physical activities.[31] Moreover, all children were evaluated in our national-multidisciplinary pediatric MS-center as part of routine medical care. Therefore, we did not have a healthy control group and used published age-related reference values of the healthy population instead. Another limitation of this study is the fact that due to

the small sample size it was not possible to correct for DMT in MS patients in the statistical analyses. However, 82% of the MS patients were on DMT, which indicates a relative high group homogeneity.

In conclusion, children with MS and post-ADEM in our study had significant problems on different domains of physical functioning. To further explore the associations between physical functioning (physical activity, exercise capacity, motor development) and psychosocial parameters such as fatigue, depression, anxiety and coping, larger (multinational) cohorts with longitudinal data are necessary. A possible next step would be to investigate whether an intervention with an exercise program can improve the physical and psychosocial functioning in children with MS and post-ADEM. Lastly, our data confirm that the EDSS lacks the sensitivity in children to reflect motor problems. This indicates that other measurements for the assessment of physical impairments, such as the MABCII, are needed.

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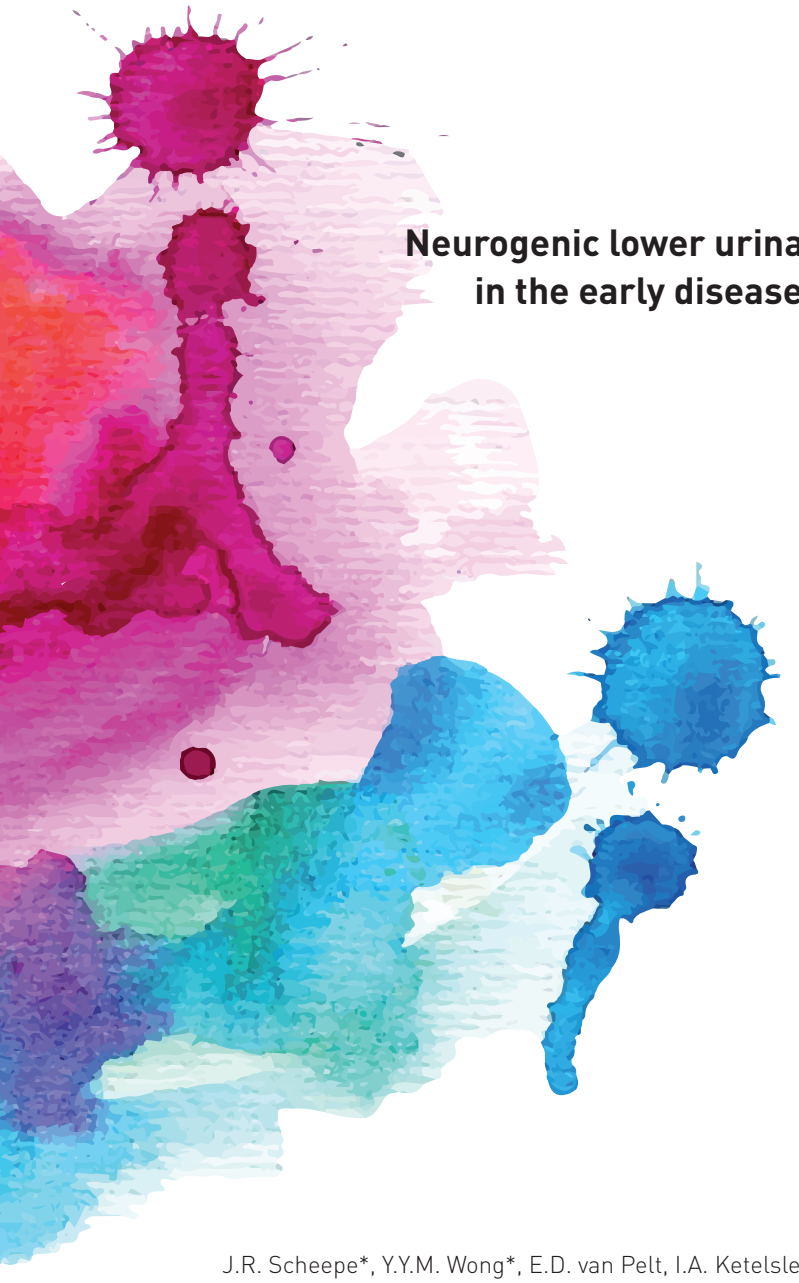

3.9

Neurogenic lower urinary tract dysfunction in the early disease phase of paediatric multiple sclerosis

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ABSTRACT

Neurogenic lower urinary tract dysfunction (LUTD) in MS is highly prevalent in adults, but has not previously been described in paediatric MS. Twenty-four consecutive children with newly diagnosed MS were prospectively assessed for bladder and bowel problems early after diagnosis. Five of 24 children (21%) showed LUTD during assessment. One of these patients did not report voiding complaints. This high prevalence of LUTD indicates that all recently diagnosed patients with paediatric MS should be evaluated early in their disease and treated for urinary problems in order to prevent potential damage to the upper urinary tract.

INTRODUCTION

Neurogenic lower urinary tract dysfunction (LUTD) is common in adults with MS. At time of MS diagnosis 10% have LUTD and after a disease duration of ten years this percentage increases to around 80%.[1,2] LUTD may not only lead to irreversible alterations and damage to the upper urinary tracts if left untreated, like vesicourethral reflux, hydronephrosis and potential renal impairment, but also causes a significant decrease in quality of life.[3,4] As children with MS face a far longer disease duration, LUTD can potentially be extra worrisome in this young patient group. At this moment, little is known about the prevalence of LUTD in children with MS. The objective of this study is to evaluate the prevalence of bladder problems in children with MS already in the very early phase of their disease.

METHODS

Study participants

All children with newly diagnosed MS, aged under 18 and who were followed-up at our paediatric MS centre, were consecutively offered a structured urological assessment (UA) between July 2012 and June 2015. We included children who had an UA within 18 months after diagnosis and who did not have a relapse during or at least two months before UA.

Paediatric MS was diagnosed in accordance with the latest International Paediatric MS Study Group (IPMSSG) criteria.[5] Children with other acquired demyelinating syndromes such as acute disseminated encephalomyelitis, neuromyelitis optica and clinically isolated syndrome were excluded. Demographic and clinical data were collected, including the Expanded Disability Status Scale (EDSS) score[6] at presentation, at time of assessment and at the most recent visit. The EDSS score obtained closest to the UA was used. The EDSS score was composed without the bladder/bowel functional score to prevent bias. This study was approved by the Medical Ethical Committee of the Erasmus MC in Rotterdam. Written informed consent was obtained from all guardians and/or children between 12-18 years old.

Urological assessment

All children were assessed by the paediatric urology team. The UA was standardised and contained a detailed medical history of voiding and bowel functions, a voiding and defecation diary, questionnaires about voiding and bowel habits, physical examination, uro-flowmetry and determination of post-voiding residue (PVR) with ultrasound. During uro-flowmetry simultaneous electromyographic (EMG) recordings of the pelvic floor muscles with surface electrodes were performed. Determinants of LUTD are PVR above 20 milliliters, increased pelvic floor muscle discharges on EMG during voiding and/or an abnormal flow-curve.

Magnetic resonance imaging

Most recent brain and spine MRI images (1.0 or 1.5 Tesla) were evaluated in consensus by two assessors (YYW and RFN). MRI spine was not available for every patient as this was not performed routinely. Available MRI were scored for hyperintense lesions on T2 or FLAIR weighted images for areas involved in micturition and MS predilection areas: periventricular, juxtacortical and infratentorial areas, pons, periaqueductal grey, cerebellum, cervical spinal cord (SC), thoracic SC and conus.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS 21.0. Chi-square and Fisher's exact test were used for categorical data. Mann-Whitney U test was used for continuous data. Results were considered significant if $p<0.05$.

RESULTS

Twenty-six patients were eligible for this study and were offered an UA. Two patients declined. In total 24 children fulfilled the inclusion criteria and were included for analysis. Patient characteristics are summarised in *Table 3.9.1*. The median age at MS diagnosis was 13,4 years and the majority of children were females (79%).

Five out of 24 children (four females and one male) showed signs of LUTD during assessment (21%). Only one of these patients did not report voiding complaints. A summary of the results of the UA of these five children is shown in *Table 3.9.2*. All symptomatic children received uro-therapy and measures for optimising defecation and eventually one patient received drug therapy for the urological complaints.

Children with LUTD more frequently had a preceding transverse myelitis (TM), although not significant. Patients with LUTD had however significantly more often pyramidal and sensory problems at time of their first clinical presentation ($p=0.028$ and $p=0.041$ respectively).

No differences were found between the location of MRI lesions in patients with and without urological symptoms. Longitudinally extended TM (≥ 3 segments) was not observed in any patient.

Children with LUTD showed higher EDSS scores at baseline, at UA and at the most recent visit, as shown in *Table 3.9.1*. No significant differences were found in annualised relapse rate.

Table 3.9.1: Patient characteristics of patients with and without lower urinary tract dysfunction (LUTD)

	LUTD present (n=5)	LUTD absent (n=19)	All (n=24)	P-value
Female gender (%)	4 (80)	15 (79)	19 (79)	NS
Onset age, median (range), years	13.7 (5.3-16.1)	14.5 (10.4-17.2)	14.4 (5.3-17.2)	NS
Time from onset to diagnosis MS, median (range), months	3.3 (0.0-29.0)	3.9 (0.0-43.9)	3.6 (0.0-43.9)	NS
Time from MS diagnosis to urological assessment, median (range), months	8.0 (2.3-15.2)	2.9 (1.6-16.4)	3.4 (1.6-16.4)	NS
Time from onset to urological assessment, median (range), months	17.2 (2.3-37.0)	8.3 (2.3-46.3)	8.9 (2.3-46.3)	NS
Time from most recent transverse myelitis to urological assessment, median (range), months	6.0 (2.3-17.3)	5.1 (2.2-8.3)	5.1 (2.2-17.3)	NS
Follow-up duration, median (range), years	3.3 (0.8-4.6)	1.3 (0.3-4.9)	1.5 (0.3-4.9)	NS
Presenting phenotype (%)				
Optic neuritis	0	5 (26)	5 (21)	NS
Transverse myelitis	3 (60)	5 (26)	8 (33)	NS
Other clinically isolated syndromes	2 (40)	9 (47)	11 (46)	NS
Clinical features at first presentation				
Pyramidal symptoms (muscle weakness, brisk reflexes, pathological reflexes)	4 (80)	4 (21)	8 (33)	0.028
Sensory problems	5 (100)	8 (42)	13 (54)	0.041
Clinical presentation of transverse myelitis prior to assessment	4 (80)	6 (32)	10 (42)	NS
MRI spine present (%)	4 (80)	16 (84)	20 (83)	NS
Time from most recent MRI to urological assessment, median (range), months	4.8 (1.5-8.1)	3.5 (1.6-23.0)	3.5 (1.5-23.0)	NS
MRI lesion locations on most recent MRI prior to urological assessment(%)				
Pontine region				
Periaquaductal grey	3 (60)	10 (53)	13 (54)	NS
Cerebellum	1 (20)	0	1 (4)	NS
Periventricular lesions	3 (60)	7 (37)	10 (42)	NS
Infratentorial lesions	5 (100)	18 (95)	23 (96)	NS
Juxtacortical lesions	5 (100)	11 (58)	16 (67)	NS
Spinal cord	3 (60)	15 (79)	18 (75)	NS
Cervical region	4/4 (100)	12/16 (75)	16/20 (80)	NS
Thoracic region	3/4 (75)	10/16 (63)	13/20 (65)	NS
Lumbar/sacral region	3/4 (75)	7/13 (54)	10/17 (59)	NS
	1/4 (25)	2/13 (15)	3/17 (18)	NS

Table 3.9.1: continued

	Bladder dysfunction present (n=5)	Bladder dysfunction absent (n=19)	All (n=24)	P-value
EDSS at baseline*	6.0 (2.0-6.0)	2.0 (1.0-6.0)	2.0 (1.0-6.0)	0.017
EDSS at urological assessment*	2.0 (1.0-6.5)	1.0 (0-3.0)	1.0 (0-6.5)	0.005
EDSS at last follow-up*	1.5 (1.0-3.0)	0 (0-2.0)	1.0 (0-3.0)	0.003

NS = not significant
*EDSS score was composed without the bladder and bowel functional scale to prevent bias

Table 3.9.2: Results of urological assessment in patients with bladder dysfunction

	I	II	III	IV	V
Gender	female	Male	female	female	Female
Age at screening	17 y	7 y	12 y	16 y	13 y
Questionnaire					
Urgency	-	+	-	+	+
Straining	+	-	-	-	-
Start difficulties	+	-	-	-	-
Incontinence (day)	-	+	-	+	+
Incontinence (night)	-	-	+	-	-
UTIs[a]	-	-	2 y ago	2 m ago	-
Voiding diary					
Voiding frequency (day)	8	5	3	10	10
Voiding frequency (night)	0	0	0	0	2
Voiding volume (min-max)	150-350	50-100	100-190	50-300	100-300
Flow					
Volume	210	175	185	360	390
Q max (cm H_2O)[b]	11	15	33	24	32
T[c]	32	19	9	25	20
PVR[d] (mL)	60	0	0	20	20
Shape[e]	plateau	Bell	tower	bell	Bell
EMG	increased	Normal	normal	normal	Normal
Constipation	yes	No	yes	yes	No

[a] UTI: urinary tract infection, [b] Qmax; maximal uroflow [cm H20], [c] T: flow time [sec], [d]PVR: post-voiding residual volume [ml]. [e]Bell-shaped flow curve indicates normal voiding phase. Plateau-shaped or a staccato flow curve might indicate an anatomical of functional obstruction. A tower shaped flow curve might indicate bladder overactivity.

DISCUSSION

Five patients (21%) in our study population showed voiding problems within the first 18 months after their paediatric MS diagnosis. This high number is in sharp contrast to the data obtained from adult patients and is higher than numbers reported in healthy school aged children.[1,7] Contributing factors to this higher prevalence of LUTD may be that children with MS show a higher relapse rate, have a higher lesion load on first MRI and have more axonal damage in early disease, leading to dysfunction in an early stage.[8,9]

In order to detect patients with LUTD, questionnaires seemed sufficient for 80% of the patients. However, one patient did not have bladder functioning related complaints and would have been missed if patients were not systematically evaluated. As expected, we observed a higher frequency of LUTD in children with a preceding TM, although this was not significant. Almost all children had a spinal cord MRI. Notably, the proportion of patients with SC lesions on MRI was equally high in patients with and without LUTD. This is in line with an earlier study in adults, that showed that only one-third of SC lesions were symptomatic.[10]

We found that patients with confirmed LUTD had a higher EDSS score. This may indicate a potential role for LUTD as a prognostic factor for future disability and should be investigated in a larger cohort. Whether higher disability is an independent risk factor for LUTD in paediatric MS needs to be investigated as well.

In conclusion treating physicians should be aware of the high prevalence of urological symptoms in children with MS, even very early in their disease. Every newly diagnosed child with MS should be assessed for LUTD and should be treated accordingly to prevent irreversible damage to the urinary tracts. Longer follow-up studies in larger cohorts and optimizing standardized and validated questionnaires to increase their diagnostic properties are the necessary next step.

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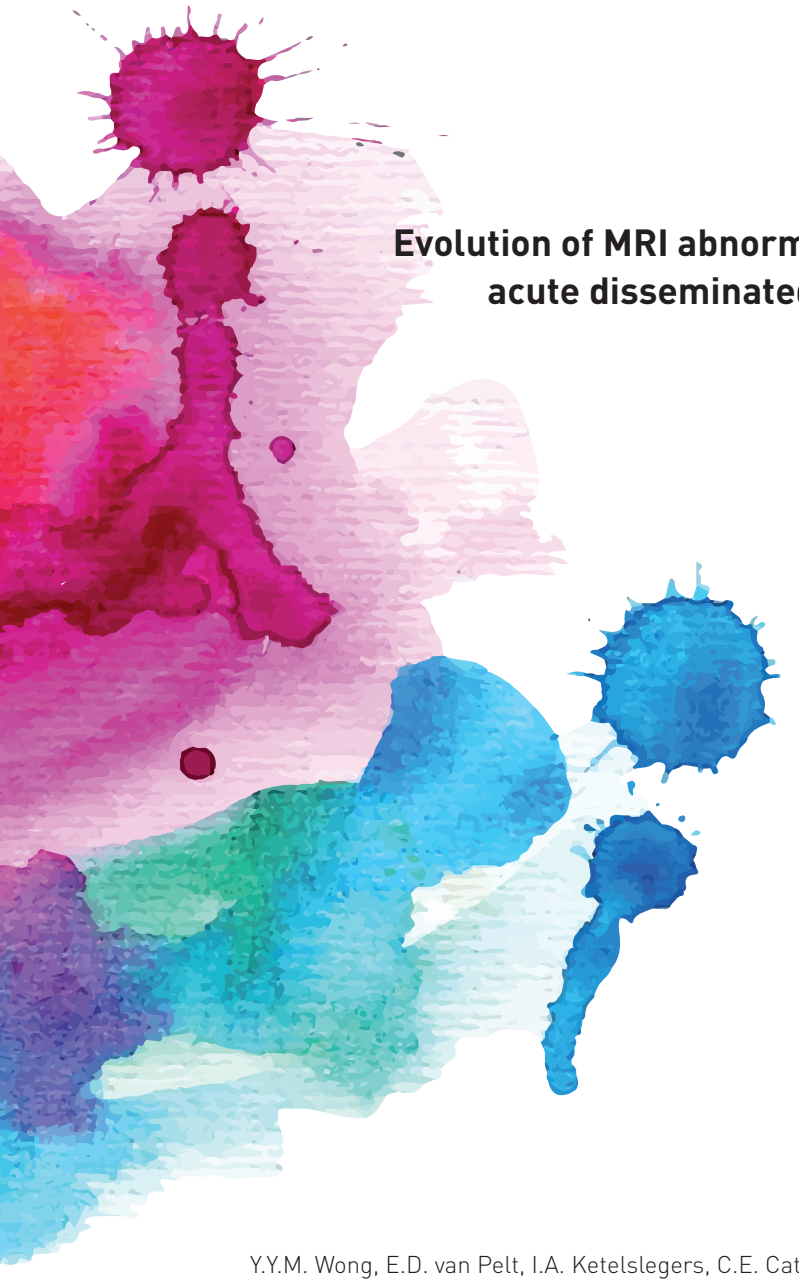

3.10

Evolution of MRI abnormalities in paediatric acute disseminated encephalomyelitis

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ABSTRACT

Objective

Acute disseminating encephalomyelitis (ADEM) is an inflammatory demyelinating disease affecting the central nervous system and mainly occurs in young children. Children who initially presented with ADEM can be diagnosed with multiple sclerosis (MS) in case new non-encephalopathic clinical symptoms occur with new lesions on MRI at least three months after onset of ADEM. We aim to study the timing of MRI abnormalities related to the evolution of clinical symptoms in our Dutch paediatric ADEM cohort.

Methods

The Dutch database for acquired demyelinating syndromes (ADS) was screened for children under age eighteen fulfilling the international consensus diagnostic criteria for ADEM. Children were eligible when the first MRI was performed within the first three months after onset of clinical symptoms and at least one brain follow-up MRI was available for evaluation. Forty-two children with ADEM were included (median age four years two months). All available MRIs and medical records were assessed and categorised as 'improved', 'deteriorated' and 'unchanged'.

Results

We found that during clinical recovery, new lesions and enlargement of existing MRI lesions occurred in the first three months in about 50% of the performed MRIs. In contrast, this was rarely seen more than three months after first onset of ADEM.

Conclusion

We recommend to perform a brain MRI as a reference scan three months after onset. Follow-up imaging should be compared with this scan in order to prevent an incorrect diagnosis of MS after ADEM.

INTRODUCTION

Acute disseminating encephalomyelitis (ADEM) is a rare immune-mediated demyelinating disease affecting the central nervous system.[1] ADEM is mainly observed in young children and usually has a monophasic disease course. A previous diagnosis of ADEM with encephalopathy is shown to be a negative predictor of a future diagnosis of multiple sclerosis (MS).[1-4] Several small studies have reported that MRI abnormalities may appear later than the clinical symptoms and progression of MRI lesions has been reported during clinical improvement.[5-7] This is a potential problem as the 2012 International Paediatric Multiple Sclerosis Study Group (IPMSSG) diagnostic criteria state that MS diagnosis can be made after ADEM, when new clinical symptoms occur with new MRI lesions at least three months after the onset of ADEM.[8] Here we aim to study the timing of MRI abnormalities related to the evolution of clinical symptoms in our Dutch paediatric ADEM cohort.

METHODS

Study participants

We included children less than 18 years old diagnosed with ADEM according to the IPMSSG criteria.[8] Patients were identified by screening the Dutch database for children with acquired demyelinating syndromes (ADS) from January 1995 to October 2015.[2,9] Children were eligible for this study when the first MRI was performed within the first three months after onset of clinical symptoms and at least one brain follow-up (FU) MRI was available for evaluation. Patients were excluded if the clinical data were incomplete. This study was approved by the Medical Ethical Committee of Erasmus MC in Rotterdam. Written informed consent was obtained from all patients and/or their families.

Demographic and clinical data

Demographical and clinical data, including the clinical status at every MRI scan, were collected. The clinical status was scored as 'improved', 'deteriorated' or 'unchanged' when compared with previous documentation of the neurological examination. FU duration was determined by the last visit or telephone contact with a neurologist or paediatrician.

MRI data

Brain MRIs were performed at 1.0 or 1.5 Tesla scanners and consisted of T1, T2, and proton density 3–5 mm images. In most cases FLAIR images were available. The MRIs were evaluated for change in size of the lesions and presence of new T2 or FLAIR lesions by two assessors (YYW and EDvP). A third assessor (RFN) was consulted in case there was no consensus. Each FU MRI was compared with the previous MRI. The change was categorised as: 1) improved: decreased amount and/or size of the lesions 2) deteriorated: increase of size and/or amount

of lesions; 3) unchanged. In case of multiphasic disease course only MRI scans preceding the second episode were evaluated.

STATISTICAL ANALYSIS

We used SPSS, version 21.0, for statistical analysis. Categorical data were analysed by Chi square test and the Fisher's exact test. Continuous data were analyzed with the Student's T-test. A p-value <0.05 was considered significant.

RESULTS

Patient characteristics

Sixty-three children with ADEM were identified of whom 42 met our inclusion criteria. In 30 children at least two MRIs were performed in the acute phase. In 25 children FU imaging after three months was available. Demographic and clinical data are shown in *Table 3.10.1*. No significant differences were found in age, gender and FU duration.

Acute treatment consisted of intravenous methylprednisolone (IvMP) for 3-5 days. In ten patients this was subsequently followed by intravenous immunoglobulins (IvIG) in case of insufficient clinical improvement. Three patients did not receive acute treatment because of mild disease severity. Eight patients were given oral prednisone taper (OPT) after acute treatment with a median duration of 2.4 months (range 0.8-5.1)

MRI abnormalities and clinical features in the acute phase

Three of the 42 patients had a normal first MRI scan at presentation (performed 3 days, 7 days and 30 days after onset). In these children MRI abnormalities were observed at the second MRI at 26, 36 and 40 days after onset respectively.

In the 30 children with multiple MRIs during the first three months, a total of 44 FU MRIs were performed. Twenty-one of the 44 FU MRIs showed deterioration (48%), of which 11 scans showed enlargement of the existing lesions and in 14 new lesions appeared. One MRI scan normalized in the acute phase. In total 16/30 patients showed radiological deterioration in the acute phase (53%). The delay of MRI abnormalities compared to clinical status is demonstrated in *Table 3.10.2*.

In this group of 30 patients only five patients were given OPT directly after acute treatment. Three out of five showed improvement on FU MRIs while being treated with OPT after previous radiological deterioration. These three patients also had FU imaging after the acute phase and after discontinuation of OPT for at least four weeks, that showed further improvement of FU MRIs.

Table 3.10.1: Demographic and clinical data

	Patients with ≥1 MRI scans in first 3 months after onset n=30	**Patients with FU MRI scans after first three months n=25**	**Patients with multiple MRI scans within and after the acute phase n=13**	**All patients n=42**
Male, n (%)	13 (43)	13 (52)	7 (54)	20 (48)
Age, y, median, IQR	5.0 (3.—6.6)	3.5 (2.2-5.7)	5.5 (3.0-7.0)	4.2 (1.0-14.6)
Neurological symptoms at presentation				
Optic neuritis	4 (13)	11 (44)	2 (15)	13 (31)
Transverse myelitis	2 (7)	4 (16)	1 (8)	5 (12)
Pyramidal signs	20 (67)	20 (80)	8 (62)	32 (76)
Cerebellar signs	13 (43)	9 (36)	5 (39)	17 (41)
Brainstem	3 (10)	5 (20)	1 (8)	7 (17)
Seizures	10 (33)	8 (32)	4 (31)	14 (33)
Admission to ICU, n (%)	9 (30)	6 (24)	5 (39)	10 (24)
Follow-up duration (years), mean (SD)	3.5 (2.9)	4.1 (2.9)	4.0 (3.1)	3.7 (2.8)
Multiphasic disease, n (%)	6 (20)	3 (12)	3 (23)	6 (14)
Time from onset to first MRI (days), median, IQR	9 (3-18)	10 (5-19)	10 (5-26)	N/A
Time from onset to last MRI (days) in the acute phase, median, IQR	37 (19-92)	N/A	44 (21-94)	N/A
Time from onset to FU MRI after acute phase (months), median, IQR	N/A	6.8 (4.9-9.4)	6.4 (4.7-9.0)	N/A

N/A = not applicable.

Two patients who also received OPT after acute treatment, showed deterioration of MRI. The first patient discontinued OPT three weeks before FU MRI in the acute phase. The second patient started OPT after the FU scan in the first three months was performed and no FU MRI after the acute phase was available.

Table 3.10.2: Comparison of clinical status and MRI evolution in the acute phase of ADEM.

44 FU MRIs in the acute phase	MRI improved, n (%)	MRI unchanged, n (%)	MRI deteriorated, n (%)
Clinically improved, n=29	20 (69)	1 (3)	8 (28)*
Clinically unchanged, n=7	0	2 (29)	5 (71)**
Clinically deteriorated, n=8	0	0	8 (100)***

Forty-four FU scans were obtained in the acute phase in 30 patients. When patients were clinically worse compared to their clinical status at the previous scan, MRI was also worse in 100% of the scans. On the other hand when patients were clinically improving at time of FU scans, MRI status was congruent in only 69% of the imaging. Twenty-eight percent showed deterioration (either enlargement of existing lesions or new lesions or both) despite of clinical improvement. The proportion of deteriorating scans with new lesions is as following: * 4/8 new lesions, ** 3/5 new lesions, *** 7/8 new lesions.

MRI abnormalities and clinical features after the acute phase

In 25 patients FU imaging was performed after the acute phase of three months. Twenty-three patients showed improvement of their MRI abnormalities. However, only one normalized. Two patients showed deterioration of MRI lesions during FU after the acute phase. The first patient was given OPT after acute treatment at onset and ceased OPT six months before FU MRI was obtained. No MRI was made between the first brain MRI and FU imaging. The second patient showed new lesions without new clinical symptoms 7 months after onset. These two children had evident encephalopathy and were 2 and 7 years old at presentation. Extensive testing was performed and these patients did not fulfill diagnostic criteria for other differential diagnosis than ADEM. During follow up of respectively 8 years and 2 years they did not fulfill the diagnostic criteria for MS.

In this group of 25 patients six were prescribed OPT. Except for the one patient mentioned above, five out of six showed improvement of MRI during FU after the acute phase. FU MRIs were all obtained after cessation of OPT for at least 4 weeks.

Patients with both MRIs in the acute phase and after the acute phase

Due to the observations made in the results shown in the previous paragraphs, a subanalysis was performed in patients who had MRIs in both the acute and post-acute phase for better comparison of the MRI-scan evolutions within each patient. For this subanalysis patients were

eligible when at least two MRIs in the acute phase and at least one FU MRI after the acute phase were available. Thirteen of the initial 42 patients were included.

A total of 24 FU MRIs were performed during the acute phase. Fourteen of these showed deterioration (58%), of which 12 showed enlargement of previously observed lesions, and 8 with observed new lesions. In total 10/13 patients showed radiological deterioration in the acute phase (77%).
When observing the MRIs after the acute phase, all patients showed radiological improvement compared to the last scan in the acute phase except for one patient. This concerned the patient with new observed lesions at 7 months after onset as previously described.

Three patients received OPT after acute treatment. These patients showed radiological improvement on FU MRIs while being treated with OPT after previous deterioration in the acute phase. The FU MRIs after the acute phase and after discontinuation of OPT were improved in all three patients.

DISCUSSION

This study confirms that evolution of MRI abnormalities in children with ADEM can be delayed compared to the evolution of clinical symptoms, as suggested by some case reports.[5-7] Also, a normal MRI in the first days after symptom onset does not rule out a diagnosis of ADEM.

The lack of a strict FU MRI protocol limited the evaluation of the exact timing of MRI abnormalities and clinical features. Due to the young age of our patients the decision to perform or not perform a FU MRI was based on individual circumstances, i.e., the need for sedation and clinical features.

It is likely that timing of discontinuation of corticosteroid treatment can potentially influence the FU MRI results. In our study this did not play a large role as only a small group received an OPT. Furthermore in most of these children FU imaging was performed at least 4 weeks after OPT was stopped.

We observed that MRI deterioration occurs often in the acute phase and rarely occurs more than three months after ADEM onset. This observation was further confirmed in the performed subanalysis of patients who had images available in both the acute and post-acute phase. The proportion of patients showing radiological deterioration is higher in the subanalysis compared to all patients who had multiple MRIs in the acute phase (77% and 53% respectively). This might be explained due to the selection bias of performing FU MRIs more often in children who showed deterioration on last MRI in the first three months.

The message that MRI deterioration rarely occurs three months after onset is important, because the latest revised diagnostic criteria for ADS including MS in children allow MS diagnosis when a first episode of ADEM is followed by a new non-encephalopathic episode with new MRI abnormalities.[8] Therefore it is important to critically assess the patient whether new clinical symptoms are truly present in case of new MRI abnormalities.

In conclusion, our study shows that new MRI abnormalities may occur in the first three months even when clinical symptoms are improving, and this rarely occurs after 3 months. Therefore we recommend to perform a brain MRI three months after onset as reference scan. Further FU imaging should be compared with this reference scan in order to avoid false positive results and as a consequence an incorrect diagnosis of MS after a first episode of ADEM.

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4.
General discussion

Acquired demyelinating syndromes (ADS) encompass a broad spectrum of inflammatory demyelinating disorders of the CNS. Accurate diagnosis and prediction of disease course is imperative for accurate counselling and treatment opportunities. This underlines the need to improve the diagnostic process by further investigating the diagnostic and prognostic value of existing biomarkers, such as anti-myelin oligodendrocyte glycoprotein antibodies (MOG-IgG), but also the need to find new biomarkers to predict the disease course accurately and early after onset. This thesis focuses on two rare groups of patients in the ADS spectrum: neuromyelitis optica spectrum disorders (NMOSD) in adults and the spectrum of childhood onset ADS. We aimed to move forward on delineating the spectrum of ADS, and investigating the disease course and outcome in children with ADS and in adults with NMOSD. Here we outline and discuss our main findings. Future directions are discussed after each specific topic and at the end of this Discussion.

PART ONE - NEUROMYELITIS OPTICA SPECTRUM DISORDERS

Clinical comparison between AQP4-IgG, MOG-IgG and seronegative NMOSD

After the discovery and validation of anti-aquaporin 4 antibodies (AQP4-IgG), the AQP4-IgG serostatus is implemented in the diagnostic criteria for NMOSD *(Table 1.1)*.[1] This broadened the clinical spectrum of NMOSD and enabled to study the differences between AQP4-IgG seropositive and AQP4-IgG seronegative patients.[1–3] In our study (**chapter 2.1)**, we confirmed that about one third of the AQP4-IgG seronegative NMOSD patients have MOG-IgG[1], including limited forms of NMOSD-like phenotypes.[4] In addition we assessed the brain MRIs of our seropositive patients for the presence of NMO-specific brain lesions, which typically occur at sites with high aquaporin-4 expression.[5,6] These lesions were not present in MOG-IgG seropositive NMOSD patients, which is likely due to the different underlying disease mechanisms. Patients with MOG-IgG are more often males in contrast to AQP4-IgG positive patients and more often have a monophasic disease course.[7–11] Despite the seemingly benign course, a proportion of patients still relapses and shows accumulation of disability. A recent retrospective cohort study showed that with a longer follow-up (FU), about 80% of the patients with MOG-IgG will develop a relapsing disease course.[12] These findings need to be validated in the future. Chronic immunosuppressive agents should be considered in MOG-IgG patients with relapsing disease, with caution of overtreatment considering the potential long disease intervals.

Epidemiological data on AQP4-IgG and MOG-IgG related disorders

In the Netherlands, AQP4-IgG and MOG-IgG are diagnostically tested in one central reference laboratory (Sanquin Diagnostic services) with specific cell-based assays. Therefore we were able to calculate the nationwide incidence figures for AQP4-IgG and MOG-IgG in the Dutch population. **Chapter 2.2** presented the Dutch incidence of AQP4-IgG associated NMOSD of 0.09 per 100.000 persons, which is about one in a million. This is within the range of previously described incidence rates from 0.05-0.4 per 100.000 people.[13–16] Differences can be explained by study design and inclusion criteria. For example two studies from comparable western countries in Denmark and the United Kingdom did not achieve nationwide coverage and also included AQP4-IgG negative NMOSD patients.[14,16]

MOG-IgG were described in demyelinating disorders including NMOSD in adults and children, and other ADS subtypes in children. In **chapter 2.3** we calculated the mean incidence for MOG-IgG seropositive ADS in the Dutch population of 0.16 per 100.000 persons per year (almost two in a million), with a higher incidence in children (0.31 per 100.000 children per year) than in adults (0.13 per 100.000 persons per year). We also observed differences in distribution of clinical phenotypes in adults and children, possibly influenced by the different impact of inflammation on myelin that is still maturing. Clinicians should be aware that a minority of

the patients have an extended time-to-first-relapse of more than 200 months. Even though we did not have a standardized serological follow-up (FU) in these patients, we observed that the majority of the patients who turned seronegative during FU, did not show further relapses. Well set-up prospective studies are needed to further investigate the association between disease course and MOG-IgG serostatus.

It should be noted that for both antibodies, the incidence estimates are minimum incidence figures, since mild cases and forme fruste types of the disease could have been missed. Yet, the longer both assays are used in clinical practice, it is likely that more rare clinical presentations will be observed, leading to a further broadening of the antibody associated clinical spectrum. One example is already given in **chapter 2.3**, where it seems that a cortical manifestation with seizures is described in a handful of MOG-IgG seropositive patients in current literature.

Future research concerning NMOSD

In NMOSD no predictive biomarkers for relapses and prognosis of disease course are identified, other than the AQP4-IgG and MOG-IgG serostatus. As disability accumulation is correlated with relapses, accurate prediction of treatment response and thus selecting the right agent to start with is essential. This is particularly important when chronic treatment options are expanding and practicing personalized medicine is desired.[17] One of the possibilities is further investigating B-cell subsets (for example surface biomarkers) or T-cell subsets.[18] Collection of paired blood and CSF in this group of patients will create opportunities to better understand the pathophysiology of NMOSD, and aid in finding new biomarkers for disease course. In addition, despite MOG-IgG can be found in one-third of the AQP4-IgG seronegative patients, the other 2/3 of patients are still unaccounted for with a double seronegative status. Some patients in this latter group might still be seropositive for one of these antibodies, but are not detected with the current CBA due to low antibody levels. The search for new antibodies in this group can provide insight in pathophysiology, customize treatment for individual patients and determine their prognosis.

The presented epidemiological figures are not only of importance for care and counselling, but also for the design potential future clinical trials. Due to the low incidence of AQP4-IgG and MOG-IgG seropositive patients, a stepwise initiation of clinical trials and international collaboration is warranted to guarantee successful enrollment of patients. Treatment agents for AQP4-IgG seropositive patients are under research in ongoing clinical trials.[19]

Main findings & Clinical implications **Part ONE**:

ADULT ACQUIRED DEMYELINATING SYNDOMRES, FOCUS ON NEUROMYELITIS OPTICA SPECTRUM DISORDERS

Main findings

The incidences of AQP4-IgG seropositive NMOSD and MOG-IgG associated ADS in the Netherlands are 0.09/100.000 persons and 0.16/100.000 persons per year respectively, with a higher MOG-IgG incidence in children than adults.

- About one-third of the Dutch AQP4-IgG seronegative patients with a clinical NMOSD phenotype are tested positive for MOG-IgG.
- Although MOG-IgG seropositive patients generally have a more favorable outcome than AQP4-IgG positive NMOSD patients, caution must be taken in relapsing MOG-IgG patients who are at risk of accumulation of disability.

Clinical implication:

MOG-IgG should be tested in parallel with AQP4-IgG in patients with suspected (limited forms of) NMOSD as part of the routine diagnostic work-up.

PART TWO – CHILDHOOD ONSET ACQUIRED DEMYELINATING SYNDROMES

SECTION TWO-A: EARLY and ACCURATE DIAGNOSIS FOR PEDIATRIC MS

Multiple sclerosis (MS) is a chronic variant of ADS and the most well-known amongst the different ADS subtypes. At the time of a first attack, it can be a challenge to distinguish MS from non-MS patients, especially in children due to a more extensive list of differential diagnoses.[20] In addition, determining the disease course after MS diagnosis can be difficult. We aimed to investigate biomarkers for an early and accurate MS diagnosis and for identification of patients with a clinically active disease.

MS diagnosis at first attack of ADS

The International Pediatric MS study group (IPMSSG) proposed the first set of diagnostic consensus criteria for children in 2007 and these were revised in 2012.[21,22] The IPMSSG 2012 consensus criteria implemented the McDonald 2010 criteria for pediatric MS, with provisions for children <12 years and patients with acute disseminated encephalomyelitis (ADEM).[23] Recently, the international panel on diagnosis of MS proposed the McDonald 2017 criteria, by reviewing and revising the prior 2010 McDonald criteria.[23,24] These revised criteria include important modifications with re-introducing CSF oligoclonal bands (OCB) into the criteria and allowing symptomatic lesions to contribute to dissemination in space (DIS) and dissemination in time (DIT). The McDonald 2017 criteria need validation before being implemented in clinical practice. The McDonald 2010 and revised McDonald 2017 criteria are displayed in *Table 1.1*.

Implications of the novel McDonald 2017 criteria

We evaluated the diagnostic accuracy of the revised 2017 criteria in 164 children (110 ADS without encephalopathy, ADS-; 54 ADEM, ADS+) with a first attack of ADS compared to the former 2010 criteria (**chapter 3.1).** Clinically definite MS (CDMS), defined as two attacks with different neurological localizations, was used as the endpoint to compare the McDonald 2010 and 2017 criteria. The 2017 criteria were more sensitive (83% vs 49%) and less specific (73% vs 87%), but the overall diagnostic accuracy was higher (77% vs 70%) than the 2010 criteria. The differences in test characteristics of the revised and former criteria are mainly caused by allowing OCB to be a substitute for DIT. Our data shows that more children will be diagnosed with MS at baseline (2017 n=48 vs 2010 n=27). Yet, the specificity decreased with the new criteria, which was caused by 7 patients. These 7 patients all have a high-risk MS profile and will likely be diagnosed with CDMS during longer FU. It is important for clinicians to realize that more children will be identified at baseline who are likely to have a less clinically active disease course.

Our data shows that the test characteristics are similar (even somewhat better) in children under age 12, and pleads for the applicability of these new criteria across the whole age-span,

in line with previously published studies.[25–27] About 10% of the ADEM patients would have been diagnosed with MS using the new MS criteria. However, no ADEM patient was diagnosed with CDMS. In order to prevent erroneous diagnosis in this group of patients, the provision to not apply these criteria on children with ADEM should be kept. Our data is in line with Fadda et al, however the figures can vary due to difference in study design, including the difference in endpoint (either CDMS or new T2 lesions on MRI).[27] Validation of these criteria in other cohorts are needed.

Biomarkers for MS at first attack of childhood-onset ADS

Predictive value of CSF soluble CD27

Soluble CD27 (sCD27) is an immunological marker that is secreted by activated T-cells, and is introduced as a potential biomarker for T-cell mediated inflammation.[28] CSF sCD27 is validated as a biomarker for intrathecal T-cell activation in MS, after using an extensive battery of biomarkers for CNS inflammation.[29] In a recent study executed in adults by our research group, high levels of sCD27 in CSF independently associate with MS diagnosis and disease course in adults with clinically isolated syndrome (CIS).[30] The predictive value of this promising biomarker in children with ADS was not investigated before.

In **chapter 3.2**, we compared the sCD27 levels in CSF of ADS subgroups sampled at time of first attack. Our data is in line with the study performed in adults showing higher levels in MS than in monophasic clinically isolated syndromes (CIS) with the highest levels among ADS-patients who were diagnosed with CDMS during FU. In addition, high sCD27 levels at baseline are predictive for CDMS diagnosis and show a shorter time to second attack in MS. This validation strengthens the conclusions from both studies. Moreover, the sCD27 levels found in pediatric MS are higher than in adult MS, which support existing data that children have a more inflammatory disease course than adults.[31–34] In contrast to the conclusions made in adult MS, we did not find a correlation between sCD27 levels and relapse rate. This finding might be explained by a ceiling effect, as the overall relapse rate is high in the pediatric MS cohort. Despite the higher disease burden in pediatric MS, the time to disease progression is slower in children than in adults.[33,35,36] A longer FU is needed to investigate a possible relationship between sCD27 levels and chronic disease progression.

Predictive value of CSF and serum neurofilament light chain

As indicated above, disease progression is slower in children than in adults.[33,35,36] Yet, neurodegeneration seems to be present early in the disease course of pediatric patients, indicated by a high proportion of T1 hypointense lesions on baseline MRI, and reduced brain-expected brain growth at baseline as reported in literature.[37,38] Axonal damage is considered one of the major causes for persisting neurological disability in MS.[39] A promising biomarker for axonal damage is neurofilament light chain (NfL), a component of the neurocytoskeleton that

is released in the extracellular space after axonal death.[40] NfL increases with age in healthy individuals, reflecting neurodegeneration as part of the physiological aging process.[41] In adults with CIS, high levels of NfL in CSF are associated with future MS diagnosis.[42]

We first investigated NfL in CSF in both adults and children (**chapter 3.3).** CSF NfL levels at first attack of ADS predicted a second attack (CDMS) both in children and adults without encephalopathy (CIS), even better than currently used markers such as asymptomatic T2 lesions on MRI and OCBs in CSF. We compared the NfL levels in children and adults, and the levels were even higher in children, implying that children not only have signs of more inflammation but also, in line with existing literature, seem to have more axonal damage than adults.[37,38] Moreover, in our pediatric MS cohort, more T1 hypointense lesions were found at the baseline scan compared to their adult counterparts. The fact that we did not find a correlation between EDSS and NfL levels is not surprising in this prospective cohort, as children with MS have a slower disease progression than adults.[33,35,36] Longer FU is needed in order to re-investigate this association.

Subsequently, we investigated NfL in serum, as serological biomarkers are more easily assessable than CSF and are likely to be preferred in the future. We show in **chapter 3.4** that CSF and serum NfL (sNfL) at baseline correlate well in the overall ADS group and correlate even better in the ADS patients without encephalopathy (ADS-) with future CDMS. The sNfL levels were highest in ADEM patients, followed by ADS- patients future CDMS diagnosis and the lowest in monophasic ADS- patients. Serum NfL was positively correlated with multiple MRI parameters, including T1 hypointense lesions, as a sign for axonal loss. This further supports sNfL as a validate marker for axonal damage. Higher levels of sNfL at baseline are associated with a shorter time to CDMS diagnosis in ADS- patients, after adjusting for age, OCB and MRI parameters.

A paradox seems to exist between the signs of more neuroaxonal loss in pediatric MS compared to adults early in the disease and yet, the rate of progression is slower in children.[33,35,36] Yet, biological differences at different ages can influence this observation, including the myelination process: this continues into early adulthood, and may cause a difference in lesion composition and in repair capacity along the myelin maturation.[43] In addition, functional compensatory mechanisms may play a role, but need to be further explored with functional MR imaging.[44]
To our surprise, ADEM patients showed rather high NfL levels in CSF and the highest among the patient subgroups in serum, as shown in **chapter 3.3** and **3.4**. These two studies complement each other and seem to indicate that neuro-axonal damage is a prominent feature in ADEM. The association between CSF/serum NfL levels and cognitive and radiological outcome measures of ADEM patients need to be investigated in the future.

Main findings & Clinical implications **Part Two – SECTION TWO-A:**

EARLY and ACCURATE PREDICTORS FOR PEDIATRIC MS

Main findings

- McDonald 2017 criteria can be applied in children across the age-span with a first attack of non-encephalopathic demyelination. Keeping in mind that the new criteria are more sensitive but less specific than the former 2010 criteria, mainly impacted by the inclusion of CSF OCBs as substitute for dissemination in time.
- With the McDonald 2017 criteria applied at baseline, more patients can be diagnosed with MS who likely have a less active disease course.

- Soluble CD27 in CSF have predictive value for future diagnosis MS at the first attack of pediatric demyelination.

- NfL in CSF at baseline predicts MS diagnosis in both adults and children. Higher levels are found in children than in adults.
- Serum NfL in children correlates well with CSF NfL and is also predictive of MS diagnosis after correction for existing predictive factors.
- High CSF and serum NfL levels in ADEM patients indicate that neuro-axonal loss is a prominent feature in in ADEM.

Clinical implication:

- McDonald 2017 criteria may be used in clinical practice for MS diagnosis in children across the whole age-span, but not in children with ADEM.
- Prompt initiation of chronic treatment in pediatric MS is supported by the presence of high levels of inflammatory and neurodegenerative biomarkers at the first attack.

SECTION TWO-B: DISEASE COURSE AND OUTCOME OF CHILDHOOD ONSET ADS

Disease course and Treatment of MOG-IgG associated relapsing disorders

MOG-IgG can be found in the spectrum of ADS in children and adults, in children up to 50% at first presentation.[45–47] The presence of MOG-IgG pleads against MS diagnosis, but is associated with a higher risk of relapsing non-MS disease.[45,46] In clinical practice these relapsing patients tend to accumulate residual deficits, sometimes despite the use of immunosuppressive treatment. Due to the rarity of MOG-IgG relapsing disorders, we collaborated with multiple European countries to increase the sample size to study the clinical spectrum, given treatments and outcome in this MOG-IgG associated relapsing demyelinating syndromes.

In **chapter 3.5**, we described the disease course, treatment response and outcome of 17 young patients presenting with ADEM followed by optic neuritis (ADEM-ON). The disease course was heterogeneous and no predictors could be found between patients with high and low relapse rates. Immunosuppressive agents were given to 59% of these patients, including azathioprine, mycophenolate and potent therapies like Rituximab, maintenance IvIG therapy and cyclophosphamide. Despite the heterogeneity in treatment, we observed that a total of 54 relapses occurred on all treatments, including more potent therapies such as Rituximab or intravenous IvIG. On the contrary, children were highly sensitive to steroids and 27/54 relapses occurred while weaning-off oral prednisone or within 4 weeks after discontinuation of prednisone. This indicates a certain degree of corticosteroid dependence in these children and may be typical of relapsing MOG-IgG associated ON.[48] However, the worrisome side effects of prednisone in these developing children[49] and the unpredictable disease course of patients warrant against long-term use of prednisone.

In **chapter 3.6** we retrospectively collected 102 children with MOG-IgG associated relapsing disorders. Above mentioned immunosuppressive agents or MS disease modifying drugs (interferon beta, glatiramer acetate) were initiated in 52/102 (51%) of these children. Despite treatment, 127 relapses still occurred in these 52 children. No difference was found in demographics between patients who did and did not relapse after initiating immunosuppressive drugs. We observed an age-dependent phenotype, with brain manifestations in younger children, and optic neuritis and/or transverse myelitis with normal brain MRI in older children. This finding may reflect the differences in white matter composition during physiologic, age-dependent maturation of the brain in childhood.[50]

In both **chapter 3.5 and 3.6**, we observed that treatments were administered heterogeneously and made it difficult to fairly compare treatment responses. Treatment consensus guidelines are urgently needed in order to make better comparison possible. As the data collection was retrospective for both studies, we were precluded from investigating the prognostic relevance

of MOG-IgG levels on disease course. Longitudinal serological FU of MOG-IgG will be important for future studies. More studies are needed to investigate the disease heterogeneity, early biomarkers for relapsing disease and optimal outcome measures for MOG-IgG associated disorders. After elucidating these aspects of MOG-IgG related disorders, clinical trials can be performed.

Outcome of childhood-onset ADS

The potential cognitive and physical sequelae of pediatric MS are mentioned in different studies.[51–53] Even though outcome studies of monophasic ADS and non-MS chronic disorders are emerging, the evidence is not as extensive as in pediatric MS patients. Additional studies on the outcomes and FU of pediatric ADS are needed.

Update on incidence and outcome of ADS

In **chapter 3.7** we provided the updated Dutch incidence of ADS and MS and is increased compared to our previous work.[52] This increase can be partially explained by increased awareness and our well-established referral network. However, we cannot exclude that the true incidence of pediatric MS is increasing in the Netherlands. Prolonged assessment of the incidence will be necessary to answer this question.

Furthermore, we studied the clinical characteristics of 243 children included in the PROUD-kids study at presentation and at last FU with a median FU time of 5 years. During FU, 37% of the ADS patients was diagnosed with MS, in line with other studies.[51,53,54] A few findings are noteworthy: In our cohort, an Expanded Disability Status scale score (EDSS) of 5.5 is only reached in 3% of the MS patients after a median of 61 months. However, the overall proportion of physicians or parents reporting residual deficits is high in our ADS patients (69%), including 83% in MS patients. This may mean that the disability measurement with EDSS, which was originally designed for adults, does not reflect disability well in the pediatric population.[55] This will be further discussed in the next section 'Fatigue and physical functioning'.

One-third of the included ADEM and MS patients who were followed in the National Pediatric MS center underwent a neuropsychological assessment in a consecutive manner. In this tested group, cognitive impairment was observed in the majority of children (>75%). The high percentage of cognitive deficits in the monophasic group (overrepresented by ADEM patients) feeds the impression that one single hit of ADEM can leave considerable intracerebral damage. As mentioned earlier, reduced age-expected brain growth is also shown in monophasic ADS patients, especially ADEM, indicating irreversible and continuing changes occurring in the CNS even in the absence of chronicity.[56] Moreover, negatively affected school performance was reported by parents in one-third of our ADS cases, some even requiring adjustments at school including extra time to complete examinations and change to special education. In a

major proportion of adult MS patients, MS negatively impacted their employment situation.[57] The effect on participation in society could even be worse in pediatric onset ADS. Adequate detection of disability and guidance of ADS patients in collaboration with other specialists, such as (pediatric) rehabilitation specialists, is important to preserve and improve societal functioning, and is essential during FU of these patients into childhood.

Fatigue and physical functioning

Fatigue is a frequently reported complaint in pediatric MS.[58,59] However, fatigue does not limit to chronic forms of demyelinating disease such as MS, but is also reported in children with ADEM.[59–61] The cause of fatigue in ADS is unclear. In a Canadian study, a correlation is found between fatigue and physical activity, where MS children were less physically active and scored higher on fatigue scales than patients with monophasic ADS.[62] We assumed that physical disturbances such as motor problems, fatigue and decreased exercise capacity affect a child's quality of life.

In the Dutch Pediatric MS center, children with ADEM and MS were consecutively referred to the pediatric physical therapist to screen for motor function, quality of life (including fatigue scales) and exercise capacity. In **chapter 3.8**, two main findings need to be discussed:
First, that children with MS and ADEM are more fatigued, have reduced exercise capacity and impaired motor performance compared to their healthy related peers. Unexpectedly, we found no correlation between these parameters. Yet, fatigue was correlated with a reduced quality of life. We hypothesize that a complex interaction exists in the process of inactivity, decreased exercise capacity and fatigue and might involve other factors, such as depression and psychosocial factors. This is supported by our finding that patients who were more fatigued, not only had more problems with physical functioning, but also in emotional, social, scholastic and psychosocial functioning. In our experience, psychological distress is often observed in ADS patients in coping with the diagnosis and residual deficits. Disease perception, disease acceptance and coping might be potential areas to investigate in order to increase our understanding of the underlying mechanisms involving fatigue, diminished physical activity and reduced exercise capacity. Future research projects in this area will need to cover all these different aspects in order to draw conclusions. Furthermore, a positive correlation was found between sports participation and exercise capacity. In adult MS patients, exercise seems to improve physical activity, depression, fatigue and quality of life.[63] The effect of high-intensity exercise programs appeared to be beneficial in adult MS patients, and are yet to be tested in children.[63,64]

Second, we found that 49% of all children showed severe or borderline deficits, which is strikingly high. Especially because only 9% of the MS patients and 6% of the ADEM patients had a EDSS score of ≥2, which reflects disability where the patient is aware of in daily life. This

discrepancy confirms that the EDSS is not an optimal metric for motor deficits in pediatric patients[65] and that the MABCII may be a more sensitive alternative. The lack of sensitivity of EDSS in the pediatric population may be explained by the requirement of adequate language perception and expression of the patient, which may not always be the case in children and young adolescents. Other metrics and tools that are sensitive to detect subtle disability in patients with pediatric MS and ADS are needed.

Bladder function in pediatric MS

Neurogenic lower urinary tract dysfunction (LUTD) is common in adults with MS with a prevalence of 10% at time of MS diagnosis, but the figures for children are unknown.[66,67] In **chapter 3.9**, we observed that 21% of our pediatric MS patients already have voiding problems in the first 18 months after initial MS diagnosis, and this figure is higher than in adults. We found that patients with confirmed LUTD had a higher EDSS score at baseline, at urological assessment and at last visit. This may indicate a potential role for LUTD as a prognostic factor for future disability. More patients with LUTD had a preceding TM, even though this was not statistically significant. Whether LUTD is an independent risk factor for disability after correction for preceding TM should be investigated in a larger cohort. Other contributing factors to the higher prevalence of LUTD may be the higher disease activity in pediatric MS, including a higher relapse rate, higher lesion load on baseline MRI and more axonal damage early in the disease.[43,68] Clinicians treating pediatric MS patients should be aware of the high prevalence of LUTD very early in the disease course. Optimizing standardized and validated questionnaires are necessary to improve LUTD detection.

MRI in children with acute disseminated encephalomyelitis

In ADEM patients, the MRI abnormalities can be very extensive.[69] The evolution of MRI lesions during FU is rarely studied, except for a few case reports.[70–72] In **chapter 3.10**, 42 children with ADEM were included for evaluation. We found that deterioration of MRI abnormalities (i.e. enlargement of existing lesions or appearance of new lesions) rarely occurs after the first three-month period. This finding is important as the latest revised diagnostic criteria for ADS including MS in children allow MS diagnosis when a first episode of ADEM is followed by a new non-encephalopathic episode with new MRI abnormalities.[22] Therefore performing a new MRI three months after symptom onset is warranted to establish a new reference point for further FU. This in order to prevent unjust confusion about potential MS diagnosis and unjustified initiation of disease modifying therapies.

Future research concerning childhood ADS

For MS risk stratification, the clinical profile, MRI, blood and CSF parameters are used in clinical practice, including MOG-IgG.[73] Finding more biomarkers and risk factors to differentiate between MS and non-MS patients is needed and has therapeutic implications.

In addition, predictive factors for future disease course in MS and other chronic forms of ADS are necessary, especially as the therapeutic possibilities will likely expand in the near future. With this in mind, a logical next step is to find easily assessable metrics for treatment response and disease progression (such as neurodegeneration). Two potential metrics for further exploration are retinal optical coherence tomography (*OCT*) and serum neurofilament. The inner layer of the retina is known as the retinal nerve fiber layer (RNFL) and can be measured with OCT. Thinning of the RNFL may lead to thinning of the macular ganglion cell layer (MCGL) and are both reflective for neurodegeneration in adults with MS.[74] OCT is non-invasive and can be of value for longitudinal monitoring of neurodegeneration in pediatric ADS. The first results of serum NfL in pediatric ADS patients are presented in this thesis, but needs validation and requires further study for clinical relevance.

Main findings & Clinical implications **Part Two – SECTION TWO-B:**

DISEASE COURSE and OUTCOME OF CHILDHOOD ONSET ADS

Main findings

- Treatment regimens used in MOG-IgG associated relapsing disorders are heterogeneous.
- MOG-IgG associated disorders are sensitive to corticosteroids and show a certain degree of corticosteroid dependence.
- Physical and cognitive sequelae are common across all subtypes of ADS, including monophasic ADS.
- EDSS is an insensitive metric to express physical disability in pediatric ADS, MABC II might be a better alternative.
- Neurogenic bladder dysfunction is common in children with MS even early in the disease course.
- In ADEM patients, a delay is observed in MRI lesions, for example enlargement and new lesions in <3 months after onset while patient is improving.

Clinical implications

- Treatment guidelines need to be established for MOG-IgG positive patients to facilitate systematic evaluation of treatment responses.
- In MOG-IgG relapsing disorders, oral corticosteroids should be tapered off very slowly, while keeping in mind that patients are prone to a relapse on low dose prednisone.
- Extra attention should be paid to long-term physical and cognitive sequelae in pediatric ADS in collaboration with pediatric rehabilitation specialists, physical therapists and neuropsychologists.
- Physicians treating pediatric MS patients should actively ask for symptoms that are related to neurogenic bladder dysfunction in order to start timely interaction.
- In ADEM patients, an MRI brain needs to be repeated after 3 months to establish a new reference point for follow-up, in order to prevent misdiagnosis of MS.

GENERAL DIRECTIONS FOR FUTURE RESEARCH

Important steps have been made in the research of NMOSD in adults and ADS in children, but additional work is needed to further explore the underlying pathophysiology of ADS, and find predictive factors for accurate diagnosis, disease course and outcome. Specific recommendations for future research regarding results presented in this thesis are described in previous paragraphs. Here are a few general suggestions for moving forward.

The value of existing cohorts with long-term follow-ups

The chapters in this thesis were made possible by the two prospective cohort studies that are currently ongoing in the ACE ErasMS center Rotterdam, including the National NMOSD and Pediatric MS expert center. The longer the FU time, the more valuable these cohorts will become, especially in children because of the long time to disease progression in this group.[33,35,36] Furthermore, collaboration between the PROUD and PROUD-kids study in Rotterdam is valuable, as both prospectively include patients with first demyelination in adults and children, respectively. Research in both adult and pediatric patients can improve our understanding of ADS in general by studying the similarities and differences, and may help in finding clues for the underlying pathophysiology.

Moving forward on the 'why' and 'when' in ADS

MS has a multifactorial etiology with an interplay between genetics and environmental factors.[75] Yet, a larger gap is present in our knowledge regarding this interplay in other ADS subtypes. Due to the rarity of ADS, including NMOSD in adults, international collaboration will allow for larger sample sizes which are required in both genetic and environmental research. Also, this collaboration may increase our knowledge on the ethnical differences in the clinical presentations of ADS subtypes. Identification of contributing (modifiable) risk factors are not only valuable for insight in pathophysiology, but also for potential prevention strategies, for example weight loss in children with increased BMI and subscribing vitamin D supplements in vitamin D deficiency in children with MS.[73,76] In addition, the role of the gut microbiome in disease pathogenesis in MS and other ADS subtypes need to be explored and might lead to complementing therapeutic strategies in the future.[77–79]

The difficulty of investigating environmental factors in general is that the relevant research parameter is often identified in a retrospective and non-population based setting, which is prone to recall-bias and is difficult to quantify. For pediatric ADS, collaboration with longitudinal prospective population-based studies in the healthy pediatric population, like the Generation R study in Rotterdam, may provide crucial insights in the effect of environmental and genetic MS risk factors on the pediatric brain development.[80,81] This provides an unique opportunity to study whether the genetic and environmental interplay generates a vulnerability in the individual

patient for future MS diagnosis. This collaboration is recently established and data shall be presented in the future.

As discussed in the first chapter of this thesis (General Introduction), the putative window of disease susceptibility is shorter in children and starts counting from birth. Yet, it is possible that the accumulation of MS risk already starts as early as in the perinatal or even in the prenatal period, as displayed in *Figure 4.1*. Despite conflicting results on the effect of prenatal maternal smoking on MS risk in the offspring, there are suggestions about increased MS risk after maternal illness and prenatal exposure to pesticides.[82–84] As the development of the immune system already starts in the embryonic phase, it will be worthwhile to set up large epidemiological studies in pregnant women (and even in the preconceptional phase) and have prospective longitudinal FU of the offspring after delivery. Knowledge on these risk factors can open up a new window for disease prevention strategies.

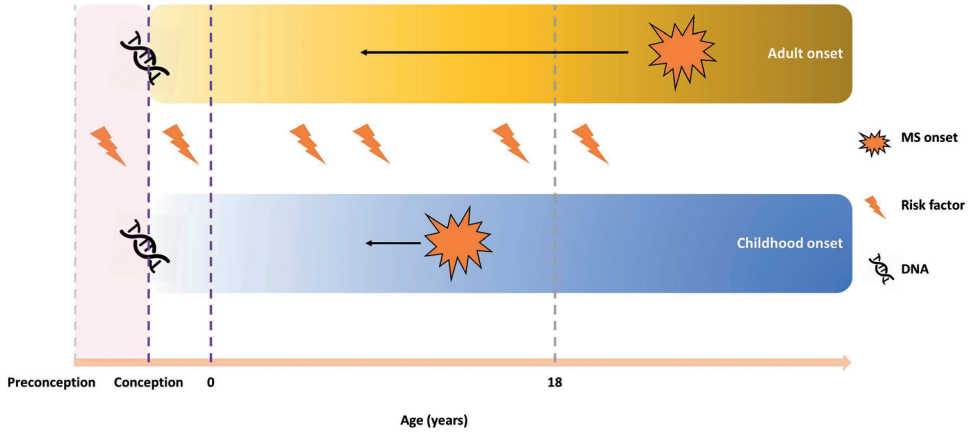

Figure 4.1: *The extended putative window of disease susceptibility.* The putative window of disease susceptibility might even start before or since the moment of conception. Investigating environmental factors before and during pregnancy adds to the knowledge on the pathophysiology of MS, and creates a new window for disease prevention strategies.

PROUD-kids 2.0: our next step

Recently, the PROUD-kids 2.0 study has been launched of children with a first attack of ADS. This study is a new and elaborated version of the current PROUD-kids study, not only aiming at identifying prognostic factors for disease course at disease onset, but also improve the (long-term) understanding of ADS including the temporal dynamics of biomarkers (such as sequential MRI parameters and biomaterial sampling like serum antibody-status and changes in immunological cell phenotypes and functionalities). Control subjects, like blood-related siblings, age-matched friends in the same environment and patients with other neurological diseases, are being included.

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5.1
Summary

Acquired demyelinating syndromes (ADS) are a group of auto-immune mediated CNS inflammatory demyelinating syndromes, of which multiple sclerosis (MS) is the most common subtype. ADS may occur as a transient illness or may represent the first attack of a chronic demyelinating disorder. At first attack it can be difficult for physicians to differentiate between the ADS subtypes due to overlapping clinical features. Yet, accurate diagnosis, prediction of disease course and outcome of different subtypes are imperative for accurate counselling and to initiate the right treatment. This thesis focuses on two rare groups of patients in the ADS spectrum: neuromyelitis optica spectrum disorders (NMOSD) in adults and the spectrum of childhood onset ADS. We aimed to move forward on delineating the spectrum of ADS, and investigating the disease course and outcome of ADS in children and in adults with NMOSD.

In **Chapter 1** the spectrum of ADS and its heterogeneity is described, and the current knowledge of NMOSD and childhood-onset ADS. The spectrum of childhood-onset ADS is outlined, including the disease course and risk factors for MS, and the treatment regimens and outcome of pediatric ADS. NMOSD was previously considered as a rare and severe subtype of MS, with predominant involvement of the optic nerves and spinal cord. Nowadays it is acknowledged as a rare subtype of ADS, and a distinct entity different from MS. The majority of patients are seropositive for the pathognonomic anti-aquaporin 4 antibodies (AQP4-IgG). In addition, the current knowledge on anti-myelin oligodendrocyte glycoprotein antibodies (MOG-IgG) associated ADS is described.

PART ONE: ACQUIRED DEMYELINATING SYNDOMRES, FOCUS ON NEUROMYELITIS OPTICA SPECTRUM DISORDERS

The first part of this thesis focuses on NMOSD in adults. In **chapter 2.1** we studied the presence of MOG-IgG in AQP4-IgG seronegative NMOSD, and compared the clinical features of MOG-IgG seropositive patients with AQP4 seropositive and 'double seronegative' patients. We found that one-third of the AQP4-IgG seronegative NMOSD patients have MOG-IgG antibodies (20/61 patients). MOG-IgG seropositive patients were more frequently males and were more often from Caucasian origin than AQP4-IgG patients. Moreover, MOG-IgG seropositive patients more frequently had a coincident presentation of optic neuritis and transverse myelitis. Overall, a monophasic disease course and favorable outcome were more frequently seen in MOG-IgG patients than AQP4-IgG seropositive patients. NMOSD patients can be classified by their serological antibody status for AQP4-IgG, MOG-IgG, or seronegativity for both antibodies.

As both antibodies are diagnostically tested in one central reference laboratory (Sanquin Diagnostic services) we had a unique opportunity to assess the nationwide incidence figures of these two antibody-associated demyelinating disorders in the Netherlands. **Chapter 2.2** reports that about 15 AQP4-IgG seropositive NMOSD patients are currently being identified per year in the Netherlands. The mean incidence of AQP4-IgG associated NMOSD of 0.09 per year per 100.000 persons, nearly one in a million. In **chapter 2.3**, the mean overall incidence of MOG-IgG seropositive ADS of 0.16 per year per 100.000 persons, with a higher incidence in children (0.31/100.000 persons per year) than in adults (0.13/100.000 persons per year). These epidemiological figures can aid in care and counselling, and the design of potential future clinical trials. In **chapter 2.3**, we additionally we studied 61 MOG-IgG seropositive patients who were known at the NMO expert center or pediatric MS center. We observed a different distribution of clinical phenotypes in children and adults. A long time-to-first-relapse >200 months was observed in a minority of the patients, suggesting that relapse can occur many years later. Lastly, it seems that a great majority of patients, who turned seronegative during follow-up (FU), do not relapse.

PART TWO – CHILDHOOD ONSET ACQUIRED DEMYELINATING SYNDROMES

The second part of this thesis focuses on childhood-onset ADS. One of the main goals of our prospective study in Rotterdam is finding biomarkers that contributes to the differentiation between MS and other ADS, allowing the physician to diagnose the patient accurately and early in the disease course. This part of the thesis is divided in two sections.

Section TWO-A: EARLY and ACCURATE PREDICTORS FOR PEDIATRIC MS

Recently, the international panel on Diagnosis of Multiple sclerosis proposed new revisions to the McDonald 2010 criteria. The revised McDonald 2017 criteria include important modifications by re-introducing CSF oligoclonal bands (OCB) into the criteria and allowing symptomatic lesions to contribute to dissemination in space and time on MRI. In **chapter 3.1** we compared the diagnostic accuracy at the first attack of the revised McDonald 2017 criteria and the former McDonald 2010 criteria of 164 ADS children. We found that the McDonald 2017 criteria were more sensitive (83% vs 49%) and less specific (73% vs 87%) for clinically definite MS (CDMS) diagnosis, but the McDonald 2017 criteria perform well across the age-span with a higher diagnostic accuracy than the 2010 criteria (77% vs 70%). Due to the higher sensitivity, the application of the 2017 criteria will lead to more MS diagnosis at baseline. But because of the decrease in specificity a subgroup of these identified children are likely to have a less active clinical disease course. In addition, these criteria should not be applied to acute disseminated encephalomyelitis patients who present with prominent encephalopathy.

Soluble CD27 (sCD27) in CSF, a protein secreted by activated T-cells, is of predictive value for MS diagnosis and disease course at first attack in adult patients. In **chapter 3.2** we evaluated this marker in 94 ADS children. High levels at baseline in patients without encephalopathy (ADS-) were predictive of CDMS diagnosis and are associated with a shorter time to a second attack of MS (hazard ratio (HR) 2.8 per 100 U/mL increase in sCD27 levels; $p<0.001$), after adjustments for age, oligoclonal bands and presence of dissemination in space on baseline MRI. No difference was seen between ADS children with encephalopathy (ADEM) and monophasic patients without encephalopathy. Moreover, the sCD27 levels found in pediatric MS are higher than in adults MS, which support existing data that children have a more inflammatory disease course than adults.

Neurodegeneration is present already early in the disease course in both adult and pediatric MS patients, despite the disease progression is still slower in children than adults. Neurofilament light chain (NfL) is a marker for neurodegeneration and high levels of NfL in CSF are associated with future MS diagnosis in adults with clinically isolated syndromes (CIS). In **chapter 3.3**, we studied the NfL levels in CSF of the first attack in 88 adult and 65 pediatric patients. We demonstrated that higher CSF NfL levels at first attack predict a second attack in both children

and adults without encephalopathy, after adjustments for currently used markers such as asymptomatic T2 lesions on MRI and OCBs in CSF (children HR 3.7; p=0.007, adults HR 2.1; p=0.032). CSF NfL levels were even higher in children than in adults, implying that children have not only signs of more inflammation but also seem to have more axonal damage than adults. This was supported by our finding that more T1 hypointense lesions were found at the baseline scan in children than in adults. We also saw high levels of CSF NfL in monophasic ADEM patients.

In adult MS, CSF and serum NfL (sNfL) are highly correlated and the levels are associated with future disease activity. We aimed to explore these associations in pediatric ADS patients in **chapter 3.4**. In total, 102 children with a first attack of demyelination and 23 pediatric controls were included. Of the 102 patients, 47 (46%) were also tested for CSF NfL. CSF and serum NfL were positively correlated in the total group (ρ 0.532, p<0.001) and even better in the subgroup of patients with future CDMS diagnosis (ρ 0.773, p<0.001). Serum NfL was higher in patients than in controls. In the ADS group, the highest levels were found in ADEM, followed by patients without encephalopathy (ADS-) with future CDMS diagnosis, and lowest levels were seen in ADS- who remained monophasic. High serum NfL levels at baseline are associated with a shorter time to CDMS diagnosis in ADS- patients (p=0.045). The HR for CDMS diagnosis was 1.09 for each 10 pg/mL increase of sNfL, after correction for age, OCB, ≥9 T2 hyperintense lesions and gadolinium enhanced lesions (p=0.012). The strong correlation between CSF and serum NfL strengthens its reliability as a serum marker of neuroaxonal damage. Moreover, the high CSF and serum NfL levels in children with ADEM suggests that neuroaxonal damage is a prominent feature in ADEM.

Section TWO-B: DISEASE COURSE AND OUTCOME OF CHILDHOOD ONSET ADS

This section focuses on the disease course and outcome of childhood-onset ADS.

Chapter 3.5 and 3.6 described the clinical phenotype and disease course of relapsing MOG-IgG seropositive children, identified by the European MOG consortium. Existing data shows that the presence of serum MOG-IgG pleads against MS diagnosis, but is associated with a higher risk for relapsing non-MS disease. In **chapter 3.5**, we described 17 patients with ADEM followed by optic neuritis (ADEM-ON); 16/17 patients were positive for MOG-IgG. The disease course was heterogeneous and no predictors could be found between patients high and low relapse rates. Twelve patients received oral prednisolone and 10 received maintenance immunosuppression (e.g. azathioprine, intravenous immunoglobulins (IvIG), Rituximab). During a FU of 5.3 years (IQR 1.8–10.2 years), 54 relapses occurred with a median of 3 relapses per patient (range 1–9 per patient). The percentage of patients with cognitive and physical sequelae is high (47%). Patients relapsed on all maintenance immunosuppressive treatments. Patients were prone to relapses when being weaned off oral prednisone, but no relapses occurred on a prednisolone

dose >10 mg/day. This suggests a degree of steroid-dependency and physicians should be aware of the risks of long-term steroid exposure.**Chapter 3.6** describes 102 children with MOG-IgG associated relapsing disorders. The observed clinical phenotype consists of NMOSD (43%), multiphasic ADEM (20%), ADEM-ON (20%) and relapsing ON (18%). Chronic immunosuppressive agents or MS disease modifying agents were given to 52 children (51%). Yet, 127 relapses still occurred in these treated children. Treated patients had more relapses (median 3.0; range 1.0-17.0) than untreated patients (median 1.0; range 0-7.0)($p<0.001$) and had higher Expanded Disability Status Scales (EDSS) scores. IvIG, as a immunomodulatory treatment, seemed to have a better effect on disease activity than other immunosuppressive agents, and MS agents did not decrease the relapse rates. We observed an age-dependent phenotype, with brain manifestations in younger children, and optic neuritis and/or transverse myelitis with normal brain MRI in older children.

Our research group reported a Dutch incidence of ADS and MS in children of 0.66/100.000 per year and 0.15/100.000 per year in the period of 2007-2010. In **chapter 3.7** we presented an updated and increased Dutch incidence of 0.80/100.000 for pediatric ADS and 0.26/100.000 for pediatric MS per year (2011-2016). Since the start of the PROUD-Kids study, 243 were included and the long-term outcome was studied in this chapter. At the end of FU (median 55 months), patients were classified into monophasic ADS (56%), MS (37%) and relapsing non-MS demyelination (7%). At least one form of residual deficit, including cognitive impairment, was found in 69% of the patients. Negatively affected school performance was reported by parents in one-third of our ADS cases, some even requiring academic adjustments.

Fatigue is a frequently reported complaint in pediatric MS and is also reported in ADEM. The cause of fatigue in ADS is unclear. Children with ADEM and MS known in Erasmus MC were consecutively referred to the pediatric physical therapist to screen for motor dysfunction, fatigue and reduced exercise capacity by administering questionnaires and physical tests. In **chapter 3.8**, we showed that children with MS and ADEM are more fatigued, have reduced exercise capacity and impaired motor performance compared to their healthy related peers. Unexpectedly no correlation was found between these three parameters. Also, ADS children had more problems in the emotional, social, scholastic and psychosocial functioning than healthy peers. These findings imply that other factors, such as depression and psychosocial factors, may play a role in the process of inactivity, decreased exercise capacity and fatigue. In addition, we found a large discrepancy between the children with severe or borderline motor deficits measured by the MABCII (49%) and the proportion of children with EDSS score of ≥2 (only 9% MS, 6% ADEM). This discrepancy confirms that the EDSS is not an optimal and sensitive metric for disability in pediatric MS and ADS patients.

Neurogenic lower urinary tract dysfunction (LUTD) is common in adults with MS. Little is known about the prevalence of LUTD in pediatric MS. In **chapter 3.9**, 24 newly diagnosed pediatric MS patients underwent an extensive urological assessment including uroflowmetry with simultaneous (non-invasive) electromyography recordings of the pelvic floor muscles. We observed a high proportion of voiding problems (21%) already in the first 18 months after initial MS diagnosis. Questionnaires about voiding seemed sufficient to detect LUTD in 80% of the patients. One patient would have been missed if patients were not systematically evaluated. Clinicians treating pediatric MS patients should be aware of the high prevalence of LUTD very early in the disease course.

The evolution of MRI lesions during FU in ADEM children is rarely studied. In **chapter 3.10**, 42 ADEM children were included for evaluation. When analyzing patients ≥2 MRI scans during the acute phase (first 3 months after symptom onset), and who had ≥1 scan after this period, we found that the evolution of MRI abnormalities in children with ADEM can be delayed compared to the evolution of clinical symptoms: a normal brain MRI was present in 3/42 patients (performed 3, 7, 30 days after onset) and MRI abnormalities were observed on scans made at day 26, 36 and 40 days respectively. In addition, deterioration of MRI abnormalities (i.e. enlargement of existing lesions or appearance of new lesions) occurs frequently in the acute phase, despite the clinical improvement of the patient. Deterioration of the scan rarely occurs after this three-month period. Therefore performing a new MRI three months after symptom onset is advised to have a new reference point for future clinical relapses.

The main findings from our studies are summarized and discussed in **Chapter 4**, including the recommendations for future research.

5.2
Samenvatting

Verworven demyeliniserende syndromen (Acquired Demyelinating Syndromes, ADS) zijn een groep auto-immuun gemedieerde inflammatoire demyeliniserende syndromen van het centrale zenuwstelsel. Multipele sclerose (MS) is hiervan de meest bekende. ADS kunnen een monofasisch of chronisch beloop hebben. Door overlappende klinische presentaties kan het voor de artsen uitdagend zijn om tijdens een eerste aanval onderscheid te maken tussen verschillende ADS subtypes. Echter is accurate diagnose, voorspelling van ziektebeloop en uitkomst van verschillende subtypes onmisbaar voor voorlichting en start van de juiste behandeling. Dit proefschrift focust zich op twee relatief zeldzame groepen van patiënten in het ADS spectrum: neuromyelitis optica spectrum ziekten (NMOSD) in volwassenen en het spectrum van ADS op de kinderleeftijd. We streven ernaar een stap vooruit te zetten door onderzoek te doen naar subtypen, ziektebeloop en uitkomsten in kinderen met ADS en in volwassenen met NMOSD.

In **hoofdstuk 1** wordt het heterogene spectrum van ADS beschreven en de huidige kennis van NMOSD en ADS op kinderleeftijd samengevat. Het spectrum van ADS op de kinderleeftijd is uiteengezet, met onder andere het ziektebeloop en risicofactoren voor diagnose MS. Tevens zijn de behandelopties en uitkomsten van kinder-ADS beschreven. NMOSD werd eerder gezien als een ernstige vorm van MS, waarbij vooral de oogzenuwen en ruggenmerg zijn aangedaan. Inmiddels wordt het erkend als een zeldzaam ADS subtype wat geheel losstaat van MS. Merendeel van deze patiënten zijn seropositief voor de pathognomonische anti-aquaporine 4 (anti-AQP4) antistoffen. In dit hoofdstuk wordt daarnaast de huidige kennis over anti-myeline oligodendrocyt glycoproteine (anti-MOG) antistoffen geassocieerde ADS beschreven.

DEEL 1: VERWORVEN DEMYELINISERENDE SYNDROMEN – FOCUS OP NEUROMYELITIS OPTICA SPECTRUM ZIEKTEN

Het eerste gedeelte van dit proefschrift focust zich op NMOSD in volwassenen. In **hoofdstuk 2.1** hebben we de aanwezigheid van anti-MOG antistoffen onderzocht in NMOSD patiënten zonder anti-AQP4 antistoffen en ook vergeleken we de klinische kenmerken van anti-MOG seropositieve met anti-AQP4 seropositieve en 'dubbel seronegatieve' patiënten. We hebben gevonden dat 1/3 van de anti-AQP4 seronegatieve NMOSD patiënten anti-MOG positief zijn (20/61 patiënten). Anti-MOG positieve patiënten zijn vaker man en van Kaukasische afkomst vergeleken met AQP4-IgG positieve patiënten. Daarnaast hebben anti-MOG positieve NMOSD patiënten vaker tegelijkertijd een neuritis optica en myelitis transversa. In het algemeen hebben patiënten met anti-MOG antistoffen vaker een monofasisch beloop en gunstigere uitkomst dan anti-AQP4 patiënten.

Omdat zowel de anti-MOG als anti-AQP4 antistoffen in Nederland worden getest in een centraal diagnostisch laboratorium (Sanquin Diagnostiek), hadden we de unieke kans om de nationale incidentie van deze twee antistof geassocieerde demyeliniserende syndromen in Nederland te onderzoeken. In **hoofdstuk 2.2** rapporteren we dat er in Nederland jaarlijks gemiddeld 15 anti-AQP4 positieve NMOSD patiënten worden gediagnosticeerd. Hiermee is de gemiddelde incidentie van anti-AQP4 geassocieerde NMOSD 0.09 per jaar per 100.000 personen; wat neerkomt op bijna 1 op de miljoen personen. In **hoofdstuk 2.3** hebben we de gemiddelde incidentie voor anti-MOG seropositieve ADS in Nederland berekend, dit was 0.16 per jaar per 100.000 personen, wat afgerond neerkomt op 2 personen per miljoen. Hierbij zagen we een hogere incidentie in kinderen (0.31/100.000 personen per jaar) dan in volwassenen (0.13/100.000 personen per jaar). Deze epidemiologische cijfers kunnen niet alleen bijdragen aan de voorlichting van patiënten, maar ook aan het opzetten van toekomstige klinische trials.

In **hoofdstuk 2.3** hebben we tevens de 61 anti-MOG positieve patiënten, die bekend waren in het nationale NMO en kinder MS centrum, bestudeerd. We zagen dat het klinische fenotype van kinderen en volwassenen verschillend is. Een minderheid van de patiënten had een relapse na meer dan 200 maanden na de eerste aanval. Dit laat zien dat een nieuwe relapse zelfs mogelijk is na een dergelijk lange periode. Ten slotte observeerden we dat bij de meerderheid van de patiënten die tijdens follow-up seronegatief werden, er geen nieuwe aanvallen meer optreden.

DEEL 2 – VERWORVEN DEMYELINISERENDE SYNDROMEN OP DE KINDERLEEFTIJD

Het tweede deel van dit proefschrift focust zich op ADS op de kinderleeftijd. Een van de hoofddoelen van de prospectieve PROUD-kids studie in Rotterdam is het vinden van biomarkers die bijdragen aan het differentiëren tussen MS en andere vormen van ADS. Dit kan artsen helpen om vroeg in het ziektebeloop een accurate diagnose te stellen. Deel 2 van dit proefschrift is verdeeld in 2 secties.

Sectie 2A – Vroege en accurate voorspellers voor diagnose MS op de kinderleeftijd

De internationale werkgroep voor het diagnosticeren van MS (International Panel on Diagnosis of MS) heeft de voormalige McDonald 2010 criteria aangepast. De belangrijkste aanpassingen in de gereviseerde McDonald 2017 criteria zijn de re-introductie van de oligoclonale banden (OCB) in liquor en de toevoeging dat symptomatische lesies kunnen bijdragen aan spreiding in tijd en plaats op de MRI-scan. In **hoofdstuk 3.1** vergelijken we de diagnostische accuraatheid van de McDonald 2010 en 2017 criteria in 164 kinderen met een eerste aanval van ADS. De McDonald 2017 criteria zijn sensitiever (83% vs 49%) en minder specifiek (73% vs 87%) voor 'clinically definite MS' (CDMS; c.q. een tweede aanval van MS). De McDonald 2017 criteria zijn toepasbaar op kinderen van alle leeftijden. Door de hogere sensitiviteit identificeren we meer kinderen met diagnose MS ten tijde van de eerste aanval. Maar door de lagere specificiteit zal er een subgroep MS patiënten zijn die een minder actief ziektebeloop heeft. De McDonald 2017 criteria niet accuraat voor kinderen met de diagnose ADEM (acuut gedissemineerde encephalomyelitis).

Soluble CD27 (sCD27) in liquor wordt uitgescheiden door geactiveerde T-cellen en is voorspellend voor MS diagnose en ziekbeloop op het moment van een eerste aanval in volwassen patiënten. In **hoofdstuk 3.2** hebben we deze biomarker bekeken in 94 ADS kinderen op het moment van de eerste aanval. Hoge levels ten tijde van de eerste aanval bij ADS kinderen zonder encefalopathie (ADS-) waren voorspellend voor de diagnose CDMS. Tevens zijn hoge levels in deze groep geassocieerd met een kortere tijd tot een tweede aanval van MS (hazard ratio (HR) 2.8 per 100 U/mL toename in sCD27 levels; $p<0.001$), na het corrigeren voor leeftijd, OCB en aanwezigheid van spreiding in plaats op de eerste MRI-scan. We zagen geen verschil in de levels bij kinderen met encefalopathie (ADEM (of evt ADS+) en monofasische ADS- kinderen. Daarnaast viel op dat sCD27 levels bij kinderen met MS hoger waren dan bij volwassenen met MS. Dit gegeven ondersteunt de bestaande literatuur dat kinderen een meer inflammatoir ziektebeloop hebben dan volwassenen.

Neurodegeneratie lijkt al vroeg in het ziektebeloop aanwezig te zijn bij zowel volwassenen als kinderen met MS, ondanks dat de ziekteprogressie bij kinderen over het algemeen trager is

dan bij volwassenen. Neurofilament light chain (NfL) is een marker voor neurodegeneratie en hoge NfL levels zijn geassocieerd met MS diagnose in patiënten met een CIS (clinically isolated syndrome). In **hoofdstuk 3.3** hebben we de NfL levels in liquor vergeleken tussen 88 volwassenen en 65 kinderen op het moment van een eerste aanval van demyelinisatie in het centraal zenuwstelsel. We tonen aan dat hogere NfL levels in liquor ten tijde van de eerste aanval, een tweede aanval voorspellen in patiënten die zich presenteren zonder encefalopathie; dit is ook het geval na correctie voor asymptomatische T2 lesies op de MRI-scan en oligoclonale banden in liquor (HR bij kinderen 3.7, p=0.007; HR bij volwassenen 2.1, p=0.032). De liquor NfL levels zijn zelfs hoger bij kinderen dan bij volwassenen. Dit zou kunnen betekenen dat kinderen niet alleen een meer inflammatoire ziekte hebben, maar ook dat kinderen meer axonale schade hebben dan bij volwassenen vroeg in het ziektebeloop. Dit wordt verder ondersteund door een groter aantal T1 hypointense lesies op de baseline MRI, die geassocieerd zijn met hogere NfL levels, in kinderen dan in volwassenen. Tevens zagen we opvallend hoge liquor NfL levels in kinderen met een monofasisch ADEM.

In volwassen MS patiënten zijn liquor en serum NfL sterk met elkaar gecorreleerd en zijn de levels geassocieerd met toekomstige ziekteactiviteit. In **hoofdstuk 3.4** hebben we deze correlatie en associatie onderzocht bij ADS kinderen. In totaal zijn er 102 kinderen met een eerste aanval van ADS en 23 symptomatische kindercontroles geïncludeerd die allen werden getest voor NfL in serum (sNfL). Van de 102 patiënten hebben we van 47 (46%) ook NfL in liquor gemeten. Er was een correlatie tussen NfL in liquor en NfL in serum in de totale groep (correlatie coëfficiënt Pearson rho 0.532, p<0.001). In de subgroep van patiënten met CDMS diagnose tijdens follow-up was deze correlatie sterker (ρ 0.773, p<0.001). Serum NfL was hoger in patiënten dan in controles. Binnen de ADS patiëntengroep zijn de hoogste levels gevonden in kinderen met ADEM, gevolgd door kinderen die zich presenteerden zonder encefalopathie (ADS-) én CDMS diagnose kregen tijdens follow-up, en ten slotte gevolgd door ADS- patiënten die monofasisch bleven. Hoge sNfL levels ten tijde van de eerste aanval zijn geassocieerd met vaker diagnose CDMS en een kortere tijd tot CDMS diagnose in ADS- patiënten (p=0.045). De HR voor CDMS was 1.09 voor elke 10 pg/mL stijging van sNfL, dit was na correctie voor leeftijd, OCB, >9 T2 hyperintense lesies en aankleurende lesies na gadolinium op de baseline MRI-scan (p=0.012).

De sterke correlatie tussen NfL in liquor en serum versterkt de betrouwbaarheid van serum NfL als een marker voor neuro-axonale schade. Daarnaast wijzen de hoge levels in zowel liquor als serum in ADEM op neuro-axonale schade bij deze aandoening.

Sectie 2B: Ziektebeloop en uitkomsten bij ADS op de kinderleeftijd

In **hoofdstuk 3.5 en 3.6** beschrijven we de klinische fenotypen en het ziektebeloop van recidiverende anti-MOG seropositieve kinderen, die geïdentificeerd zijn door het Europese MOG consortium. Uit bestaande literatuur blijkt dat de aanwezigheid van anti-MOG tegen MS diagnose pleit en dat de aanwezigheid van anti-MOG is geassocieerd met een hoger risico op een recidiverende aandoening anders dan MS. In **hoofdstuk 3.5** beschrijven we 17 kinderen die na een ADEM recidiverend een of meerdere neuritis optica hebben doorgemaakt (ADEM-ON); 16/17 patiënten zijn positief getest voor anti-MOG. Het ziektebeloop is heterogeen en er zijn geen voorspellers gevonden voor patiënten die veel of weinig relapsen hadden tijdens follow-up. Twaalf patiënten werden behandeld met oraal prednison en 10 patiënten met prednison-sparende immuunsuppressie (o.a. azathioprine, intraveneuze immunoglobulines (IvIG), Rituximab. Tijdens een follow-up van 5.3 jaar (IQR 1.8-10.2), deden zich 54 aanvallen voor, met een mediaan van 3 aanvallen per patiënt (range 1-9). Een groot percentage patiënten had last van cognitieve en fysieke problemen (47%). Geen van de prednison-sparende immuunsuppressiva was effectief genoeg om patiënten aanvalsvrij te houden. Daarentegen zijn er geen relapsen waargenomen tijdens prednisongebruik van meer dan 10 mg per dag, maar zijn patiënten bij het afbouwen van prednison onder deze dosering gevoelig voor een relapse. Dit wijst op enige mate van prednison-afhankelijkheid, waarbij men bewust dient te zijn van de risico's van lange termijn blootstelling aan corticosteroïden bij kinderen. In **hoofdstuk 3.6** beschrijven we 102 kinderen met een anti-MOG geassocieerde recidiverende ziekte. De klinische fenotypen bestonden uit NMOSD (43%), multifasisch ADEM (20%), ADEM-ON (20%) en recidiverende neuritis optica (18%). Totaal werden 52 kinderen (51%) behandeld met chronische immunosuppressiva of MS medicatie. Desondanks traden er in deze behandelde kinderen 127 aanvallen op. Behandelde patiënten hadden meer relapsen dan onbehandelde patiënten (mediaan 3.0, range 1-17 vs mediaan 1.0, range 0-7.0)($p<0.001$) en hadden daarnaast een hogere score voor invaliditeit (EDSS). IvIG, dat een immunomodulatoir effect heeft, leek een beter effect te hebben op ziekteactiviteit dan andere immunoosuppressiva. MS middelen lieten geen effect op de ziekteactiviteit zien. We observeerden een leeftijdsafhankelijk fenotype, waarbij jongere kinderen vaker een klinische presentatie met betrokkenheid van de hersenen hadden. Oudere kinderen daarentegen hadden juist vaker een neuritis optica en/of een myelitis transversa met een normale MRI hersenen.

Onze onderzoeksgroep heeft eerder een Nederlandse incidentie voor ADS en MS bij kinderen gerapporteerd van respectievelijk 0.66 per 100.000 per jaar en 0.15 per 100.000 per jaar in de periode van 2007-2010. In **hoofdstuk 3.7** presenteren we een update van de toegenomen Nederlandse incidentie van 0.80/100.000 voor kinderen met ADS en 0.26/100.000 voor kinderen met MS (2011-2016). Sinds de start van de PROUD-kids study zijn 243 kinderen geïncludeerd en zijn de lange termijn uitkomsten bestudeerd. Bij een mediane follow-up van 55 maanden zijn patiënten onderverdeeld in monofasisch ADS (56%), MS (37%) en recidiverende non-

MS aandoeningen (7%). Van de patiënten heeft 69% ten minste één vorm van restklachten, inclusief cognitieve beperkingen. Negatieve impact op schoolprestaties is gerapporteerd in een derde van de ADS patiënten, waarbij soms schoolaanpassingen nodig waren.
Vermoeidheid is een frequent gerapporteerde klacht in kinderen met MS en ADEM. De oorzaak voor vermoeidheid is onbekend. Kinderen met ADEM en MS die werden behandeld in het Erasmus MC zijn systematisch verwezen naar de kinderfysiotherapeut voor een screening van motore dysfunctie, vermoeidheid en verminderde conditie door middel van het afnemen van vragenlijsten en fysieke testen. In **hoofdstuk 3.8** laten we zien dat kinderen met MS en ADEM meer en vaker vermoeid zijn en daarnaast een slechtere conditie en motore functie hebben vergeleken met gezonde leeftijdsgenootjes. We hebben geen correlatie gevonden tussen deze drie parameters. Bovendien hadden kinderen met ADS meer problemen in zowel het functioneren op emotioneel, sociaal en psychosociaal gebied als in het functioneren op school. Daarnaast hebben we een grote discrepantie waargenomen tussen het percentage kinderen met ernstige of borderline motorische dysfunctie bij de MABCII test (49%) en het percentage kinderen dat een EDSS score van twee of hoger heeft (9% MS, 6% ADEM). Deze discrepantie bevestigt dat de EDSS schaal geen optimaal en sensitief meetinstrument is voor het uitdrukken van beperkingen in kinderen met MS en ADS.

Neurogeen blaaslijden komt vaak voor bij volwassenen met MS. Er is weinig bekend over deze klachten bij kinderen met MS. In **hoofdstuk 3.9** hebben 24 nieuw gediagnosticeerde kinderen met MS een uitgebreide urologische screening gekregen inclusief uroflowmetrie, waarbij tegelijkertijd een (niet-invasieve) elektromyografie van de bekkenbodemspieren werd verricht. Een groot aantal kinderen had reeds mictieproblemen in de eerste 18 maanden na diagnose(21%). Mictievragenlijsten waren voldoende om 80% van de patiënten met een neurogene blaas te detecteren; één patiënt zou gemist zijn wanneer deze kinderen niet systematisch werden doorverwezen voor de urologische screening. Artsen die kinderen met MS behandelen dienen alert te zijn op het voorkomen van neurogeen blaaslijden vroeg in het ziektebeloop.

De verandering van MRI lesies tijdens follow-up van ADEM patiënten is weinig onderzocht. In **hoofdstuk 3.10** werden 42 kinderen met een ADEM geïncludeerd voor analyse. Bij ADEM kinderen die ≥2 MRI scans hadden in de acute fase (eerste 3 maanden na het ontstaan van klachten) en ≥1 scan na de acute fase zagen we dat de veranderingen van MRI afwijkingen achterloopt ten opzichte van de klinische symptomen: in 3/42 patiënten was de MRI normaal bij debuut (MRI verricht 3, 7 en 30 dagen na onset) en werden MRI afwijkingen zichtbaar op follow-up scans gemaakt op respectievelijk dag 26, 36 en 40. Daarnaast zagen we dat verslechtering van MRI afwijkingen (e.g. toename in grootte van bestaande lesies of ontstaan nieuwe lesies) vaak voorkomt in de acute fase, ondanks verbetering in de kliniek. Toename van afwijkingen (in grootte en/of aantal) gebeurt zeer zelden na de eerste drie maanden. Derhalve adviseren

we 3 maanden na het ontstaan van symptomen een nieuwe MRI te verrichten als nieuwe uitgangswaarde voor eventuele nieuwe aanvallen.

De belangrijkste bevindingen van onze studies zijn samengevat en bediscussieerd in **hoofdstuk 4.** Hier worden ook aanbevelingen gedaan voor toekomstig onderzoek.

6.
Epilogue

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Many thanks to all co-authors of the European MOG consortium. I hope this collaborative effort will be a stepping stone towards an expansion in knowledge on MOG associated demyelinating syndromes. I look forward to the future projects!

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ABOUT THE AUTHOR

Yu Yi M. Wong was born on October 17th 1987 in Nijmegen and was raised in Druten, The Netherlands. In 2006 she started her studies in Medicine at the Radboud University Medical Center in Nijmegen. After obtaining her Bachelor in Medicine, she initiated and executed a research project about predictive factors for dementia in Parkinson's disease in 2009-2010 at the Prince of Wales hospital, Chinese University of Hong Kong in Hong Kong (supervisor prof. dr. Vincent C.T. Mok). After her return she continued on her Master in Medicine with clinical rotations.

For her master thesis she temporarily moved to Rotterdam in 2013 to study the MRI patterns in pediatric acute disseminated encephalomyelitis at the department of Pediatric Neurology at Erasmus MC-Sophia, Rotterdam (supervisor: dr. R.F. Neuteboom). After graduation in November 2013, she worked as a resident at the Department of Neurology at the St. Antonius Hospital in Nieuwegein.

In June 2014 she started her PhD research at Erasmus MC Rotterdam, including the National Neuromyelitis Optica and National Pediatric MS center, under supervision of Prof. dr. R.Q. Hintzen. She succeeded dr. Daniëlle van Pelt as coordinator of the PROUD-kids study (PRedicting the OUtcome of a Demyelinating event), the Dutch multicenter and prospective study for acquired demyelinating syndromes in children. The results of these 4 years of research are presented to you in this thesis. Currently she is working as a resident in Neurology at Erasmus MC Rotterdam (head: prof. dr. P.A.E. Sillevis Smitt).

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CL de Mol*, **YYM Wong***, ED van Pelt, BHA Wokke, TAM Siepman, RF Neuteboom, D Hamann, RQ Hintzen. The Spectrum of anti-MOG associated demyelinating syndromes. *Submitted*

YYM Wong, CL de Mol, RM van der Vuurst de Vries, ED van Pelt, IA Ketelslegers, CE Catsman-Berrvoets, RF Neuteboom**, RQ Hintzen**, On behalf of the Dutch Pediatric MS and ADEM study group. Real world validation of the 2017 McDonald criteria for pediatric multiple sclerosis. *Accepted, Neurology: Neuroimmunology & Neuroinflammation 2018*

RM van der Vuurst de Vries, JY Mescheriakova, **YYM Wong**, TF Runia, N Jafari, JP Samijn, JWK de Beukelaar, BHA Wokke, TAM Siepman, RQ Hintzen. Application of the 2017 revised McDonald criteria for multiple sclerosis to patients with a typical clinically isolated syndrome. *Accepted, Jama Neurology 2018*

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PHD PORTFOLIO

PhD training		
Courses	**Year**	**Workload (ECTS)**
Expanded Disability Status scale training	2014	0.2
Introduction into Data analysis (NIHES)	2014	0.7
Excel basic workshop (MolMed)	2014	0.3
Basic genetics (MolMed)	2014	0.6
Basic SNPs course (MolMed)	2014	2.0
Research Integrity	2015	0.3
Introduction in GraphPad Prism	2015	0.3
Basiscursus Regelgeving Klinisch onderzoek (BROK)	2016	1.4
Survival Analysis (MolMed)	2016	0.6
Microbiome (MolMed)	2016	0.5
Advanced Immunology (MolMed)	2017	1.5
Biomedical English writing and communication	2017	4.0
Oral presentations		
Childhood-onset MS; Regionaal MS symposium; Maasstad ziekenhuis, Rotterdam	2014	1.2
Neuromyelitis optica spectrum disorders; ErasMS Rotterdam	2014, 2017	1.2
Disease course and outcome of children with ADEM followed by optic neuritis; International Child Neurology Congress, Amsterdam	2016	1.2
Prediction of pediatric MS: bands do aid; Meeting of the Dutch MS Research Foundation	2016	1.2
Disease course and outcome of children with ADEM followed by optic neuritis; Meeting of the Dutch MS Research Foundation	2016	1.2
Soluble CD27 in pediatric ADS; Voorjaarsvergadering NVKN	2017	1.2
Validation of the McDonald 2017 criteria for MS in children; Voorjaarsvergadering NVKN	2018	1.2
Poster presentations		
ECTRIMS (5 posters)	2015-2017	5.0
International Child Neurology Congress, Amsterdam	2016	1.0
Meeting of the Dutch MS Research foundation, Amsterdam	2016	1.0
Wetenschappelijke vergadering, Nunspeet	2017	1.0

(Inter)national conferences		
Congress of the European Committee of Treatment and Research in MS (ECTRIMS); Boston (2014), Barcelona (2015), London (2016), Paris (2017)	2014-2017	4.0
Meeting of the Dutch MS Research foundation	2015, 2016	1.0
International Child Neurology Congress, Amsterdam	2016	1.0
MS symposium VUMC, Amsterdam	2016, 2017	1.0
Wetenschappelijke vergadering NVN	2017	0.5
Voorjaarsvergadering NVKN	2017, 2018	1.0
2. Teaching		
Lecturing		
Lectures 'Acquired demyelinating syndromes in childhood' and 'Treatment of childhood-onset MS', medical students, minor in pediatric neurology, Erasmus MC, Rotterdam	2014, 2015, 2017	1.0
Supervising/guiding (master) students		
Guiding pre-university students in their final school year with their research paper on pediatric MS (Aleid Bax, Sammie Yam)	2015	1.0
C. Louk de Mol, January 2017-May 2018, Clinical Research Master (NIHES), Rotterdam	2017-2018	10.0
3. Other		
International investigators meeting PARADIGMS (Fingolimod vs Avonex) trial, Frankfurt, Germany	2014	1.0
Organizer of the 'Symposium for neuroimmunological disorders in children', Rotterdam	2016	1.5
Sub-investigator in clinical trials for pediatric MS Fingolimod (PARADIGMS), Teriflunomide (Aubagio)	2014-2018	2.0
Co-investigator in clinical trials for adult RRMS and PPMS (Fingolimod, Ocrelizumab, BIIB033)	2014-2018	1.5
Total ECTS		**36.4**

LIST OF ABBREVIATIONS

ACE	Academic centers of Excellence
ADEM	acute disseminated encephalomyelitis
ADEM-ON	acute disseminated encephalomyelitis followed by (recurrent) optic neuritis
ADS	Acquired demyelinating syndromes
AID	Auto-immune disease
AQP4	Anti-aquaporin 4
ARR	Annualized relapse rate
CBA	Cell-based assay
CDMS	Clinically definite multiple sclerosis
CIS	Clinically isolated syndrome
CNS	Central nervous system
CSF	Cerebrospinal fluid
DIS	Dissemination in space
DIT	Dissemination in time
DMT	Disease modifying therapy
DMFI	Delta mean fluorescence intensity
EDSS	Expanded disability status scale
ELISA	Enzyme-Linked Immuno Sorbent Assay
FACS	Fluorescence activated cell sorting
FLAIR	Fluid-attenuated inversion recovery
FU	Follow-up
HEK293	Human embryonic kidney cell line
HR	Hazard ratio
HRQoL	Health-related quality of life
ICU	Intensive care unit
IgG / IgM	Immunoglobulin G and immunoglobulin M
IMT	Immunomodulatory treatment
IPMSSG	International Pediatric Multiple Sclerosis Study Group
IvIG	Intravenous immunoglobulins
IvMP	Intravenous methylprednisolone
LETM	Lontigudinally extensive transverse myelitis
LN18	Human malignant glioma cell line
LP	Lumbar puncture
LUTD	Lower urinary tract dysfunction
MDEM	Multiphasic ADEM
MMF	Mycophenolate mofetil

MOG	Myelin oligodendrocyte glycoprotein
Mono-ADS	Monophasic ADS
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NfL	Neurofilament Light chain
NMOSD	Neuromyelitis optica spectrum disorders
NPA	Neuropsychological assessment
OCB	Oligoclonal bands
ON	Optic neuritis
OND	Other neurological diseases
OPT	Oral prednisone taper
PROUD-kids	PRedicting the OUtcome of Demyelinating syndromes in children study
SC	Spinal cord
sCD27	Soluble CD27
SD	Standard deviation
SPMS	Secondary progressive multiple sclerosis
RDS	Relapsing demyelinating disorder
RTX	Rituximab
RON	Relapsing optic neuritis
RR	Relative risk
RRMS	Relapsing remitting multiple sclerosis
TM	Transverse myelitis
TTFR	Time to first relapse
VA	Visual acuity
WBC	White blood count

MOVING FORWARD ON ACQUIRED DEMYELINATING SYNDROMES

DIAGNOSIS, DISEASE COURSE AND OUTCOME

Acquired demyelinating syndromes (ADS) encompass a broad spectrum of inflammatory demyelinating disorders of the central nervous system. This thesis focuses on two rare groups of patients in the ADS spectrum: neuromyelitis optica spectrum disorders (NMOSD) in adults and the spectrum of childhood onset ADS. Accurate diagnosis and prediction of disease course is imperative for accurate counselling and treatment opportunities. We aimed to move forward on the spectrum of ADS by investigating predictive factors for an accurate diagnosis and by describing the disease course and outcome of these patients.